UNIVERSIDADE FEDERAL DE LAVRAS

ANDREA JARAMILLO MESA

ANATOMICAL, COMPUTATIONAL AND MOLECULAR ANALYSES OF VASCULARIZATION IN COFFEE PEDUNCLES OF REPRODUCTIVE BUDS AND FRUITS: FUTURE PERSPECTIVES FOR THE *Coffea arabica* L.

LAVRAS-MG
2024

ANDREA JARAMILLO MESA

ANATOMICAL, COMPUTATIONAL AND MOLECULAR ANALYSES OF VASCULARIZATION IN COFFEE PEDUNCLES OF REPRODUCTIVE BUDS AND FRUITS: FUTURE PERSPECTIVES FOR THE *Coffea arabica* L.

Thesis submitted for the Degree of Doctor of Philosophy in Agronomy concentration area of Plant Physiology Postgraduate Program of Federal University of Lavras

Prof. Antonio Chalfun Junior, Ph.D
Supervisor

Ph.D. Raphael Ricon de Oliveira
Co-Supervisor

LAVRAS-MG
2024

Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

JARAMILLO MESA, ANDREA.
Anatomical, computational and molecular analyses of vascularization in coffee peduncles of reproductive buds and fruits; future perspectives for the *Coffea arabica* L. / ANDREA JARAMILLO MESA. - 2024.
135 p. : il.

Orientador(a): Antonio Chalfun-Junior.
Coorientador(a): Raphael Ricon de Oliveira.
Tese (doutorado) - Universidade Federal de Lavras, 2024.
Bibliografia.

1. *Coffea arabica* L. 2. Vascularizatio in reproductive peduncles. 3. Gene characterization. 4. RNAseq/RT-qPCR expression analyses. I. Chalfun-Junior, Antonio. II. de Oliveira, Raphael Ricon. III. Título.

ANDREA JARAMILLO MESA

ANATOMICAL, COMPUTATIONAL AND MOLECULAR ANALYSES OF VASCULARIZATION IN COFFEE PEDUNCLES OF REPRODUCTIVE BUDS AND FRUITS: FUTURE PERSPECTIVES FOR THE *Coffea arabica* L.

ANÁLISES ANATÔMICAS, COMPUTACIONAIS E MOLECULARES DA VASCULARIZAÇÃO EM PEDÚNCULOS DE GEMAS FLORAIS E FRUTOS DE CAFÉ: PERSPECTIVAS FUTURAS PARA O *Coffea arabica* L.

Thesis submitted for the Degree of Doctor of Philosophy in Agronomy concentration area of Plant Physiology Postgraduate Program of Federal University of Lavras

Approved on July 30th 2024
Stefan de Folter, Ph.D. Cinvestav, Mexico
Luciano Pereira, Ph.D. Universität Ulm, Institut für Botanik, Germany
Prof. Thiago Alves Magalhães, Ph.D. UFLA, Brazil
Prof. Paulo Eduardo Ribeiro Marchiori, Ph.D. UFLA, Brazil

Prof. Antonio Chalfun Junior, Ph.D.
Supervisor

Ph.D. Raphael Ricon de Oliveira
Co-Supervisor

LAVRAS-MG
2024

To Baguira, Beatriz, Carlos, David, Gloria, Thales and my beloved daughter
Sarah Cherubino-Jaramillo

Agradecimentos

Gostaria de agradecer ao Brasil por me acolher durante meus estudos, à Universidade Federal de Lavras (UFLA), aos membros do Programa de Pós-Graduação em Fisiologia Vegetal e ao Laboratório de Fisiologia Molecular em Plantas (LFMP) pela grande oportunidade. Agradeço a orientação do professor Antonio Chalfun-Junior e a co-orientação do Dr. Raphael Ricon de Oliveira. Da mesma forma, agradeço a cada um dos membros do LFMP pelo seu apoio e cooperação durante a minha estadia.

Agradeço á Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) e ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) pelo apoio financeiro. Agradeço também ao PhD. Blake Meyers por me permitir o uso de seus servidores e do professor Dr. José Donizeti Alves por me abrir uma porta cheia de oportunidades.

Agradeço a todos os meus amigos, Mayra, Zulma, Isa bebê, Héctor, Dayana, Karen, Viviana, Janet, Robert e Dawyson pelo grande apoio, compreensão, momentos cheios de risadas e sincera amizade durante minha estadia no Brasil e a Tokyo, Artemis e Kiara por ser eles. Minha imensa gratidão às minhas amigas, minhas "mujeres medicina" Stephany, Ana María e Marian, que foram um apoio muito importante em momentos transcendentais da minha vida.

Agradeço sinceramente aos meus anjos neste planeta, meus pais Gloria e Carlos, que têm sido o pilar da minha vida. Agradeço também ao meu irmão David pelo apoio incondicional, às minhas avós Beatriz e Rosaura que ainda me acompanham e me guiam em alma e à minha linda felina e curandeira energética e companheira Baguira.

Agradeço também muito o apoio do meu parceiro Thales, que tem sido uma luz no meu caminho. Agradeço à nossa bebê Sarah, que é minha mestra de vida e que em muitos momentos difíceis, com sua alegria e ternura, me deu forças para continuar com meu doutorado.

Agradeço também ao universo e a cada uma das pessoas com quem convivi ao longo deste percurso, especialmente a todos aqueles que acreditaram em mim, familiares, amigos, colegas, professores, e a quem corresponda.

Agradeço à "Pacha-mama", à natureza e especialmente a todas aquelas plantas de café que me forneceram suas flores e frutos para o nosso trabalho. Por fim, gostaria de agradecer cada momento vivido e cada aprendizado. Mas o que mais valorizo e aprecio são as lições de vida. Obrigada!

RESUMO GERAL

Os grãos de café são uma *commodity* agrícola predominantemente cultivada em regiões tropicais, sustentando os meios de subsistência de milhões de pessoas. O processo de floração das plantas de café é indispensável para o desenvolvimento dos grãos, mas frequentemente sofre com o desenvolvimento assincrônico, resultando em maturação inconsistente dos frutos. Esse problema é particularmente pronunciado no Brasil, onde a colheita mecanizada mistura grãos verdes e maduros, afetando negativamente a qualidade. Além disso, a floração não uniforme complica o manejo de pragas e doenças. Dada a sensibilidade dos botões florais à disponibilidade de água, compreender o desenvolvimento vascular nos órgãos reprodutivos, especialmente nos pedúnculos que conectam inflorescências aos ramos, é necessário. Nosso estudo investigou os aspectos anatômicos e moleculares do desenvolvimento vascular nos órgãos reprodutivos do café por meio de uma combinação de análises anatômicas, moleculares e computacionais. Realizamos análises *in silico* para identificar genes-chave envolvidos no desenvolvimento vascular, como os ortólogos pertencentes a família Homeodomain Leucine Zipper Class III (*HB Zip III*) e *OCTOPUS* (*OPS*) de *Arabidopsis thaliana*, e *VASCULAR-RELATED NAC-DOMAIN7* (*VND7*) de *Populus trichocarpa*. Em seguida, quantificamos os níveis de expressão de genes pertencentes aos grupos ortólogos (*HB*, *OPS* e *VND7*) no café. Por fim, combinamos a análise *in silico* com um estudo abrangente da vasculatura dos pedúnculos em 16 estágios, incluindo o desenvolvimento de gemas florais, flores e frutos. Nossas descobertas aprofundam a compreensão do desenvolvimento vascular no café, fornecendo novas percepções que podem melhorar as práticas de cultivo, otimizando as condições para floração e frutificação. Este estudo visa preencher a lacuna de conhecimento sobre o desenvolvimento anatômico e vascular dos pedúnculos de café, oferecendo uma visão abrangente de seu papel no suporte ao crescimento e maturação das flores e frutos do cafeeiro.

Palavras-chave: gemas florais e frutos de café; desenvolvimento vascular do pedúnculo; caracterização gênica; análise de expressão de RNA-seq/RT-qPCR.

GENERAL ABSTRACT

Coffee beans are an agricultural commodity predominantly cultivated in tropical regions, supporting the livelihoods of millions. The flowering process of coffee plants is indispensable for bean development but often suffers from unevenness, resulting in inconsistent fruit maturation. This issue is particularly pronounced in Brazil, where mechanized harvesting blends unripe and ripe beans, negatively affecting quality. Moreover, non-uniform flowering complicates pest and disease management. Given the sensitivity of floral buds to water availability, understanding vascular development in reproductive organs, especially peduncles that connect inflorescences to branches, is necessary. Our study investigates the anatomical and molecular aspects of vascular development in coffee's reproductive organs through a combination of anatomical, molecular, and computational analyses. We performed *in silico* analyses to identify orthologous key genes involved in vascular development, such as Class III Homeodomain-Leucine Zipper (*HB Zip III*) and *OCTOPUS* (*OPS*) from *Arabidopsis thaliana*, and *VASCULAR-RELATED NAC-DOMAIN7 (VND7)* from *Populus trichocarpa*. Subsequently, we quantified the expression levels of these orthologous group members (*HB*, *OPS*, and *VND7*). Finally, we combined *in silico* analyses with a comprehensive study of peduncle vasculature across 16 stages, encompassing the development of flower buds, flowers, and fruits. Our findings deepen the understanding of vascular development in coffee, providing insights that could enhance cultivation practices by optimizing conditions for flowering and fruiting. This study aims to fill the knowledge gap regarding the anatomical and vascular development of coffee peduncles, offering a comprehensive view of their role in supporting the growth and maturation of coffee flowers and fruits.

Keywords: coffee floral buds and fruits; peduncle vascular development; gene characterization; RNAseq/RT-qPCR expression analyses.

Impactos sociais, tecnológicos, econômicos e culturais

O estudo teve como objetivo investigar o desenvolvimento vascular dos pedúnculos do cafeeiro, buscando otimizar as condições de floração e frutificação, o que pode gerar impactos tecnológicos e econômicos significativos, especialmente para as populações em regiões produtoras de café, que podem ser afetadas pelas mudanças no regime hídrico provocadas pelo aquecimento global. Este trabalho elucidou anatomicamente a progressão da vascularização em 16 estágios do desenvolvimento das flores e frutos do café, além de avaliar a distribuição e abundância de 55 genes reconhecidos em outras espécies vegetais por seu papel no controle e manutenção da vascularização. Ao aprofundar a compreensão dos mecanismos anatômicos e moleculares que influenciam a floração das plantas de café, espera-se contribuir para a melhoria da qualidade dos grãos colhidos no Brasil e auxiliar no desenvolvimento de estratégias e tecnologias que tornem a produção mais resiliente às imprevisibilidades climáticas. A floração assincrônica, um problema comum no país devido à colheita mecanizada, compromete a qualidade do café e aumenta os desafios de manejo de pragas e doenças. Assim, ao investigar os genes envolvidos no desenvolvimento vascular dos órgãos reprodutivos do cafeeiro, nosso estudo visa fornecer novos conhecimentos que podem ser aplicados para melhorar a eficiência do cultivo e tornar a produção de café mais sustentável em relação ao uso da água. O impacto potencial dessa pesquisa está alinhado aos Objetivos de Desenvolvimento Sustentável (ODS) da ONU, especialmente em relação à inovação, infraestrutura sustentável e desenvolvimento de práticas agrícolas mais resilientes, apoiando pequenos agricultores e reduzindo perdas durante a produção. O desenvolvimento futuro de aplicações práticas oriundas deste estudo beneficiará diretamente as populações envolvidas no cultivo de café, melhorando seus meios de subsistência ao aumentar a qualidade e o valor agregado do produto. O trabalho, portanto, apresenta um potencial caráter extensionista ao envolver o setor cafeeiro e os produtores como público-alvo. As melhorias tecnológicas derivadas desta pesquisa podem ter implicações diretas na sustentabilidade da produção agrícola, especialmente nas áreas de tecnologia, produção, trabalho e economia, promovendo melhores condições de manejo e aumentando a renda agrícola. Por fim, estudos futuros devem integrar membros da comunidade acadêmica com parceiros institucionais e comunidades produtivas localizadas no sul de Minas Gerais, contribuindo para a preservação ambiental, crescimento econômico e uso eficiente dos recursos hídricos.

Social, technological, economic and cultural impacts

The study aimed to investigate the vascular development of coffee peduncles, seeking to optimize flowering and fruiting conditions, which could lead to significant technological and economic impacts, particularly for populations in coffee-producing regions that may suffer from water regime changes caused by global warming. This research anatomically elucidated the progression of vascularization across 16 stages of flower and fruit development in coffee, as well as assessed the distribution and abundance of 55 genes known in other plant species for regulating and maintaining vascularization. By deepening the understanding of the anatomical and molecular mechanisms influencing coffee plant flowering, the study aims to contribute to the improvement of coffee bean quality in Brazil and aid in developing strategies and technologies to make production more resilient to climate unpredictability. Asynchronous flowering, a problematic issue in Brazil due to mechanized harvesting, compromises coffee quality and increases challenges in pest and disease management. Therefore, by investigating the genes involved in the vascular development of coffee's reproductive organs, our study aims to provide new knowledge that can be applied in the field to improve cultivation efficiency, making coffee production more sustainable in terms of water use. The potential impact of this research aligns with the United Nations Sustainable Development Goals (SDGs), particularly in fostering innovation, sustainable infrastructure, and developing more resilient agricultural practices, supporting small farmers and reducing losses during coffee production. The future development of practical applications stemming from this study will directly benefit populations involved in coffee cultivation by improving their livelihoods through increased product quality and added value. The work, therefore, has a potential extensionist character by involving the coffee sector and producers as target audiences. The technological improvements resulting from this research could have direct implications for the sustainability of agricultural production, particularly in the areas of technology, production, labor, and economy, promoting better management practices and increasing agricultural income. Finally, future studies should aim to integrate members of the academic community with institutional partners and productive communities in southern Minas Gerais, contributing to environmental preservation, economic growth, and efficient use of water resources.

SUMMARY

GENERAL INTRODUCTION

Coffee beans stand as a significant global commodity, grown across more than 70 tropical countries, spanning roughly 10 million hectares. The majority of global coffee production consists of *Coffea arabica* (69%), followed by *C. canephora* (30%), and *C. liberica* (1%). For the 2023/24 period, worldwide coffee production is estimated at 178 million bags, with South America leading the output at 89.3 million bags, and Brazil alone contributing 59.1 million bags (Arcila et al., 2001; Nascimento et al., 2008; Alves et al., 2011; Volsi et al., 2019; Deshmukh, 2021; Zanetti et al., 2021; CONAB, 2024).

C. arabica L. (2n = 44), the main source of coffee beans, is an allopolyploid species arising from an interspecific cross between *C. canephora* Pierre ex Froehner and *C. eugenioides* Moore (Lashermes et al., 1999). The *Coffea* genus is the most well-known within the Rubiaceae family (Charrier and Berthaud, 1985), comprising a total of 124 species, with 66 natives to the Indian Ocean Islands (Meyer, 1965; Berthaud and Charrier, n.d.; Yu et al., 2011; Hamon et al., 2015).

Botanically, *Coffea* plants exhibit a tree-like structure with a single robust trunk, dense wood, and horizontal or semi-horizontal plagiotropic branching. Plant growth originates from meristematic cells located at the stem apex, branch tips, and leaf axils, where primordia develop into nodes, leaves, branches, and flowers. The stem apex drives vertical orthotropic growth, while the branch apex promotes lateral plagiotropic growth. In the leaf axils, 4 or 5 buds form, differentiating into flowers or branches. The oldest bud on the main stem develops into horizontal primary branches, while subsequent buds may either form vertical shoots or remain dormant. During the fruiting phase, branches at least one year old produce fruits, enabling the plant to bear crops for over 20 years, with peak productivity occurring between 5 and 10 years of age (Dedecca, 1957; Arcila et al., 2001; Davis et al., 2006; Arcila Pulgarín, 2007).

The *C. arabica* plant takes two years to complete its phenological cycle, undergoing a succession of vegetative and reproductive phases. Camargo and Camargo (2001) described the coffee plant's phenological cycle for the tropical climatic conditions of Brazil in six distinct moments corresponding to the plant's two phenological years. Under specific conditions, some of the coffee plant meristems transition from an undifferentiated state to the reproductive phase. During this phase, environmental stimuli, such as photoperiod, become crucial signals that set the plant for the flowering process (Pezzopane et al., 2003; Camargo and Camargo, 2001). A recent model, revised by López *et al.* (2021), proposed modifications to the phenological cycle described by Camargo and Camargo (2001). Thus, the vegetative and reproductive development in *C. arabica* cultivars was presented in relation to the time of flowering for cultivars considered early or late. After the March equinox—when the duration of the day is reduced to less than 13 hours of light—photoperiod acts as a signal for promoting the development of floral buds. Thus, the transition from axillary vegetative buds to reproductive buds—due to photoperiodicity—defines the stage of floral evocation (López et al., 2021).

Changes in photoperiod, temperature, and water availability trigger morphophysiological alterations in coffee plants, leading to floral development and allowing for phenotypic distinction between vegetative and reproductive shoots (Nunes et al., 2010). The reproductive phase in coffee plants features a gradient of floral bud stages, from undifferentiated buds to flowering, fruiting, and ripening.

Floral development is complex, involving both the inflorescence in the leaf axils and the flowers within each inflorescence. Each node has two axles with opposite leaves, each containing 3 to 4 inflorescences with 4 or 5 flowers each, potentially totaling 24 to 32 floral buds per node forming an inflorescence known as a glomerule (Dedecca, 1957). The bud takes about 12 weeks to develop floral buds.

At the G4 stage, floral buds, measuring 4 - 6 mm in length (Morais et al., 2008), enter a reproductive arrest phase, commonly called dormancy (Alvim, 1958; Alvim, 1960) (Magalhaes and Angelocci, 1976; Crisosto et al., 1992; Drinnan and Menzel, 1994; Wintgens, 2012; Penfield, 2024). This phase is induced by water deficit or endogenous factors and coincides with the dry and winter season in southeastern Brazil. Dormancy persists after cold exposure and continues until the onset of rains, which resumes bud development and leads to flowering (Crisosto et al., 1992; Arcila Pulgarín, 2007; López et al., 2021). However, this dormancy of the floral buds can be disrupted by insufficient cold during winter or by sporadic rains, resulting in multiple episodes of lower-intensity flowering (Hoeberichts et al., 2017). This phenomenon results in non-uniformity in the phenological timing of flowering and subsequent fruit maturation. Consequently, this lack of uniformity in coffee bean maturation can hinder pest and disease control efforts and negatively impact overall yield (DaMatta et al., 2007).

In Brazil, the non-uniformity in fruit ripening is a problem exacerbated by the predominantly mechanized and non-selective harvesting system. This system results in fruits at different phenological stages of development being harvested at the same time, resulting in a beverage known as "hard," meaning when the beverage is derived from a mixture of beans at different stages of ripeness. In addition to reducing quality, non-uniformity also increases the likelihood of flower and fruit abortion, leading to a decrease in the quantity of coffee beans harvested (DaMatta et al., 2007). It is known that both flowering and fruiting of the coffee plant suffer significant losses in the number of buds and cherries until fruit development is completed and harvesting takes place (Dedecca, 1957; Mayer et al., 2013; Garcia and Matiello, 2013; Yilma and Kufa, 2020). However, little is known about which stages are most affected by this loss and the underlying causes of bud and fruit abortion.

In this regard, it is of paramount importance for Brazilian coffee farming to understand the reasons that could impair production, whether due to non-uniformity in flowering and flower and fruit abortion. Identifying which stages of the coffee plant's development this non-uniformity can be controlled is of fundamental interest to the coffee market. To address this, Oliveira *et al*. (2014) observed that non-uniformity in reproductive bud development may increase not only during bud development but also prior to the floral meristem differentiation. Given that the molecular processes involved in vegetative and reproductive development are markedly distinct, it is plausible that the mechanisms driving these asynchronies are genetically independent and collectively contribute to the non-uniformity of coffee flowering. Consequently, coffee flowering is a complex and multifaceted process, occurring at various stages of both reproductive and vegetative development, even among buds originating from the same node (de Oliveira et al., 2014).

Understanding vascular development in coffee flower and fruit peduncles is essential because these structures are key for transporting water, nutrients, and energy needed for growth and maturation. Although the anatomy of coffee flowers and fruits is well studied, the changes and vascularization of peduncles are less understood. Since (1) water availability and the plant's ability to transport it to the buds impact flowering, and (2) flower buds from the same node can develop at different rates, it suggests there are anatomical differences that are not immediately visible but affect coffee plant non-uniformity (Pereira Gardin, 2006; Voltan et al., 2011).

How could anatomical differences in bud vascularization influence the non-uniformity of flowering? This study aims to address this question by analyzing anatomical and molecular changes in *C. arabica* peduncles at different growth stages. Our objectives were to: (1) Identify the developmental stage of the peduncle where vascularity emerges and understand how the bud connects to the rest of the plant. (2) Determine the stages at which vascularity differentiates through the various steps of vascular development. (3) Identify coffee orthologs governing vascular development at the transcriptional level. (4) Use gene expression analyses to pinpoint the stages where these orthologs are upregulated. By focusing on these objectives, we linked changes in vascular tissue with morphological transitions in floral buds, flowers, and fruits, providing insights into the molecular mechanisms governing vascular development in this economically significant species.

This work is divided into two chapters, each one a draft of research papers that will be submitted to peer-review journals. The first chapter is a review paper that dives into the labyrinth pathways of both anatomical and molecular layers of plant vascularity. The second chapter is a research paper which focuses on a subset of ortholog genes that are potentially governing peduncle vascular development. By publishing these works, we expect that our contributions will guide future research on the role of peduncle vascularization not only in coffee but also in other important crops.

REFERENCES

Alves, H.M.R., Volpato, M.M.L., Vieira, T.G.C., Borém, F.M., Barbosa, J.N., 2011. **Características ambientais e qualidade da bebida dos cafés do estado de Minas Gerais**. Inf. Agropecuário Belo Horiz. 32, 18–29.

Alvim, P. de T., 1960. **Moisture stress as a requirement for flowering of coffee**. Science 132, 354–354.

Alvim, P. de T., 1958. **Estímulo de la floración y fructificación del cafeto por aspersiones con ácido giberélico**. Turrialba 8, 64–72.

Arcila, J., Buhr, L., Bleiholder, H., Hack, H., Wicke, H., 2001. **Aplicación de la escala BBCH ampliada para la descripción de las fases fenológicas del desarrollo de la planta de café *Coffea* sp.**

Arcila Pulgarín, J., 2007. **Crecimiento y desarrollo de la planta de café**. Sist. Prod. Cafe En Colomb. Cenicafe Chinchina Colomb. 21–60.

Berthaud, J., Charrier, A., n.d. **Genetic resources of *Coffea***.

Camargo, Â.P. de, Camargo, M.B.P. de, 2001. **Definição e esquematização das fases fenológicas do cafeeiro arábica nas condições tropicais do Brasil**. Bragantia 60, 65–68.

Charrier, A., Berthaud, J., 1985. **Botanical Classification of Coffee**, in: Clifford, M.N., Willson, K.C. (Eds.), Coffee. Springer US, Boston, MA, pp. 13–47. https://doi.org/10.1007/978-1-4615-6657-1_2

CONAB, 2024. **Conab - Safra de grãos 2023/2024 está estimada em 294,1 milhões de toneladas** [WWW Document]. URL https://www.conab.gov.br/ultimas-noticias/5478-safra-de-graos-2023-2024-esta-estimada-em-294-1-milhoes-de-toneladas (accessed 7.15.24).

Crisosto, C.H., Grantz, D.A., Meinzer, F.C., 1992. **Effects of water deficit on flower opening in coffee (*Coffea arabica* L.)**. Tree Physiol. 10, 127–139.

DaMatta, F.M., Ronchi, C.P., Maestri, M., Barros, R.S., 2007. **Ecophysiology of coffee growth and production**. Braz. J. Plant Physiol. 19, 485–510.

Davis, A.P., Govaerts, R., Bridson, D.M., Stoffelen, P., 2006. **An annotated taxonomic conspectus of the genus *Coffea* (Rubiaceae)**. Bot. J. Linn. Soc. 152, 465–512.

de Oliveira, R.R., Cesarino, I., Mazzafera, P., Dornelas, M.C., 2014. **Flower development in *Coffea arabica* L.: new insights into MADS-box genes**. Plant Reprod. 27, 79–94.

Dedecca, D.M., 1957. **Anatomia e desenvolvimento ontogenético de *Coffea arabica* L. var. typica Cramer**. Bragantia 16, 315–366. https://doi.org/10.1590/S0006-87051957000100023

Deshmukh, A., 2021. **The World's Top Coffee Producing Countries** [WWW Document]. Vis. Capital. URL https://www.visualcapitalist.com/worlds-top-coffee-producing-countries/ (accessed 2.7.22).

Drinnan, J., Menzel, C.M., 1994. **Synchronization of anthesis and enhancement of vegetative growth in coffee (*Coffea arabica* L.) following water stress during floral initiation**. J. Hortic. Sci. 69, 841–849.

Garcia, A.L.A., Matiello, J.B., 2013. **Índice de abortamento, ao longo do desenvolvimento dos frutos, de flores com antese inibida pelo déficit hídrico em *C. arabica***.

Hamon, P., Hamon, S., Razafinarivo, N.J., Guyot, R., Siljak-Yakovlev, S., Couturon, E., Crouzillat, D., Rigoreau, M., Akaffou, S., Rakotomalala, J.-J., De Kochko, A., 2015. ***Coffea* Genome Organization and Evolution, in: Coffee in Health and Disease Prevention**. Elsevier, pp. 29–37. https://doi.org/10.1016/B978-0-12-409517-5.00004-8

Hoeberichts, F.A., Povero, G., Ibañez, M., Strijker, A., Pezzolato, D., Mills, R., Piaggesi, A., 2017. **Next generation sequencing to characterise the breaking of bud dormancy using a natural biostimulant in kiwifruit (*Actinidia deliciosa*)**. Sci. Hortic. 225, 252–263.

Lashermes, P., Combes, M.-C., Robert, J., Trouslot, P., D'Hont, A., Anthony, F., Charrier, A., 1999. **Molecular characterisation and origin of the *Coffea arabica* L. genome**. Mol. Gen. Genet. MGG 261, 259–266. https://doi.org/10.1007/s004380050965

López, M.E., Santos, I.S., de Oliveira, R.R., Lima, A.A., Cardon, C.H., Chalfun-Junior, A., 2021. **An overview of the endogenous and environmental factors related to the *Coffea arabica* flowering process**. Beverage Plant Res. 1, 1–16.

Magalhaes, A., Angelocci, L., 1976. **Sudden alterations in water balance associated with flower bud opening in coffee plants**. J. Hortic. Sci. 51, 419–423.

Mayer, J.L.S., Carmello-Guerreiro, S.M., Mazzafera, P., 2013. **A functional role for the colleters of coffee flowers**. AoB PLANTS 5, plt029. https://doi.org/10.1093/aobpla/plt029

Meyer, F.G., 1965. **Notes on wild *Coffea arabica* from southwestern Ethiopia, with some historical considerations**. Econ. Bot. 19, 136–151. https://doi.org/10.1007/BF02862825

Morais, H., Caramori, P.H., Koguishi, M.S., Ribeiro, A.M. de A., 2008. **Escala fenológica detalhada da fase reprodutiva de *Coffea arabica***. Bragantia 67, 257–260.

Nascimento, M.N. do, Alves, J.D., Soares, Â.M., Castro, E.M. de, Magalhães, M.M., Alvarenga, A.A. de, Silva, G.H., 2008. **Alterações bioquímicas de plantas e morfológicas de gemas de cafeeiro associadas a eventos do florescimento em resposta a elementos meteorológicos**. Ciênc. Rural 38, 1300–1307.

Nunes, F.L., Camargo, M.B.P. de, Fazuoli, L.C., Rolim, G. de S., Pezzopane, J.R.M., 2010. **Modelos agrometeorológicos de estimativa da duração do estádio floração-maturação para três cultivares de café arábica**. Bragantia 69, 1011–1018.

Penfield, S., 2024. **Beyond floral initiation: the role of flower bud dormancy in flowering time control of annual plants**. J. Exp. Bot. erae223. https://doi.org/10.1093/jxb/erae223

Pereira Gardin, J.P., 2006. **Desenvolvimento reprodutivo do cafeeiro: anatomia de vasos do xilema e dinâmica de carboidratos** (Tesis). UFLA, Lavras, MG.

Pezzopane, J.R.M., Pedro Júnior, M.J., Thomaziello, R.A., Camargo, M.B.P. de, 2003. **Escala para avaliação de estádios fenológicos do cafeeiro arábica**. Bragantia 62, 499–505.

Volsi, B., Telles, T.S., Caldarelli, C.E., Camara, M.R.G. da, 2019. **The dynamics of coffee production in Brazil**. PloS One 14, e0219742.

Voltan, R.B.Q., Fahl, J.I., Carelli, M.L.C., 2011. **Diferenciação de gemas florais em cultivares de cafeeiro**.

Wintgens, J.N., 2012. **Coffee - Growing, Processing, Sustainable Production: A Guidebook for Growers, Processors, Traders and Researchers**. John Wiley & Sons.

Yilma, A., Kufa, T., 2020. **Coffee Peaberry as A Potential Seed Source for Production**. Int. J. Res. Stud. Sci. Eng. Technol. 7, 30–35.

Yu, Q., Guyot, R., de Kochko, A., Byers, A., Navajas-Pérez, R., Langston, B.J., Dubreuil-Tranchant, C., Paterson, A.H., Poncet, V., Nagai, C., Ming, R., 2011. **Micro-collinearity and genome evolution in the vicinity of an ethylene receptor gene of cultivated diploid and allotetraploid coffee species (*Coffea*)**. Plant J. 67, 305–317. https://doi.org/10.1111/j.1365-313X.2011.04590.x

Zanetti, W.A.L., da Silva Marques, M., do Amaral, A.M.S., da Silva, A.B., Putti, F.F., Goes, B.C., 2021. **Analysis of the Technological Evolution of Coffee Production in Brazil**. J. Agric. Stud. 9, 352–362.

CHAPTER 1

1. Molecular programming of vascular development in angiosperm plants, future perspectives for the *Coffee arabica* L.

(Draft Version)
Article prepared for submission to the Annual Review of Plant Biology Journal

Andrea Jaramillo Mesa[1], Raphael Ricon de Oliveira[1], Stefan de Folter[2], Antonio Chalfun-Junior[1]*

[1]Laboratory of Plant Molecular Physiology, Plant Physiology Sector, Institute of Biology, Federal University of Lavras (UFLA), Lavras, Minas Gerais, Brazil.
[2]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (Cinvestav), CDMX, México.

*Corresponding author: chalfunjunior@ufla.br

Abstract

Vascularization is a fundamental process in land plants, for water and nutrient transport, structural support, long distance signaling, growth and development. During embryogenesis, the procambium, a meristematic tissue, lays the foundation for the establishment of the vascular pattern. This initial phase is orchestrated by intricate interactions primarily between auxins and cytokinins, which also play critical roles in vascular patterning during the establishment of the plant primary axis. As seedlings progress in their development, the spatial arrangement becomes essential for the formation of conductive tissues. In this way, a complex regulatory network governs vascular differentiation, where multiple transcription factors emerge as key players in this process. Thus, the vascular system comprises two primary tissues: the phloem, mainly responsible for transporting organic compounds, and the xylem, which conducts water and minerals. In addition, the cambium is a layer of actively dividing cells (secondary meristem cells), between xylem and phloem responsible for the radial growth of additional layers as the secondary xylem and phloem. In the face of climate change, particularly the rise in global temperatures and droughts, there is an urgent need for a more efficient use of water in agricultural practices. Hence, understanding the vascular procambium/cambium regulatory network during primary and secondary growth is essential to deciphering the complexities of vascular plant development and guarantee food production. Here, we used *Coffea* sp. L., a socioeconomic important crop with a complex phenological cycle responsible for environmental cues, as a model to explore this issue. Thus, our work contributes to understanding vascular development in crops, guiding breeding programs toward higher productivity while promoting sustainable agriculture.

Keywords: procambium; cambium; phloem; xylem; coffee plant.

1.1 Introduction

Vascular development is a fundamental aspect in the growth of both land and aquatic plants, playing a crucial role in defining their structure and allowing their permanence on Earth. The formation and development of conductive vessels are essential for land plants, providing not only mechanical support but also enabling the efficient transport of water, essential mineral nutrients, sugars, signal molecules, and amino acids over long-distances. This vascular system operates as an effective communication network throughout the entire plant, creating a channel for the transmission of both biotic and abiotic information across distant tissues (143).

The vascular system of plants, consisting of a cohesive and continuous network of threads known as vascular bundles, represents a well-organized and flexible development process. This intricate system undergoes various changes throughout the plant's life cycle and in response to interactions with the environment. These changes include adaptations to growth, environmental stimuli, and developmental stages, making the vascular system a dynamic and responsive component of plant physiology. In plant embryos, following the asymmetric cell division, pro-vascular cells emerge. These cells undergo differentiation into pre-cambial vascular cells, exhibiting characteristics akin to stem cells (198, 79).

Once the vascular system initiates its formation through the growth of expanding tissues and structures, a functional communication network is established between each part of the aerial component and the root system. This intricate communication process orchestrates the organization and differentiation of the phloem and xylem. Therefore, the vascular tissues of plants, arranged as a network of continuous threads, exhibit interconnected longitudinal and radial patterns, allowing the integration of diverse signals to drive the plant architecture (198).

During vegetative growth, the apical meristem serves as a continuous source of initial procambial cells, which subsequently give rise to the vascular procambium, leading to the formation of the primary xylem and the primary phloem (198, 78). In secondary growth, the vascular cambium, also originating from procambium, is present in plants with radial thickening, such as gymnosperms and dicots (198). The vascular cambium acts as a reservoir of cells producing cells of the xylem and phloem. As a result, the vascular bundles represent the organization of vascular tissues. Within these bundles, both phloem and xylem consist of various types of specialized vascular cells, including conductive elements such as tracheary elements (TE) in the xylem and sieve elements (SE) in the phloem (97). Additionally, parenchyma cells and sclerenchyma contribute to the structural and functional complexity of the vascular tissues (198, 78).

Coffee, as a vascular and woody plant, is extensively cultivated across over 70 tropical countries, spanning approximately 10 million hectares (11, 49). *Coffea arabica* L., recognized as the most important commercial species in this genus, requires two years to complete its phenological cycle, going from an undifferentiated meristem to the reproductive stage, and ending with the senescence of the reproductive tertiary and quaternary branches (138). Environmental stimuli such as photoperiod (31), water availability (134, 139), and temperature (43) play a crucial role in the induction of flowering and subsequent fruiting. Consequently, environmental changes can trigger morphophysiological alterations that affect the development of the coffee flower and subsequent development and maturation of the fruit (163). Given the critical function of conductive tissues in facilitating water, signals, and nutrient transport, research focusing on diverse molecular mechanisms influencing vascular function, particularly during the coffee tree's productive season, holds significant importance in optimizing cultivation practices to enhance production (226).

While the molecular mechanisms underlying plant development have been extensively studied in model plants like *Arabidopsis* and others such as *Populus*, it's important to note that these mechanisms are diverse and tailored to the specific phenological cycles of different species, yet remain poorly characterized in crops like coffee. In this review, we delve into this topic by summarizing scientific advancements on the understanding of the regulatory networks governing vascular development. Through this exploration, we aim to gain a deeper understanding of crop vascularization development like coffee, offering insights to enhance agricultural practices and guide breeding programs towards more sustainable food production and efficient water usage.

1.2 The importance of vascularization in plants

The development of vascular tissues such as xylem and phloem, emerged in early land plants, such as vascular cryptogams like lycophytes, horsetails, and ferns, a crucial evolutionary feature that marked the origin of tracheophytes as gymnosperms and angiosperms (54). The primary function of this vascularization is to distribute water and solutes throughout the plant, and its emergence allowed plants to become increasingly larger, particularly when compared to bryophytes. This evolutionary adaptation involves a well-organized, flexible development process that takes on different conformations throughout the organism's life, responding to various interactions and responses to environmental signals (198).

The development of vascularization occurs in two phases, giving rise to the formation of primary and secondary vascular systems (198, 80). The primary vascular system extends throughout the plant from roots, stems, and lateral branches to leaves, flowers, and fruits. Its formation is determined by the activity of the procambium, which differentiates early in embryo development (258). In plants that exhibit radial thickening, such as woody plants, the cambium acts as a reservoir of cells that can differentiate into various vascular structures. For instance, the (pro)cambium can produce secondary xylem and phloem cells, shaping the plant architecture through divisions in interconnected longitudinal and radial patterns in response to a multitude of signals. Thus, the development of the vascular system is subject to complex control, where changes in hormonal balance and the involvement of regulatory genes are decisive for its differentiation and establishment (198).

The secondary vascular system, the cambium, continues to give rise to xylem and phloem cells throughout the plant's life. Therefore, the evolution of secondary growth has allowed the emergence of large trees and long-lived plants, as well as species of economic interest for wood production, additionally enabling dense growth in the areas where the plant requires it (88). Although the understanding of this phenomenon is continually evolving, regulatory mechanisms that control key aspects of secondary growth have been identified and partially characterized. These include the initiation and maintenance of the cambium, tissue patterning, and the delicate balance between cell division and differentiation (221). While there is still much to uncover, genomic studies have yielded comprehensive lists of genes expressed during secondary growth and illuminated the functions of various regulators, such as transcription factors, signaling peptides, and receptors (101, 28, 183). For example, it's been observed that plant hormones, including cytokinins, ethylene, gibberellic acid, brassinosteroids, and auxins, play important roles in different facets of secondary growth and the establishment of vascular patterns in plants (221, 143).

1.3 Procambium

Vascular bundles, integral components of plant anatomy, constitute a highly organized series of tissues, where the primary root and hypocotyl already exhibit a predetermined vascular pattern from embryogenesis (82). Meristematic tissues, essential for the continuous plant development, harboring stable populations of pluripotent cells, the procambial cells serve as a reservoir, ensuring the continual vascular development across all plant organs (26, 28).

While root apical meristem (RAM) and shoot apical meristem (SAM) cells, both originating from meristematic cells, differ from each other, the structural characteristics of procambial cells remains consistent regardless of their location within the plant. These cells have two potential fates: to become xylem or phloem cells (28). The procambium can be recognized as continuous threads of dense and narrow cytoplasmic cells, with their axes oriented parallel to the plant's axis of inclination. Pre-procambial cells, typically polygonal and isodiametric, exhibit distinctive characteristics. They serve as precursors to the procambium and undergo transformations as the vascular strand develops. Cambial cells, responsible for secondary growth, divide parallel to the axis of the developing vascular strand (198).

In the early stages of cell differentiation, procambial filaments are not clearly distinguishable from adjacent cells in the meristematic region. Morphological differences between procambium and adjacent parenchyma gradually develop. Initially, procambial filaments increase in size by adding new cells towards the periphery, and this process coincides with non-procambial adjacent cell divisions (59, 60). In this context, although these adjacent cells are not morphologically differentiated, their regulatory pathways programming are distinct (191).

1.3.1 The first establishment of vascular pattern occur during embryogenesis

A common feature of procambium regulatory pathways and meristems, in general, is the rigorous control of the rate of cell division, differentiation, and cellular identity throughout plant growth (198). In the case of *Arabidopsis*, the vascular pattern is established from the earliest stages of embryo development. During the globular stage, all vascular tissues in the plant axis (root and hypocotyl) derive from only four initial cells (143, 82). Following their specification as initial procambial cells, a longitudinal (or periclinal) division occurs to increase the number of cell layers in the mature embryo. Moreover, these periclinal divisions also occur post-embryonically in the root's procambium, phloem, and xylem (44). In the early torpedo stage, the embryonic root already has established provascular cells that maintain the vascular pattern in post-embryonic stages. Additionally, at the end of embryogenesis gene expression analyses suggest that cellular identities in the nascent vascular tissues are already well-established (26, 28).

Two genes encoding for receptor-like protein kinases, *RECEPTOR-LIKE PROTEIN KINASE 1* (*RPK1*), and *TOADSTOOL 2* (*TOAD2*) have been identified in *Arabidopsis* as crucial for the formation of the vascular pattern from the embryo (161). An auxin-responsive transcription factor, *MONOPTEROS*/*AUXIN RESPONSE FACTOR 5* (*MP*/*ARF5*), is also of significant importance in maintaining the specification of the pro-vascular tissue during and after embryogenesis (92, 200). Moreover, the expression of *MP* is gradually confined to the provascular tissues, coinciding with an accumulation of auxins promoted by the efflux throughout PIN-FORMED carriers. Consequently, *MP* auxin-inducible regulates the expression of *PIN-FORMED* (*PIN*), which, in turn, forms a positive feedback loop resulting in the maintenance of constant and high auxin levels in the provascular tissues (92, 200, 191, 29).

The loss of function of *MP*/*ARF5* or *PIN1* eliminates auxin accumulation, disrupting the vascular pattern in the early embryo and the initiation of the seedling's body axis, resulting in severe developmental defects including root absence (92, 29). Therefore, the expression of *MP*/*ARF5* and *PIN1*, mediating auxin accumulation is considered a crucial signal for establishing pro-vascular tissues and maintaining the vascular pattern in the *Arabidopsis* early embryonic and post-embryonic stages. *MP* also positively regulates the expression of *ATHB8*, a member of the Class III Homeodomain Leucine Zipper proteins (HD-ZIP III), which are involved in the proliferation of procambial cells and the organization of vascular tissue (129, 143, 191).

On the other hand, the *BODENLOS* (*BDL*) gene, which encodes an auxin response regulator *AUXIN/INDOLE-3-ACETIC ACID 12* (*AUX/IAA12*) represses the transcriptional activation of target genes mediated by MP (191, 29). In this way, mutations in both the *BDL* and *MP* genes impact the development of the hypophysis (the founding cell of the root stem cell system) in the embryo, altering the orientation of the zygote division. This means that the hypophysis fails to undergo the asymmetric division that gives rise to the precursors of the quiescent center and the mother cells of the lower levels of the RAM (89, 200). This same phenotype was observed in double mutants *bdl mp*, suggesting that the two proteins, BDL and MP, act in the same developmental pathway. While the *MP* gene encodes *ARF5*, a transcription factor of the ARF family (auxin response factor) that activates target genes responsive to auxins, on the other hand, *bdl* mutant seedlings are insensitive to the auxin analog 2,4-dichlorophenoxyacetic acid (2,4-D). This implies that *BDL* is also involved in auxin response (89).

Several targets of *MP* are exclusively expressed in vascular tissue, such as the transcription factors *TARGET OF MONOPTEROS 5* (*TMO5*) and *LONESOME HIGHWAY* (*LHW*), which form a basic helix-loop-helix (bHLH) heterodimer to promote periclinal divisions in the provascular tissue of *Arabidopsis*. The *TMO5/LHW* complex is necessary to initiate periclinal cell division, primarily controlling vascular development in the plant (45). It has been observed that tissue-specific expression of *TMO5* is sufficient to largely rescue vascular defects in *mp* mutants, which fail to produce an embryonic root (164, 200, 45, 171). It is important to note that during early embryogenesis, *TMO5* is expressed within the four initial cells of the procambium. However, later in plant development, it becomes restricted solely to the xylem, which does not undergo periclinal division. While *LHW* is broadly expressed in vascular cells, the expression of *TMO5* and its homolog *TMO5-LIKE1* (*T5L1*) is confined to precursor cells of the xylem (171).

In the post-embryonic stages of *Arabidopsis*, the proliferation of procambial cells and the differentiation of xylem precursor cells begin in the RAM (171). The formation of heterodimers LHW-TMO5 and LHW-T5L1 promotes the co-expression of key genes involved in cytokinin production, such as *LONELY GUY* (*LOG*), and *ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 6* (*AHP6*), resulting in specific proliferation of vascular cells and the organization of vascular tissue itself (191). While the *TMO-LHW* complex induces the expression of *LOG3* and *LOG4*, leading to increased levels of bioactive cytokinins (CK) in surrounding cells and stimulating periclinal divisions, the activity of *AHP6*, regulated by *LHW-T5L1*, promotes the differentiation of protoxylem cells by suppressing cytokinin signaling (171). Additionally, the *SUPPRESSOR OF ACAULIS-LIKE* (*SACL)* family members can form heterodimers with *LHW*, competing with *TMO5* and counteracting the promotion of periclinal divisions by the *TMO5*/*LHW* complex (171, 191).

The *ACAULIS 5* (*ACL5*) gene, specifically expressed in provascular cells and positively regulated by *TMO5*/*LHW*, encodes a thermospermine synthase (EC: 2.5.1.79) in *Arabidopsis* (108, 29). In angiosperms, thermospermine acts as a growth regulator by promoting stem elongation and suppressing xylem differentiation through the limitation of auxin signaling (247). The loss of function in *ACL5* leads to deficient thermospermine biosynthesis, resulting in excessive xylem differentiation, defects in stem internode elongation, severe dwarfism phenotypes, and leaf vein thickening in the *tkv* mutation due to increased vascularization (90, 37). Thermospermine signaling is mediated by the *SUPPRESSOR OF ACAULIS 51* (*SAC51*), which encodes a bHLH transcription factor, and the related protein *SAC51-LIKE*. *SAC51* regulates xylem differentiation and promotes stem elongation. Therefore, the phenotype of shorter and thicker stems results from the loss of translational activation of *SAC51*, interfering with thermospermine signaling (247, 115).

Thermospermine also negatively regulates auxin signaling, which promotes xylem differentiation. An improvement in xylem vessel differentiation was observed in *Arabidopsis acl5* mutant seedlings when treated with synthetic pro-auxins, but not in wild-type ones. However, this inductive effect of pro-auxins could be suppressed by thermospermine. Thus, *ACL5* expression may be positively regulated by auxins but negatively regulated by thermospermine. Regarding the *ATHB8* gene, which directly activates *ACL5* expression, it may act in a negative feedback loop against auxin-induced xylem differentiation (248).

Another known compound called xylemin, an antagonist of spermidine, can act as an inhibitor of thermospermine biosynthesis and counteract its effects on vascular development. Exogenously applied xylemin has the ability to overstimulate xylem production in *Arabidopsis thaliana*, *Zinnia elegans*, and *Nicotiana benthamiana*, resulting in a phenotype similar to that of *Arabidopsis acl5* mutants. Additionally, the application of a combination of xylemin and thermospermine to wild-type seedlings neutralizes the effect of xylemin, while a combined application of xylemin with a synthetic pro-auxin has a synergistic inductive effect on xylem differentiation. This supports the involvement of auxins in vascular development, as their interaction with other signals can spatially and temporally regulate xylem vessel differentiation. Therefore, procambium formation and xylem differentiation in vascular plants are directed by auxins (248).

Previous studies showed that the expression of two promoters of the *Dof* gene, *AtDof2.4* and *AtDof5.8*, are sequentially activated during procambium formation in *Arabidopsis* leaf primordia, suggesting a potential involvement of *Dof* transcription factors in the vascular development of higher plants. *AtDof5.8* coincides with *PIN1* expression in *Arabidopsis* leaf primordia during procambium development, while the expression timing of *AtDof2.4* was quite similar to *ATHB8* gene in processes following primary procambium formation (129). In the *Arabidopsis thaliana* genome, 36 putative *Dof* genes have been identified of which only nine have been characterized. Out of these nine, it has been demonstrated that they are involved in salicylic acid response, seed germination, phytochrome signaling, flowering, and glucosinolate biosynthesis (240, 241, 109, 229, 210, 129).

The above illustrates how vascular development in higher plants is highly coordinated by key genes and its relation with plant hormones and other signals, initiating procambium and subsequently differentiating of xylem and phloem. It also highlights the importance of initial cell divisions in the zygote, which determine the cell identities that will give rise to vascular bundles. These identities establish the initial architecture for root and hypocotyl formation, maintaining the vascular pattern during the development of various plant organs. However, although the molecular pathways underlying vascularization is well described in model species, this aspect is poorly explored in woody species and especially crops. Hence, considering the diversity and plasticity of plants, understanding the regulatory networks directing and promoting vascularization is crucial to regulate plant development and improve agricultural practices.

1.3.2 Auxin-cytokinin interactions drive vascular patterning during embryogenesis and primary root axis formation in Arabidopsis

As previously mentioned, cytokinins are also key molecules involved in vascularization, and their combined signaling with auxins converges in the formation of the vascular bundle pattern (21, 105, 45, 44, 191, 28). One of the earliest indications of cytokinin signaling involvement in vascular development was the discovery of the *wooden-leg* (*wol*) mutant. The *WOL* is expressed in vascular precursors during embryogenesis and the primary root development of *Arabidopsis thaliana*. The WOL locus was identified by a recessive mutation resulting in defective periclinal divisions towards the end of embryogenesis, leading to fewer vascular initials. Additionally, all procambial cells differentiate into protoxylem at the expense of phloem in the root (199, 146, 28). The *WOL* is a two-component cytokinin receptor allele; *CYTOKININ RESPONSE 1* (*CRE1*) and *ARABIDOPSIS HISTIDINE KINASE 4* (*AHK4*). In *Arabidopsis*, cytokinin perception occurs through three homologous histidine kinase receptors (HK): AHK2, AHK3, and CRE1/WOL/AHK4 (CRE family receptors), which initiate phosphorylation through *ARABIDOPSIS* HISTIDINE PHOSPHOTRANSFER PROTEIN (AHP) and *ARABIDOPSIS* RESPONSE REGULATOR (ARR), allowing the signaling circuit for cytokinin production at the cellular level (146, 94, 52, 191).

This signaling is initially driven by ligand-sensor molecular interactions; the activation pathway begins with self-phosphorylation at a conserved His residue of hybrid His kinases in the N-terminal sensor-kinase domain, which is subsequently transferred to a conserved Asp in the C-terminal receptor domain. In this regard, *AHK2*, *AHK3*, and *AHK4*/*CRE1*/*WOL* are activated by cytokinins through the binding of a specific ligand to their transmembrane CHASE domains upstream of the His kinase domain. Subsequently, activation of the hybrid His kinase occurs; membrane-associated *CKI1* possesses constitutive His kinase activity, and its overexpression triggers the entire cytokinin signaling pathway in cells. The aforementioned signals converge in the activation of *AHP* (*AHP*1–5), which mediates the transfer of cytoplasmic signals to nuclear signals. These signals activate nuclear *ARR* type B (positive regulators of cytokinins), which are DNA-binding transcription factors. *ARR* type B directly promotes the nuclear expression of *ARR* type A (negative regulators of cytokinins) as primary cytokinin target genes and negative feedback regulators (105, 52).

A transcriptional profile of all putative phosphorelay signaling components at the heart stage of embryogenesis revealed the expression of genes *AHK4*, *AHP2*, *AHP3*, *AHP5*, and *ARR* type A and B, providing evidence of cytokinin signaling during *Arabidopsis* embryogenesis (156). At this stage, it was observed that auxin signaling antagonizes cytokinin signaling by inducing *ARR* type A. While cytokinins promote periclinal divisions and inhibit protoxylem differentiation in the root (171, 191), conversely, the auxin-dependent cytokinin signaling repressor *AHP6* promotes protoxylem differentiation by restricting cytokinin activity. In turn, cytokinin influences auxin activity by regulating the distribution of efflux proteins PIN1, PIN3, and PIN7 in the procambial cells (208, 45, 29, 191).

Finally, the antagonistic interaction between auxins and cytokinins allows the regulation of embryonic and post-embryonic vascular development; auxins promote protoxylem differentiation and cytokinins promote periclinal divisions of the procambium. This occurs because PIN proteins promote auxin efflux creating a cellular environment with low levels of auxin and high levels of cytokinins. With the auxin flow towards the cells surrounding the procambium, the specification of protoxylem identity occurs, also promoting the expression of *AHP6* and reducing cytokinin signaling in these cells. Despite *AHP6* repressing cytokinin signaling, newly synthesized cytokinin can diffuse to procambial cells where its cytokinin signaling is not repressed, allowing cytokinin to act as a non-autonomous mobile regulator of cells, enabling periclinal divisions of the procambium. In this way, mutually inhibitory auxin-cytokinin interactions between adjacent cells maintain the bisymmetric vascular pattern and drive the development of the vascular pattern in the primary root of *Arabidopsis* (21, 45, 44, 29).

1.3.3 Spatial organization of vascularization in the establishment of the seedling

The vascular strands are composed of rows of interconnected cells. It is crucial that vascular strands are spaced and shaped properly within tissues, and that cell types functionally coordinate differentiation to produce interconnected conduits for transport. In this sense, the formation of the vascular system in higher plants is a well-structured and flexible developmental process in response to environmental changes. Due to its crucial role in adapting to environmental conditions, the arrangement of xylem and phloem in various plant organs can vary significantly among different species (143, 167).

Various molecular and anatomical studies have provided evidence of the origin of vascularization at embryogenesis, as well as the spatial organization of the vascular system in plant architecture (214, 53, 27, 33, 166, 171, 172, 79). In this way, although it has initially been observed that cell identities for post-embryonic vascular development are determined from the embryo, the timing of when these identities are established may differ between species. In the *Arabidopsis* embryo, vascular cells develop from eight procambial cells in the late globular stage, whereas in *Nicotiana* embryos, this occurs at the 20-cell stage, and in *Trifolium* embryos, it happens at the 120-cells (84, 171).

While procambial cells that will give rise to the phloem and xylem of the primary root and hypocotyl are established during embryogenesis, the onset of vascular development can also be observed with the emergence of new lateral organs, such as leaves and roots, from the meristems (168). Thus, as the plant grows and develops new organs, it is important for vascular bundles to maintain continuity and plasticity while preserving the specific anatomy and morphology to each structure. Due to the action of phytohormones and other long-distance signaling molecules, growing organs can develop vascular tissues that allow for the continuity of communication with the older parts of the plant (5, 258, 79).

For several years, it has been evidenced how the biosynthesis of certain phytohormones is essential for shaping the vascular pattern in the plant body and developing organs, as well as for the division and differentiation of the cellular identities composing vascular tissues. Evidence of this is the way auxins are transported through cells, a determining factor for establishing the vascular pattern and acquiring polarity in xylem and phloem cells (32, 174, 92, 211, 103, 18, 116). Additionally, cytokinins, along with auxins, signal vascular patterning from embryogenesis and act as growth regulators throughout the plant's life (3, 4, 156, 94, 22, 208, 52). On the other hand, gibberellin signaling pathways can promote xylem differentiation and influence cambial activity. Along with auxin production signaling pathways, they induce the differentiation of vascular fibers (51, 1, 58, 19, 24, 150, 151, 181, 42, 67, 104). Other phytohormones known to influence vascular development are brassinosteroids, which can act in cell expansion and impact plant morphogenesis (114, 202, 30). There is also evidence that the ethylene biosynthesis pathway interacts with vascular development, specifically in the production of radial vascular rays and tension wood generation in poplar (141, 176, 64).

In addition to phytohormones, sugar transport is also important for establishing cellular identity and organizing the vascular system (230, 232, 2). The first evidence of sucrose influence on plant vascularization came from tissue culture experiments. Works with callus cultures of Syringa, Daucus, and Soy demonstrated that the addition of the auxin indole-3-acetic acid (IAA) and sucrose to the culture media could influence the production of xylem and phloem cells. It was observed that while increasing levels in IAA concentration promoted the number of sieve elements and tracheary elements, a constant concentration of IAA and sucrose did not alter vascular development. However, an increase in sucrose concentration resulted in an increase in the amount of callose deposited on sieve plates (2).

After the identities of vascular cells are established in the embryo, they are maintained during the development of the plant's main axis in post-embryonic stages. The vascular system exhibits two levels of organization: a radial or transverse pattern, which is the spatial arrangement of xylem and phloem, and a longitudinal pattern, which pertains to the organization of vascular bundles within specific organs such as roots, stems, leaves, flowers, and fruits (198).

The organization of vascular bundles in plant architecture initiates with the differentiation of proto-phloem and proto-xylem in the primary root and hypocotyl. In *Arabidopsis*, the vascular tissues of the hypocotyl form pre-existing procambial cells, while in the root, they derive from the activity of the root apical meristem (RAM). In the hypocotyl, the initiation of vascular cell differentiation is first observed as two rows of proto-phloem elements located on opposite sides of the vascular cylinder, and proto-xylem developing towards the opposite sides of the cylinder behind the proto-phloem development. In this sense, xylem development itself proceeds from the two poles on the outside of the cylinder towards the center until the xylem forms a continuous plate. This xylem plate divides the vascular cylinder and separates the developing phloem into two regions (27, 217, 80).

In *Arabidopsis* primary roots, the organization of vascular tissue reveals the presence of protoxylem in a diarch conformation. This configuration indicates that vascular tissue development progresses from the base towards the apex (acropetal). Consequently, the early stages of vascular development are prominently observed near the root tip (53, 27, 146, 218). Derived from initials near the quiescent center, rows of xylem cells form an axis typically composed of 4 or 5 cells. This xylem axis resembles a plate, similar to the structure observed in the hypocotyl. It serves to partition the vascular cylinder into two distinct domains. Each of these domains comprises undifferentiated procambial and phloem cell lineages. However, the precise number and pattern of cell divisions in procambial cells may differ between species (146, 218, 80). It is postulated that the diameter of meristems, RAM and SAM, can influence the number of cell lineages and the organization of vascular bundles. Studies, such as those conducted in peas, suggest a correlation between the number of xylem poles and root diameter (214).

In most cases, the vascular bundle follows a collateral pattern with xylem on the inside and phloem on the outside. However, in some monocotyledons, vascular bundles have an amphivasal organization where phloem cells are surrounded by xylem cells. On the other hand, in some angiosperms and ferns, the vascular pattern is arranged amphicribral, where xylem cells are surrounded by phloem cells (82, 98). Despite variations in vascular system configuration across plant structures and species, the procambium, xylem, and phloem are always present and remain fundamental components throughout the developmental stages of higher plants.

1.4 Regulatory network of vascular differentiation

During the final stages of vascular differentiation, phloem and xylem precursor cells undergo distinct developmental programs and differentiate into mature phloem and xylem elements. However, to understand the different regulatory networks governing vascular development, it's important to consider how vascular tissues initially emerged and specialized. As evolutionary processes allowed plants to colonize land, adaptations were necessary for the formation of structures that would enable their survival in the new habitat. As multicellular plant bodies of increased complexity and size emerged, new forms of efficient long-distance transport for water, nutrients, metabolites, and small signaling molecules became necessary (143, 234).

In early land plants and extant bryophytes, water and nutrients were transported from water conducting cells (WCC), and carbohydrates and amino acids through food conducting cells (FCC). Currently, it is known that plant vascularization originated from these specialized cells, with WCC being very similar in function and structure to xylem tracheary elements and FCC resembling phloem sieve cells. Moreover, through various molecular studies, it has been determined that the transcriptional networks governing the differentiation of WCC and FCC, along with the regulatory networks for the differentiation of tracheary and sieve elements, are largely conserved among existing land plants (143, 172, 234).

Nowadays, different types of more specialized WCC and FCC can now be recognized, such as hydroids and leptoids of some *Bryophytes*. At the cellular level, hydroids undergo several structural modifications in the cell wall and eventually die off. Thus, their cell wall may thicken in some cases, although they lack lignin. When hydroids form hydromas, they enable vertical growth of up to 50 centimeters and have been considered precursors of true conducting vessels like xylem, as their cell walls are highly permeable to water, allowing for its transport through the plant's main axis (133, 143, 172, 234). However, tracheophytes, more complex vascular plants, also have other specialized types of WCC. Tracheophytes have lignified WCC known as tracheids, and their conducting tissues are organized into vascular bundles consisting of xylem with tracheary elements and phloem with sieve elements (234). Similarly, to hydroids, the leptoids found in some moss species exhibit characteristics similar to the sieve elements of the protophloem, as they display loss of large vacuoles and cytoplasmic polarization, along with a high content of plasmodesmata in the terminal cell walls. This allows the transport of nutrients through the plant body, similar to phloem cells, which undergo organelle degradation and form plasmodesmata for the proper transport of photoassimilates (133, 143, 172).

In accordance with the foregoing, although it is suggested that WCCs and FCCs in non-vascular plants share, to some extent, a similar developmental program with the tracheary elements and sieve elements of vascular plants (237), it is known that the origin of xylem and phloem cells comes from the procambium and/or vascular cambium, and their relation it’s not clear yet. Therefore, it is important to understand the molecular basis that has been unveiled in the process of vascular tissue differentiation, as well as the formation of WCC and FCC. In the case of *Arabidopsis*, the differentiation of vessel elements is initiated by the master regulator *VASCULAR-RELATED NAC-DOMAIN* (*VND*), which activates the expression of genes regulating programmed cell death (PCD), and also induces *MYB* transcription factors that are key intermediaries for activating secondary cell wall (SCW) formation. On the other hand, the master regulator for phloem development, *ALTERED PHLOEM DEVELOPMENT* (*APL*), regulates *NAC45* and *NAC86*, which activate nuclear degradation through positive regulation of the expression of the gene that encodes the exonuclease domain protein NAC45/86-DEPENDENT EXONUCLEASE-DOMAIN PROTEIN 1 to 4 (NEN1 to NEN4) (26, 130, 83, 172).

Meanwhile, there are many gene families that are key to vascular development, and it's important to note that these families are evolutionarily older than the appearance of tracheophytes. In this sense, it's important to consider that during the evolutionary development of vascular plants, there were gene duplication events, gene modifications, or genes that are jointly necessary to direct and regulate vascular development. In this case, many regulatory networks that govern vascularization underlie the biochemical processes necessary for the production of vascular tissues and lignification of secondary cell walls (178, 143).

1.4.1 HD-ZIP transcription factors

Transcription factors (TFs) containing homeodomains (HD) were first discovered in *Drosophila melanogaster* in 1983, due to anomalies caused in homeotic genes (85, 86). They were subsequently identified in other species across all eukaryotic kingdoms. Thus, one of the first genes discovered in plants with a homeobox was the *Knotted1* gene in *Zea mays* (225). Subsequently, 14 families were identified based on the sequence similarity of the HDs and their unique co-domains. One of these classes, termed Homeodomain-Leucine Zipper (HD-Zip), comprises proteins that have an HD adjacent to a leucine zipper (LZ) domain. Thus, LZ allows the proteins to form homo- or heterodimers, while the HD, immediately upstream of LZ, is responsible for the protein's specific DNA recognition and interaction (185).

It is important to note that this particular structural association with both motifs, HD and LZ, in a single molecule is exclusive to plants. Thus, *HD-Zip* TFs are classified into four subfamilies, termed Class I, II, III, and IV, based on the conservation of the HD-Zip domain and the presence of additional conserved motifs outside this structure (185).

1.4.1.1 Class III HD-ZIP transcription factors are essential in vascular development

The *HD-ZIP Class III* gene family is a group of transcription factors that play an essential role in vascular development in land plants. Characteristically, they encode proteins that bind to specific DNA sequences, regulating the expression of genes involved in vascular bundle formation. This gene family expanded and diversified in land plant lineages, acquiring new expression patterns and functions over time. They predate the appearance of vasculature; therefore, the TFs encoding them are also evolutionarily ancient and found in all land plants. Additionally, the miRNAs that negatively regulate them are also highly conserved (143). Their significance lies in their functions in embryo patterning, meristem maintenance, leaf development, inflorescence architecture, ovule development, growth response to environmental signals, and senescence (143, 183).

The Class III HD-ZIP family comprises approximately 100 genes in *Arabidopsis thaliana* and a similar number in other land plants. These genes are divided into two main subgroups: Class IIIA and Class IIIB. Class IIIA genes are primarily expressed in the xylem, regulating the expression of key genes involved in tracheary elements and vessels elements formation, while Class IIIB genes are mainly expressed in the phloem, regulating the expression of genes involved in sieve elements and companion cell formation (143, 183).

From the IIIA and IIIB subgroups, five subclades are known in *Arabidopsis*: REVOLUTA (REV)/INTERFASCICULAR FIBRELESS 1 (IFL1)/AMPHIVASAL VASCULAR BUNDLES 1, PHABULOSA (PHB)/ATHB14, PHAVOLUTA (PHV)/ATHB9, HOMEOBOX 8 (ATHB8), and CORONA (CNA)/INCURVATA 4/ATHB15, all of which play an essential role in all stages of vascular development, from procambium induction to vascular cell differentiation. In the procambium induction phase, *REV*, *PHB*, and *PHV* genes indicate to the meristematic cells that they should begin differentiating into vascular cells. In the differentiation phase, *ATHB8* and *CNA/ATHB15* genes regulate the expression of genes encoding proteins involved in the formation of distinctive vascular cell features, such as the sieve elements of phloem cells and the lignified cell wall of xylem cells (143, 183).

Mutations in *Class III HD-ZIP* genes have been observed to cause the development of plants with defects in the vascular system. These plants may exhibit reduced growth, low environmental stress resistance, and loss of the ability to transport water and nutrients, suggesting the essential role played by TFs of the Class III HD-ZIP family in the vascular development of land plants (213, 183).

1.4.2 Phloem

The phloem primarily facilitates the mass transport of water and carbohydrates from sites of synthesis to demand organs. Additionally, it enables the passage of amino acids, mRNA, phytohormones, and other compounds crucial for pathogen response. Moreover, it plays a role in signaling for flowering, exemplified by *FLOWERING LOCUS T* (*FT*) in *Arabidopsis* (236, 245, 23, 246). From the region adjacent to the meristem, the phloem connects leaves, stems, and roots throughout the plant, as its cells are sequentially established forming a continuous pathway resembling tubes (216). In apical regions, phloem formation is crucial for the continuous growth and development of new organs, as meristems are areas with highly active cellular metabolism, requiring a high demand for photosynthetic compounds to provide the necessary energy for cell division and wall synthesis (162, 258, 91).

In this context, the phloem, as a highly specialized conducting tissue, is primarily composed of two equally specialized cell types: sieve elements (SE), companion cells (CC), phloem parenchyma, and phloem fiber cells, all originating from the procambium. Characteristically, mature SE are elongated and slender cells aligned along the longitudinal axis, forming a sieve tube. The long-distance distribution of photoassimilates is achieved through the establishment of this tube. To facilitate this long-distance transport, during early phloem development, SE undergoes significant changes in their morphology. These changes are driven by selective autolysis involving the degradation of organelles such as vacuoles, rough endoplasmic reticulum (ER), Golgi apparatus, combined with enucleation in the final stage of differentiation. Thus, mature SE exhibit a significant reduction in their cellular contents, necessary for establishing an efficient transport route through the vascular conduit. However, SE remains alive, preserving some organelles in modified form such as the plasma membrane, mitochondria, smooth ER, phloem plastids, and P proteins (phloem protein material) (61, 209, 122, 143, 188, 187, 98, 97, 91). Additionally, SE remains interconnected with neighboring CC via residual ER located in the plasmodesmata, providing a pathway for protein exchange (205).

In addition to organelle degradation, SEs also undergo significant modifications in their cell wall. Initially, during phloem differentiation, SEs experience thickening of the cell wall, providing mechanical support against the high turgor pressure resulting from the elevated sugar concentration throughout the sieve tube. To achieve this, callose deposits in the form of plates are generated around the PD, replacing the cellulose already present. Overall, callose accumulation at the PD is controlled by callose synthases (CALS; EC 2.4.1.34), which are endogenous enzymes mediating callose synthesis, along with the hydrolytic enzymes β-1,3-glucanases (BG; EC 3.2.1.39), which specifically degrade callose (250, 224).

As SEs mature, the deposited callose along with the middle lamella (connecting the cell walls between SEs) are degraded, giving the cells a sieve plate appearance with enlarged pores. The lateral cell walls also develop specialized pore areas derived from the PD, known as lateral sieve areas. Thus, both the sieve plates and newly formed lateral sieve areas allow each individual SE to compose an integrated sieve tube system, facilitating effective fluid transport through the phloem. Consequently, this transport becomes more effective over time, as vascular tissues age, the pores considerably increase in size, allowing for greater transport potential (142, 143, 97).

As SEs are differentiated by a specialized autolysis mechanism involving enucleation, during this process, nuclear contents are released and degraded in the cytoplasm while other organelles reorganize and the cytosol degrades. These cellular rearrangements, in *Arabidopsis*, are orchestrated by TFs with NAC domain; *NAC45* and *NAC86*. The specific expression of these two genes has been observed in some SEs before enucleation, as well as in the pericycle cells of the phloem pole. Thus, *NAC45/86* activate a family of nucleases, the NAC45/86-DEPENDENT EXONUCLEASE DOMAIN PROTEIN 1-4 (NEN1-4), where *NEN1* and *NEN2*, and *NEN4* are specifically expressed in proto phloem cells to induce nucleus degradation initiation and subsequent SE differentiation (82, 187).

Regarding the CCs, they are specialized parenchyma cells with densified cytoplasm, partly due to an increased number of plastids, numerous mitochondria, a large nucleus, a small vacuole, and abundant ER with many attached ribosomes. Since the CCs are connected to their adjacent SEs by numerous branched PD, this allows them to receive energy, assimilate, and macromolecular compounds such as proteins and RNA. In this sense, the PDs serve as structural bridges allowing the transport of signaling molecules related to cell communication. Therefore, from the onset of phloem differentiation, the proper establishment of the SE-CC complex as a functional unit is essential (61, 143, 97).

In previous studies (205), it was observed in *Gossypium hirsutum* L. plants how the establishment of PDs in CCs also allowed them to be more direct with phloem parenchyma cells. Given that SEs showed very limited symplastic transport with phloem parenchyma cells, it was proposed that CCs play a role in mediating communication along the radial axis consisting of phloem parenchyma cells, CCs, and SEs. Generally, phloem parenchyma cells have larger vacuoles and fewer ribosomes compared to CCs, besides being essential for the structure and functioning of the vascular conduit.

Accordingly, a highly integrated regulatory network with specific patterns of gene expression allows the differentiation and characteristic morphology of the cells composing the phloem. Currently, two key genes for its development and maintenance are recognized: *ALTERED PHLOEM DEVELOPMENT* (*APL*) and *OCTOPUS* (*OPS*). *APL* is a MYB family TF, localized in the nucleus, and essential for proper differentiation of SEs and CCs. This TF participates during the late stage of phloem development, where cell wall architecture and callose accumulation depend on it. Moreover, *APL* contributes to spatial confinement during xylem differentiation, through its expression in both cell types (SE and CC) (26, 143, 191).

The gen *APL* is expressed from embryogenesis, at the torpedo stage for *Arabidopsis*, onwards. Mutants *APL* are often seen to be lethal, as they exhibit a specific defect where meta-phloem cells are absent and proto-phloem cells develop xylem cell characteristics (146, 215). In *Arabidopsis* seedlings with *APL* mutation, various observations indicate that shoot development halts after the formation of a few true leaves, while in the root, neither SEs nor CCs are evident at the phloem poles' location. Additionally, at the hypocotyl-cotyledon junction, the characteristic callose deposition in SEs is absent (26).

In earlier work, aiming to investigate the pattern and differentiation of phloem cells during *Arabidopsis* early development, five specific phloem differentiation markers called PHLOEM DIFFERENTIATION 1–5 (PD1–PD5) were identified in genetic trap mutants.

Among these, PD4 was identified as *BREVIS RADIX* (*BRX*), and PD5 as *OCTOPUS* (*OPS*), respectively. Regarding *BRX*, it was predominantly observed to be expressed in proto-phloem cells and identified as one of the targets of *MP*. Roots with *brx* mutations exhibit discontinuity in *APL* expression and defects in phloem differentiation, where the number of cells in the transition area to proto-phloem is reduced (15).

Regarding *OPS*, it is a transcription factor that encodes a protein associated with the polar membrane in procambial and phloem cells. The *OPS* protein has a size of 686 amino acids with a glycine-rich domain at its C-terminal end, although it is not classified as a glycine-rich protein. Also, it belongs to a family of five genes in *Arabidopsis* that share a domain of unknown function (DUF740) (157, 215). Regarding its expression, it initially occurs in procambial cells and initial proto-phloem cells, where once the vascular cell type is specified, *OPS* is restricted to the phloem cell lineage during plant development. Thus, in *Arabidopsis*, it has been observed initially at the heart stage during embryogenesis. Additionally, genetic and molecular studies have shown that *OPS* interacts with other transcription factors and signaling pathways to coordinate phloem development. In this regard, *OPS* can interpret signals from *APL* for cell differentiation, contributing to longitudinal signaling by inducing the specification of SEs through their still undifferentiated precursor cells. Linked to this, *OPS* mutants show affected vascular patterns in cotyledons and discontinuous phloem differentiation in roots, demonstrating its importance in vascular development. It is also worth noting that similar genes to *OPS* have been observed in higher plants, and in higher plants where their genome has been sequenced, the presence of *OPS* is usually consistent (215, 143, 8, 191).

During the establishment and specification of proto-phloem cells, *OPS* also interacts with brassinosteroids (BR) signaling pathways. Initially, hormonal perception of BR at the plasma membrane occurs through the BR INSENSITIVE 1 (BRI1) kinase receptor, and its nuclear response is mediated by the transcription factors *BRI1-EMS-SUPPRESSOR 1* (*BES1*) and *BRASSINAZOLE-RESISTANT 1* (*BZR1*). The non-phosphorylated active forms of *BES1* and *BZR1* accumulate in the nucleus and bind to their target genes to induce the cellular BR response, promoting cell expansion or participating in negative feedback of BR biosynthesis. In this regard, *BES1* and *BZR1*, when expressed in proto-phloem cells, participate in their differentiation (8, 194, 87).

In previous work, Anne *et al.* (2015) observed that *OPS* directly represses the *GSK3 BIN2* by sequestering it in the plasma membrane. *BR INSENSITIVE 2* (*BIN2*) is a glycogen synthase kinase 3 (*GSK3*) that phosphorylates *BES1* and *BZR1*, inducing their degradation and limiting their accumulations in the nucleus. However, the presence of BRs through the phosphatase *BRI1 SUPPRESSOR 1* (*BSU1*) represses *BIN2* expression and releases the repression mechanism. Similarly, in yeast and plants, it was observed that *OPS* releases the repression of the key TFs *BES1* and *BZR1* by inhibiting *BIN2*, paving the way for phloem differentiation. Consequently, *OPS* can activate BR signaling responses through the accumulation of non-phosphorylated *BES1*. As known, BRs are also important regulators of the vascular balance between xylem and phloem differentiation; therefore, loss of function in *brl1* causes abnormal proportions of phloem and xylem differentiation, affecting the vascular conduit (30, 8, 87).

From the above, it can be appreciated how different molecular mechanisms lead to the morphological modifications experienced by the main cells that make up the phloem. From these modifications, the constitution of highly specialized vascular conduits for long-distance transport in the plant is initiated. Various studies discuss the theory of pressure flow, which explains the transport mechanisms through the phloem, where the massive flow in the SE is driven by a difference in osmotic pressure, which is usually high in photosynthetic/source tissues, where sucrose is loaded, and low pressure in draining/sink tissues, where sucrose is unloaded (121). In the case of xylem, the tension-cohesion theory also provides an explanation for the upward translocation of water through the tracheary elements, proposing that water evaporation on the leaf surface due to transpiration generates a negative hydrostatic pressure that drives sap ascent through the vascular conduit (119, 97). Although the present paper does not aim to delve into these long-distance transport theories, it is worth noting that these hypotheses complement the different explanations of phloem and xylem tissue ontogeny.

1.4.3 Xylem

The xylem comprises the pathway through which water and nutrients are absorbed by the roots and transported to the sites of evapotranspiration in the shoot system. During its development, the cells of the vascular conduits undergo specialized differentiation whereby internal flow resistance is minimized and cell walls thicken, enabling more efficient flow through the vessels, in addition to providing mechanical support. While the sieve elements (SE) of the phloem, through their cellular differentiation, allow the transport of molecules through their symplastic space, on the other hand, the tracheary elements of the xylem, during their differentiation, undergo complete removal of their cellular components allowing an apoplastic transport route (97).

In both root and shoot vascular tissues, the xylem is established through the differentiation of procambium/cambium into tracheary elements (TE), xylem fiber cells, and xylem parenchyma cells. In this way, the various types of xylem cells differentiated in the shoot apical meristem (SAM) and root apical meristem (RAM) act as conduits, which not only connect different parts of the plant and provide physical support to aerial organs but also enable long-distance transport of signaling molecules, maintaining cellular communication (143, 162).

In this regard, cell-to-cell communication becomes essential for the development of vascular tissues. Different molecules act as signaling agents directing the differentiation, formation, and organization of xylem and phloem from procambial and cambial cells. Among these signaling molecules, phytohormones such as auxins, cytokinins, and brassinosteroids are primarily known. However, an inhibitor of tracheary element differentiation (TDIF) also plays a role in xylem cell differentiation and proliferation (77, 112, 126). TDIF is a small peptide composed of twelve amino acids with hydroxylation at two proline residues. It belongs to the CLAVATA3/EMBRYO SURROUNDING REGION-related (CLE) family, some of whose members are known to act in cell-to-cell communication within plant meristems. The TDIF sequence is encoded by two genes, *CLE41* and *CLE44*, and its receptor, the membrane protein kinase RECEPTOR/PHLOEM INTERCALATED WITH XYLEM (TDR/PXY), belongs to the Class XI LEUCINE-RICH REPEAT RECEPTOR-LIKE KINASE (LRR-RLK) family (125, 112, 72, 102, 99, 100, 101, 78).

The significance of the TDIF signaling pathway perceived by TDR lies in its crucial role in maintaining vascular stem cells, as it promotes the proliferation of procambial cells and inhibits xylem differentiation. While *TDR* expression predominantly occurs in the procambium and vascular cambium, *CLE41* and *CLE44* are expressed in the phloem and surrounding cells. It has been observed that defects in *TDR* or *CLE41* in the hypocotyl lead to depletion of procambial cells, resulting in the formation of xylem vessels adjacent to phloem cells (102, 99, 143). Based on this, Lucas *et al*. (2013) propose that *TDIF* produced in phloem cells is perceived in procambial cells by *TDR*. Consequently, *TDR*, upon perceiving *TDIF*, promotes the self-renewal of procambial cells through *WOX4* and, on the other hand, inhibits tracheary element differentiation, likely by interfering with the expression of TF *VND6*/*7*.

The HOMEOBOX gene (*WOX4*), related to *WUSCHEL* (*WUS*), a homeodomain TF essential for maintaining stem cells in the SAM, is predominantly expressed in the procambium and cambium, being a key target of the *TDIF* signaling pathway as previously discussed. However, in *Arabidopsis*, it was observed that plants treated with *TDIF* application showed an increase in *WOX4* expression regardless of *TDR* perception. Thus, these genetic analyses demonstrated that although *WOX4* is necessary for the proliferation of procambium and cambium cells, it does not directly repress xylem differentiation in response to the *TDIF* signal (99). Hence, Hirakawa *et al*. (2010) proposes that the *TDIF*-*TDR*-*WOX4* signaling pathway is crucial in maintaining the procambium and vascular cambium during secondary growth.

In the same vein, ethylene signaling pathways (ERF) can also regulate the cell division activity of the procambium and cambium, in addition to functioning in parallel with the *CLE41*-*TDR*/*PXY* pathway (63). Consequently, the two intracellular signaling pathways, *TDR*/*PXY* and *ERF*, after *TDIF* recognition by *TDR*, diverge, independently regulating the behavior of vascular stem cells (143). Similarly, other regulatory pathways of plant phytohormones also interfere with xylem development. It has been observed that the interaction between the CLE peptide and cytokinin signaling can interfere with xylem differentiation. CLE inhibits protoxylem vessel formation in *Arabidopsis* roots by activating cytokinin signaling (112, 145, 127).

In *Arabidopsis*, the response to cytokinins has been formulated as the *AHK–AHP–ARR* phosphorelay signaling, initiated by the cytokinin receptor histidine protein kinases (*AHK2*, *AHK3*, and *AKH4*/*CRE1*/*WOL*) (244). In this regard, previous studies observed how treatment with the CLE9 and CLE10 peptides, which encode the same CLE peptide, affected protoxylem cell differentiation in roots by reducing the expression of *ARR5* and *ARR6*, which are type A ARRs (negative regulators of cytokinin signaling). Thus, *arr5* and *arr6* mutants exhibited defective protoxylem vessel formation, while *arr10* and *arr12* mutants, which are type B ARRs (positive regulators of cytokinin signaling), showed ectopic protoxylem vessel formation (127).

As the vascular system is built through specific modifications of the cell wall and subsequent specialization of each cell, during the formation of tracheary elements (TE) and xylem fibers, two important events take place: programmed cell death (PCD) and the formation of secondary walls known as lignification. After the loss of the nucleus and cellular content, xylem cells are formed following PCD, where thickened secondary cell walls remain to form conduits that allow the transport of water and nutrients from the root to the aerial part (93, 25, 162). However, these processes require a highly specialized gene network, where different transcription factors direct the various processes leading to the specific morphology of xylem cells.

Initially, the process leading to the origin of xylem involves *Class III HD-ZIP* genes, which positively regulate its cellular differentiation. This can be evidenced as, in *Arabidopsis* roots, miR165/166 promotes the differentiation of protoxylem vessels while degrading transcripts of *Class III HD-ZIP* that promote metaxylem. Additionally, it has also been reported that *KANADI* can interfere with *Class III HD-ZIP* TFs by jointly regulating the differentiation of vascular cambium cells. On one hand, *KANADI* inhibits auxin transport, and on the other hand, *Class III HD-ZIP*s promote xylem cell differentiation (165, 143, 162, 191).

It's also important to highlight that member of a subgroup of NAM/ATAF/CUC (NAC) domain proteins, the *VASCULAR-RELATED NAC-DOMAIN* (*VND*) proteins, and the *SECONDARY WALL THICKENING PROMOTING FACTOR*s/*SECONDARY WALL-ASSOCIATED NAC DOMAIN PROTEIN*s (*NST*s/*SND*s), function as specific plant TFs in inducing xylem cell differentiation through their ectopic expression (48). The TFs *VND6* and *VND7* are recognized here for initiating differentiation of protoxylem and metaxylem vessels respectively, while *SND1*/*NST3* and *NST1* are known to induce xylem fiber differentiation (130, 238, 239, 252, 251, 191, 169).

In this pathway, the transcriptional network primarily regulating xylem cell differentiation is constituted by the TFs *SND2*, *MYB46*, *MYB103*, and *KNOTTED1-LIKE HOMEODOMAIN PROTEIN 7*, as direct targets of *NST3*/*SND1*/*ANAC012* and possibly *NST1*, *VND6*, and *VND7*. Thus, during primary growth in vascular plants, xylem, originating from the procambium, initially differentiates into protoxylem, characterized by annular and spiral secondary wall thickenings, which are established prior to cell elongation or growth, during the initiation of new organ development. Subsequently, metaxylem is derived from protoxylem, composed of reticulate and pitted secondary walls, which is established once cell elongation ceases, i.e., once organ growth stops (239).

For this purpose, the master regulators *VND6*, *VND7*, and *SND1*/*NST3* induce the formation of secondary walls in xylem vessels and fibers through a hierarchical gene network. Initially, *VND6*, *VND7*, and *SND1* induce the expression of *MYB46*, *MYB83*, and *MYB103*. Since the TFs *MYB46* and *MYB83* regulate the biosynthetic pathways of three main components constituting the secondary wall: cellulose, lignin, and xylan, then *MYB58*, *MYB63*, and *MYB85*, which could be target genes of *MYB46* and/or *MYB83*, specifically regulate genes related to the lignin biosynthetic pathway, the main constituent of wood and fibers (255, 153). In this regard, both *SND1*/*NST3* and *VND6* and *VND7* can alter their hierarchy at the top of the gene network by positively regulating TFs like *MYB*. Furthermore, within this hierarchy, *MYB* TFs can act as secondary or tertiary regulators for the positive expression of other genes encoding enzymes that catalyze secondary wall thickening during specific stages of xylem fiber differentiation (170, 239, 143).

Consequently, during the genetic control for xylem differentiation, *VND6* and *VND7* together can form subclades with *VND1* to *VND5* for proto and metaxylem vessel differentiation (130). Functional suppression of *VND6* and *VND7* results in the inhibition of vascular element formation; thus, it is suggested that these TFs act as master switches for TE formation (130, 238). According to the above, the *Arabidopsis* TF *VASCULAR-RELATED NAC-DOMAIN 7* (*VND7*), acting as a master regulator of xylem vessel differentiation, interacts with *VND-INTERACTING 2* (*VNI2*), expressed in both xylem and phloem cells in roots and shoot inflorescence stems. In this regard, *VNI2* overlaps with *VND7* expression, suppressing its ability to activate transcription, causing a repression in xylem vessel differentiation. Therefore, the transcriptional activity of *VND7* may be regulated by its interactions with other VND proteins (239, 57).

In plants, several developmental processes involve PCD, a process that can be independently regulated as it involves its own specific developmental steps. In the case of mature TE formation, the onset of PCD begins with signals of cell death, followed by the accumulation of autolytic enzymes in the vacuole. Thus, vacuole swelling and collapse release hydrolytic enzymes such as cysteine proteases XCP1 and XCP2, Zn^{2+}-dependent nuclease ZEN1, RNases, as well as nucleases, proceeding to degrade nuclear content. In this regard, PCD and secondary wall deposition, where cortical microtubules regulate the spatial pattern of the cell wall by orienting cellulose deposition, are two closely related processes necessary for xylem cell differentiation (76, 143, 81).

For the former, the master TF in TE formation, *VASCULAR-RELATED NAC-DOMAIN 6* (*VND6*), coordinately regulates some of the genes involved in PCD processes, while *SECONDARY WALL-ASSOCIATED NAC DOMAIN PROTEIN 1* (*SND1*) regulates xylem fiber formation as previously mentioned. What happens is that although *VND6* and *SND1* govern different aspects of xylem formation, they can still act together to regulate common genes, specifically those related to secondary cell wall formation (252, 170, 239, 143).

In previous studies, Ohashi-Ito *et al*. (2010) observed that only *VND6* positively regulated genes involved in PCD for TE formation, identifying a specific cis element present in its promoter, the tracheary element-regulating cis-element (TERE). In this regard, both *VND6* and *VND7* bind to the TERE sequence, which is present in many gene sequences expressed in xylem vessel cells, to simultaneously regulate the expression of genes involved in both secondary wall formation and PCD. On the other hand, *SND1* binds to a specific sequence, the *SECONDARY WALL NAC BINDING ELEMENT* (*SNBE*), for xylem fiber formation (170, 143).

All the foregoing elucidates how the differentiation of xylem cells is regulated in a highly organized manner, where different transcription factors orchestrate the signaling cascade for TE differentiation and xylem fiber formation. It is worth noting that, although these two processes are distinct, they converge harmoniously to establish the vascular structure through autonomous and non-autonomous cellular processes (143).

1.5 Leaf vascular development

Conducting tissues in leaves are crucial for proper solute translocation. Vascularization is established in veins, and at least seven distinct cell types make up the leaf organ to carry out specific functions of transport, metabolism, and signaling. Thus, the different cells that will form the vascular system are first specified in the aerial part in cotyledons and subsequently in the main axis of the seedling as provascular tissues. Initially, in the leaf blade, vascularization forms a network resulting in connected vascular strands. Provascular tissues can thus be observed initially in the cotyledon midvein before vascular differentiation, as continuous procambial strands. Leaves initiate at the flanks of the shoot apical meristem developing a specialized upper (adaxial or dorsal) side for light capture and a lower (abaxial or ventral) side for gas exchange. Consequently, vascular strands in leaves are typically positioned where the adaxial and abaxial domains meet, respectively (198, 183, 29, 120).

In the *Arabidopsis* embryo, two phloem strands form along the main longitudinal axis, symmetrically flanking the xylem axis. At the cotyledonary node, each phloem strand bifurcates into two strands, with each entering a cotyledon. Thus, in the mid-cotyledonary vein, there are two phloem strands in the proximal part. In the distal part of the midvein, as well as in secondary and higher-order veins, only a single abaxial phloem strand has been found (15, 98).

The final differentiation of vascular precursor cells, in general, is not a synchronous process and may initiate in different locations within the plant. For instance, after germination in *Arabidopsis*, the differentiation of proto-phloem precursor cells into mature SEs in leaves begins simultaneously in two locations: the mid veins of cotyledons and the cotyledonary node. Subsequently, the growth of provascular cells progresses from the midvein along the cotyledon veins and from the cotyledonary node toward the hypocotyl and root, establishing a continuous network of functional vascular cells (27, 15).

Given that auxin transport and accumulation are the primary parameters for establishing vascular patterning, because of the PIN1 cellular efflux transporter activity, auxin is channeled to the provascular regions, activating the expression of the preprocambial marker *AtHB8*. The *AtHB8* gene is expressed prior to procambial identity in developing veins during vascular strand formation in leaves. The resulting auxin accumulation activates *MP*, which in turn regulates PIN1 to produce a positive feedback loop that restricts the expression of *MP* and PIN1 to a point of convergence (197, 143, 29). However, *AtHB8* expression can regulate *ACL5* along with a gene involved in spermine biosynthesis, *BUSHY AND DWARF 2* (*BUD2*). In this regard, *ACL5* can inhibit the expression of *AtHB8* and its related genes, creating a negative feedback loop (13).

After the initial differentiation, the vasculature develops into bundles. In leaves, being lateral and flattened organs, these bundles exhibit a radial pattern where the phloem is abaxial (ventral) and the xylem is adaxial (dorsal), divided by the active procambium. The juxtaposition of adaxial and abaxial characteristics allows the leaf to grow into a flattened lateral organ. This radial pattern is established during embryogenesis by the key radial pattern TFs *KANADI* (*KAN*1-4) and *Class III HD-ZIP*s: *PHB*, *PHV*, *REV*, and *ATHB15*. The *KAN* TF family is expressed in the phloem and represses procambial identity, and they are believed to antagonize and complement the activity of *Class III HD-ZIP* TFs, with their effects likely mediated through changes in auxin transport via PIN proteins. In this regard, *KAN* activity appears to promote the differentiation of abaxial/phloem tissue, while *REV*, *PHB*, and *PHV* activity are necessary to maintain adaxial/xylem differentiation (16, 113, 107, 183, 29).

Once vascularization is established in the leaf, its conformation is determined by the phloem tissue with enucleated sieve elements (SE) as actual conduits, coupled with companion cells (CC) alongside phloem parenchyma (PP), and on the other hand, the xylem tissue with dead tracheary elements accompanied by xylem parenchyma (XP), in addition to the vascular parenchyma (VP) located at the interface between the phloem and xylem (120). Additionally, the vascular system is also surrounded by non-conducting cells, such as parenchyma, sclerenchyma, and fibers. Thus, during leaf development, the formation of vascular precursor cells ceases following the differentiation of adjacent mesophyll cells, indicating that vascular patterning occurs within a highly regulated developmental window (197, 143).

1.6 Steam vascular development

In dicotyledonous plants, vascular bundles in the stem are arranged radially, where each bundle contains a procambium that produces phloem outward and xylem inward. In establishing the polarity of cells composing the conducting tissues, *Class III HD-ZIP* TFs play a crucial role in determining the collateral pattern. Additionally, these *Class III HD-ZIP* genes are differentially regulated in their functions by miRNA165 and miRNA166. Generally, miRNAs regulate the functions of their target genes by binding to complementary sequences in target transcripts and subsequently causing translational attenuation or transcript cleavage (254, 29).

Thus, in previous studies, it was demonstrated through *in situ* mRNA localization, along with promoter-GUS analyses, that the *REV* gene is expressed in interfascicular fibers and vascular bundles of *Arabidopsis* inflorescence stems. It was observed that the spatial specification of interfascicular fiber differentiation is mainly regulated by the *INTERFASCICULAR FIBERLESS1* (*IFL1*) gene, as its mutation suppresses the formation of normal interfascicular fibers in stems (253). In this regard, the mutation of *IFL1*/*REV* revealed phenotypes of amphivasal vascular bundle formation and altered organ polarity, similar to what occurs with overexpression of miRNA165. Consequently, transverse sections of stems that overexpressed miR165 showed a reduced number of vascular bundle cells that developed fewer interfascicular fibers compared to wild-type plants. Similarly, it was observed that overexpression of miRNA166, which negatively regulates the expression of three *Class III HD-ZIP* genes, *PHV*, *PHB*, and *ATHB15*, led to a reduction in their transcription level, recapitulating the phenotypes of simultaneous loss of their function (254).

Initially, various hormones coordinate the initiation of vascular pattern formation by promoting procambial cells, which influences the number and size of vascular bundles. In this regard, auxin production correlates with the organization of vascular bundles in the stem, as it drives the establishment of the primary pattern, similar to what occurs in the primary root. Additionally, auxins can act as temporal regulators of xylem formation from the vascular cambium in tree trunks (193, 198, 29). Brassinosteroids, on the other hand, can also stimulate early divisions in the procambium once primary growth is initiated through the *GSK3-BES1* signaling pathway (106).

Before the final determination of cell fate in the conducting tissues, GLYCOGEN SYNTHASE KINASE3/SHAGGY-LIKE KINASE (GSK3/SK) proteins regulate vascular cell differentiation by maintaining procambial and cambial cells, suppressing their differentiation into xylem or phloem cells (195). In this regard, a member of the GSK3 protein, *BRASSINOSTEROID INSENSITIVE2* (*BIN2*), initially binds to the cytoplasmic kinase domain of *TDR*. Subsequently, extracellular *TDIF* induces the release of *BIN2* from *TDR* into the cytoplasm, activating *BIN2* signaling to inhibit xylem differentiation (128, 195, 79).

As mentioned earlier, *TDIF* is produced and secreted from phloem cells, generating the *TDIF-TDR* signal for the maintenance of procambial cell populations through the regulation of two main processes: first, the promotion of procambial cell division, and second, the suppression of procambial cell differentiation into tracheary elements (TE). For the maintenance of vascularization in the stems, *TDIF* signaling induces the differentiation of stem cells into transit-amplifying cells during the procambial stage. In transit-amplifying cells, the *TDIF-WOX4* and *TDIF-GSK3*/*SK*s/*BES1*/*BZR1* signaling pathways regulate the promotion of cell division and the inhibition of differentiation into xylem and phloem cells, respectively (79).

On the other hand, in previous studies, Kondo *et al*. (2014), through the design of an *in vitro* culture system to analyze vascular cell differentiation called VISUAL (Vascular Cell Induction Culture System Using *Arabidopsis* Leaves), found that the TF *BRI1-EMS-SUPPRESSOR 1* (*BES1*), acting downstream of the *TDIF-TDR-GSK3* pathway and genetically independent of the *TDIF-TDR-WOX4* pathway, is involved in maintaining procambial cells. Thus, *BES1* functions as a downstream target of *GSK3* during xylem formation, promoting bidirectional differentiation of procambial cells into xylem and phloem cells. It is noteworthy that the *GSK3-BES1* signaling pathway also participates in the brassinosteroids (BR) signaling pathway. Perception of BR by *BRASSINOSTEROID-INSENSITIVE 1* (*BRI1*) leads to the inactivation of *GSK3*, subsequently activating the TFs of the *BES1* family to control downstream gene expression. In *Arabidopsis*, six members of the *BES1* family are known, of which, along with the closest homolog of *BES1*, *BRASSINAZOLE RESISTANT 1* (*BZR1*), are considered the main mediators of BR signaling (195).

Other phytohormones known to facilitate the establishment of vascular bundles during embryogenesis and maintain cambial activity in the stem are cytokinins. Although their role in the initial establishment of stem vascular bundles is unclear, trans-zeatin-type cytokinins are synthesized primarily in the root vascular bundles and transported via xylem to the shoot, regulating shoot growth and vice versa (117, 123).

1.7 Root vascular development

The vascular system in the roots of higher plants is primarily constituted by a cylinder formed by the vascular tissues procambium, phloem, and xylem. However, the root system is also surrounded by radially symmetric layers of pericycle differentiated into cells of the xylem pole and cells of the phloem pole, the endodermis, the cortex, and the epidermal cells. During root growth, different cellular patterns are established through asymmetric cell divisions and maintained through self-renewal of meristematic cells with pluripotent activity. Thus, different cellular identities originate from stem cells around the quiescent center (QC), from which initial cells of the provascular, stele, endodermis, epidermis, and columella arise. In this context, in the tissue organization of the conducting vessels of the *Arabidopsis* root, the xylem exhibits a diarch form with peripheral protoxylem and central metaxylem, flanked by two phloem tissue poles (143, 183).

The centripetal pattern of conducting tissues in the *Arabidopsis* root is the result of highly regulated cell division and specification events. Thus, the interaction between the vascular cylinder and the surrounding endodermis, mediated by the movement of transcription factors (TFs) between cells and microRNAs, influences vascular bundle differentiation. In this regard, the transcription factor *SHORT-ROOT* (*SHR*) is expressed in the vascular cylinder, where its protein moves to the endodermis, which surrounds the stele, activating the TF *SCARECROW* (*SCR*). Together, *SHR* and *SCR* direct the pattern of fundamental tissues, mainly that of the endodermis, as they jointly activate the transcription of the three genes encoding miR165 and miR166 in roots; MIR165A, MIR166A, and MIR166B. Thus, the miR165/166 derived from the endodermis diffuses to the center of the stele to form a gradient that restricts the domain of activity of the mRNA of the *HD-ZIP Class III* TFs, mainly *PHB*, which positively regulates *MP*, *IAA20*, and *IAA30* to stabilize the auxin response in the root xylem axis. Consequently, the degradation of *HD ZIP III* is activated, resulting in a gradient that drives the TFs towards the center of the xylem, initiating specification into protoxylem and metaxylem (95, 34, 183).

In this regard, the gradients of miRNA of *HD ZIP Class III* TFs in the root overlap with the balanced signaling between auxins and cytokinins, which establish the identity of xylem and procambium cells initially. On the one hand, cytokinins maintain the differentiation of procambial cells, while auxins, restricted to the xylem axis by the presence of cytokinins, are the main promoters of protoxylem and metaxylem establishment respectively. Therefore, during the establishment of the vascular pattern of the *Arabidopsis* root, multiple intersections occur between auxins, cytokinins, and *HD ZIP Class III* transcription factors shaping the vascular cylinder. However, inhibition of cytokinin signaling in the xylem axis is necessary for vessel formation, and the presence of cytokinin signaling in procambial cells is necessary to maintain them in their undifferentiated state (21, 220, 183, 191).

In general, the aforementioned *HD-ZIP Class III* TFs; *PHB*, *PHV*, *REV*, *COR*, and *ATHB8*, act redundantly to regulate the initial differentiation fate of xylem cells and are sufficient to regulate the xylem pattern in the *Arabidopsis* root (143, 183).

1.7.1 Root xylem

During the early development of the *Arabidopsis* root, two outer protoxylem cells and three inner metaxylem cells can be observed, which can be distinguished based on the characteristics of their secondary cell wall primarily. Morphologically, protoxylem exhibits a helical or annular pattern, while metaxylem cells generate a wall deposition pattern resembling pits. Similarly, they can be distinguished at the molecular level by specific cell type markers, such as *AHP6* for protoxylem and *ACAULIS5* for metaxylem. Before the surrounding root tissues elongate, protoxylem vessels mature and are destroyed during cell expansion. Therefore, metaxylem vessels act as the primary conducting tissue in the main axis of the plant once organs are established (143, 191).

Thus, the differentiation of metaxylem cells is temporally separated from the differentiation of protoxylem cells, in the sense that the outer metaxylem cells differentiate only after the protoxylem cells have differentiated and the surrounding tissues have completed their expansion. Additionally, the inner metaxylem tissue differentiates later than the outer metaxylem tissue (143).

In the protoxylem domain, the peak of auxins activates an inhibitor of cytokinin signaling, *ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 6* (*AHP6*), which is possibly one of the earliest regulators, but not the only one, for protoxylem cell specification. In this regard, while *AHP6* suppresses and spatially restricts cytokinin signaling, allowing the signaling pathway for the establishment of protoxylem cells, cytokinins repress the expression of *AHP6*. Additionally, two transcription factors expressed in the *Arabidopsis* root, *AT-HOOK MOTIF NUCLEAR LOCALIZED PROTEIN 3* (*AHL3*) and *AHL4*, are essential for regulating the boundaries between differentiating xylem and procambium. *AHL4* moves from the procambium to the xylem in the RAM as a heteromeric complex with *AHL3* (145, 143, 220, 183, 256).

In the primary root, the identity of the two types of xylem vessels is determined by the bidirectional signaling pathway *SHR–SCR*–miRNA165/166–*HD-ZIP III*. High levels of miRNA165/166 expression and low levels of *HD-ZIP III* gene expression promote protoxylem identity, whereas metaxylem is specified when *HD-ZIP III Class III* expression levels increase. However, several members of the *MYB* and *NAC* TF families are also known to act in the signaling pathway during vascular differentiation in the *Arabidopsis* root. In this regard, the *MYB46* gene, one of the TFs necessary for synthesizing various components of secondary cell walls in the root's protoxylem and metaxylem, is expressed toward the end of the elongation zone in protoxylem cells and later in metaxylem cells. Additionally, the formation of xylem vessels requires programmed cell death signaling, where nuclear content is initially degraded and then perforated plates connecting each vessel cell are formed. In this context, the formation of secondary cell walls, with patterns of deposition featuring holes or spirals, is initially controlled by the *NAC* TFs; *VND6*, *VND7*, *SND1*, *NST1*, and *NST2* (252, 238, 143, 191).

1.7.2 Root phloem

The differentiation of proto- and meta-phloem can be detected during seedling establishment in independent locations such as cotyledons, shoots, and roots. Additionally, different genes are expressed in each plant organ to regulate the differentiation and establishment of vascular bundles specific to that organ. In the case of *Arabidopsis* roots, where the central vascular bundle branches into lateral roots, *LATERAL ROOT DEVELOPMENT 3* (*LRD3*) plays a crucial role in regulating early phloem development and controlling its transport function. Moreover, it is essential for maintaining a balance between primary and lateral root growth. *LRD3* encodes a LIM-domain protein specifically expressed in companion cells (15, 110).

In this regard, proto-phloem differentiation in the *Arabidopsis* root initiates from a locus independent of the hypocotyl. In the root apical meristem (RAM), phloem cell lineages arise from two initial cell domains through asymmetric cell divisions, where periclinal divisions establish companion cells (CCs) and tangential divisions establish sieve elements (SEs). Although both proto- and meta-phloem SEs within a phloem pole ultimately derive from the same mother cell in the RAM, CCs develop from a different adjacent cell lineage to the sieve tube elements. Thus, when phloem cells have already differentiated, prior to secondary growth, the vascular cylinder predominantly exhibits proto-phloem SEs on the exterior, meta-phloem SEs on the interior, and CCs, responsible for sucrose loading within the SE-CC complex, flanking the two types of SEs respectively. It is through the establishment of proto- and meta-phloem that the actual conducting units of the phloem within the root are composed (146, 26, 143, 190, 118).

The proto-phloem and meta-phloem sieve elements (SEs) are essential for transporting sugars and other solutes towards the root meristem. To meet the energy demand of the growing meristem, proto-phloem SEs differentiate closer to the root tip than all other cell types. Thus, while proto-phloem SEs lose their transport capacity in the more differentiated parts of the root, meta-phloem SEs differentiate further away from the root tip and remain functional for longer periods. Consequently, the meta-phloem transports solutes towards the root tip where they are transferred to the proto-phloem SEs (190).

The maturation of proto-phloem and meta-phloem sieve tubes can occur similarly. Transcriptionally, phloem development is initially coordinated by the transcription factor *SHR*. On one hand, *SHR*'s movement towards the endodermis regulates asymmetric cell divisions for companion cell (CC) formation by activating microRNA165/166. On the other hand, *SHR*'s movement towards the phloem regulates asymmetric cell divisions generating phloem sieve elements (SEs). Consequently, in the phloem, *SHR* sequentially activates *NAC-REGULATED SEED MORPHOLOGY 1* (*NARS1*) and *SECONDARY WALL-ASSOCIATED NAC DOMAIN PROTEIN 2* (*SND2*). Thus, these three genes, *SHR*, *NARS1*, and *SND2*, form a positive circuit, where *NARS1*, generated in the CCs of the root differentiation zone, establishes a top-down signal that drives asymmetric divisions for phloem SE differentiation in the root meristem (118).

Based on the aforementioned signals, in *Arabidopsis* root, cellular differentiation from the initiation of proto-phloem to the formation of mature sieve elements (SEs) is initiated by cell elongation, followed by callose deposition and subsequent thickening of the cell wall longitudinally. For this, the differentiated SEs undergo different morphological processes, where their cellular content is reorganized through modification and realignment of organelles. This process culminates in the breakdown of the nucleus and the formation of pores in the sieve plates that connect each enucleated SE (143).

In this context, *OCTOPUS* (*OPS*), *BREVIX RADIX* (*BRX*), and *COTYLEDON VASCULAR PATTERN 2* (*CVP2*) act as positive regulators of proto-phloem specification and early SE differentiation. *OPS* represses *BIN2* in the brassinosteroids signaling pathway, known for its involvement in vascular differentiation, specifically proto-phloem differentiation. On the other hand, *BRX* suppresses the expression of *BARELY ANY MERISTEM 3* (*BAM3*), also implicated in early proto-phloem development akin to *OPS*. *BRX* restricts *BAM3* expression, the receptor for the small peptide ligand *CLAVATA3/ENDOSPERM SURROUNDING REGION 45* (*CLE45*), which inhibits proto-phloem differentiation. Additionally, *CVP2* is involved in maintaining phosphoinositide levels, which also play a role in proto-phloem differentiation (188, 191).

Among the genes known to regulate phloem in *Arabidopsis*, *OPS* is expressed earlier in the root phloem cell lineage, from the phloem lineage stem cells. The *OPS* mutants exhibit discontinuous development of proto-phloem cells, with gaps of undifferentiated cells interrupting the continuity of SEs. Additionally, the altered differentiation of proto-phloem sieve tube cells also appears to affect the function of CCs, as *OPS* mutants do not express the gene encoding the sucrose transporter *AtSUC2*, responsible for active sucrose loading into the phloem, despite not showing visible alterations in their differentiation (215). Conversely, loss of function of *BRX* and *CVP2*, along with their homolog *CVL1*, also results in the appearance of gap cells and undifferentiated cells in the mature root proto-phloem (188, 190). In this regard, previous studies identified a new protein similar to *OPS*, *OCTOPUS-LIKE 2* (*OPL2*), a homolog of *OPS* that, together with *OPS*, serves as an important regulator of vascular patterning, root growth, and proper phloem development. Hence, *OPS* and *OPL2* were identified as the first genes to act in the differentiation of meta-phloem sieve tubes (190).

In addition to the above, the *APL* gene is also required in the later stages of differentiation of SEs and CCs, through the regulation of the expression of *NAC45* and *NAC86* as discussed in previous sections. However, *APL* is negatively regulated by *NAC20*. Therefore, the key regulators of nuclear degradation in SEs, *NEN1*, *NEN2*, and *NEN4*, act downstream of *NAC45* and *NAC86* for this process. Thus, *APL* along with *OPS* are known to be the main players in the development and maintenance of the phloem; *OPS* regulating proto-phloem differentiation and along with *OPL2* the differentiation of meta-phloem, and *APL* regulating the timing of asymmetric cell divisions that typically give rise to SEs and CCs in the proto-phloem and meta-phloem of *Arabidopsis* roots (26, 143).

1.8 Cambium, the secondary growth

Upon completion of primary growth, both in the root and stem, the cambium is generated from the vascular procambium. In regard to this point, the cambium is the second meristematic tissue that gives rise to the conducting tissues. Additionally, it is primarily responsible for the plant body's diameter growth and its occurrence in nature, dating back to the emergence of gymnosperms, remains conserved (144, 172, 29). However, the origin of the cambium differs in the root and the shoot because each organ presents a different radial cellular organization. In the shoot, the vascular cambium can originate from the fascicular cambium, formed from the procambium of vascular bundles produced during primary growth, and from the interfascicular cambium, which develops later from the parenchyma cells found between each vascular bundle. In herbaceous plants such as *Arabidopsis*, secondary growth from the cambium primarily initiates locally in the basal region of the inflorescence shoot, mainly serving to provide support to the plant (131, 152, 29).

Consequently, two types of meristems are recognized in the formation of the plant's secondary body: the vascular cambium which gives rise to secondary xylem and secondary phloem, and the cork cambium which produces a layer of bark that replaces the epidermis and protects the stem from mechanical damage and pathogens. The onset of vascular cambium activity also leads to a high production of secondary xylem tissue resulting in wood deposition. Therefore, from the cambium, plants also generate wood, which is recognized worldwide for its multiple functions. Thus, depending on the properties of the wood, the cambium, through regular renewal of functional xylem and phloem, provides support as well as adequate nutrient transport to trees and woody plants, ensuring perennial life. Therefore, the appearance of the cambium was a significant evolutionary event in the history of terrestrial plants, especially those that gained greater height and thickness. Moreover, besides being fundamental in wood development, the vascular cambium also lays the groundwork for solute transport support in other plant organs, such as the peduncle of buds, leaves, flowers, and fruits (131, 16, 221).

1.8.1 Secondary growth in stems and roots

In both the root and hypocotyl, cambium initiation occurs when the vascular parenchyma begins to divide, even before the primary xylem has fully matured. Thus, in stems, the vascular cambium forms a continuous cylinder of meristematic cells, producing secondary phloem tissue on the outside and secondary xylem or wood on the inside. Meanwhile, in the trunks of forest trees, cork cambium can be observed depositing just beneath the bark. Regarding roots, asymmetric divisions and cell expansions of the pericycle cause the phloem to be pushed toward the center, away from the xylem until a cambium ring is formed, thus, the cambium is surrounded by phloem inwardly and xylem outwardly. Since xylem constitutes the majority of biomass in roots and stems, its secondary growth will be determined by the rate of cell production in the cambium, eventually leading to the thickening of plant organs. Hence, a large proportion of the Earth's carbon resides in the stems and roots of plants (131, 148).

Although *Arabidopsis* plants do not produce wood or medullary rays like trees, the anatomy of secondary growth at the base of the hypocotyl closely resembles that of wood-producing plants. Thus, since one of the obstacles hindering molecular research on wood formation in trees is the lengthy growth periods required to assess cambial activity, as well as the difficulties in obtaining mutants, previous studies have used *Arabidopsis* as a model for initial investigations into vascular cambium development (36, 181, 29).

In this way, it has been described that in the *Arabidopsis* hypocotyl, secondary growth consists of two main phases: an earlier phase, the first one, where the formation of vessels and secondary xylem parenchyma occurs, and a later phase, the second one, where the secondary cell walls of xylem fibers thicken and lignify to provide structural support. Therefore, during the second phase of *Arabidopsis* secondary growth, there is an increased rate of xylem differentiation, leading to its expansion and resulting in an increase in the hypocotyl base diameter. Additionally, another important aspect of *Arabidopsis* secondary growth is that the transition between the early and late phases in the hypocotyl is triggered along with the flowering process. Thus, the induction of flowering, not the formation of flowers or elongation of the main inflorescence, is considered a sufficient signal to trigger xylem expansion (206) (181). As for the root, it also undergoes phase changes for secondary growth, but unlike the hypocotyl, it is not uniform along the cylinder. This occurs because the root widens near the hypocotyl, which is the oldest part that has undergone more extensive secondary growth over a longer period, in contrast to the tissue nearer to the RAM, which is considerably narrower. Additionally, at this point, the arrangement of vascular bundles is similar to how they are organized in the hypocotyl (17, 29).

1.8.2 Hormonal regulation during cambium development

The second phase of secondary growth is also known as the xylem expansion phase. In previous studies, Ragni *et al*. (2011) observed that during the second phase in *Arabidopsis*, gibberellin (GA) was a crucial signaling molecule mobilized from the shoot to the hypocotyl and root to induce xylem expansion. Furthermore, this process was similar to secondary growth in trees. Accordingly, in various species, the GA pathway in combination with the auxin pathway has been recognized to promote the induction of xylem fiber differentiation and xylogenesis (51, 58, 19, 111, 41, 151, 181, 147), as well as promoting primary differentiation of phloem fibers (1). However, GAs can also have a general growth-promoting effect, suggesting that elevated GA synthesis might enhance xylem fiber production while also promoting growth (181). This phenomenon was also noted in earlier investigations involving transgenic *Populus tremula (P. tremula* × *tremuloides)* trees, wherein the overexpression of a GA biosynthetic gene not only enhanced tree growth but also augmented both the rate and length of total xylem fibers in comparison to wild-type (WT) trees (58).

Auxins are recognized to have a broader role in secondary vascular patterning and are essential in wood formation. Given their peak concentration in the cambium, it's been proposed that auxin flows basipetally within cambial cells, subsequently distributing to the phloem and xylem, respectively (219, 160, 29). Auxin transport within the cambium has been documented in *Arabidopsis* inflorescence stems. Initially, auxins accumulated from differentiated cells of the interfascicular parenchyma, initiating the development of the interfascicular cambium. This process involved an initial auxin accumulation triggered by PIN1 expression, followed by the induction of periclinal divisions by the polar PIN1 protein, specifically localized to the basal plasma membrane of the parenchyma. Therefore, cellular events were initiated within parenchyma cells adjacent to the vascular bundles and subsequently extended from that point towards the middle region of the interfascicular area, situated between neighboring vascular bundles, where the cambial ring closure within the stem ultimately took place (152).

Furthermore, given the synergistic action of GAs with auxins in cambium development, it has been noted that the combined application of both phytohormones yields a more pronounced effect on the cambium compared to the use of either hormone individually (24). In various studies using decapitated poplar stems, it was observed that when treated with GA, the division and expansion of cambial zone cells were stimulated, akin to the effect of auxin treatments. However, when auxins and GAs are applied together, a more pronounced synergistic effect on cambial growth has been observed, similar to growth in primary stems (231, 137, 233, 24, 104). Building on this, Björklund *et al.* (2007) also demonstrated that GAs stimulates auxin transport, as GA applications to poplars stimulated cambial cell division and increased auxin levels in tree stems by enhancing polar auxin transport, likely through increased expression of its transporter PIN1 (24). Thus, while maximum auxin levels are found in the cambium, GA peaks within early xylem differentiation. In this regard, studies on mutant and transgenic plants have shown that GA and its signaling pathway are necessary and sufficient to directly trigger enhanced xylogenesis (111, 181).

Cytokinins also regulate cambium activity and stimulate wood formation. Initially, it was proposed that cytokinins, being a known factor for cell division, acted as a signaling molecule during nuclear divisions followed by cytokinesis, when fiber formation occurred (3). Due to the stimulating effect of cytokinins on cell division, in other studies Nieminen *et al.* (2008) observed that their activity was a direct determinant of radial growth in poplar (*Populus trichocarpa*) and birch (*Betula pendula*). This was demonstrated using transgenic trees ectopically expressing an enzyme that degrades cytokinins in the cambial zone. Thus, in both transgenic poplar and birch, decreased cytokinin signaling was observed, where both apical and radial growth were compromised, showing a reduction in the number of cell divisions in the vascular cambium (159). Likewise, researchers also noted that the *Arabidopsis thaliana* quadruple mutant *atipt1;3;5;7*, which disrupts four genes encoding cytokinin biosynthetic isopentenyl transferases, failed to develop cambium and displayed diminished root and stem thickening. However, following the application of exogenous cytokinins, these plants exhibited augmented secondary growth, thereby restoring the thickening of previously affected organs. Additionally, grafting of the quadruple mutant onto WT plants resulted in normal growth of both root and shoot, where it was determined that shoot-derived trans-zeatin-type cytokinins, but not isopentenyladenine-type cytokinins, restored cytokinin levels from WT plants. Therefore, cytokinins were shown to influence the regulation of cell division rate but not the direction or pattern of cell differentiation in the vascular cambium (148).

On the other hand, multiple studies have demonstrated how different forms of physical stress, such as bending stems or branches in woody plants, induce ethylene biosynthesis (196, 55, 6, 141, 243, 204, 203). In this context, trees and woody plants frequently produce two types of reactive wood in response to mechanical and gravitational forces exerted by the wind. The influence of wind typically stimulates increased stem diameter growth to prevent breakage, resulting in the formation of compression wood. On the other hand, when the trunk is statically inclined, it triggers a localized growth reaction known as tension wood. Consequently, in gymnosperms, compression wood, which is rich in lignin, usually forms in the lower section of inclined stems or branches. Conversely, in dicotyledonous angiosperm trees, tension wood develops in the upper portion of inclined stems. This results in fibers characterized by an inner gelatinous secondary cell wall layer, devoid of lignin but abundant in cellulose. This asymmetrical growth aids in correcting the stem's orientation (96, 7, 56).

Several studies have explored how mechanical stresses in stem wood locally stimulate cambium activity, showing a positive correlation with ethylene biosynthesis (158, 136, 141, 242). Initially, in vegetative tissues in response to many environmental signals, ethylene synthesis starts from S-adenosylmethionine, through the action of 1-Aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) and oxidase (ACO). Savidge *et al.* (1988) noted ACC activity in the cambium of *Pinus contorta* trees during compression wood formation, with ACC synthesis observed during xylem expansion and maturation (196). Similarly, induced tension wood, as studied in *Metasequoia glyptostroboides* and *Aesculus turbinata*, showed a parallel trend between cambial activity, quantified by wood formation, and ethylene production evolution in both species (56). While it was confirmed in poplar that cambial cell division is induced by ethylene when tension wood is generated (141), high levels of ethylene production can also occur in response to either compression or tension wood formation (6, 204).

Considering that ethylene application stimulates cambial growth in both trees and herbaceous species (20) and is naturally produced in wood-producing tissues, additional studies provide support for the notion that exogenously applied ethylene affects the pattern and rate of xylem development (6). In cell cultures of *Zinnia elegans*, ethylene was found to stimulate the differentiation of tracheary elements (TE), while the application of inhibitors of ethylene biosynthesis prevented this differentiation. These findings led the authors to conclude that ethylene synthesis takes place in the apoplast of xylem elements and also plays a role in regulating the size of cambial mother cells by promoting local auxin biosynthesis during secondary xylem formation (176, 249). The relationship between secondary xylem development and ethylene biosynthesis is evident from the impact of elevated levels of exogenous ethylene (or its precursors) on cambial growth, xylem morphology, and the ontogeny of vessels, fibers, and rays during wood formation (137).

Furthermore, in conjunction with the synergistic effects of auxin, ethylene's role in secondary root growth has been delineated. It has been proposed that ethylene enhances auxin levels in the cambium adjacent to the xylem, thus sustaining cambial activity (249). Consequently, ethylene can induce auxin biosynthesis and basipetal auxin transport to the root elongation zone, triggering a local auxin response that inhibits cell elongation. Consistently, in mutants with impaired auxin perception or transport, it was noted that ethylene failed to trigger the auxin response or regulate root growth. Given that ethylene is recognized for modulating the transcription of several auxin transport components, it facilitates the local activation of the ethylene-regulated auxin signaling pathway to regulate root growth (132, 177, 182, 192).

1.8.3 Regulatory network of vascular cambium during secondary growth

Plants modulate their cambial activity in response to environmental cues like photoperiod, temperature fluctuations, and the availability of water and nutrients. Hence, the vascular cambium needs to adeptly generate various cell types constituting the xylem and phloem, diverging in opposite directions and with varying rates over the plant's lifespan, all while ensuring a consistent pool of stem cells in equilibrium. Given the influence of both genetic predispositions and environmental conditions on cambial activity, directing newly divided cells along distinct developmental trajectories, the cambium necessitates responsiveness to external stimuli like climate fluctuations to initiate and cease periods of dormancy. Moreover, it must remain receptive to internal shifts, such as transitioning from the xylem expansion phase due to flowering cues, facilitating plant and organ thickening growth. Hence, the gene networks governing the establishment and upkeep of the vascular cambium are highly specialized. Previous research in *Arabidopsis* has shed light on these gene networks, particularly during secondary growth in wood-producing plants such as poplar (148, 29).

During the regulation of stem cell proliferation in the cambium, the TDIF peptide moves from the phloem to the cambium, binding to its receptor, the *PHLOEM INTERCALLATED WITH XYLEM* (*PXY*) gene, a leucine-rich repeat receptor-like kinase. *PXY* is activated by its ligands, the small peptides CLV3/ESR1-LIKE 41 (CLE41) and CLE44, to positively regulate the expression of *WOX4* and *WOX14*. Activation of *WOX4/14* leads to the maintenance of stem cell proliferation resulting in vascular cambium cell proliferation. Additionally, the *PXY* signaling pathway also activates *BIN2*, the GSK3 protein that inhibits the expression of the TF *BES1*, allowing xylem differentiation. Furthermore, through another signaling pathway, ethylene controls vascular cambium cell proliferation in the absence of *WOX4/14*. Therefore, *ETHYLENE RESPONSE FACTOR* (*ERF*) induced by ethylene, along with *ANT* and *CYCD3* (the CYCLIN D3 cell cycle regulator group) induced by cytokinins, also participate in cambial cell proliferation (207, 73, 149, 64, 63, 227, 143, 184, 29).

In this regard, *PXY* and *CLE41* form a multifunctional ligand-receptor pair that regulates vascular cell division, vascular organization, and xylem differentiation in vascular tissues. Additionally, *WOX4* acts redundantly with *WOX14* in regulating vascular cell division, albeit not in its organization (63). Furthermore, the ethylene signaling pathway also interacts with *PXY*, in parallel to *PXY/CLE41*, to regulate cell division in developing vascular tissue. Therefore, *PXY* and *WOX4* may work together with various *ERF* TFs and the ethylene signaling pathway to maintain regulated cell divisions of the vascular cambium during tissue development. In this context, the central regulation of cell division and organization by *TDIF-PXY-WOX4/14* in the cambium can occur in both poplar and *Arabidopsis* (65, 64, 62, 228).

On another note, while *HD ZIP Class III* TFs have been implicated in determining cell polarity, patterning in lateral organs, differentiation of primary vascular tissues, and the initiation and function of shoot apical meristems, during secondary growth in *Populus*, another function of these TFs has been demonstrated during cambial development and secondary vascular tissue formation (124, 38, 257, 183). In this regard, it was observed that the *popREVOLUTA* (*PRE*) gene, an ortholog of *Arabidopsis thaliana REVOLUTA*, is expressed both in the shoot apical meristem and in the woody stems of *Populus* trees. Robischon *et al*., (2011) demonstrated that *PRE* is normally expressed during secondary growth, being essential for cambium initiation and secondary vascular tissue patterning. Moreover, erroneous expression of *PRE* leads to cambium initiation at abnormal positions, resulting in vascular pattern defects in derived secondary tissues, including complete polarity inversions (186). Therefore, in woody plants, during secondary growth, *HD ZIP Class III* TFs have also been recognized to act in the establishment and differentiation of vascular cambium (201, 124, 149, 186, 38, 257).

In another aspect, during leaf development in *Arabidopsis*, three main gene families have been recognized for establishing the adaxial/abaxial pattern; *KANADI*, *HD ZIP Class III*, and *YABBY*. Studies on mature poplar leaves observed that the expression of an *AtKAN1* homolog (*PttKAN1*) showed high expression towards the phloem side of the cambial zone, similar to the abaxial expression of *AtKAN1* during *Arabidopsis* leaf development. In the same vein, poplar homologs for the *PHAVOLUTA*/*PHABULOSA* genes (*PttHB9*) and *ATHB15* (*PttHB15*) also exhibited increased expression on the xylem side of the cambial zone, indicating their involvement during secondary growth. Additionally, considering that *ATHB8* expression is noted in the initial phases of vascular differentiation, its homolog in poplar, *PttHB8*, was specifically identified on the xylem side of the cambial zone and in the neighboring expanding xylem. Considering this, since poplar homologs of *KANADI* and *HD ZIP III* genes could be involved in the radial pattern of secondary tissues, *YABBY* family genes might express more specifically in leaves and other organs such as flowers (201).

Expanding on earlier discoveries, it has been shown that gene expression profiles, such as *PttKAN1* and *PttHB9* in poplar stems, closely match the reported expression patterns of their *Arabidopsis thaliana* counterparts in leaves. Given that leaf vasculature is contiguous with that of the stem, it is highly probable that they arise from a shared patterning mechanism. Additionally, since there seems to be a level of conservation in the radial patterning system between the shoot apex and the cambial meristem, various transcriptomic analyses in *Arabidopsis* and *Populus* have revealed that certain genes linked to vascular development during primary growth are also active during secondary growth. Moreover, genes such as *SHR* and *SCR*, which primarily function in vascular development in the *Arabidopsis* RAM, also exhibit expression towards the phloem differentiation side in the cambial zone in their poplar homologs, *PttSHR1* and *PttSHR2*. However, the expression of the closest *SCR* homolog was less pronounced, possibly indicating its limited involvement in early differentiation events (201, 16).

1.8.4 Cambial area anatomy

Conceptually, the region encompassing the cambium consists of stem cells or their derivatives along with initial cells. Anticlinal divisions of these initial cells lead to an expansion of the cambial cylinder's circumference, while periclinal divisions give rise to stem cells or their derivatives destined for differentiation into phloem and xylem. These derivative cells, responsible for secondary conducting tissues, also serve to establish the initial cells in the meristems. Consequently, these cambial derivative cells can undergo multiple divisions before committing to vascular tissue differentiation, thereby preserving a balance between undifferentiated initial cells and differentiated derivatives. As a result, the term "cambial zone" encompasses all cell types within the cambium (223). In this context, the vascular cambium comprises two morphologically distinct types of initial cells: elongated axial fusiform initials, responsible for forming the axial system including tracheids, vessel elements, fibers, axial parenchyma cells, SE and CC (phloem); and isodiametric ray initial cells, which are smaller and contribute to the development of radially oriented parenchyma rays (16).

Therefore, during axial growth, as the cambium develops near photosynthetic tissues, xylem cells typically exhibit relatively shorter lengths, thinner cell walls, and axes oriented away from the longitudinal axis of the stem. Moreover, xylem cells originating in the cambium near the upper part of the tree, or crown, often contain cellulose microfibrils that are less oriented towards the cell's longitudinal axis, and their cell walls typically have a higher lignin content compared to cellulose. Consequently, the wood derived from these cells is generally less dense and referred to as juvenile wood. In contrast, mature wood, formed by older cambial tissues located further from the leaves, is denser and exhibits superior strength properties, making it economically valuable (140). Therefore, cambial cells divide in a systematic fashion along their longitudinal axis, resulting in well-defined rows of cells as observed in tree growth rings and also in higher plants such as *Arabidopsis* (36).

1.9 Anatomical aspects and vascular development in coffee plants

1.9.1 Botanical aspects overview of the coffee tree

Botanically, plants of the *Coffea* genus can be recognized as trees with a single main trunk (orthotropic) of hard and dense wood, with horizontal or semi-horizontal branches (plagiotropic branching) and paired axillary inflorescences (11). The growth of the coffee tree is generated from meristematic cells located at the apex of the stem, branches, and in the leaf axils, in which the primordia of nodes, leaves, branches, and flowers develop (47, 11). The stem apex is responsible for node and leaf formation and for the vertical growth of the plant (orthotropic growth). At the branch apex, node and leaf formation occur along with lateral growth (plagiotropic growth) (47, 10, 40). In coffee, as in other dicotyledonous plants, the first root axis emerges from the radicle and is referred to as the taproot, from which the root system is composed mainly of lateral roots, feeder roots, and root hairs (71).

At a specific distance from the stem apex and branches, 4 or 5 buds form in a series within the leaf axils, from which either flowers or branches will differentiate (11). When these buds are situated on the main stem, the eldest one exclusively produces horizontal shoots, serving as primary branches. Consequently, in the coffee tree, a pair of opposite branches emerges at each node. Subsequent buds in the series generate vertical shoots, while the remaining buds stay dormant or may potentially develop cauline flowers. However, when positioned on the branches, their primary purpose is to flower. Buds that don't transition into flowers have the potential to develop into secondary or tertiary branches. As the fruiting phase ensues, branches aged at least one year will bear fruit. The plant sustains its productivity for 20 years or longer, with peak yield typically occurring between 5 and 10 years of age (47, 10, 40, 11).

1.9.2 The first establishment of vascular pattern during coffee embryogenesis

The vascular pattern in coffee plants is established during embryogenesis, marking the initial development of fundamental conducting tissues. Therefore, from this process, meticulous cellular differentiation and organization during the embryonic stage lay the groundwork for the development and subsequent functioning of the plant's vascular system, which is essential for its growth and development.

The mature coffee fruit, considered an indehiscent drupe, is anatomically composed of a pericarp differentiated into the exocarp, mesocarp, and endocarp, which constitute the layers surrounding the seed, which in turn consists of an embryo, endosperm, and perisperm. Initially, the exocarp, which comprises the outermost part of the fruit, consists of a layer of compact polygonal parenchymal cells. The mesocarp, also known as mucilage or pulp, is the fleshy part of the fruit between the parchment and the skin, consisting of parenchymal cells and vascular bundles. As for the endocarp, more commonly known as parchment, it is composed of sclerenchyma cells and cellulose material that completely envelops the seed (71).

Regarding the seed, it is surrounded on its outermost part by the perisperm, composed mainly of sclerenchyma cells. Next, in layers, follows the endosperm, considered a living tissue, which represents the main reserve tissue for the initial growth of the seedling once germination begins. Additionally, on the innermost part, is the embryo composed of a hypocotyl attached to two cotyledons, located near the convex surface of the seed. Furthermore, the coffee embryo initially has few storage reserves and, therefore, relies on the endosperm to obtain nutrients during its initial growth (71).

The morphological and cellular changes occurring during *in vitro* embryogenesis have been well described, supported by histological observations of the different cellular conformations emerging at each stage of development. Previous research by Bartos *et al.* (2018) provided important histological insights into the development of somatic embryos obtained from *C. arabica* leaves. Initially, they observed that somatic embryos were primarily composed of protoderm surrounding the fundamental meristem. Subsequently, at the torpedo stage, tissue polarization was observed, leading to early differentiation of procambial strands. Later, when cotyledonary somatic embryos were obtained, they were observed that at this stage, the initiation of vascular element differentiation occurred, a characteristic also observed in mature zygotic embryos (Figure 1) (14).

Building upon the aforementioned, from the generation of embryogenic callus of coffee plants, the somatic embryos obtained progress through typical developmental stages; globular, heart-shaped, torpedo, and cotyledon stages. Although various studies addressing somatic and/or zygotic embryo production do not focus on the anatomical description of provascular cell development, different works have shed light on the stages at which the differentiation of cells with vascular identity begins during coffee embryogenesis (154, 180, 66, 14, 12, 69).

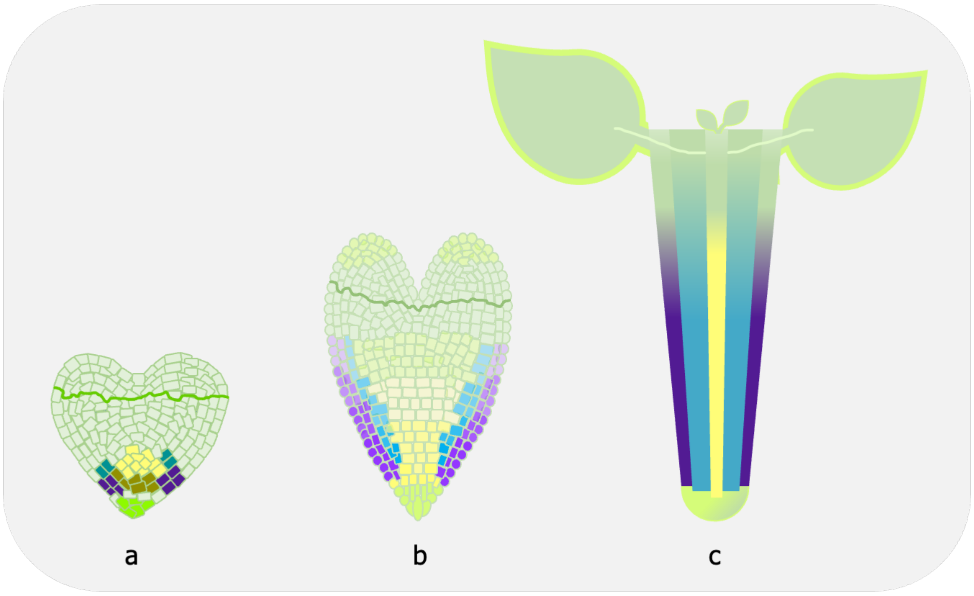

Figure 1. The schematic provides an approximate reconstruction depicting the initial establishment of provascular cellular identities during embryogenesis and the formation of the vascular pattern in the first coffee root and cotyledons. Cells shaded in dark and light yellow represent procambium identity, while purple and dark blue cells indicate pro-vascular and initial vascular tissues, such as xylem and phloem. Lemon green cells denote the identity of the root apical meristem (RAM). **a**. Heart-shaped; embryos primarily consist of a protoderm enveloping the fundamental meristem **b**. Torpedo; early differentiation of procambial cells. **c**. The cotyledonary embryo comprises a hypocotyl attached to two cotyledons, each exhibiting its respective cotyledonary veins. Initiation of vascular element differentiation.

1.9.3 Development of vascular tissues of the coffee leaf

The development of leaves in the coffee plant initiates three crucial physiological processes essential for growth, vegetative development, and reproductive development: photosynthesis, respiration, and transpiration. The leaf originates from the apical bud, which, when observed in a longitudinal section, appears as a protuberance composed of several layers of cells. Some of these cells possess the capacity to divide, generating new cells that will contribute to the formation of other plant organs. Leaf development commences with a series of divisions in one of the three outermost cell layers near the apical bud, leading to the formation of a lateral protuberance or leaf primordium (11, 235).

While current studies on the origin and establishment of conductive tissues during leaf primordia growth in coffee plants are scarce, Fournier (1965) provided insights into the origin and differentiation of vascular tissues in *Coffea arabica* L. cv. *bourbon* Choussy. It was observed that procambium and phloem differentiation proceeds continuously and acropetally in all leaf veins, while xylem differentiation begins in isolated cords. Initially, the first xylem elements appear in the middle third of the main vein, where the first procambium cells differentiate into secondary veins. Consequently, the differentiation of secondary and minor vein elements forms a complex venous network, where any increase in area results from intercalary growth. However, no phloem elements were observed at the ends of smaller veins (47, 75).

1.9.4 The vascular tissues of the stem and root of the coffee plant

Coffee, being a vascular and woody plant, initiates the development of its conductive tissues from the procambium during its primary growth phase, while its secondary growth is driven by the cambium cells. Radial expansion in coffee stems primarily originates from two lateral meristems commonly found in woody dicotyledons: the vascular cambium and the cortical cambium (16).

Following seed germination and during the initial growth phase of the stem, the procambium generates centripetal xylem and centrifugal phloem, forming vascular bundles arranged in a ring-like pattern within the tissue (46). Cross-sections of orthotropic and plagiotropic branches of coffee plants undergoing secondary growth reveal a well-structured composition of tissues. From outermost to innermost, these tissues include the periderm (cortex, phellogen and phelloderm), cortical parenchyma, secondary phloem, cambium, secondary xylem, primary xylem and pith (Figure 2) (47, 74, 70).

Initially, the cortex comprises the periderm, consisting of the outermost layer of cork or phellem, the phellogen (a meristematic tissue characterized by periclinal divisions in the subepidermal layer), and the phelloderm (70). Subsequently, the formation of the secondary phloem can be observed, which extends towards the cortex, enveloping the secondary xylem as it grows inward within the trunk. Notably, cells in both the xylem and phloem exhibit predominantly vertical orientation, with the vascular cambium serving as a reservoir of stem cells, defining the growth boundary for both cell types. As coffee stems mature, the secondary phloem and xylem give rise to fiber bands, observable as horizontal parenchyma intersected by medullary rays serving as starch reservoirs. The activation of both the phellogen and vascular cambium, responsible for generating protective and secondary vascular tissues respectively, facilitates the expansion of the plant stem's girth. Consequently, the initiation of the phellogen in coffee plants results in the formation of periderm tissue (47, 16, 70). Conversely, the pith, found exclusively in stems and absent in roots, primarily comprises parenchyma tissue. Typically, parenchyma serves as the predominant constituent of ground tissue, pervading all plant organs as a continuous structure composed of isodiametric cells featuring thin cell walls (9, 70).

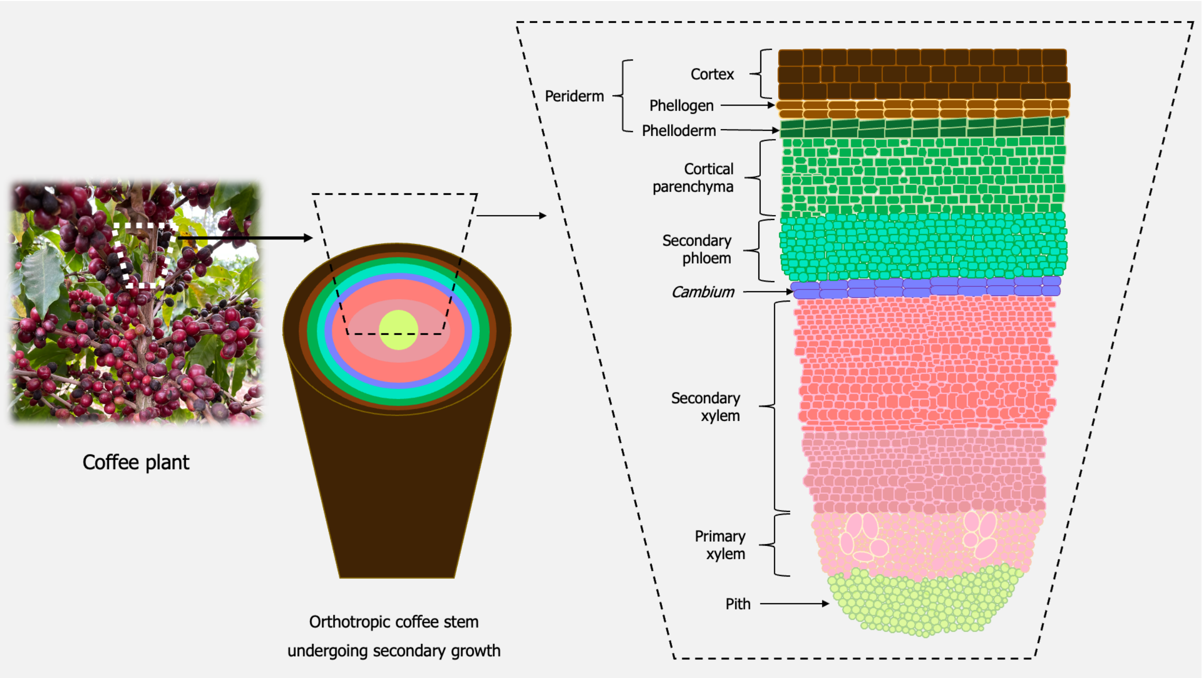

Figure 2. The schematic is an approximate reconstruction of the structures mostly of the conducting tissues, during the secondary growth of orthotropic stems of *Coffea arabica*. Based on Dedecca (1957).

On the other hand, the root development in angiosperms initiates from a meristem positioned at the base of the hypocotyl of the seed embryo, which generates the embryonic root. Upon germination, the initial structure to emerge is the radicle or primary root. In gymnosperms, magnoliophyta, and eudicots, the primary root extends vertically downward, evolving into the taproot, from which branches or lateral roots emerge, establishing the taproot system (11).

The typical roots of angiosperms, including the coffee plant, consist anatomically of several distinct regions. Firstly, the calyptra or “coifa” serves as an apical tissue protecting the root tip. Above this lies the meristematic region or growth point, composed of actively dividing small, prismatic cells, which give rise to the fundamental tissues of the root: the protoderm (the precursor of the epidermis), cortex, and the procambium responsible for the development of xylem, phloem, pericycle, and cambium. Subsequently, the region of elongation encompasses the apical meristematic zone, followed by the region of differentiation or maturation. In this final region, specialized epidermal cells differentiate into absorbent roots (47, 11).

Above the elongation zone in the main coffee root, a radial structure emerges from a cross-section, comprising the epidermis, the cortex (divided into exodermis, central cortex, and endodermis), and the vascular cylinder or stele. The vascular tissues, enclosed by the endodermis, include the pericycle, xylem, and phloem (Figure 3). The pericycle consists of closely connected, thick-walled cells without intercellular spaces, serving as the origin of lateral roots. Moreover, the pericycle forms in direct association with the protophloem and protoxylem, retaining its meristematic potential to give rise to both the vascular cambium and the suberous or phellogen cambium. According to Dedecca (1957), the differentiation of conductive tissues initiates approximately 3.5 mm from the root apex, as the cells of the procambium undergo differentiation. This process leads to the formation of the first protoxylem elements, characterized by relatively thin cell walls. Concurrently, protophloem initials emerge between these proto-xylematic elements. Both primary xylem and phloem differentiate centripetally. As xylem differentiation continues, a solid xylem core forms at the center of the vascular cylinder, imparting a proto-stelic radial structure to the root. This solid core gives rise to branches that extend towards the pericycle, interspersed with strips of phloem (47, 11).

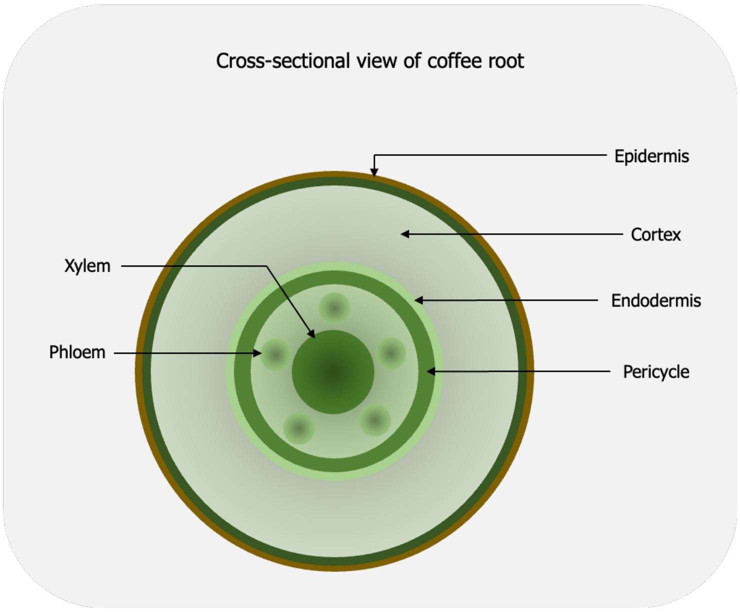

Figure 3. The schematic provides an approximate cross view of the tissues during root growth located near the base of the hypocotyl of *Coffea arabica* L.

The coffee root is considered polyarchic, with a radial variation in the number of vascular bundles ranging from 6 to 11 bundles of primary xylem and phloem. Protoxylem mainly comprises non-septate woody fibers, spiral-type tracheids, and small vessels with simple perforated plates and walls featuring punctate to alternating spaces. Upon formation of the metaxylem, cells become enriched with woody parenchyma and develop thick cell walls. The protophloem exhibits a typical configuration of sieve elements-companion cells (SE-CC) and phloem parenchyma (47).

While comprehensive studies specifically targeting the vascular development of coffee plants are limited, previous research has explored the development of conductive tissues in a manner more focused on the crop's physiology. Various studies have investigated vascularization directly, such as when coffee plants are subjected to water stress (39), or indirectly through anatomical alterations observed under boron deficiency (189), heat stress (135), induced sprouting of microcuttings (46), changes in the foliar anatomy implanted using hydro retainer polymers (173), and nutritional variations. It has been noted that soil fertility can influence the development of stem xylem (35).

1.9.5 Insights into the regulatory network governing vascular development in coffee trees

Several investigations have made significant strides in unraveling the molecular mechanisms underlying vascular development, particularly in *Arabidopsis* for both primary and secondary growth, and Populus for secondary growth and wood formation (27, 36, 218, 16, 143, 152, 191, 212). Hormonal signaling has emerged as a crucial regulator in the proliferation of procambium and vascular cambium cells, employing a peptide-receptor-transcription factor regulatory mechanism akin to that governing the activity of the shoot apical meristem (SAM) (102, 99, 100, 227). Additionally, the differentiation of procambium and vascular cambium into phloem and xylem cells is orchestrated by plant hormones and *HD-ZIP Class III* TF, while the coordinated activation of genes involved in secondary wall biosynthesis for xylem establishment, and wood formation is primarily mediated by a transcriptional network encompassing *NAC*s and *MYB* master switches, along with their downstream TF (33, 254, 252, 153, 101, 186, 221, 83, 171, 183, 97, 79). Given the availability of genomic sequences from various vascular species, key genes involved in vascular bundle development are now known. This knowledge holds promise for elucidating the genetic network regulating the establishment of conductive tissues in specific plant organs, such as flowers and fruits. Moreover, understanding the genetic network governing vascular development during the reproductive phases, particularly in economically important plants like the coffee plant, is imperative for enhancing productivity.

Recent studies have advanced our understanding of the genes underlying vascularization in coffee, an important agricultural commodity. Much of this knowledge has been derived from model plants like *Arabidopsis thaliana*, which may share common gene expression patterns with coffee plants. In this way, transcriptome analyses during somatic embryogenesis induction in *Coffea canephora* have revealed the involvement of *ARF* and *Aux/IAA* genes (179). Additionally, research on microRNAs has elucidated regulatory mechanisms in vascular development, particularly in woody plants (50, 68). Similarly, gene expression studies in *Coffea arabica* have explored genes related to the two-component system and cytokinin signaling pathways, uncovering interactions with morphogenic genes like *WUSCHEL*, *WOX5*, *BABY-BOOM*, *LEC1*, *FUS3*, and *AGL15* (222). These insights contribute to a comprehensive understanding of the molecular mechanisms governing vascular development in coffee plants, particularly during somatic and zygotic embryo generation (155, 14).

Previous studies have provided insights into the vascular tissue formation in different organs of the coffee plant, identifying distinct patterns of conductive tissues at the anatomical level. For instance, Bartos *et al*. (2018) elucidated the histological characteristics of somatic embryos derived from *Coffea arabica* leaves, shedding light on vascular tissue differentiation during embryogenesis. Additionally, Deddeca (1957) contributed valuable information through comparative anatomy regarding the organization of vascular cells and tissues in each organ of *Coffea arabica*. Moreover, Fournier (1965) demonstrated the continuous differentiation of procambium and phloem, along with the initiation of xylem formation in coffee leaves. However, while emphasis has been placed on understanding the processes involved in the secondary growth of stems, leaves, and roots to enhance agricultural practices and crop yield (70, 175, 71, 222, 35), studies addressing the gene network directly involved in the development of procambium/cambium, xylem, and phloem during primary and secondary growth, as well as reproductive organ development in coffee, are lacking. Therefore, building upon previous knowledge of regulatory gene networks in *Arabidopsis* and Populus, especially regarding vascular cambium cell proliferation, a similar gene network is proposed with genes that would be orthologous in *Coffee*, during the development of conductive tissues in secondary growth of coffee stems (Figure 4).

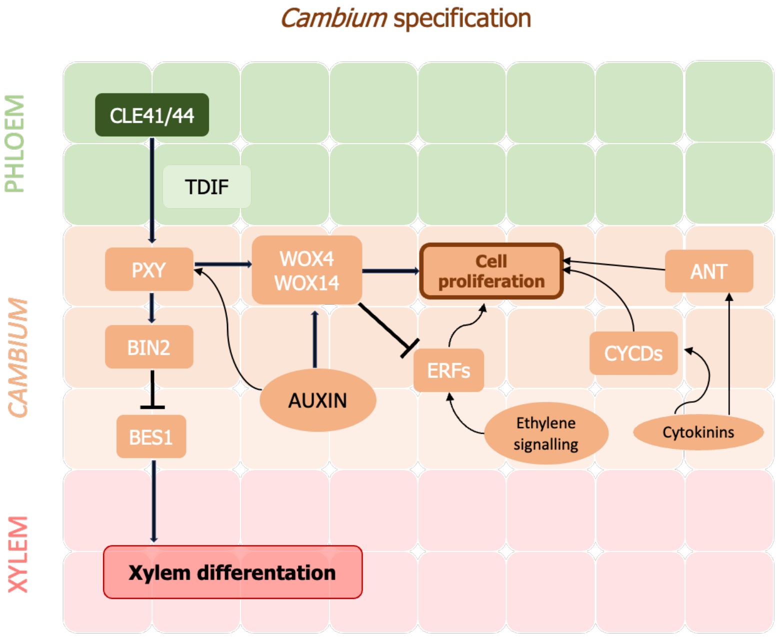

Figure 4. The schematic provides an approximate reconstruction of the regulatory networking during the secondary growth of *Arabidopsis thaliana* stems. It serves as a model for understanding the regulatory network involved in the cambium proliferation in coffee plant stems. In this context, it is hypothesized that during the secondary growth of coffee plants, the TDIF peptide orthologous gene translocates from the phloem to the cambium, where it interacts with its corresponding *PXY* receptor ortholog. Activation of the *WOX4*/*14* ortholog then maintains stem cell proliferation, while a distinct *BIN2*-*BES1* pathway regulates xylem differentiation. Additionally, ethylene signaling modulates cell proliferation when the *WOX4*/*14* ortholog is absent, enhancing the robustness of the pathway. As well, cytokinin-induced *ANT* and *CYCD*3;1 are involved in cambial cell proliferation. Adapted from Lucas *et al*., (2013) and Campbell and Simon Turner, (2017).

Given the aforementioned points and in conjunction with various anatomical studies on vascular development in coffee plants, there is a notable importance in conducting future research to study and propose orthologous genes and signaling pathways in vascular patterns, specifically the interactions of the TDIF peptide ortholog and the *PXY* receptor, which have been implicated in vascular cambium cell proliferation, as well as the *WOX4*/*14* orthologous pathway that may be involved in regulating and maintaining stem cells during secondary growth as in *Arabidopsis* (207, 73, 65, 99, 64, 63). These findings could provide insights into the intricate regulatory networks governing vascular development in coffee plants, thus offering valuable information to enhance crop productivity.

1.10 Concluding remarks and future perspectives

Land plants have developed intricate vascular systems to address diverse physiological requirements. These systems facilitate the efficient distribution of photoassimilates from leaves to roots, ensuring a sufficient provision of vital nutrients for the organism. Moreover, cellular differentiation plays an important role in this process, as cells need to acquire distinct identities to execute specific functions within the vasculature. This differentiation is orchestrated through the regulation of diverse transcriptional networks, dictating the specialization of each cell type. Furthermore, beyond its transport function, the vasculature acts as a conduit for communication among various organs, enabling the synchronization of metabolic and developmental processes across the plant.

In this sense, while coffee plants and other crops may not be a primary focus in botanical research, its developmental diversity and economic significance cannot be overstated. The quality and quantity of coffee yield are not only reliant on sound agronomic practices but also heavily dependent on the meticulous development of conductive tissues originating from the vascular procambium/cambium. This intricate process ensures the efficient transportation of water and vital nutrients throughout the plant. Despite the essential role these tissues play, our understanding of vascular development in various organs of the coffee plant, such as reproductive organs, remains limited. Thus, further exploration and research in this domain are imperative to refine management strategies and augment globally coffee production. Such advancements would yield valuable insights into crops anatomy and pave the way for strategies to plant breeding and enhance food production.

REFERENCES

1. Aloni R. 1979. Role of Auxin and Gibberellin in Differentiation of Primary Phloem Fibers 1. *Plant Physiol.* 63(4):609–14
2. Aloni R. 1980. Role of auxin and sucrose in the differentiation of sieve and tracheary elements in plant tissue cultures. *Planta*. 150(3):255–63
3. Aloni R. 1982. Role of Cytokinin in Differentiation of Secondary Xylem Fibers. *Plant Physiol.* 70(6):1631–33
4. Aloni R. 1995. The induction of vascular tissues by auxin and cytokinin. *Plant Horm. Physiol. Biochem. Mol. Biol.*, pp. 531–46
5. Aloni R. 2013. The Role of Hormones in Controlling Vascular Differentiation. In *Cellular Aspects of Wood Formation*, ed J Fromm. 20:99–139. Berlin, Heidelberg: Springer Berlin Heidelberg
6. Andersson-Gunnerås S, Hellgren JM, Björklund S, Regan S, Moritz T, Sundberg B. 2003. Asymmetric expression of a poplar ACC oxidase controls ethylene production during gravitational induction of tension wood. *Plant J.* 34(3):339–49
7. Andersson-Gunnerås S, Mellerowicz EJ, Love J, Segerman B, Ohmiya Y, *et al.* 2006. Biosynthesis of cellulose-enriched tension wood in Populus: global analyses of transcripts and metabolites identifies biochemical and developmental regulators in secondary wall biosynthesis. *Plant J.* 45(2):144–65
8. Anne P, Azzopardi M, Gissot L, Beaubiat S, Hématy K, Palauqui J-C. 2015. *OCTOPUS* Negatively Regulates *BIN2* to Control Phloem Differentiation in *Arabidopsis* thaliana. *Curr. Biol.* 25(19):2584–90
9. APEZZATO-DA-GLÓRIA B, CARMELLO-GUERREIRO SM. 2006. Anatomia vegetal. atual. *Universidade Fed. Viçosa*
10. Arcila J, Buhr L, Bleiholder H, Hack H, Wicke H. 2001. *APL*icación de la escala BBCH ampliada para la descripción de las fases fenológicas del desarrollo de la planta de café Coffea sp
11. Arcila P. J, Farfán V. F, Moreno B. A, Salazar G. LF, Hincapié G. E. 2007. Sistemas de producción de café en Colombia. Cenicafé, Chinchiná, Caldas, Colombia
12. Awada R, Campa C, Gibault E, Déchamp E, Georget F, *et al.* 2019. Unravelling the Metabolic and Hormonal Machinery During Key Steps of Somatic Embryogenesis: A Case Study in Coffee. *Int. J. Mol. Sci.* 20(19):4665
13. Baima S, Forte V, Possenti M, Peñalosa A, Leoni G, *et al.* 2014. Negative Feedback Regulation of Auxin Signaling by ATHB8/ACL5–BUD2 Transcription Module. *Mol. Plant*. 7(6):1006–25
14. Bartos PMC, Gomes HT, Gomes SM, Vasconcelos Filho SC, Teixeira JB, Scherwinski-Pereira JE. 2018. Histology of somatic embryogenesis in Coffea arabica L. *Biologia (Bratisl.)*. 73(12):1255–65
15. Bauby H, Divol F, Truernit E, Grandjean O, Palauqui J-C. 2007. Protophloem Differentiation in Early *Arabidopsis* thaliana Development. *Plant Cell Physiol.* 48(1):97–109
16. Baucher M, El Jaziri M, Vandeputte O. 2007. From primary to secondary growth: origin and development of the vascular system. *J. Exp. Bot.* 58(13):3485–3501
17. Benfey PN, Scheres B. 2000. Root development. *Curr. Biol.* 10(22):R813–15
18. Berleth T, Mattsson J. 2000. Vascular development: tracing signals along veins. *Curr. Opin. Plant Biol.* 3(5):406–11
19. Biemelt S, Tschiersch H, Sonnewald U. 2004. Impact of Altered Gibberellin Metabolism on Biomass Accumulation, Lignin Biosynthesis, and Photosynthesis in

Transgenic Tobacco Plants. *Plant Physiol.* 135(1):254–65

20. Biro R, Hunt Jr E, Erner Y, Jaffe M. 1980. Thigmomorphogenesis: changes in cell division and elongation in the internodes of mechanically-perturbed or ethrel-treated bean plants. *Ann. Bot.* 45(6):655–64
21. Bishopp A, Help H, El-Showk S, Weijers D, Scheres B, *et al.* 2011. A Mutually Inhibitory Interaction between Auxin and Cytokinin Specifies Vascular Pattern in Roots. *Curr. Biol.* 21(11):917–26
22. Bishopp A, Lehesranta S, Vatén A, Help H, El-Showk S, *et al.* 2011. Phloem-Transported Cytokinin Regulates Polar Auxin Transport and Maintains Vascular Pattern in the Root Meristem. *Curr. Biol.* 21(11):927–32
23. Bishopp A, Lehesranta S, Vatén A, Help H, El-Showk S, *et al.* 2011. Phloem-Transported Cytokinin Regulates Polar Auxin Transport and Maintains Vascular Pattern in the Root Meristem. *Curr. Biol.* 21(11):927–32
24. Björklund S, Antti H, Uddestrand I, Moritz T, Sundberg B. 2007. Cross-talk between gibberellin and auxin in development of Populus wood: gibberellin stimulates polar auxin transport and has a common transcriptome with auxin. *Plant J.* 52(3):499–511
25. Bollhöner B, Prestele J, Tuominen H. 2012. Xylem cell death: emerging understanding of regulation and function. *J. Exp. Bot.* 63(3):1081–94
26. Bonke M, Thitamadee S, Mähönen AP, Hauser M-T, Helariutta Y. 2003. *APL* regulates vascular tissue identity in *Arabidopsis*. *Nature*. 426(6963):181–86
27. Busse JS, Evert RF. 1999. Vascular Differentiation and Transition in the Seedling of *Arabidopsis thaliana* (Brassicaceae). *Int. J. Plant Sci.* 160(2):241–51
28. Campbell L, Turner S. 2017. Regulation of vascular cell division. *J. Exp. Bot.* 68(1):27–43
29. Campbell L, Turner S. 2017. Regulation of vascular cell division. *J. Exp. Bot.* 68(1):27–43
30. Caño-Delgado A, Yin Y, Yu C, Vafeados D, Mora-García S, *et al.* 2004. BRL1 and BRL3 are novel brassinosteroid receptors that function in vascular differentiation in *Arabidopsis*. *Development*. 131(21):5341–51
31. Cardon CH, de Oliveira RR, Lesy V, Ribeiro THC, Fust C, *et al.* 2022. Expression of coffee florigen *CaFT1* reveals a sustained floral induction window associated with asynchronous flowering in tropical perennials. *Plant Sci.* 325:111479
32. Carland FM, McHale NA. 1996. LOP1: a gene involved in auxin transport and vascular patterning in *Arabidopsis*. *Development*. 122(6):1811–19
33. Carlsbecker A, Helariutta Y. 2005. Phloem and xylem specification: pieces of the puzzle emerge. *Curr. Opin. Plant Biol.* 8(5):512–17
34. Carlsbecker A, Lee J-Y, Roberts CJ, Dettmer J, Lehesranta S, *et al.* 2010. Cell signalling by microRNA165/6 directs gene dose-dependent root cell fate. *Nature*. 465(7296):316–21
35. Carréra JC, Resende TB, Vicente Campos AA, De Souza RR, De Oliveira IMM, *et al.* 2023. Anatomic characteristics of branches related to the vegetative growth of coffee tree (*Coffea arabica* L., Rubiaceae) under nutritional variation. *J. Plant Nutr.* 46(19):4594–4605
36. Chaffey N, Cholewa E, Regan S, Sundberg B. 2002. Secondary xylem development in *Arabidopsis*: a model for wood formation. *Physiol. Plant.* 114(4):594–600
37. Clay NK, Nelson T. 2005. *Arabidopsis thickvein* Mutation Affects Vein Thickness and Organ Vascularization, and Resides in a Provascular Cell-Specific Spermine Synthase Involved in Vein Definition and in Polar Auxin Transport. *Plant Physiol.* 138(2):767–77
38. Côté CL, Boileau F, Roy V, Ouellet M, Levasseur C, *et al.* 2010. Gene family

structure, expression and functional analyses of HD-Zip III genes in angiosperm and gymnosperm forest trees. *BMC Plant Biol.* 10(1):273

39. Crisosto CH, Grantz DA, Meinzer FC. 1992. Effects of water deficit on flower opening in coffee (Coffea arabica L.). *Tree Physiol.* 10(2):127–39
40. Davis AP, Govaerts R, Bridson DM, Stoffelen P. 2006. An annotated taxonomic conspectus of the genus Coffea (Rubiaceae). *Bot. J. Linn. Soc.* 152(4):465–512
41. Dayan J, Schwarzkopf M, Avni A, Aloni R. 2010. Enhancing plant growth and fiber production by silencing GA 2-oxidase. *Plant Biotechnol. J.* 8(4):425–35
42. Dayan J, Voronin N, Gong F, Sun T, Hedden P, *et al*. 2012. Leaf-Induced Gibberellin Signaling Is Essential for Internode Elongation, Cambial Activity, and Fiber Differentiation in Tobacco Stems. *Plant Cell*. 24(1):66–79
43. de Oliveira RR, Ribeiro THC, Cardon CH, Fedenia L, Maia VA, *et al*. 2020. Elevated Temperatures Impose Transcriptional Constraints and Elicit Intraspecific Differences Between Coffee Genotypes. *Front. Plant Sci.* 11:
44. De Rybel B, Mähönen AP, Helariutta Y, Weijers D. 2016. Plant vascular development: from early specification to differentiation. *Nat. Rev. Mol. Cell Biol.* 17(1):30–40
45. De Rybel B, Möller B, Yoshida S, Grabowicz I, Barbier de Reuille P, *et al*. 2013. A bHLH Complex Controls Embryonic Vascular Tissue Establishment and Indeterminate Growth in *Arabidopsis*. *Dev. Cell*. 24(4):426–37
46. De Silva Angelo PC. 2019. Study on the Anatomy of Young Coffee Plants from Micro-Cuttings and Induced to Sprout. *Plant Tissue Cult. Biotechnol.* 29(2):185–94
47. Dedecca DM. 1957. Anatomia e desenvolvimento ontogenético de Coffea arabica L. var. typica Cramer. *Bragantia*. 16(unico):315–66
48. Demura T, Fukuda H. 2007. Transcriptional regulation in wood formation. *Trends Plant Sci.* 12(2):64–70
49. Deshmukh A. 2021. *The World's Top Coffee Producing Countries*. Visual Capitalist. https://www.visualcapitalist.com/worlds-top-coffee-producing-countries/
50. Devi KJ, Chakraborty S, Deb B, Rajwanshi R. 2016. Computational identification and functional annotation of microRNAs and their targets from expressed sequence tags (ESTs) and genome survey sequences (GSSs) of coffee (Coffea arabica L.). *Plant Gene*. 6:30–42
51. Digby J, Wareing PF. 1966. The Effect of Applied Growth Hormones on Cambial Division and the Differentiation of the Cambial Derivatives. *Ann. Bot.* 30(3):539–48
52. Dobisova T, Hrdinova V, Cuesta C, Michlickova S, Urbankova I, *et al*. 2017. Light Controls Cytokinin Signaling via Transcriptional Regulation of Constitutively Active Sensor Histidine Kinase CKI11[OPEN]. *Plant Physiol.* 174(1):387–404
53. Dolan L, Janmaat K, Willemsen V, Linstead P, Poethig S, *et al*. 1993. Cellular organisation of the *Arabidopsis* thaliana root. *Development*. 119(1):71–84
54. Doyle JA. 2017. Phylogenetic Analyses and Morphological Innovations in Land Plants. In *Annual Plant Reviews online*, pp. 1–50. John Wiley & Sons, Ltd
55. Du S, Yamamoto F. 2003. Ethylene evolution changes in the stems of Metasequoia glyptostroboides and Aesculus turbinata seedlings in relation to gravity-induced reaction wood formation. *Trees - Struct. Funct.* 17(6):522–28
56. Du S, Yamamoto F. 2007. An Overview of the Biology of Reaction Wood Formation. *J. Integr. Plant Biol.* 49(2):131–43
57. Endo H, Yamaguchi M, Tamura T, Nakano Y, Nishikubo N, *et al*. 2015. Multiple Classes of Transcription Factors Regulate the Expression of VASCULAR-RELATED NAC-DOMAIN7, a Master Switch of Xylem Vessel Differentiation. *Plant Cell Physiol.* 56(2):242–54
58. Eriksson ME, Israelsson M, Olsson O, Moritz T. 2000. Increased gibberellin

biosynthesis in transgenic trees promotes growth, biomass production and xylem fiber length. *Nat. Biotechnol.* 18(7):784–88

59. Esau K. 1943. Vascular differentiation in the vegetative shoot of Linum. III. The origin of the bast fibers. *Am. J. Bot.*, pp. 579–86
60. Esau K. 1954. Primary vascular differentiation in plants. *Biol. Rev.* 29(1):46–86
61. Esau K. 1969. *The Phloem*. Stuttgart, Germany: Schweizerbart Science Publishers
62. Etchells JP, Mi*SHR*a LS, Kumar M, Campbell L, Turner SR. 2015. Wood formation in trees is increased by manipulating PXY-regulated cell division. *Curr. Biol.* 25(8):1050–55
63. Etchells JP, Provost CM, Mishra L, Turner SR. 2013. WOX4 and WOX14 act downstream of the PXY receptor kinase to regulate plant vascular proliferation independently of any role in vascular organisation. *Development*. 140(10):2224–34
64. Etchells JP, Provost CM, Turner SR. 2012. Plant Vascular Cell Division Is Maintained by an Interaction between PXY and Ethylene Signalling. *PLOS Genet.* 8(11):e1002997
65. Etchells JP, Turner SR. 2010. The PXY-CLE41 receptor ligand pair defines a multifunctional pathway that controls the rate and orientation of vascular cell division. *Development*. 137(5):767–74
66. Etienne H, Bertrand B, Georget F, Lartaud M, Montes F, *et al.* 2013. Development of coffee somatic and zygotic embryos to plants differs in the morphological, histochemical and hydration aspects. *Tree Physiol.* 33(6):640–53
67. Falcioni R, Moriwaki T, de Oliveira DM, Andreotti GC, de Souza LA, *et al.* 2018. Increased Gibberellins and Light Levels Promotes Cell Wall Thickness and Enhance Lignin Deposition in Xylem Fibers. *Front. Plant Sci.* 9:1391
68. Fang L, Wang Y. 2021. MicroRNAs in Woody Plants. *Front. Plant Sci.* 12:
69. Ferrari IF, Marques GA, Junior WLS, Biazotti BB, Pena Passos M, *et al.* 2021. Comparative ontogenesis of Coffea arabica L. somatic embryos reveals the efficiency of regeneration modulated by the explant source and the embryogenesis pathway. *Vitro Cell. Dev. Biol.-Plant*. 57:796–810
70. Ferreira INM, dos Santos CS, Flho JTC. 2014. CaracterizaÃ § Ã£ o AnatÃ´ mica dos Ã? rgÃ£ os Vegetativos do Cafeeiro ArÃ¡ bica (Coffea Arabica L.). *Rev. Fragm. Cult.-Rev. Interdiscip. Ciênc. Humanas*. 24(1):153–61
71. Ferreira T, Shuler J, Guimarães R, Farah A. 2019. Introduction to Coffee Plant and Genetics
72. Fiers M, Ku K, Liu C. 2007. CLE peptide ligands and their roles in establishing meristems. *Curr. Opin. Plant Biol.* 10(1):39–43
73. Fisher K, Turner S. 2007. PXY, a Receptor-like Kinase Essential for Maintaining Polarity during Plant Vascular-Tissue Development. *Curr. Biol.* 17(12):1061–66
74. Fournier LA. 1964. Desarrollo del vástago vegetativo en Coffea arabica L. cv. bourbon Choussy. I Ontogenia del ápice de la plántula. *Rev. Biol. Trop.* 12(2):237–55
75. Fournier LA. 1965. Desarrollo del vástago vegetativo en Coffea arabica L. cv. bourbon Choussy. IV. Desarrollo del procambium y los tejidos vasculares de la hoja. *Rev. Biol. Trop.* 13(2):229–37
76. Fukuda H. 2000. Programmed cell death of tracheary elements as a paradigm in plants. In *Programmed Cell Death in Higher Plants*, ed E Lam, H Fukuda, J Greenberg, pp. 1–9. Dordrecht: Springer Netherlands
77. Fukuda H. 2004. Signals that control plant vascular cell differentiation. *Nat. Rev. Mol. Cell Biol.* 5(5):379–91
78. Fukuda H, Hardtke CS. 2020. Peptide Signaling Pathways in Vascular Differentiation[OPEN]. *Plant Physiol.* 182(4):1636–44
79. Fukuda H, Ohashi-Ito K. 2019. Vascular tissue development in plants. In *Current*

Topics in Developmental Biology. 131:141–60. Elsevier

80. Fukuda H, Ohashi-Ito K. 2019. Vascular tissue development in plants. *Curr. Top. Dev. Biol.* 131:141–60
81. Funada R, Yamagishi Y, Begum S, Kudo K, Nabeshima E, *et al*. 2016. Xylogenesis in trees: from cambial cell division to cell death. In *Secondary xylem biology*, pp. 25–43. Elsevier
82. Furuta KM, Hellmann E, Helariutta Y. 2014. Molecular Control of Cell Specification and Cell Differentiation During Procambial Development. *Annu. Rev. Plant Biol.* 65(1):607–38
83. Furuta KM, Yadav SR, Lehesranta S, Belevich I, Miyashima S, *et al*. 2014. *Arabidopsis* NAC45/86 direct sieve element morphogenesis culminating in enucleation. *Science*. 345(6199):933–37
84. Gahan PB. 1988. Xylem and Phloem Differentiation in Perspective. In *Vascular Differentiation and Plant Growth Regulators*, pp. 1–21. Berlin, Heidelberg: Springer Berlin Heidelberg
85. Gehring WJ. 1993. Exploring the homeobox. *Gene*. 135(1–2):215–21
86. Gehring WJ, Affolter M, Bürglin T. 1994. Homeodomain proteins. *Annu. Rev. Biochem.* 63(1):487–526
87. Greenwood KN, King CL, Melena I, Stegemann KA, Donnelly M, *et al*. 2023. The brassinosteroid-responsive protein *OCTOPUS* is a novel regulator of *Arabidopsis* thaliana immune signaling. *Plant Direct*. 7(8):e524
88. Groover AT, Nieminen K, Helariutta Y, Mansfield SD. 2010. Wood Formation in Populus. In *Genetics and Genomics of Populus*, ed S Jansson, R Bhalerao, A Groover, pp. 201–24. New York, NY: Springer New York
89. Hamann T, Benkova E, Bäurle I, Kientz M, Jürgens G. 2002. The *Arabidopsis BODENLOS* gene encodes an auxin response protein inhibiting MONOPTEROS-mediated embryo patterning. *Genes Dev.* 16(13):1610–15
90. Hanzawa Y, Takahashi T, Michael AJ, Burtin D, Long D, *et al*. 2000. ACAULIS5, an *Arabidopsis* gene required for stem elongation, encodes a spermine synthase. *EMBO J.* 19(16):4248–56
91. Hardtke CS. 2023. Phloem development. *New Phytol.* 239(3):852–67
92. Hardtke CS, Berleth T. 1998. The *Arabidopsis* gene MONOPTEROS encodes a transcription factor mediating embryo axis formation and vascular development. *EMBO J.* 17(5):1405–11
93. Hartung W, Sauter A, Hose E. 2002. Abscisic acid in the xylem: where does it come from, where does it go to? *J. Exp. Bot.* 53(366):27–32
94. Hejátko J, Ryu H, Kim G-T, Dobešová R, Choi S, *et al*. 2009. The Histidine Kinases CYTOKININ-INDEPENDENT1 and *ARABIDOPSIS* HISTIDINE KINASE2 and 3 Regulate Vascular Tissue Development in *Arabidopsis* Shoots. *Plant Cell*. 21(7):2008–21
95. Helariutta Y, Fukaki H, Wysocka-Diller J, Nakajima K, Jung J, *et al*. 2000. The SHORT-ROOT Gene Controls Radial Patterning of the *Arabidopsis* Root through Radial Signaling. *Cell*. 101(5):555–67
96. Hellgren JM, Olofsson K, Sundberg B. 2004. Patterns of auxin distribution during gravitational induction of reaction wood in poplar and pine. *Plant Physiol.* 135(1):212–20
97. Heo J, Blob B, Helariutta Y. 2017. Differentiation of conductive cells: a matter of life and death. *Curr. Opin. Plant Biol.* 35:23–29
98. Heo J, Roszak P, Furuta KM, Helariutta Y. 2014. Phloem development: Current knowledge and future perspectives. *Am. J. Bot.* 101(9):1393–1402

99. Hirakawa Y, Kondo Y, Fukuda H. 2010. TDIF Peptide Signaling Regulates Vascular Stem Cell Proliferation via the WOX4 Homeobox Gene in *Arabidopsis*. *Plant Cell.* 22(8):2618–29
100. Hirakawa Y, Kondo Y, Fukuda H. 2010. Regulation of Vascular Development by CLE Peptide-receptor Systems. *J. Integr. Plant Biol.* 52(1):8–16
101. Hirakawa Y, Kondo Y, Fukuda H. 2011. Establishment and maintenance of vascular cell communities through local signaling. *Curr. Opin. Plant Biol.* 14(1):17–23
102. Hirakawa Y, Shinohara H, Kondo Y, Inoue A, Nakanomyo I, *et al.* 2008. Non-cell-autonomous control of vascular stem cell fate by a CLE peptide/receptor system. *Proc. Natl. Acad. Sci.* 105(39):15208–13
103. Hobbie L, McGovern M, Hurwitz LR, Pierro A, Liu NY, *et al.* 2000. The axr6 mutants of *Arabidopsis* thaliana define a gene involved in auxin response and early development. *Development.* 127(1):23–32
104. Hu J, Su H, Cao H, Wei H, Fu X, *et al.* 2022. AUXIN RESPONSE FACTOR7 integrates gibberellin and auxin signaling via interactions between DELLA and AUX/IAA proteins to regulate cambial activity in poplar. *Plant Cell.* 34(7):2688–2707
105. Hwang I, Sheen J, Müller B. 2012. Cytokinin Signaling Networks. *Annu. Rev. Plant Biol.* 63(1):353–80
106. Ibañes M, Fàbregas N, Chory J, Caño-Delgado AI. 2009. Brassinosteroid signaling and auxin transport are required to establish the periodic pattern of *Arabidopsis* shoot vascular bundles. *Proc. Natl. Acad. Sci.* 106(32):13630–35
107. Ilegems M, Douet V, Meylan-Bettex M, Uyttewaal M, Brand L, *et al.* 2010. Interplay of auxin, KANADI and Class III HD-ZIP transcription factors in vascular tissue formation. *Development.* 137(6):975–84
108. Imai A, Hanzawa Y, Komura M, Yamamoto KT, Komeda Y, Takahashi T. 2006. The dwarf phenotype of the *Arabidopsis* acl5 mutant is suppressed by a mutation in an upstream ORF of a bHLH gene. *Development.* 133(18):3575–85
109. Imaizumi T, Schultz TF, Harmon FG, Ho LA, Kay SA. 2005. FKF1 F-Box Protein Mediates Cyclic Degradation of a Repressor of *CONSTANS* in *Arabidopsis*. *Science.* 309(5732):293–97
110. Ingram P, Dettmer J, Helariutta Y, Malamy JE. 2011. *Arabidopsis* Lateral Root Development 3 is essential for early phloem development and function, and hence for normal root system development. *Plant J.* 68(3):455–67
111. Israelsson M, Sundberg B, Moritz T. 2005. Tissue-specific localization of gibberellins and expression of gibberellin-biosynthetic and signaling genes in wood-forming tissues in aspen. *Plant J. Cell Mol. Biol.* 44(3):494–504
112. Ito Y, Nakanomyo I, Motose H, Iwamoto K, Sawa S, *et al.* 2006. Dodeca-CLE Peptides as Suppressors of Plant Stem Cell Differentiation. *Sci. New Ser.* 313(5788):842–45
113. Izhaki A, Bowman JL. 2007. KANADI and Class III HD-Zip Gene Families Regulate Embryo Patterning and Modulate Auxin Flow during Embryogenesis in *Arabidopsis*. *Plant Cell.* 19(2):495–508
114. Jang J-C, Fujioka S, Tasaka M, Seto H, Takatsuto S, *et al.* 2000. A critical role of sterols in embryonic patterning and meristem programming revealed by the fackel mutants of *Arabidopsis* thaliana. *Genes Dev.* 14(12):1485–97
115. Katayama H, Iwamoto K, Kariya Y, Asakawa T, Kan T, *et al.* 2015. A Negative Feedback Loop Controlling bHLH Complexes Is Involved in Vascular Cell Division and Differentiation in the Root Apical Meristem. *Curr. Biol.* 25(23):3144–50
116. Kerstetter RA, Bollman K, Taylor RA, Bomblies K, Poethig RS. 2001. KANADI regulates organ polarity in *Arabidopsis*. *Nature.* 411(6838):706–9

117. Kiba T, Takei K, Kojima M, Sakakibara H. 2013. Side-Chain Modification of Cytokinins Controls Shoot Growth in *Arabidopsis*. *Dev. Cell*. 27(4):452–61
118. Kim H, Zhou J, Kumar D, Jang G, Ryu KH, *et al*. 2020. SHORTROOT-Mediated Intercellular Signals Coordinate Phloem Development in *Arabidopsis* Roots[OPEN]. *Plant Cell*. 32(5):1519–35
119. Kim HK, Park J, Hwang I. 2014. Investigating water transport through the xylem network in vascular plants. *J. Exp. Bot.* 65(7):1895–1904
120. Kim J-Y, Symeonidi E, Pang TY, Denyer T, Weidauer D, *et al*. 2021. Distinct identities of leaf phloem cells revealed by single cell transcriptomics. *Plant Cell*. 33(3):511–30
121. Knoblauch M, Knoblauch J, Mullendore DL, Savage JA, Babst BA, *et al*. 2016. Testing the Münch hypothesis of long distance phloem transport in plants. *eLife*. 5:e15341
122. Knoblauch M, Oparka K. 2012. The structure of the phloem – still more questions than answers. *Plant J.* 70(1):147–56
123. Ko D, Kang J, Kiba T, Park J, Kojima M, *et al*. 2014. *Arabidopsis* ABCG14 is essential for the root-to-shoot translocation of cytokinin. *Proc. Natl. Acad. Sci.* 111(19):7150–55
124. Ko J-H, Prassinos C, Han K-H. 2006. Developmental and seasonal expression of PtaHB1, a Populus gene encoding a class III HD-Zip protein, is closely associated with secondary growth and inversely correlated with the level of microRNA (miR166). *New Phytol.* 169(3):469–78
125. Kondo T, Sawa S, Kinoshita A, Mizuno S, Kakimoto T, *et al*. 2006. A Plant Peptide Encoded by *CLV3* Identified by in Situ MALDI-TOF MS analyses. *Science*. 313(5788):845–48
126. Kondo Y, Fujita T, Sugiyama M, Fukuda H. 2015. A novel system for xylem cell differentiation in *Arabidopsis* thaliana. *Mol. Plant*. 8(4):612–21
127. Kondo Y, Hirakawa Y, Kieber JJ, Fukuda H. 2011. CLE Peptides can Negatively Regulate Protoxylem Vessel Formation via Cytokinin Signaling. *Plant Cell Physiol.* 52(1):37–48
128. Kondo Y, Ito T, Nakagami H, Hirakawa Y, Saito M, *et al*. 2014. Plant GSK3 proteins regulate xylem cell differentiation downstream of TDIF–TDR signalling. *Nat. Commun.* 5(1):3504
129. Konishi M, Yanagisawa S. 2007. Sequential activation of two Dof transcription factor gene promoters during vascular development in *Arabidopsis* thaliana. *Plant Physiol. Biochem.* 45(8):623–29
130. Kubo M, Udagawa M, Nishikubo N, Horiguchi G, Yamaguchi M, *et al*. 2005. Transcription switches for protoxylem and metaxylem vessel formation. *Genes Dev.* 19(16):1855–60
131. Lachaud S, Catesson A-M, Bonnemain J-L. 1999. Structure and functions of the vascular cambium. *Comptes Rendus Académie Sci.-Ser. III-Sci. Vie*. 322(8):633–50
132. Lee JS, Chang W-K, Evans ML. 1990. Effects of ethylene on the kinetics of curvature and auxin redistribution in gravistimulated roots of Zea mays. *Plant Physiol.* 94(4):1770–75
133. Ligrone R, Duckett JG, Renzaglia KS. 2000. Conducting tissues and phyletic relationships of bryophytes. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 355(1398):795–813
134. Lima AA, Santos IS, Torres MEL, Cardon CH, Caldeira CF, *et al*. 2021. Drought and re-watering modify ethylene production and sensitivity, and are associated with coffee anthesis. *Environ. Exp. Bot.* 181:104289
135. Lima RB, dos Santos TB, Vieira LGE, Ferrarese M de LL, Ferrarese-Filho O, *et al.*

2013. Heat stress causes alterations in the cell-wall polymers and anatomy of coffee leaves (Coffea arabica L.). *Carbohydr. Polym.* 93(1):135–43

136. Little C, Eklund L. 1999. Ethylene in relation to compression wood formation in Abies balsamea shoots. *Trees*. 13:173–77
137. Little C, Savidge R. 1987. 7. The role of plant growth regulators in forest tree cambial growth. *Plant Growth Regul.* 6:137–69
138. López ME, Santos IS, de Oliveira RR, Lima AA, Cardon CH, Chalfun-Junior A. 2021. An overview of the endogenous and environmental factors related to the Coffea arabica flowering process. *Beverage Plant Res.* 1(1):1–16
139. López ME, Silva Santos I, Marquez Gutiérrez R, Jaramillo Mesa A, Cardon CH, *et al.* 2022. Crosstalk Between Ethylene and Abscisic Acid During Changes in Soil Water Content Reveals a New Role for 1-Aminocyclopropane-1- Carboxylate in Coffee Anthesis Regulation. *Front. Plant Sci.* 13:
140. Lorenz WW, Dean JF. 2002. SAGE profiling and demonstration of differential gene expression along the axial developmental gradient of lignifying xylem in loblolly pine (Pinus taeda). *Tree Physiol.* 22(5):301–10
141. Love J, Björklund S, Vahala J, Hertzberg M, Kangasjärvi J, Sundberg B. 2009. Ethylene is an endogenous stimulator of cell division in the cambial meristem of Populus. *Proc. Natl. Acad. Sci. U. S. A.* 106(14):5984–89
142. Lucas WJ, Ding B, Van Der Schoot C. 1993. Plasmodesmata and the supracellular nature of plants. *New Phytol.* 125(3):435–76
143. Lucas WJ, Groover A, Lichtenberger R, Furuta K, Yadav S, *et al.* 2013. The plant vascular system: evolution, development and functions f. *J. Integr. Plant Biol.* 55(4):294–388
144. Lucas WJ, Groover A, Lichtenberger R, Furuta K, Yadav S-R, *et al.* 2013. The plant vascular system: evolution, development and functions f. *J. Integr. Plant Biol.* 55(4):294–388
145. Mähönen AP, Bishopp A, Higuchi M, Nieminen KM, Kinoshita K, *et al.* 2006. Cytokinin Signaling and Its Inhibitor *AHP6* Regulate Cell Fate During Vascular Development. *Science*. 311(5757):94–98
146. Mähönen AP, Bonke M, Kauppinen L, Riikonen M, Benfey PN, Helariutta Y. 2000. A novel two-component hybrid molecule regulates vascular morphogenesis of the *Arabidopsis* root. *Genes Dev.* 14(23):2938–43
147. Mäkilä R, Wybouw B, Smetana O, Vainio L, Solé-Gil A, *et al.* 2023. Gibberellins promote polar auxin transport to regulate stem cell fate decisions in cambium. *Nat. Plants*. 9(4):631–44
148. Matsumoto-Kitano M, Kusumoto T, Tarkowski P, Kinoshita-Tsujimura K, Václavíková K, *et al.* 2008. Cytokinins are central regulators of cambial activity. *Proc. Natl. Acad. Sci.* 105(50):20027–31
149. Matte Risopatron JP, Sun Y, Jones BJ. 2010. The vascular cambium: molecular control of cellular structure. *Protoplasma*. 247:145–61
150. Mauriat M, Moritz T. 2009. Analyses of GA20ox- and GID1-over-expressing aspen suggest that gibberellins play two distinct roles in wood formation. *Plant J.* 58(6):989–1003
151. Mauriat M, Sandberg LG, Moritz T. 2011. Proper gibberellin localization in vascular tissue is required to control auxin-dependent leaf development and bud outgrowth in hybrid aspen. *Plant J.* 67(5):805–16
152. Mazur E, Kurczyńska EU, Friml J. 2014. Cellular events during interfascicular cambium ontogenesis in inflorescence stems of *Arabidopsis*. *Protoplasma*. 251(5):1125–39

153. McCarthy RL, Zhong R, Ye Z-H. 2009. MYB83 Is a Direct Target of *SND1* and Acts Redundantly with MYB46 in the Regulation of Secondary Cell Wall Biosynthesis in *Arabidopsis*. *Plant Cell Physiol.* 50(11):1950–64
154. Menéndez-Yuffá A, García de García E. 1997. Morphogenic events during indirect somatic embryogenesis in coffee "Catimor." *Protoplasma*. 199:208–14
155. Mi*SHR*a MK, Slater A. 2012. Recent Advances in the Genetic Transformation of Coffee. *Biotechnol. Res. Int.* 2012:e580857
156. Müller B, Sheen J. 2008. Cytokinin and auxin interplay in root stem-cell specification during early embryogenesis. *Nature*. 453(7198):1094–97
157. Nagawa S, Sawa S, Sato S, Kato T, Tabata S, Fukuda H. 2006. Gene Trapping in *Arabidopsis* Reveals Genes Involved in Vascular Development. *Plant Cell Physiol.* 47(10):1394–1405
158. Nelson N, Hillis W. 1978. Ethylene and tension wood formation in Eucalyptus gomphocephala. *Wood Sci. Technol.* 12(4):309–15
159. Nieminen K, Immanen J, Laxell M, Kauppinen L, Tarkowski P, *et al*. 2008. Cytokinin signaling regulates cambial development in poplar. *Proc. Natl. Acad. Sci.* 105(50):20032–37
160. Nilsson J, Karlberg A, Antti H, Lopez-Vernaza M, Mellerowicz E, *et al*. 2008. Dissecting the molecular basis of the regulation of wood formation by auxin in hybrid aspen. *Plant Cell*. 20(4):843–55
161. Nodine MD, Yadegari R, Tax FE. 2007. RPK1 and TOAD2 Are Two Receptor-like Kinases Redundantly Required for *Arabidopsis* Embryonic Pattern Formation. *Dev. Cell*. 12(6):943–56
162. Notaguchi M, Okamoto S. 2015. Dynamics of long-distance signaling via plant vascular tissues. *Front. Plant Sci.* 6:161
163. Nunes FL, Camargo MBP de, Fazuoli LC, Rolim G de S, Pezzopane JRM. 2010. Modelos agrometeorológicos de estimativa da duração do estádio floração-maturação para três cultivares de café arábica. *Bragantia*. 69:1011–18
164. Ohashi-Ito K, Bergmann DC. 2007. Regulation of the *Arabidopsis* root vascular initial population by LONESOME HIGHWAY. *Dev. Camb. Engl.* 134(16):2959–68
165. Ohashi-Ito K, Fukuda H. 2003. HD-Zip III Homeobox Genes that Include a Novel Member, ZeHB-13 (Zinnia)/ATHB-15 (*Arabidopsis*), are Involved in Procambium and Xylem Cell Differentiation. *Plant Cell Physiol.* 44(12):1350–58
166. Ohashi-Ito K, Fukuda H. 2010. Transcriptional regulation of vascular cell fates. *Curr. Opin. Plant Biol.* 13(6):670–76
167. Ohashi-Ito K, Fukuda H. 2014. Initiation of vascular development. *Physiol. Plant.* 151(2):142–46
168. Ohashi-Ito K, Fukuda H. 2014. Initiation of vascular development. *Physiol. Plant.* 151(2):142–46
169. Ohashi-Ito K, Iwamoto K, Fukuda H. 2018. LOB DOMAIN-CONTAINING PROTEIN 15 positively regulates expression of *VND7*, a master regulator of tracheary elements. *Plant Cell Physiol.* 59(5):989–96
170. Ohashi-Ito K, Oda Y, Fukuda H. 2010. *Arabidopsis* VASCULAR-RELATED NAC-DOMAIN6 Directly Regulates the Genes That Govern Programmed Cell Death and Secondary Wall Formation during Xylem Differentiation. *Plant Cell*. 22(10):3461–73
171. Ohashi-Ito K, Saegusa M, Iwamoto K, Oda Y, Katayama H, *et al*. 2014. A bHLH Complex Activates Vascular Cell Division via Cytokinin Action in Root Apical Meristem. *Curr. Biol.* 24(17):2053–58
172. Ohtani M, Akiyoshi N, Takenaka Y, Sano R, Demura T. 2017. Evolution of plant conducting cells: perspectives from key regulators of vascular cell differentiation. *J.*

Exp. Bot. 68(1):17–26

173. Oliveira NK, Castro E de, Guimarães RJ, Pieve LM, Baliza DP, *et al.* 2014. Foliar anatomy of coffee plants implanted using hydro retainer polymers.
174. Oyama T, Shimura Y, Okada K. 1997. The *Arabidopsis* HY5 gene encodes a bZIP protein that regulates stimulus-induced development of root and hypocotyl. *Genes Dev.* 11(22):2983–95
175. Pereira MA, Silva JRM da, Rabelo GF, Andrade AC de A. 2014. Propriedades anatômicas, químicas e de densidade da madeira de Coffea arabica L. *Cerne*. 20:363–67
176. Pesquet E, Tuominen H. 2011. Ethylene stimulates tracheary element differentiation in Zinnia elegans cell cultures. *New Phytol.* 190(1):138–49
177. Pickett FB, Wilson AK, Estelle M. 1990. The aux1 mutation of *Arabidopsis* confers both auxin and ethylene resistance. *Plant Physiol.* 94(3):1462–66
178. Pires ND, Dolan L. 2012. Morphological evolution in land plants: new designs with old genes. *Philos. Trans. R. Soc. B Biol. Sci.* 367(1588):508–18
179. Quintana-Escobar AO, Nic-Can GI, Galaz Avalos RM, Loyola-Vargas VM, Gongora-Castillo E. 2019. Transcriptome analyses of the induction of somatic embryogenesis in Coffea canephora and the participation of ARF and Aux/IAA genes. *PeerJ*. 7:e7752
180. Quiroz-Figueroa F, Fuentes-Cerda C, Rojas-Herrera R, Loyola-Vargas V. 2002. Histological studies on the developmental stages and differentiation of two different somatic embryogenesis systems of Coffea arabica. *Plant Cell Rep.* 20:1141–49
181. Ragni L, Nieminen K, Pacheco-Villalobos D, Sibout R, Schwechheimer C, Hardtke CS. 2011. Mobile Gibberellin Directly Stimulates *Arabidopsis* Hypocotyl Xylem Expansion. *Plant Cell*. 23(4):1322–36
182. Rahman A, Amakawa T, Goto N, Tsurumi S. 2001. Auxin is a positive regulator for ethylene-mediated response in the growth of *Arabidopsis* roots. *Plant Cell Physiol.* 42(3):301–7
183. Ramachandran P, Carlsbecker A, Etchells JP. 2017. Class III HD-ZIPs govern vascular cell fate: an HD view on patterning and differentiation. *J. Exp. Bot.* 68(1):55–69
184. Randall RS, Miyashima S, Blomster T, Zhang J, Elo A, *et al.* 2015. AINTEGUMENTA and the D-type cyclin CYCD3; 1 regulate root secondary growth and respond to cytokinins. *Biol. Open*. 4(10):1229–36
185. Ribone PA, Capella M, Arce AL, Chan RL. 2016. What Do We Know about Homeodomain–Leucine Zipper I Transcription Factors? Functional and Biotechnological Considerations. In *Plant Transcription Factors*, pp. 343–56. Elsevier
186. Robischon M, Du J, Miura E, Groover A. 2011. The Populus Class III HD ZIP, popREVOLUTA, Influences Cambium Initiation and Patterning of Woody Stems. *Plant Physiol.* 155(3):1214–25
187. Rodriguez-Villalon A. 2016. Wiring a plant: genetic networks for phloem formation in *Arabidopsis* thaliana roots. *New Phytol.* 210(1):45–50
188. Rodriguez-Villalon A, Gujas B, Kang YH, Breda AS, Cattaneo P, *et al.* 2014. Molecular genetic framework for protophloem formation. *Proc. Natl. Acad. Sci.* 111(31):11551–56
189. Rosolem CA, Leite VM. 2007. Coffee leaf and stem anatomy under boron deficiency. *Rev. Bras. Ciênc. Solo*. 31(3):477–83
190. Ruiz Sola MA, Coiro M, Crivelli S, Zeeman SC, Schmidt Kjølner Hansen S, Truernit E. 2017. *OCTOPUS*-LIKE 2, a novel player in *Arabidopsis* root and vascular development, reveals a key role for *OCTOPUS* family genes in root metaphloem sieve tube differentiation. *New Phytol.* 216(4):1191–1204
191. Ruonala R, Ko D, Helariutta Y. 2017. Genetic networks in plant vascular development.

Annu. Rev. Genet. 51:335–59

192. Růžička K, Ljung K, Vanneste S, Podhorská R, Beeckman T, *et al.* 2007. Ethylene Regulates Root Growth through Effects on Auxin Biosynthesis and Transport-Dependent Auxin Distribution. *Plant Cell.* 19(7):2197–2212
193. Sachs T. 1981. The Control of the Patterned Differentiation of Vascular Tissues. In *Advances in Botanical Research*. 9:151–262. Elsevier
194. Saito M, Kondo Y, Fukuda H. 2018. *BES1* and BZR1 Redundantly Promote Phloem and Xylem Differentiation. *Plant Cell Physiol.* 59(3):590–600
195. Saito M, Kondo Y, Fukuda H. 2018. *BES1* and BZR1 Redundantly Promote Phloem and Xylem Differentiation. *Plant Cell Physiol.* 59(3):590–600
196. Savidge RA. 1988. Auxin and ethylene regulation of diameter growth in trees. *Tree Physiol.* 4(4):401–14
197. Scarpella E, Marcos D, Friml J, Berleth T. 2006. Control of leaf vascular patterning by polar auxin transport. *Genes Dev.* 20(8):1015–27
198. Scarpella E, Meijer AH. 2004. Pattern formation in the vascular system of monocot and dicot plant species. *New Phytol.* 164(2):209–42
199. Scheres B, Laurenzio LD, Willemsen V, Hauser M-T, Janmaat K, *et al.* 1995. Mutations affecting the radial organisation of the *Arabidopsis* root display specific defects throughout the embryonic axis. *Development*. 121(1):53–62
200. Schlereth A, Möller B, Liu W, Kientz M, Flipse J, *et al.* 2010. MONOPTEROS controls embryonic root initiation by regulating a mobile transcription factor. *Nature*. 464(7290):913–16
201. Schrader J, Nilsson J, Mellerowicz E, Berglund A, Nilsson P, *et al.* 2004. A High-Resolution Transcript Profile across the Wood-Forming Meristem of Poplar Identifies Potential Regulators of Cambial Stem Cell Identity. *Plant Cell.* 16(9):2278–92
202. Schrick K, Mayer U, Horrichs A, Kuhnt C, Bellini C, *et al.* 2000. FACKEL is a sterol C-14 reductase required for organized cell division and expansion in *Arabidopsis* embryogenesis. *Genes Dev.* 14(12):1471–84
203. Seyfferth C, Wessels BA, Gorzsás A, Love JW, Rüggeberg M, *et al.* 2019. Ethylene signaling is required for fully functional tension wood in hybrid Aspen. *Front. Plant Sci.* 10:1101
204. Seyfferth C, Wessels B, Jokipii-Lukkari S, Sundberg B, Delhomme N, *et al.* 2018. Ethylene-Related Gene Expression Networks in Wood Formation. *Front. Plant Sci.* 9:
205. Shih CY, Currier HB. 1969. FINE STRUCTURE OF PHLOEM CELLS IN RELATION TO TRANSLOCATION IN THE COTTON SEEDLING. *Am. J. Bot.* 56(4):464–72
206. Sibout R, Plantegenet S, Hardtke CS. 2008. Flowering as a Condition for Xylem Expansion in *Arabidopsis* Hypocotyl and Root. *Curr. Biol.* 18(6):458–63
207. Sieburth LE. 2007. Plant development: PXY and polar cell division in the procambium. *Curr. Biol.* 17(15):R594–96
208. Šimášková M, O'Brien JA, Khan M, Van Noorden G, Ötvös K, *et al.* 2015. Cytokinin response factors regulate PIN-FORMED auxin transporters. *Nat. Commun.* 6(1):8717
209. Sjolund R. 1997. The Phloem Sieve Element: A River Runs through It. *Plant Cell.* 9(7):1137–46
210. Skirycz A, Reichelt M, Burow M, Birkemeyer C, Rolcik J, *et al.* 2006. DOF transcription factor AtDof1.1 (OBP2) is part of a regulatory network controlling glucosinolate biosynthesis in *Arabidopsis*. *Plant J.* 47(1):10–24
211. Steinmann T, Geldner N, Grebe M, Mangold S, Jackson CL, *et al.* 1999. Coordinated Polar Localization of Auxin Efflux Carrier PIN1 by GNOM ARF GEF. *Science*. 286(5438):316–18

212. Takata N, Awano T, Nakata MT, Sano Y, Sakamoto S, *et al*. 2019. Populus NST/SND orthologs are key regulators of secondary cell wall formation in wood fibers, phloem fibers and xylem ray parenchyma cells. *Tree Physiol.* 39(4):514–25
213. Talbert PB, Adler HT, Parks DW, Comai L. 1995. The REVOLUTA gene is necessary for apical meristem development and for limiting cell divisions in the leaves and stems of *Arabidopsis* thaliana. *Development*. 121(9):2723–35
214. Torrey JG. 1955. ON THE DETERMINATION OF VASCULAR PATTERNS DURING TISSUE DIFFERENTIATION IN EXCISED PEA ROOTS. *Am. J. Bot.* 42(2):183–98
215. Truernit E, Bauby H, Belcram K, Barthélémy J, Palauqui J-C. 2012. *OCTOPUS*, a polarly localised membrane-associated protein, regulates phloem differentiation entry in *Arabidopsis* thaliana. *Development*. 139(7):1306–15
216. Turgeon R, Oparka K. 2010. The secret phloem of pumpkins. *Proc. Natl. Acad. Sci. U. S. A.* 107(30):13201–2
217. Turner S, Sieburth LE. 2003. Vascular patterning. *Arab. BookAmerican Soc. Plant Biol.* 2:
218. Turner S, Sieburth LE. 2003. Vascular Patterning. *Arab. Book Am. Soc. Plant Biol.* 2:e0073
219. Uggla C, Magel E, Moritz T, Sundberg B. 2001. Function and Dynamics of Auxin and Carbohydrates during Earlywood/Latewood Transition in Scots Pine. *Plant Physiol.* 125(4):2029–39
220. Ursache R, Miyashima S, Chen Q, Vatén A, Nakajima K, *et al*. 2014. Tryptophan-dependent auxin biosynthesis is required for HD-ZIP III-mediated xylem patterning. *Development*. 141(6):1250–59
221. Ursache R, Nieminen K, Helariutta Y. 2013. Genetic and hormonal regulation of cambial development. *Physiol. Plant.* 147(1):36–45
222. Valencia-Lozano E, Ibarra JE, Herrera-Ubaldo H, De Folter S, Cabrera-Ponce JL. 2021. Osmotic stress-induced somatic embryo maturation of coffee Coffea arabica L., shoot and root apical meristems development and robustness. *Sci. Rep.* 11(1):9661
223. van Raemdonck D, Pesquet E, Cloquet S, Beeckman H, Boerjan W, *et al*. 2005. Molecular changes associated with the setting up of secondary growth in aspen. *J. Exp. Bot.* 56(418):2211–27
224. Vatén A, Dettmer J, Wu S, Stierhof Y-D, Miyashima S, *et al*. 2011. Callose Biosynthesis Regulates Symplastic Trafficking during Root Development. *Dev. Cell.* 21(6):1144–55
225. Vollbrecht E, Veit B, Sinha N, Hake S. 1991. The developmental gene Knotted-1 is a member of a maize homeobox gene family. *Nature*. 350(6315):241–43
226. Voltan RBQ, Fahl JI, Carelli MLC. 2011. Diferenciação de gemas florais em cultivares de cafeeiro
227. Wang J, Kucukoglu M, Zhang L, Chen P, Decker D, *et al*. 2013. The *Arabidopsis* LRR-RLK, PXC1, is a regulator of secondary wall formation correlated with the TDIF-PXY/TDR-WOX4 signaling pathway. *BMC Plant Biol.* 13:1–11
228. Wang R, Ji Y, Wang J, Yang S, Song Y. 2015. Vascular expression of Populus LRR-RLK genes and the effects of their overexpression on wood formation. *Mol. Breed.* 35:1–12
229. Ward JM, Cufr CA, Denzel MA, Neff MM. 2005. The Dof Transcription Factor OBP3 Modulates Phytochrome and Cryptochrome Signaling in *Arabidopsis*. *Plant Cell.* 17(2):475–85
230. Wardlaw IF, Mortimer D. 1970. Carbohydrate movement in pea plants in relation to axillary bud growth and vascular development. *Can. J. Bot.* 48(2):229–37

231. Wareing P. 1958. Interaction between indole-acetic acid and gibberellic acid in cambial activity. *Nature*. 181(4625):1744–45
232. Wilson JW. 1978. The position of regenerating cambia: auxin/sucrose ratio and the gradient induction hypothesis. *Proc. R. Soc. Lond. B Biol. Sci.* 203(1151):153–76
233. Wolbang CM, Ross JJ. 2001. Auxin promotes gibberellin biosynthesis in decapitated tobacco plants. *Planta*. 214:153–57
234. Woudenberg S, Renema J, Tomescu AMF, De Rybel B, Weijers D. 2022. Deep origin and gradual evolution of transporting tissues: Perspectives from across the land plants. *Plant Physiol.* 190(1):85–99
235. Xiao Z, Ma G, Bai X, Li J, Zhao M, *et al*. 2024. The influence of leaf anatomical traits on photosynthesis in Catimor type Arabica coffee. *Beverage Plant Res.* 4(1):0–0
236. Xoconostle-Cázares B, Xiang Y, Ruiz-Medrano R, Wang H-L, Monzer J, *et al.* 1999. Plant Paralog to Viral Movement Protein That Potentiates Transport of mRNA into the Phloem. *Science*. 283(5398):94–98
237. Xu B, Ohtani M, Yamaguchi M, Toyooka K, Wakazaki M, *et al.* 2014. Contribution of NAC Transcription Factors to Plant Adaptation to Land. *Science*. 343(6178):1505–8
238. Yamaguchi M, Kubo M, Fukuda H, Demura T. 2008. VASCULAR-RELATED NAC-DOMAIN7 is involved in the differentiation of all types of xylem vessels in *Arabidopsis* roots and shoots. *Plant J.* 55(4):652–64
239. Yamaguchi M, Ohtani M, Mitsuda N, Kubo M, Ohme-Takagi M, *et al.* 2010. VND-INTERACTING2, a NAC Domain Transcription Factor, Negatively Regulates Xylem Vessel Formation in *Arabidopsis*. *Plant Cell*. 22(4):1249–63
240. Yanagisawa S. 2002. The Dof family of plant transcription factors. *Trends Plant Sci.* 7(12):555–60
241. Yanagisawa S. 2004. Dof Domain Proteins: Plant-Specific Transcription Factors Associated with Diverse Phenomena Unique to Plants. *Plant Cell Physiol.* 45(4):386–91
242. Yang S, Wang S, Li S, Du Q, Qi L, *et al.* 2020. Activation of ACS7 in *Arabidopsis* affects vascular development and demonstrates a link between ethylene synthesis and cambial activity. *J. Exp. Bot.* 71(22):7160–70
243. Ye Z-H, Zhong R. 2015. Molecular control of wood formation in trees. *J. Exp. Bot.* 66(14):4119–31
244. Yokoyama A, Yamashino T, Amano Y-I, Tajima Y, Imamura A, *et al.* 2007. Type-B ARR Transcription Factors, ARR10 and ARR12, are Implicated in Cytokinin-Mediated Regulation of Protoxylem Differentiation in Roots of *Arabidopsis* thaliana. *Plant Cell Physiol.* 48(1):84–96
245. Yoo B-C, Kragler F, Varkonyi-Gasic E, Haywood V, Archer-Evans S, *et al*. 2004. A Systemic Small RNA Signaling System in Plants. *Plant Cell*. 16(8):1979–2000
246. Yoo S-C, Chen C, Rojas M, Daimon Y, Ham B-K, *et al.* 2013. Phloem long-distance delivery of FLOWERING LOCUS T (FT) to the apex. *Plant J.* 75(3):456–68
247. Yoshimoto K, Noutoshi Y, Hayashi K, Shirasu K, Takahashi T, Motose H. 2012. Thermospermine suppresses auxin-inducible xylem differentiation in *Arabidopsis* thaliana. *Plant Signal. Behav.* 7(8):937–39
248. Yoshimoto K, Takamura H, Kadota I, Motose H, Takahashi T. 2016. Chemical control of xylem differentiation by thermospermine, xylemin, and auxin. *Sci. Rep.* 6:21487
249. Yu Q, Cheng C, Zhou X, Li Y, Hu Y, *et al.* 2023. Ethylene controls cambium stem cell activity via promoting local auxin biosynthesis. *New Phytol.* 239(3):964–78
250. Zavaliev R, Ueki S, Epel BL, Citovsky V. 2011. Biology of callose (β-1,3-glucan) turnover at plasmodesmata. *Protoplasma*. 248(1):117–30
251. Zhong R, Lee C, Ye Z-H. 2010. Global analysis of Direct Targets of Secondary Wall

NAC Master Switches in *Arabidopsis*. *Mol. Plant*. 3(6):1087–1103

252. Zhong R, Lee C, Zhou J, McCarthy RL, Ye Z-H. 2008. A Battery of Transcription Factors Involved in the Regulation of Secondary Cell Wall Biosynthesis in *Arabidopsis*. *Plant Cell*. 20(10):2763–82
253. Zhong R, Ye Z-H. 1999. IFL1, a Gene Regulating Interfascicular Fiber Differentiation in *Arabidopsis*, Encodes a Homeodomain-Leucine Zipper Protein. *Plant Cell*. 11(11):2139–52
254. Zhong R, Ye Z-H. 2007. Regulation of HD-ZIP III Genes by MicroRNA 165. *Plant Signal. Behav.* 2(5):351–53
255. Zhou J, Lee C, Zhong R, Ye Z-H. 2009. MYB58 and MYB63 Are Transcriptional Activators of the Lignin Biosynthetic Pathway during Secondary Cell Wall Formation in *Arabidopsis*. *Plant Cell*. 21(1):248–66
256. Zhou J, Wang X, Lee J-Y, Lee J-Y. 2013. Cell-to-Cell Movement of Two Interacting AT-Hook Factors in *Arabidopsis* Root Vascular Tissue Patterning[W]. *Plant Cell*. 25(1):187–201
257. Zhu Y, Song D, Sun J, Wang X, Li L. 2013. PtrHB7, a class III HD-Zip Gene, Plays a Critical Role in Regulation of Vascular Cambium Differentiation in Populus. *Mol. Plant*. 6(4):1331–43
258. Zúñiga-Sánchez E, Martínez-Barajas E, Zavaleta-Mejía E, Gamboa-de-Buen A. 2017. El floema y la ruta simplástica durante la formación de órganos de demanda. *Rev. Fitotec. Mex.* 40(3):249–59

CHAPTER 2

2. Anatomical, computational and molecular analyses of vascularization in peduncles of reproductive buds, flowers and fruits of *Coffea arabica* L.

(Draft Version)

Article prepared for submission to the Plant Gene Journal

Andrea Jaramillo Mesa[1], Raphael Ricon de Oliveira[1], Lillian Magalhães Azevedo[1], Thales Henrique Cherubino Ribeiro[2], Thiago Alves Magalhães[3], Antonio Chalfun-Junior[1]*

[1]Laboratory of Plant Molecular Physiology, Plant Physiology Sector, Institute of Biology, Federal University of Lavras (UFLA), Lavras, Minas Gerais, Brazil.
[2]Genome Center, University of California, Davis, USA.
[3]Laboratory of Structural Botany at the Federal University of Lavras (UFLA), Lavras, Minas Gerais, Brazil.

*Corresponding author: chalfunjunior@ufla.br

Abstrac

Coffee beans are an important agricultural product primarily grown in tropical countries. The flowering of coffee plants is necessary for bean development but can be uneven, leading to inconsistent fruit maturation. In Brazil, where mechanized harvesting mixes green and ripe beans, this phenomenon can impact quality. In addition, non-uniform flowering complicates pest and disease control. The reproductive period of coffee plants depends on photoperiod and water availability. Once induced, floral organs progress through developmental stages classified based on size and coloration. Floral buds enter a reproductive arrest period, usually called dormancy, when they reach a length between 4 - 6 mm in size that coincides with dry periods in Minas Gerais, Brazil. Sporadic rains during this period can trigger growth in larger buds, exacerbating developmental differences between buds, even in the same node. Key transcription factors in model species are known to initiate vascular bundle development. Here we identified, characterized, and evaluated three key orthogroups: *HD14* (*HD-ZIP Class III*) for procambium initiation and formation, *OCTOPUS* for phloem cell identity, and *VND7* for protoxylem and metaxylem development. Our integrated approach of anatomical peduncle characterization and RNAseq/RT-qPCR analyses unravels molecular control mechanisms in coffee vascular development. This research aims to enhance coffee production and improve strategies for other globally important crops.

Keywords: flowering and fruiting of coffee; peduncle vascular development; gene characterization; RNAseq/RT-qPCR expression analyses.

Supplemental material can be accessed using the link:
https://drive.google.com/drive/u/0/folders/1HhYwcEviMOmtu06T2PsMKdvt9NQy-etF

2.1 Introduction

Due to the significant socioeconomic importance of coffee production, sale, and consumption worldwide, various agronomic strategies have been developed to meet the need for plants with greater productivity, disease resistance, and superior cup quality (Arcila-Pulgarin et al., 2001; Ferreira et al., 2019). Knowledge of the coffee plant and its agronomic characteristics has been fundamental to understanding practical issues of coffee cultivation, as well as the interaction of this crop with the environment (Krishnan, 2017). The *Coffea* genus, belonging to the Rubiaceae family, also holds great scientific value (Anthony et al., 2010). Studies at physiological, anatomical, and molecular levels have enriched scientific knowledge of the species, which has also helped to improve its production. The allopolyploidy of *Coffea arabica* L. ($2n = 44$), the most economically important species of the *Coffea* genus, arises from an interspecific cross between *Coffea canephora* Pierre ex Froehner and *Coffea eugenioides* Moore (Lashermes et al., 1999). This genomic feature makes it an interesting candidate for evolutionary studies aimed at understanding regulatory networks in polyploids (Cherubino Ribeiro et al., 2023).

The coffee bean production is centered around three species; *Coffea arabica* (69%), followed by *Coffea canephora* (30%) and *Coffea liberica* (1%). For the 2023/24 period, world coffee production is projected at 178 million bags, with South America contributing 89.3 million bags, led by Brazil at 59.1 million bags (CONAB, 2024). This global market involves not only production centers but also consumption and trade routes over 60 countries supporting the livelihood of millions (Waller et al., 2007).

Members of the *Coffea* genus are perennial woody plants. *C. arabica* and its close relative *C. canephora* take two years to transition from a vegetative meristem at a node to produce flowers and fruits, leading up to the senescence of fruitful branches (Camargo and Camargo, 2001). The reproductive phase is marked by the emergence of numerous floral buds, many of which fail to develop into fruit due to abortion. In addition, the coffee plants must maintain the shoot apical meristem in a vegetative state to enable simultaneous yet spatially separated growth and reproductive development (López et al., 2021). The combination of these and other factors likely leads to the non-uniformity in coffee plants' flowering and fruit ripening process, a serious economic problem that impairs the coffee market as a whole.

Previous studies have addressed the non-uniformity of coffee flowering and ripening in terms of the spatiotemporal expression *MADS-box* genes (de Oliveira et al., 2014), floral induction timing (Cardon et al., 2022), small RNAs regulation (Cherubino Ribeiro et al., 2024), ethylene signaling (Lima et al., 2021a), and water availability (López et al., 2021) (López et al., 2022). However, few studies have focused on the anatomical aspects of coffee plant vascularization, particularly the peduncles connecting floral buds and fruits with the plant, especially in the context of non-uniformity.

Peduncles are considered extensions of stems and are responsible for the support and conduction of sap to the flowers and fruits (Beentje and Williamson, 2010). The peduncle of coffee reproductive organs has been little studied. The available literature has examined the coffee fruit peduncle primarily to determine the mechanical and physical properties to improve mechanized harvesting (Brandão et al., 2016; De Melo et al., 2023). Nevertheless, little is known about the anatomy and vascular development of the peduncle during the reproductive phase of coffee.

This study aims to fill this gap by providing a detailed analysis of the anatomical and molecular changes in the peduncles during different growth stages of *C. arabica*. By combining anatomical analyses, molecular techniques, bioinformatic, and statistical approaches we were able to identify, characterize and evaluate expression profiles of potential candidate genes orchestrating the peduncle vascular development at key steps of coffee floral bud development. We show a high degree of cellular organization in the woody plant *C. arabica* potentially orchestrated by known genes in vascular plants, such as those found in *Arabidopsis* and *Populus*. These include Class III homeodomain-leucine-zipper (*HD-Zip III*) (Emery et al., 2003), *OCTOPUS* (*OPS*) (Bauby et al., 2007; Truernit et al., 2012), *VASCULAR-RELATED NAC-DOMAIN6* and *7* (*VND6* and *7*) (Kubo et al., 2005) and *SECONDARY WALL-ASSOCIATED NAC DOMAIN PROTEIN1* (*SND1*) (Zhong et al., 2006). Our findings not only enhance the understanding of vascular development in coffee, and potentially other crop species, but also offer valuable insights that could improve coffee cultivation practices, particularly in optimizing conditions for synchronous flowering and fruiting.

2.2 Materials and methods

2.2.1 Plant material for anatomical analyses

We collected the plant material at the Cafuá Farm in the municipality of Ijací, Minas Gerais, Brazil (21º9’52’’S and 44º59’31’’W, 940 m.a.s.l.). We sampled floral buds, flowers, and fruits of *C. arabica*, Var. Catuaí vermelho IAC 144, with 8 years old, at different developmental stages from the plagiotropic branches located in the middle part of the coffee plant, during the reproductive phase, facing east. To determine the stage of development at which the vascular bundles begin to form in the peduncles of the coffee tree's reproductive organs, we analyzed 16 reproductive stages of 18 adapted from Morais *et al.* (2008) classification, including the shoot apical meristem (SAM) and stages G1 through G6, as well as the floral stages. Additionally, we collected samples that we classified as “shedding of floral whorls”, “beads-like”, “fruit filling”, “green fruit”, “green-cane fruit”, and “red fruit” stages (Figure 1). The samples were then processed at the Laboratory of Molecular Physiology in Plants (LFMP) and the Laboratory of Structural Botany at the Federal University of Lavras (UFLA), Minas Gerais, Brazil.

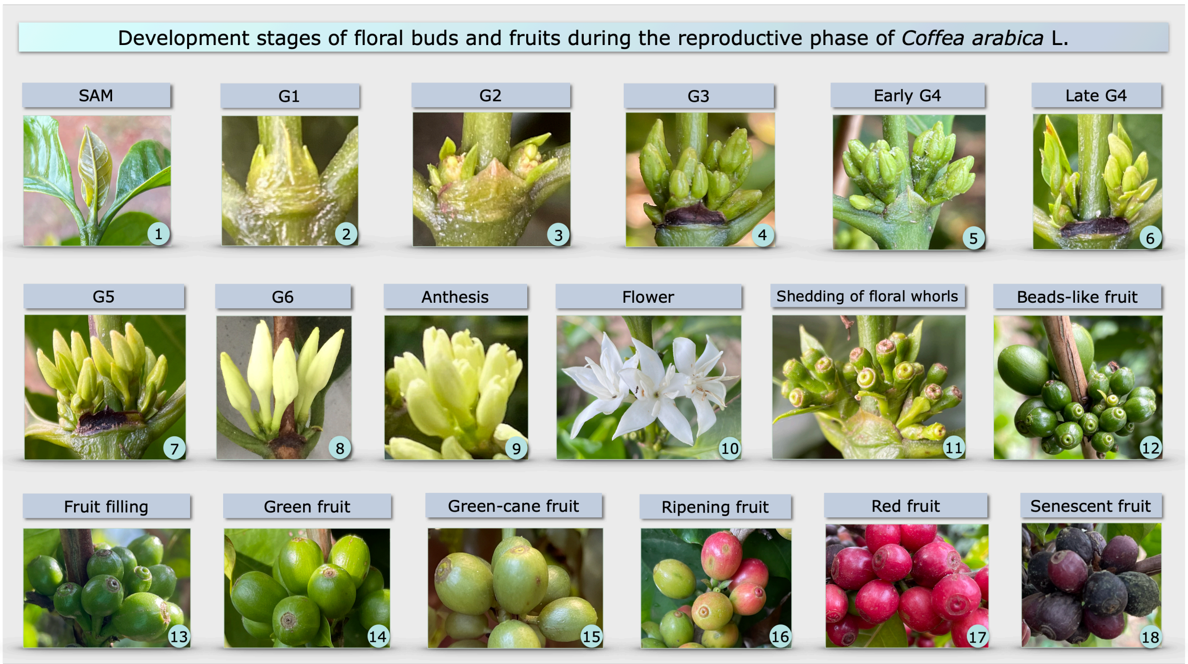

Figure 1. The diagram outlines the developmental stages of floral buds and fruits in *C. arabica*. **SAM**; Shoot apical meristem. Development of flower buds adapted from Morais *et al*., (2008): **G1**; nodes with apparently undifferentiated buds, **G2**; nodes with swollen buds, **G3**; buds measuring up to 3 mm in length, **G4**; buds measuring from 3.1 to 6 mm in length and green color (**Earlier G4**: before rains, and **Late G4**: after rains), **G5**; buds from 6.1 to 10 mm in length and light green color, almost white,

G6; buds larger than 10 mm with chandelier appearance and white color, **Anthesis**; beginning of flower opening and **Flower;** once anthesis is completed. Development and maturation of coffee fruit according to our description: **Shedding of floral whorls**; Abscission of floral whorls, the peduncles do not appear to grow, **Beads like**; as the fruit begins to fill, the grains assume a beads-like appearance (from 1 to 1.5 cm in length), **Fruit filling**; fruits measuring larger than 1.5 and before complet fruit filling, **Green**; this stage marks the point at which the fruit is fully filled and exhibits a uniform green coloration throughout, **Green-cane**; fruits with a light green color similar to the color of the cane almost yellow, **Ripening fruit**; fruits with a color between green, yellow and red, **Red**; fully ripe fruits with a strong red color, and **Senescence fruit**; dark purple fruit withering.

2.2.2 Histochemical preparation and histological analyses

We removed the leaves from each sampled branch and collected nodes containing organs such as floral buds, flowers, or fruits. We placed the samples into Falcon tubes containing 70% ethanol and stored them at 4°C for preservation and subsequent analyses. For the anatomical observations, we processed and analyzed at least five biological replicates of each developmental stage. Then, we used a scalpel to make cuts from the inflorescence insertion point to the node, separating each inflorescence, including a small portion of the node connected to the sample and then removing the bracts of each one.

We treated the samples with a graded ethanol series (80%, 90%, and 98%), allowing approximately one day for dehydration at each step, this procedure was adapted from Johansen (1940). Then, we immersed the samples in an activated resin solution (histological resin Leica, 500 mL, catalog code: 7592) following the manufacturer's instructions. For pre-infiltration, we used a mixture of absolute ethanol and liquid base resin (1:1), leaving the samples in suspension for at least 7 days. Subsequently, the samples were transferred to a pure activated resin solution for an additional 7 days, with vacuum applications for 40 minutes during each dehydration and embedding stage, at least twice a day. Finally, we embedded the samples in histomolds using the HistoResin kit (Leica Microsystems, Heidelberg, Germany). Thin cross and longitudinal sections (7-8 µm) were cut using a semi-automatic microtome and stained with 0.05% Toluidine blue O in pH 4.7 acetate buffer (O'Brien et al., 1964) for approximately 15 seconds.

Finally, we mounted the samples on glass microscope slides and examined and photographed them using a light microscope equipped with a camera (Olympus CX31 Capture system BEL) connected to a computer, at 4x and 10x objective magnification. Subsequently, we analyzed the images using ImageJ software (Stossi and Singh, 2023) to evaluate the development of the vascular area.

2.2.3 In silico analyses for the identification and characterization of vascular identity genes in C. arabica

We retrieved all *in silico* predicted protein sequences of *C. arabica* var. Caturra previously described by (Ribeiro et al., 2023). We utilized OrthoFinder v2.5.5 (Emms and Kelly, 2019) to identify orthologous groups (orthogroups) of protein coding genes in this set of *C. arabica* sequences in addition to other 24 representative plant species, which encompassed members from taxonomic groups such as monocots, rosids, asterids, and included *Selaginella,* a lycophyte (Banks et al., 2011), or *Amborella* the most basal lineage in the clade of angiosperms (Qiu et al., 1999) - as outgroups (Supplementary material 1). The parameters used were: -a 40 -M msa -os. The orthologs of the VND6 protein - POPTR_0015s14770 (Lin et al., 2017), OPS protein - At3g09070 (Truernit et al., 2012), and a member of HD Zip Class III family - UniProt ID Q9ZU11 (Prigge and Clark, 2006) were used to identify the sequences of each orthogroup. Sequences that were smaller than 70% of the length of the orthologs described in these studies, lacked expected conserved domains, or exhibited less than 50% similarity were excluded from the analyses. We manually evaluated the characteristic conserved domains of each group of orthologs using hmmscan from the HMMER v3.3.2 software.

To characterize each protein ortholog, we conducted multiple sequence alignment using MAFFT (Katoh et al., 2002) (v. 7.490) and inferred phylogenetic trees with PHYLIP (Felsenstein, 1993) (v. 3.696) employing 1000 bootstrap replicates. For inferring evolutionary relationships among proteins, we utilized the Jones – Taylor – Thornton substitution model (Jones et al., 1992) and the neighbor-joining clustering method (Saitou and Nei, 1987). Consensus trees were determined using majority rule and generated using the Interactive Tree of Life (iTOL v. 6.5.8) (Letunic and Bork, 2021).

2.2.4 Gene expression analyses by RNA-seq

We retrieved quality-controlled RNA-seq libraries from coffee buds, including their peduncles, at stages G2, late G4, and G5 from the Sequencing Read Archive (Supplementary material 2). We mapped these libraries to the *C. arabica* Var. Caturra genome using the STAR aligner v. 2.7.10b. We then sorted the alignment files using Samtools and quantified the reads uniquely mapped to gene-exons as characterized by Cherubino-Ribeiro et al. (2023) using the HT-seq count script.

For differential accumulation analyses, we used edgeR with the glmQLFit function (Robinson et al., 2010). We selected glmQLFit because it is particularly suited for handling libraries derived from different experimental settings. We deemed a protein-coding gene differentially expressed (DE) between stages if its false discovery rate (FDR) was less than 0.05 and the fold change was ≥2 in any of the contrasts.

2.2.5 Primer design

Primers for RT-qPCR were designed using the Primer3 tool (https://primer3.ut.ee/). Subsequently, the intraspecific specificity of the primers was evaluated with the blastn program v2.14.0+ using the parameters: -word_size 7-evalue 10 -task megablast. The interspecific specificity was performed using the online PrimerBlast tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). Primers were designed according to standard guidelines, ensuring an amplicon length between 80 and 150 bases. We avoided sequences within conserved domains, targeted a GC content of 40 – 60%, and adhered to additional recommendations as outlined by MIQE (Bustin et al., 2009). The full list of primers are available in supplementary material 3.

2.2.6 RNA extraction and cDNA synthesis

We collected plant material from buds at stages early G4 (G4E) and late G4 (G4L) on 15/09/2022 and 04/10/2022, respectively, and immediately kept them in liquid nitrogen until storage at -80°C. We extracted about 2.5 µg (sd ± 0.32) of total RNA from each biological sample, following the method of de Oliveira *et al.*, (2015) (Oliveira et al., 2015). We measured RNA concentration and purity using spectrophotometric analyses (GE NanoVue™ Spectrophotometer, Boston, MA), ensuring all samples had the proportion OD260/280 between 1.8 and 2.2. Additionally, we assessed RNA integrity number (RIN) using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA), selecting only samples with a RIN above 6 for further analyses.

We treated all samples with the Ambion DNase I (RNase-free) kit (Thermo Fisher, Waltham, MA) and synthesized cDNA using the High-Capacity cDNA Reverse Transcriptase

Kit (Applied Biosystems®, Foster City, CA). We used an RNA/reaction volume ratio of 1 μg per 20 μL, following the manufacturer's instructions. For RT-qPCR analyses, we used 15 ng of cDNA in a 15 μL reaction volume with the QuantiNova PCR kit (Qiagen, Venlo, Netherlands, Cat. No. / ID: 208256) on a Rotor Gene-Q (Qiagen) thermocycler. Each reaction consisted of 1.5μL of cDNA, 7.5 μL of SYBR (QuantiNova SYBR Green), 1.5 μL of each primer at a final concentration of 1μM, and 3.0 μL of RNase-free water, totaling 15 μL of final volume. We conducted the amplification with three biological replicates and three technical repetitions each, following the manufacturer's instructions. We assessed primer efficiencies for the target genes *OPS*, *VND*, and *HB*, and the reference genes *MDH* and *RPL39* (Martins et al., 2017), using a standard curve method. These dilution curves were also used to adjust RNA concentration. No-template controls (NTCs) were used to check for contamination.

2.2.7 Relative expression analyses by RT-qPCR

We calculated relative expression differences using the base 2 logarithm of fold change and performed statistical analyses, including confidence intervals, using a Linear Mixed Model as described by Steibel (Steibel et al., 2009). Threshold cycle (Ct) values were corrected based on primer efficiency. Residuals were verified to be normally distributed, and graphics were generated using R software. Following the MIQE guidelines (Bustin et al., 2009), a gene would be considered differentially expressed if the fold change is greater than 2 (upregulated) or less than 0.5 (downregulated), the confidence intervals do not include zero, and the p-values are less than 0.05.

2.3 Results

2.3.1 Early vascular differentiation in coffee floral buds and fruit peduncles

During our anatomical analyses, we elucidated the presence and primary characteristics of vascular bundles at each developmental stage in the flower bud and fruit peduncles, especially in the area connecting to the branch of *C. arabica* plants. Our observations suggest that, from stages G1 and G2 onwards, early vascular structures are present and the vascular area is already undergoing differentiation. Therefore, given that the procambium area is observable in longitudinal sections of G1 and G2, we propose that once the buds of *C. arabica* plants acquire reproductive identity and begin to sprout at the node, the peduncles establish a vascular connection with the branch. In this process, the vascularization of the branch node extends to the area connecting with the floral bud, and upon reaching the floral bud, we observe a procambial region (Figure 2).

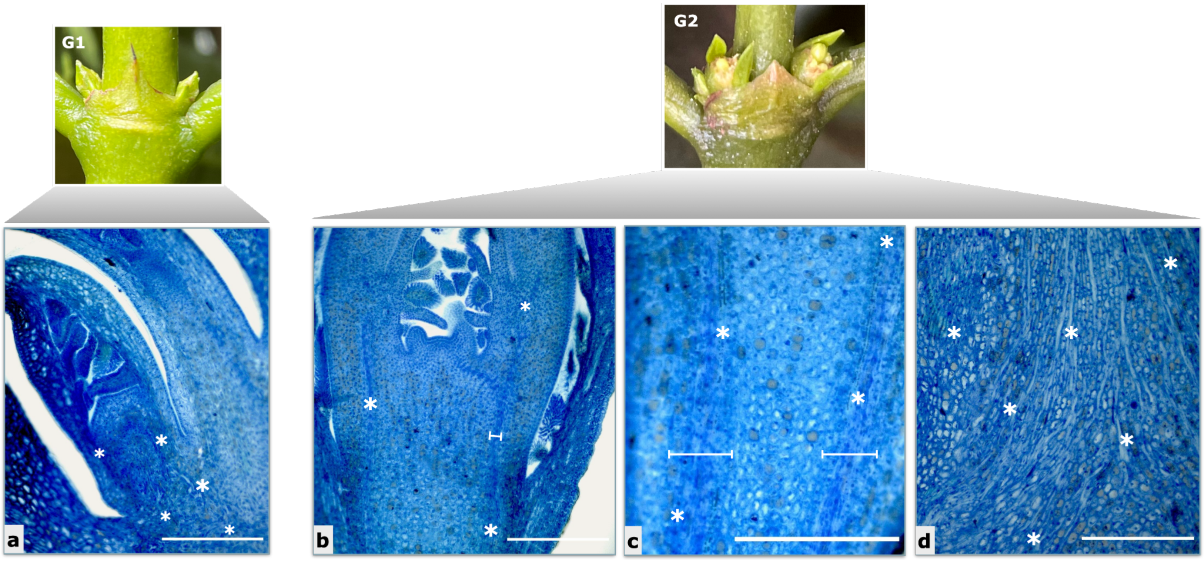

Figure 2. Longitudinal sections of the coffee flower bud at the G1 and G2 development stages. In the longitudinal section of G1, we observed the pathway marked by vascular bundles originating from the node region, which exhibited continuity along the floral bud. Similarly, at G2, the vascular area was observed extending from the base of the floral bud at its connection with the branch, showing a continuous presence of these procambium throughout the bud. Specifically: (**a**): G1 bud in connection with the branch; (**b**): G2 floral bud; (**c**): midsection of G2; (**d**): area encompassing the anatomical connection of the G2 with the branch. The white asterisks and lines are pointing at the formation of vascular bundles. Bar scale: 50 µm.

Next, in both longitudinal and cross sections at G3, the vascular area was found to be larger compared to G1 and G2, with vascular cells still undergoing differentiation and parenchyma and pith cells more distinctly defined. In G4 cross sections, we observed parenchymatous cells with better defined walls and a rounded shape. Furthermore, vascularization showed further differentiation in the areas destined to form the xylem and phloem. Based on our observations, we propose that starting from G4, in buds approximately 4 to 5 mm in length, the anatomical stage of development shows marked differentiation of vascular cells (Figure 3).

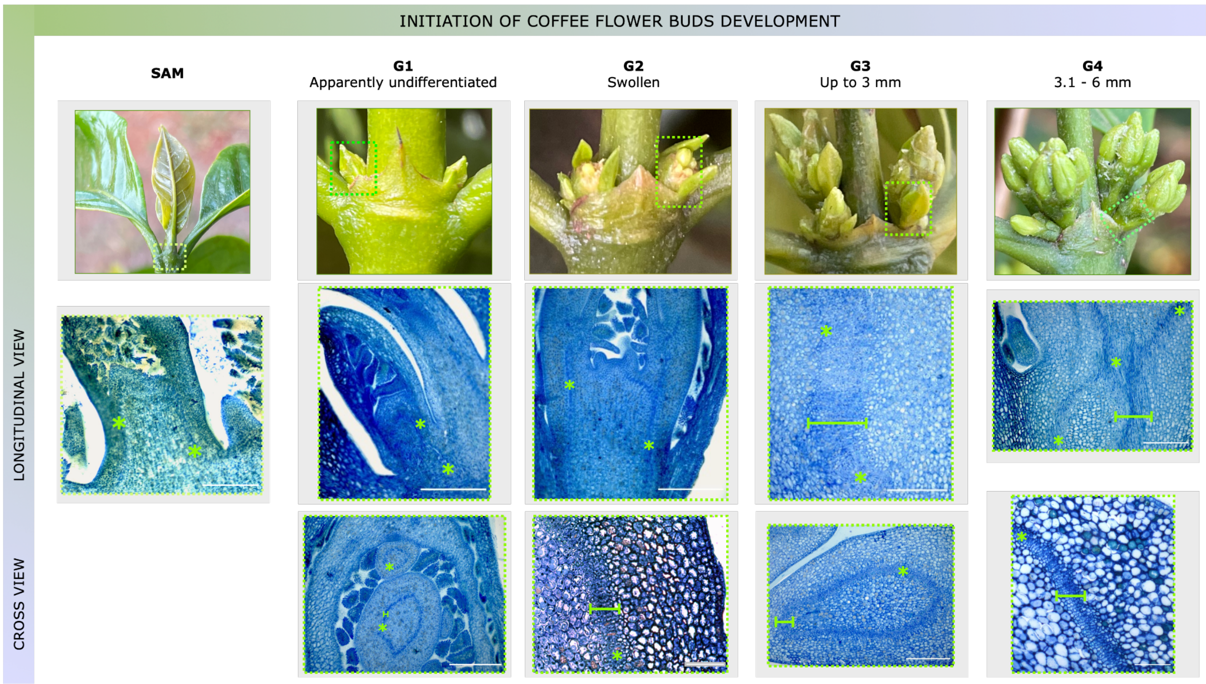

Figure 3. Vascular development in longitudinal section of *C. arabica* shoot apical meristem (SAM), and longitudinal and cross sections of G1 to G4 flower buds. In the SAM, we observed a procambial area. At the G1 and G2 development stages, the differentiation of vascular tissue is still in progress. By G3, the vascular area has expanded in comparison to earlier stages. From G4 peduncles onward, development and cellular differentiation occurred within the vascular area. The lime-green dotted rectangles outline the floral bud peduncle while the lime-green asterisks and lines highlight the development of the vascular bundles. Bar scale: 50 µm.

At the transition from the G4 to G5 stage, we noted enhanced differentiation in the vascular area compared to earlier developmental stages, indicating progressive vascular development in the peduncles. Longitudinal sections showed cell walls with punctate markings similar to those of phloem sieve cells. These punctate walls had been seen in some longitudinal sections of the peduncles at G4 when they measured 6 mm in length and at the G5 stage; however, they were more clearly observable at G6. These findings align with anatomical descriptions by Marchand (1864), who described these structures as punctuated and striped vessels within the phloem area in *C. arabica*, and by Crisosto *et al*. (1992), who noted greater differentiation of the phloem and xylem in cross-sections of peduncles of coffee flower buds, similar to our descriptions for the G4 stage. In the longitudinal sections of G5 and G6, the parenchyma, vascular area, and pith were more clearly defined. Similarly, at the G6 stage, cross-sections revealed that the cell walls in the vascular area were thicker (Figure 4).

Furthermore, we observed spiral-shaped cells resembling tracheary elements in longitudinal sections of both the floral peduncle and the peduncle after shedding of floral whorls. In the transverse sections during the shedding of floral whorls stage, increased differentiation of the xylem zone was observed at the base of the peduncle, connected with the branch. We identified this in longitudinal views by the presence of spiral-shaped cells and in cross-sections by the presence of xylem vessels (Figure 4).

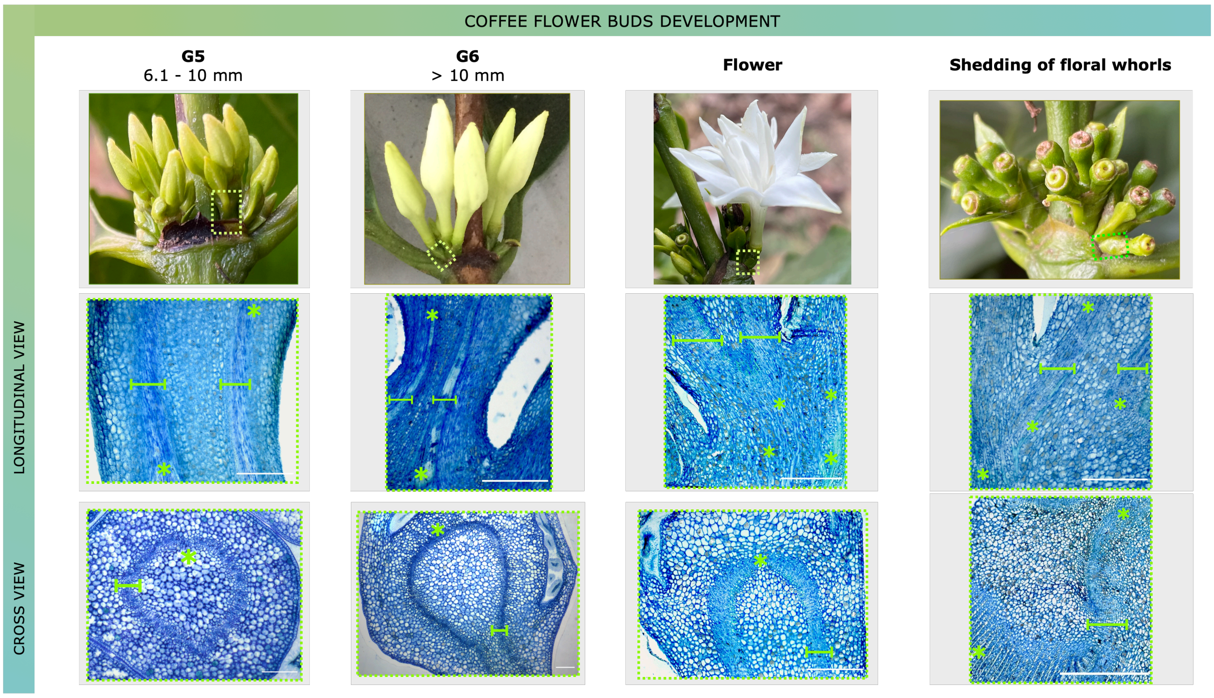

Figure 4. Vascular development in longitudinal and cross sections of *C. arabica* peduncles at G5, G6, flower and shedding of floral whorls stages. From G5 to the shedding of floral whorls stage, the vascular area exhibits substantial development and remains in a continuous process of growth. During this period, the xylem area begins to differentiate and can be identified in anatomical analyses. The lime-green dotted rectangles outline the floral bud peduncle while the lime-green asterisks and lines highlight the development of the vascular bundles. Bar scale: 50 µm.

During the development of floral organs, we observed a significant thickening of the cell walls in the xylem region after rainfall, beginning in the late G4 and G5 stages. This suggests a notable structural adaptation that may enhance the peduncles' ability to transport water and nutrients in response to post-precipitation conditions. Additionally, thickened cell walls within the xylem may provide greater mechanical strength, further supporting flower buds and later fruit growth (Figure 5).

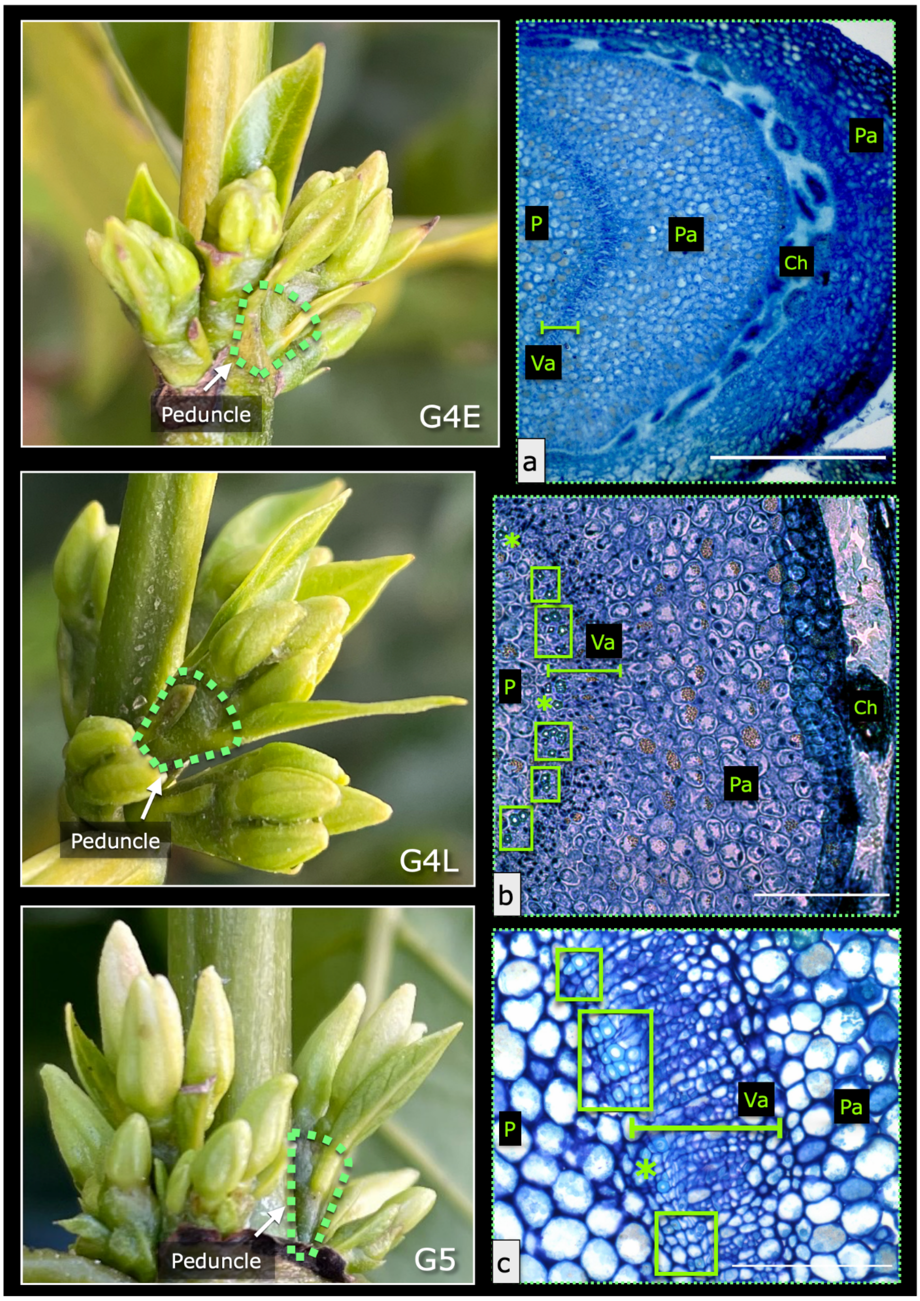

Figure 5. Cross-sections of *C. arabica* floral bud peduncles at development stage early G4 (**a**), late G4 (**b**) and G5 (**c**). From late G4 (G4L) and G5, we observed thickening of the cell walls in the xylem area, a feature not seen in the images of younger flower buds, such early G4 (G4E), before rains. **Ch**: Colleters; **Pa**: Parenchyma; **Va**: Vascular area; **P**: Pith. In (**a**, **b**, and **c**); the lime-green lines are delimiting the vascular area while the lime-green asterisks and squares highlight cells with thickening walls, especially the xylem cells. Image (**a** and **b**) is in a 10x objective, and image (**c**) is in a 20x objective. Bar scale: 50 µm.

From the G6 stage onwards, the flower bud is fully developed. Successive transverse sections of the peduncles revealed that, from the distal part of the peduncle connected to the branch to the base of the terminal flower, different cell conformations are reorganized, especially the radial ones. The cellular organization at the base and middle part of the peduncle are similar to the stem organization observed in previous works on *C. arabica* by Dedecca (1957), Ferreira *et al.* (2014) and Carréra *et al.*, (2023). However, as the cross-sections approach the flower, characteristic areas such as the gynoecium, corolla, style, and anthers become evident. Extending continuously towards the interior of the flower bud, we observed the characteristic whorls of the flower as reported by (Dedecca, 1957), similar to the flower bud structure of *Canephora madagascariensis* (De Block and Vrijdaghs, 2013), also a Rubiaceae. Despite this cellular reorganization throughout the floral bud, we observed the continuity of the vascular bundles from the branch, through the peduncle, to the flower. Specifically, in the peduncles, the vascular bundles formed a closed structure encircling the pith. However, beyond the gynoecium zone, the vascular bundles bifurcate, supplying each whorl of the coffee flower (Figure 6).

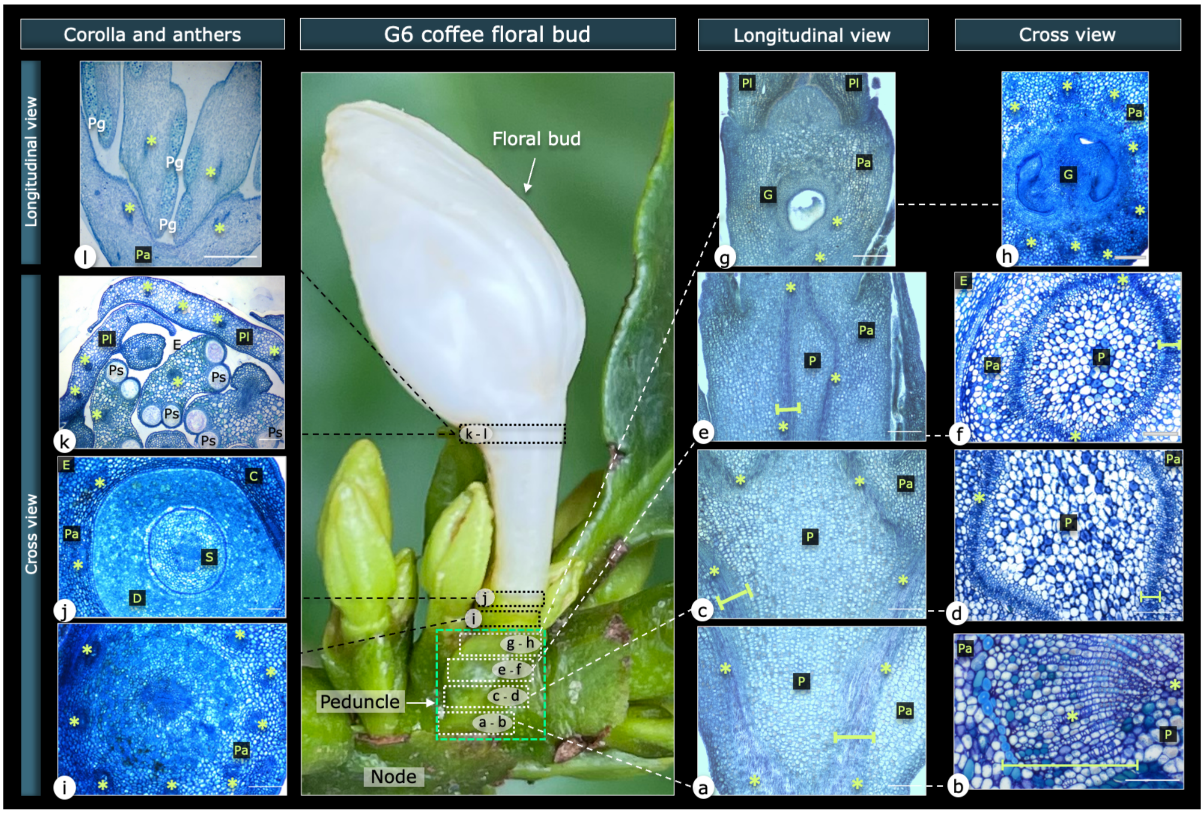

Figure 6. Successive longitudinal and cross sections through the G6 floral bud. The green dotted rectangle highlights the analyzed region of the peduncle. The white dashed rectangles indicate the longitudinal and cross sections made at various heights along the peduncle. The black dashed rectangles indicate the longitudinal and cross sections made in the floral bud. Specifically, on the right side of the image: (**a** and **b**): encompass the area of the peduncle connected to the branch. (**c** and **d**): covers the middle section of the flower bud peduncle. (**e** and **f**): covers the area just before the gynoecium. (**g** and **h**): shows the area of the gynoecium. On the left side of the image: (**i**): cross-section after gynoecium. (**j**): cross-section of the base of the corolla of the floral bud. (**k**) and (**l**): a cross and longitudinal section of the upper area of the floral bud, showing structures like pollen sacs.

E: Epidermis; **Pa**: Parenchyma; **Pl**: Petals; **P**: Pith; **C**: Corolla; **D**: Disc; **S**: Style; **Ps**: Pollen sacs; **Pg**: Pollen grains. The lime-green asterisks and lines delimit the vascular area. Bar scale: 50 µm.

Once the flower whorls undergoes senescence and the fruit begins to develop, anatomical analyses of the fruit peduncles reveal progressive growth in the vascular area as the fruit organ expands. In both the peduncles of inflorescences connected to the branch and those of individual fruits, there was notable development of xylem cells, primarily reflected in an increased vessel diameter. During the beads-like and fruit-filling stages, we observed that the vascular bundles were denser compared to those in the peduncles shedding of floral whorls stage. This cellular expansion led to an increase in diameter of the vascular cylinder, associated with the expansion of the fruit peduncle and continued growth and development until the fruit turned red. Similarly, in the green and red fruit peduncles, the xylem area was further developed, and the spiral-shaped cells were yet clearly identifiable (Figure 7), resembling tracheary elements (Malpighi, 1968).

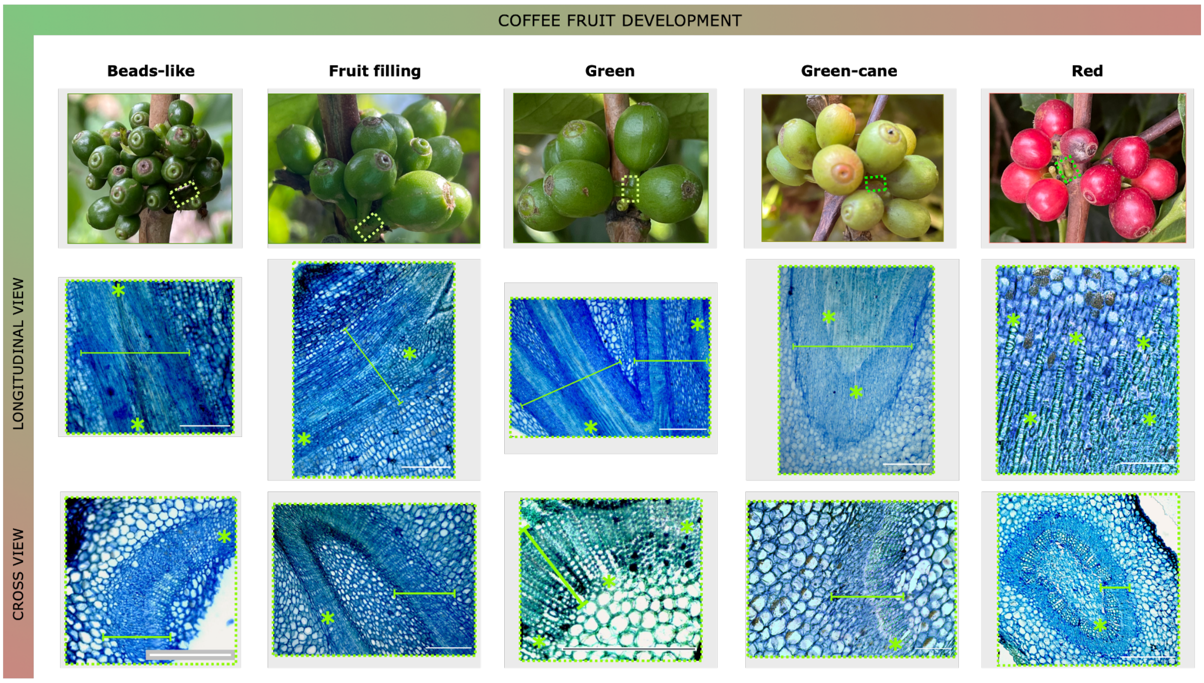

Figure 7. Vascular growth and development in longitudinal and cross sections of peduncles of *C. arabica* fruits at different stages. Once fruit filling and subsequent ripening begins, the formation of vascular bundles is clearly visible. The lime-green dotted rectangles point to the peduncle of the fruit. The lime-green asterisks and lines point at the development of the vascular bundles. Bar scale: 50 µm.

Our anatomical observations revealed expressive changes in the vascular area of the peduncles in relation to the developmental stage of the coffee reproductive organ. At early G4, peduncles exhibited a notable degree of differentiation in the vascular area, primarily in phloem development, with increased xylem cell differentiation observed following substantial rainfall. The xylem was the most differentiated tissue from late G4 to the red fruit stage, as determined by this technique. As the fruit matures, the xylem in the fruit peduncle develops further, enhancing the connection between the peduncle and the branch. Additionally, cross-sections of the peduncle in red fruit showed well-differentiated xylem and phloem vessels (Figure 8).

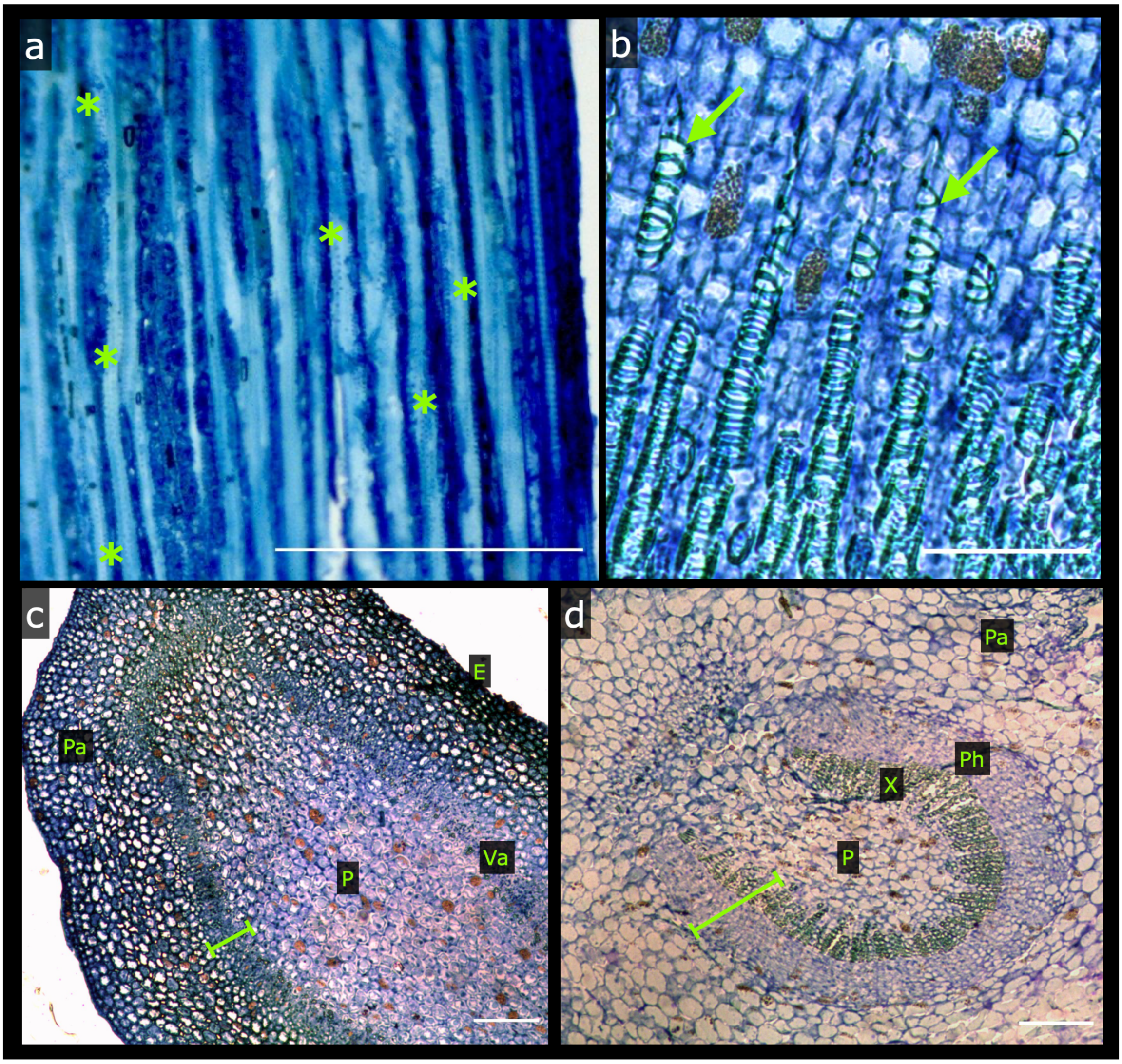

Figure 8. Comparison of the vascular area in peduncles at G4 (6 mm in length) and red fruit of *C. arabica*. Longitudinal-sections; (**a**)**:** G4 peduncle, the cell walls display punctuated marks highlighted by green asterisks, and, (**b**)**:** peduncle of the red fruit, where the tracheary elements (xylem) are shown by green arrows. Cross-sections; (**c**)**:** G4 peduncle, showing the radial organization composed of **E**: Epidermis, **Pa**: Parenchyma, **Va**: Vascular area, and **P**: Pith, and (**d**): peduncle of the red fruit, where the differentiation of the vascular area was observed; **X**: Xylem and **Ph**: Phloem. The green lines are delimiting the vascular area. Scale bars: (**a** and **b**), 30 µm; (**c** and **d**), 50 µm.

We also observed that the peduncles exhibited common characteristics across all stages of development, including a radial organization from the outermost to the innermost layers, consisting of the epidermis, parenchyma, vascular area, and pith. These radial organizations are common in orthotropic and plagiotropic stems (Dedecca, 1957; Ferreira et al., 2014), and the fruit peduncle reflects this caulinar structure. At all development stages, the vascularity connections with the node were continuous. Peduncles of the reproductive coffee organs follow a collateral pattern with xylem on the inside and phloem on the outside (amphicribral). Between the phloem and xylem is the procambial area, while the central region contains rounded parenchymatous cells in the pith (Furuta et al., 2014a; Heo et al., 2014).

These findings clarify the anatomical aspects of vascular development in the peduncles of *Coffea* reproductive organs. Next, we focused on the molecular identification and characterization of key transcription factors in *C. arabica* known to orchestrate vascular development in model plants.

2.3.2 Identification of orthologs and phylogenetic inference

Understanding the phylogenetic relationships between gene sequences is crucial for comparative biological research. This approach lays the foundation for studying the evolution and diversity of life on Earth and allows us to transfer biological insights across different organisms (Harvey and Pagel, 1991). To identify groups of orthologs (orthogroups) of vasculature identity genes in several plants, including *C. arabica*, and to correctly characterize their respective sub-groups, we utilized OrthoFinder (Emms and Kelly, 2019) and the PHYLIP (Felsenstein, 1993) package.

We identified 140 genes across 24 plant species as orthologs of the Homeobox-leucine zipper protein *AT*HB-15, UniProt ID Q9ZU11 (Figure 9, Supplementary material 4), along with 4 paralogs in *Arabidopsis thaliana*. Our phylogenetic analyses revealed that this orthogroup is subdivided into three sub-groups with representative sequences identified as *HB8* or *HB15/CORONA* (*HB8*/*HB15*), *REVOLUTA* (*REV*/*IFL-1*/*AVB-1*), and *HB14/PHABULOSA* or *HB9/PHAVOLUTA* (*HB14*/*HB9*) (Prigge and Clark, 2006). The genome of *Selaginella moellendorffii*, an extant lineage of early vascular plants (Banks et al., 2011), (identified by the prefix *Smo* in the tree) encodes only two members belonging to *HB8*/*HB15*, with no representatives in the *HB14*/*HB9* and *REV* sub-groups, suggesting that

HB8/*HB15* is the ancestral lineage. The *C. arabica* genome encodes two members in the *HB8*/*HB15* group, both identified as *HB8* orthologs, each from a different sub-genome. It also contains one *REV* member present exclusively in the *Coffea arabica* sub-genome inherited from *C. eugenioides* (*CaE*) and two *HB14*/*HB9* members, one from each sub-genome.

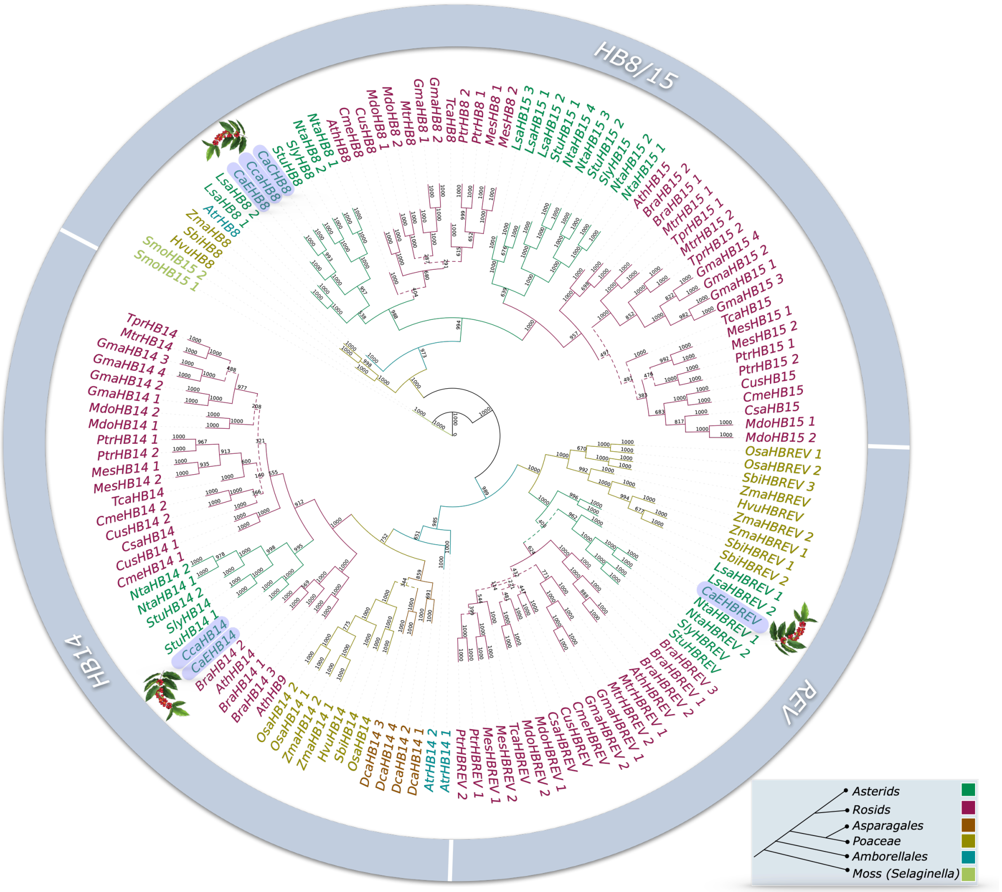

Figure 9. Neighbor-joining consensus tree depicting the evolutionary relationships of members of the *HD-Zip Class III* family orthogroup, which includes sub-groups of the procambium identity master regulators *HB8*, *HB14*, and *HB15*. *HB8* is present in two copies in *C. arabica*, with each copy from a different sub-genome. Additionally, there is one copy in the extant *C. canephora* lineage. No ortholog of *HB15* was detected in coffee. For the *REVOLUTA* gene, a single ortholog was detected in *C. arabica*, localized in the sub-genome inherited from *C. eugenioides*. A single copy of the *HB14* ortholog was detected in the sub-genome inherited from the *C. eugenioides* ancestral parent, and one copy was detected in the extant *C. canephora* lineage. Node numbers indicate the total occurrences of grouped pairs or individual sequences that clustered together in 1000 bootstrap replicates; dashed lines represent nodes where group pairs are clustered in fewer than 500 (50%) bootstraps, while continuous lines indicate nodes with more than 500 (50%) bootstrap support.

Our phylogenetic analyses of the *OPS* orthogroup, characterized by its similarity to *Arabidopsis thaliana OCTOPUS* At3g09070 (Truernit et al., 2012), which includes 73 members across 22 of the 25 evaluated species, identified that it is subdivided into two sub-groups, *OPS1* and *OPS2* (Figure 10, Supplementary material 5). No members were found in

Selaginella moellendorffii, *Hordeum vulgare*, or *Cannabis sativa*. Only one orthologue sequence, *OPS1*, was detected in *Amborella trichopoda* (*Art* in the tree), suggesting that this is the ancestral lineage. In addition, no *OPS1* members were detected in the Poales and Asparagales lineages. Conversely, the *OPS2* group seems to have expanded, with a total of 47 identified orthologs. In *Coffea arabica*, we identified two copies of each sub-group, with each one encoded by a different sub-genome.

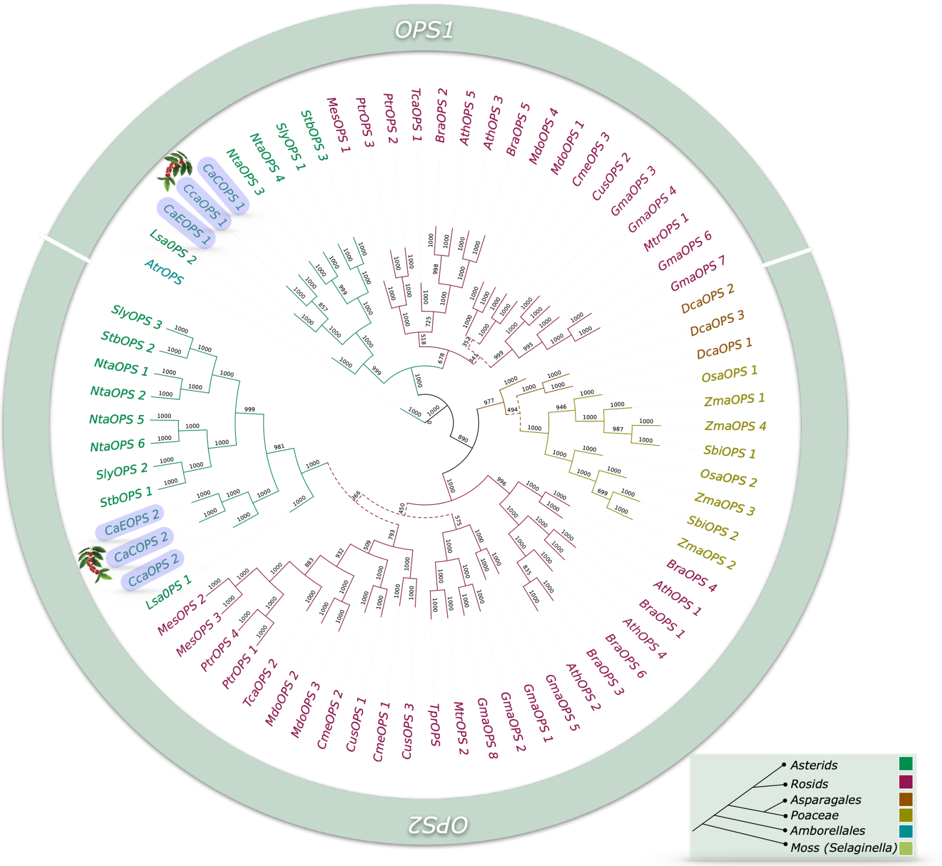

Figure 10. Neighbor-joining consensus tree depicting the evolutionary relationships of the *OPS* gene orthogroup, which potentially orchestrates phloem identity. *OPS* is present in four copies in *C. arabica*, with each pair of *OPS1* and *OPS2* originating from its respective sub-genome. Additionally, a pair of *OPS1* and *OPS2* was detected in the extant *C. canephora* lineage. Node numbers indicate the total occurrences of grouped pairs or individual sequences that clustered together in 1000 bootstrap replicates; dashed lines represent nodes where group pairs are clustered in fewer than 500 (50%) bootstraps, while continuous lines indicate nodes with more than 500 (50%) bootstrap support.

Finally, we identified a total of 628 orthologous sequences of *Populus trichocarpa VND6*-A1 (POPTR_0015s14770) (Lin et al., 2017), including sub-groups characterized by the presence of *VND6*, *VND7*, *SDN1* transcription factors, and other NAC subgroups, across 23 plant species. We also identified 33 paralogs of *VND6*-A1 in *Populus trichocarpa*, totaling an orthogroup with 662 sequences (Figure 11, Supplementary material 6). No ortholog was detected in *Selaginella moellendorffii*. In regard to *Coffea arabica*, characterization of the *VND6* gene found 3 copies in the sub-genome inherited from *C. canephora* (*CaC*) and 5 copies from the sub-genome inherited from *C. eugenioides*. Additionally, for the *VND7* gene, we identified 2 copies in the sub-genome inherited from *C. canephora* (*CaC*), 2 copies in the extant *C. canephora* (*Cca*), and, despite its conservation, no copy in the sub-genome inherited from *C. eugenioides* (*CaE*).

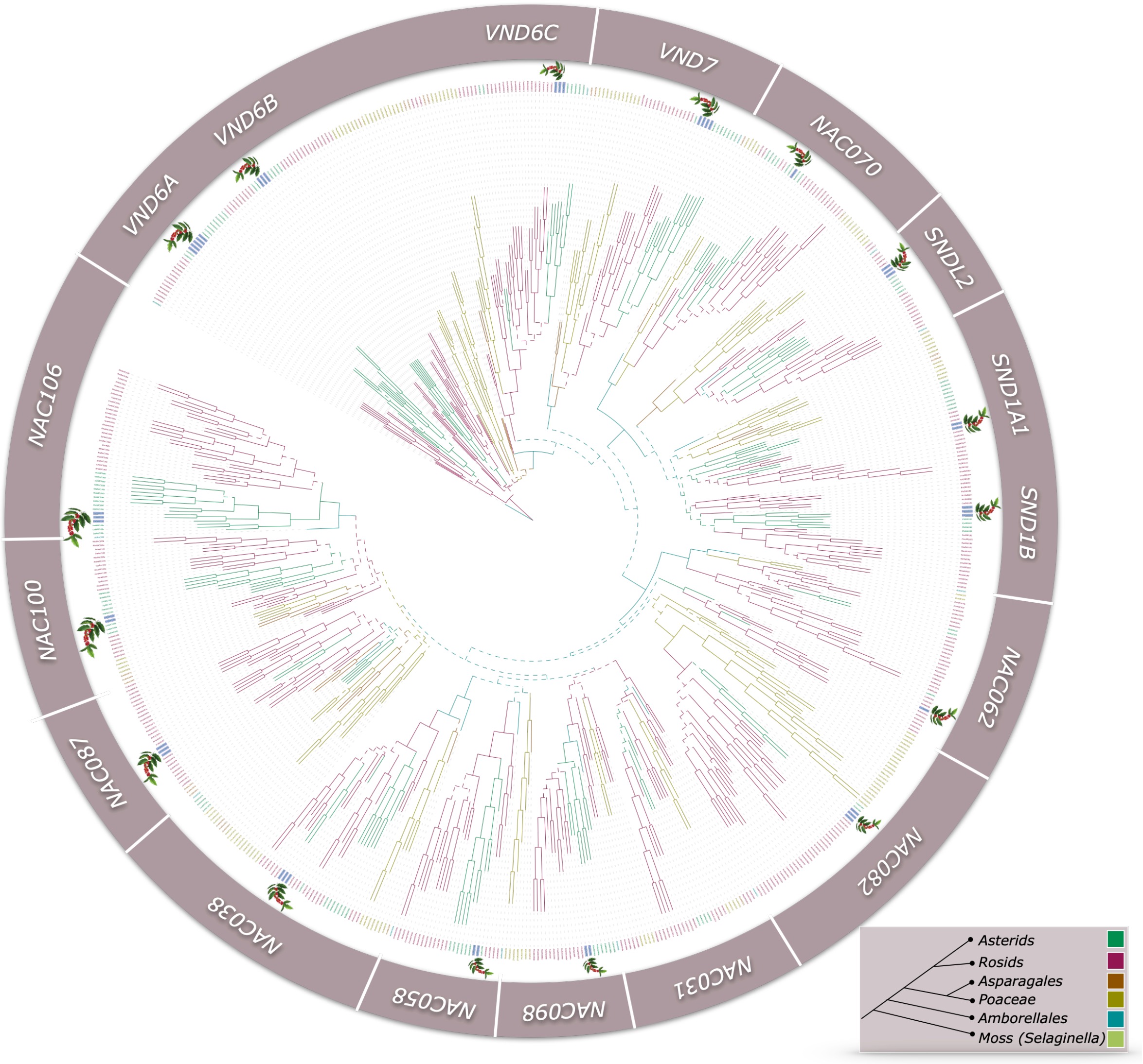

Figure 11. Neighbor-joining consensus tree depicting the evolutionary relationships of a *NAC* gene family orthogroup, which includes sub-groups of the xylem identity master regulators *VND6* and *VND7*. *VND6* is present in three copies in the *C. arabica* sub-genome inherited from *C. canephora* (*CaC*), five copies in the sub-genome inherited from *C. eugenioides* (*CaE*), and three copies in the extant *C. canephora* (*Cca*) lineage. For the *VND7* gene, we identified two copies in the *C. arabica* sub-genome inherited from *C. canephora* (*CaC*) and two copies in *C. canephora* (*Cca*), with no detectable copy in the sub-genome inherited from *C. eugenioides* (*CaE*). Dashed lines represent nodes where group pairs are clustered in fewer than 500 (50%) bootstraps, while continuous lines indicate nodes with more than 500 (50%) bootstrap support.

The characterization of these *C. arabica* orthologs into sub-groups enabled us to identify potential candidate regulatory network genes involved in controlling vascular development in buds and peduncles during stages G2, late G4 (G4L) and G5 (Figure 12). This insight allows us to scrutinize these genes through RNA-seq and RT-qPCR analyses, focusing on key developmental stages of bud development.

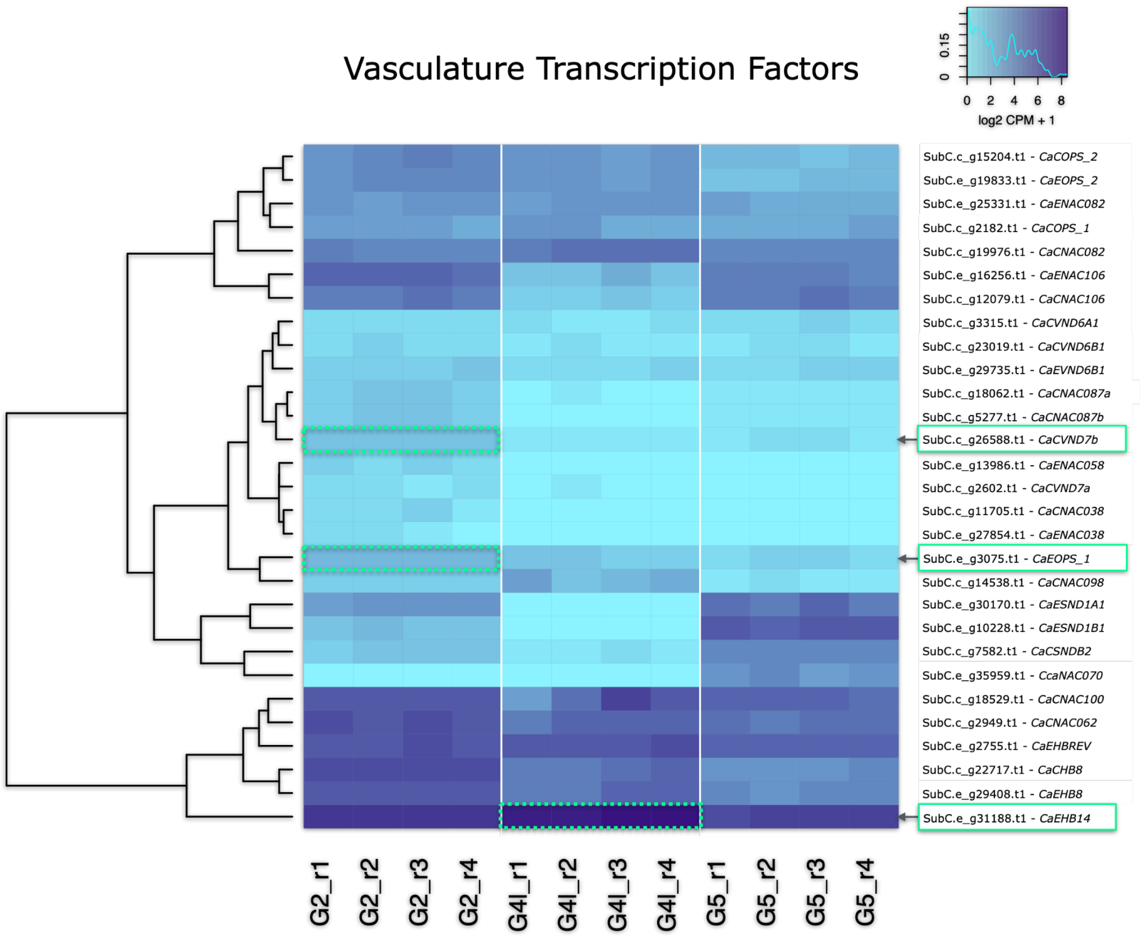

Figure 12. RNA-seq-based expression profile of vascular identity genes *VND7, OPS*, and *HB14* orthologs in *C. arabica* for stages G2, G4L, and G5, with 4 biological replicates for each stage. The heat map depicts the abundance of reads mapped to exons of the evaluated vascularity-related orthogroup members, presented as the logarithm base 2 (log2) of counts per million plus 1 (CPM +1). Lighter blue shades indicate lower abundance, while darker purple shades indicate higher abundance. Dotted area selections highlight the upregulation of three key candidate genes potentially controlling procambial (*HB14*), xylem (*VND7b*), and phloem (*OPS1*) identity in *C. arabica*.

2.3.3 RNA-seq gene expression analyses

A total of 23.403 protein-coding genes were found to be differentially expressed (DE) across the three evaluated developmental stages, namely, G2, late G4, and G5 (Supplementary material 7). This high number of DE genes highlights the pronounced transcriptional changes during the transition from G2 to G5 development stages. We focused our analyses on important transcription factor orthogroups known to orchestrate procambial, phloem, and xylem development in model plant species.

Four members of the *HD-ZIP Class III* family in coffee were found to be highly expressed across stages G2, late G4, and G5 (Figure 13). Two homeologs of *HB8* were significantly upregulated in G2 compared to G5 and, to a lesser extent, late G4. The *HBREV* ortholog, presented as a single copy in the *C. arabica* genome, was not DE. *HB14*, also a single copy locus, was the *HD-ZIP Class III* member with the highest expression, being significantly upregulated in late G4. This suggests an enhanced proliferation of procambial cells after the rains.

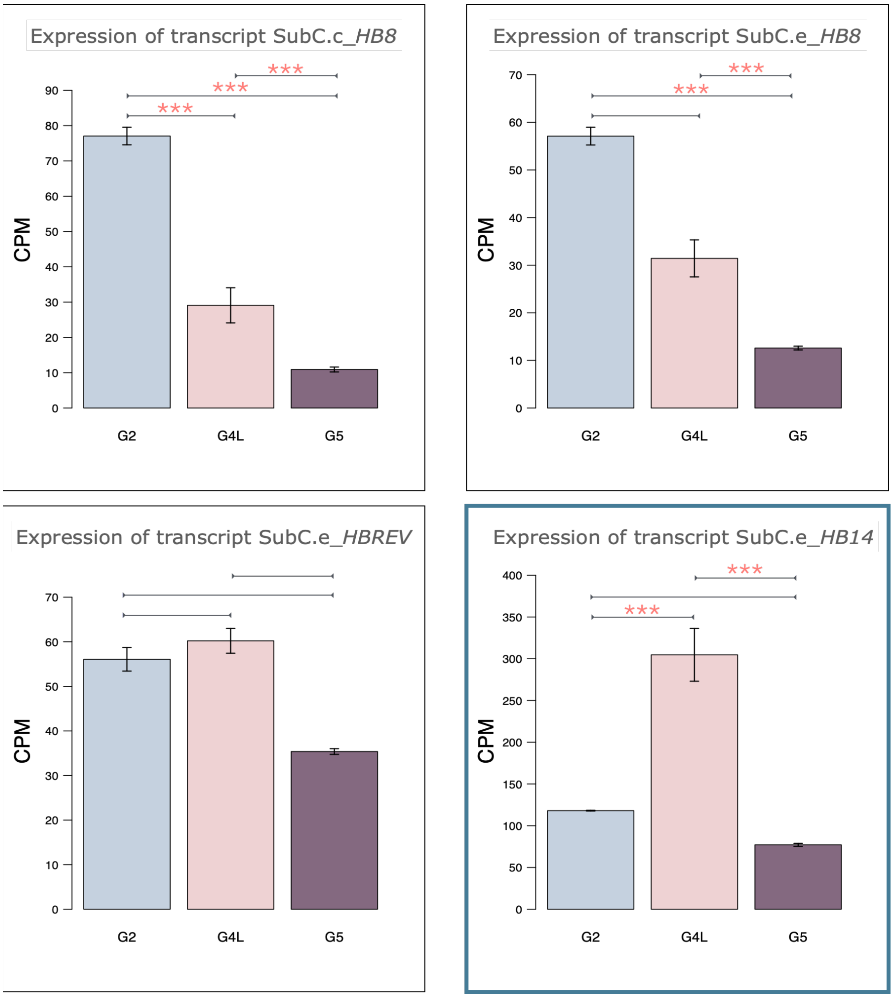

Figure 13. Bar plots depicting the expression profile of members of the *HD-ZIP Class III* sub-group potentially related to procambial cell identity in *C. arabica*. The y-axis represents Counts Per Million (CPM), while the x-axis represents the G2, G4L, and G5 stages. The highlighted box for *HB14* found in the *C. eugenioides* sub-genome (*SubC.e_HB14*) indicates that this gene was selected for RT-qPCR analyses. Whiskers on the bars indicate the standard error of the mean. Horizontal lines represent

specific contrasts between sampled stages, and asterisks denote significance levels following FDR analyses (* p < 0.05, ** p < 0.01, *** p < 0.001).

The four members of *OPS* characterized in *C. arabica* were primarily upregulated in G2 (Figure 14), with the exception of a copy clustered in the *OPS1* group, detected in the *C. canephora* sub-genome (*SubC.c_OPS_1*), for which we could not find statistical evidence of differential expression. The general trend of *OPS* family members' upregulation in G2 indicates that phloem development is an active process in the early stages of bud development.

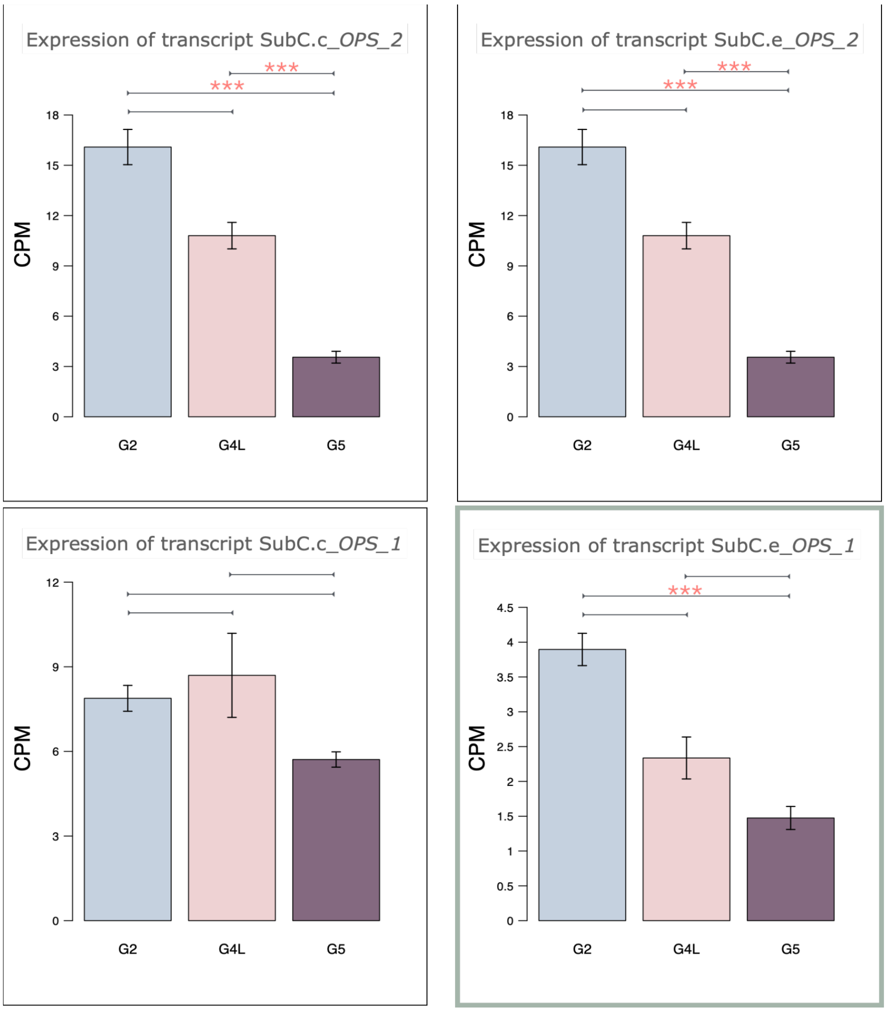

Figure 14. Bar plots depicting the expression profile of members of the *OPS* orthogroup potentially related to phloem cell identity in *C. arabica*. The y-axis represents Counts Per Million (CPM), while the x-axis represents the G2, G4L, and G5 stages. The highlighted box for *OPS1* found in the *C. eugenioides* sub-genome (*SubC.e_OPS_1*) indicates that this gene was selected for RT-qPCR analyses. Whiskers on the bars indicate the standard error of the mean. Horizontal lines represent specific contrasts between sampled stages, and asterisks denote significance levels following FDR analyses (* p < 0.05, ** p < 0.01, *** p < 0.001).

Although we identified eight copies of the *VND6* ortholog in the *C. arabica* genome, we could detect transcriptional signals from only three loci, none of which showed evidence of differential expression (Figure 15 - first row). Nevertheless, the two *VND7* orthologs, exclusively found in the *C. canephora* sub-genome (*SubC.c*), presented significant expression changes, being upregulated at the G2 stage (Figure 15 - second row). This finding suggests that the machinery necessary for xylem differentiation is active in the early stages of bud development.

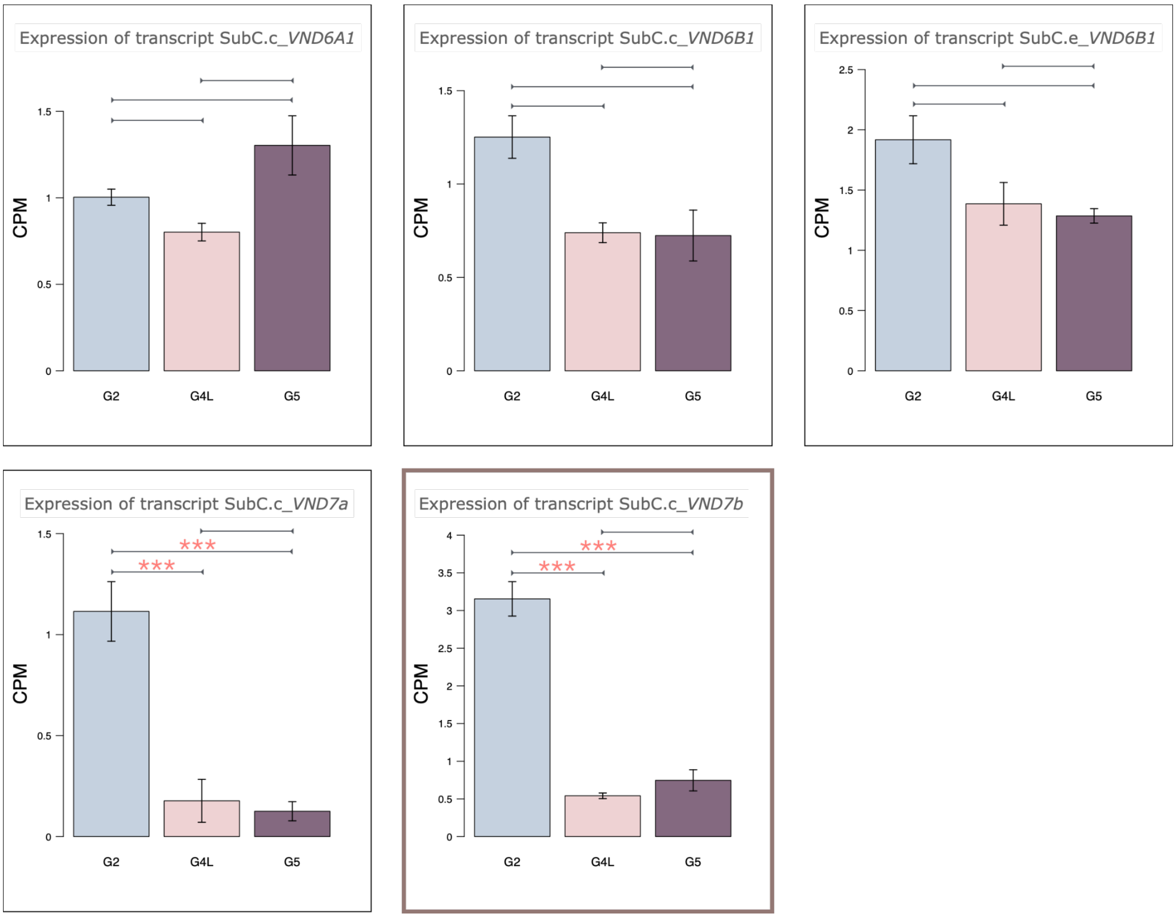

Figure 15. Bar plots depicting the expression profile of members of the *VND6* and *VND7* sub-groups potentially related to xylem cell identity in *C. arabica*. The y-axis represents Counts Per Million (CPM), while the x-axis represents the G2, G4L, and G5 stages. The highlighted box for *VND7* found in the *C. canephora* sub-genome (*SubC.c_VND7b*) indicates that this gene was selected for RT-qPCR analyses. Whiskers on the bars indicate the standard error of the mean. Horizontal lines represent specific contrasts between sampled stages, and asterisks denote significance levels following FDR analyses (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

We detected a contrasting trend from *VND7* in *SND1* expression profile. *SND1* members were clustered within the same orthogroup as *VNDs* (Figure 11) but they orchestrated another step of xylem development (Zhong et al., 2006; Zhong et al., 2007a). We found that all three expressed copies of *SND1* were significantly upregulated in G5 compared to G4L and, to a lesser extent, G2 (Figure 16). This result, together with our anatomical observations (Figure 5), suggests an enhancement in secondary cell wall thickening following the resumption of development triggered by rains.

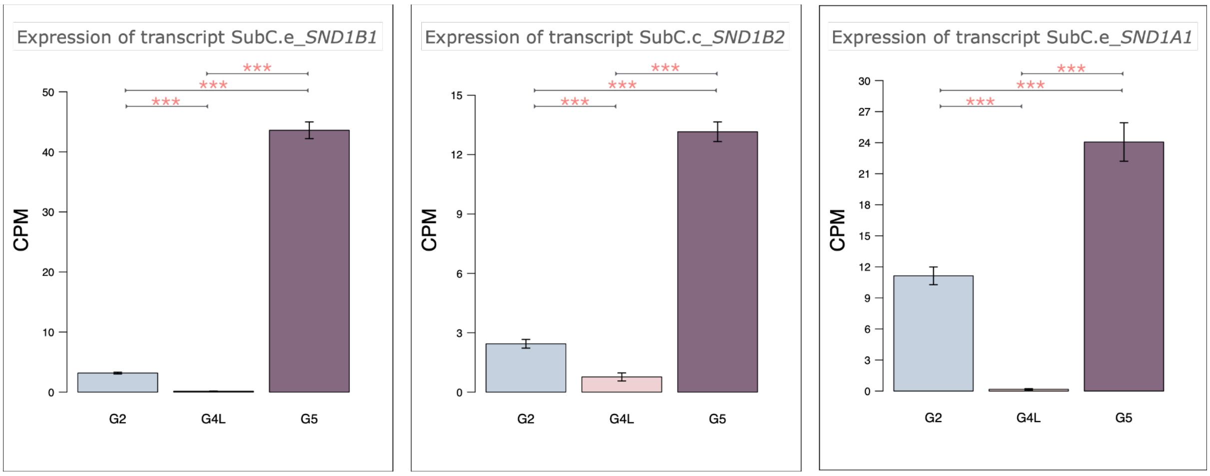

Figure 16. Bar plots depicting the expression profile of members of the *SND1* sub-group potentially related to xylem cell wall thickening in *C. arabica*. The y-axis represents Counts Per Million (CPM), while the x-axis represents the G2, G4L, and G5 stages. Whiskers on the bars indicate the standard error of the mean. Horizontal lines represent specific contrasts between sampled stages, and asterisks denote significance levels following FDR analyses (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

2.3.4 RT-qPCR analyses

To test the hypothesis that transcriptional changes in genes controlling vascular identity are directly involved in the development of buds at stage G4 before (early G4) and after substantial rainfall (late G4), we performed RT-qPCR analyses. The samples were collected on 15/09/2022 (after a long period of droughts) and 04/10/2022 (when about 100 mm of rainfall accumulated). Tables with raw cycle threshold values (Ct) are available in supplementary material 8. We investigated the orthologs *HB14*, *OPS*, and *VND7* transcripts for expression changes, applying a linear mixed-effects model to account for variability among the technical replicates by including a random effect for the replicates within each biological sample.

Our results were inconclusive if we consider the usual threshold of a doubling expression change between conditions. Although the evaluated genes displayed a trend of being upregulated in late G4 compared to early G4—*VND7* with a fold change of 1.36, *HB14* with a fold change of 1.27, and *OPS* with a fold change of 1.14 (Figure 17)—these values were not greater than the expected fold change value of 2. However, their confidence intervals never included zero, and the p-values (Pr > |z|) were significant: *VND7* at 6.71e^{-05}, *HB14* at 1.05e^{-05}, and *OPS* at 2.5e^{-03}.

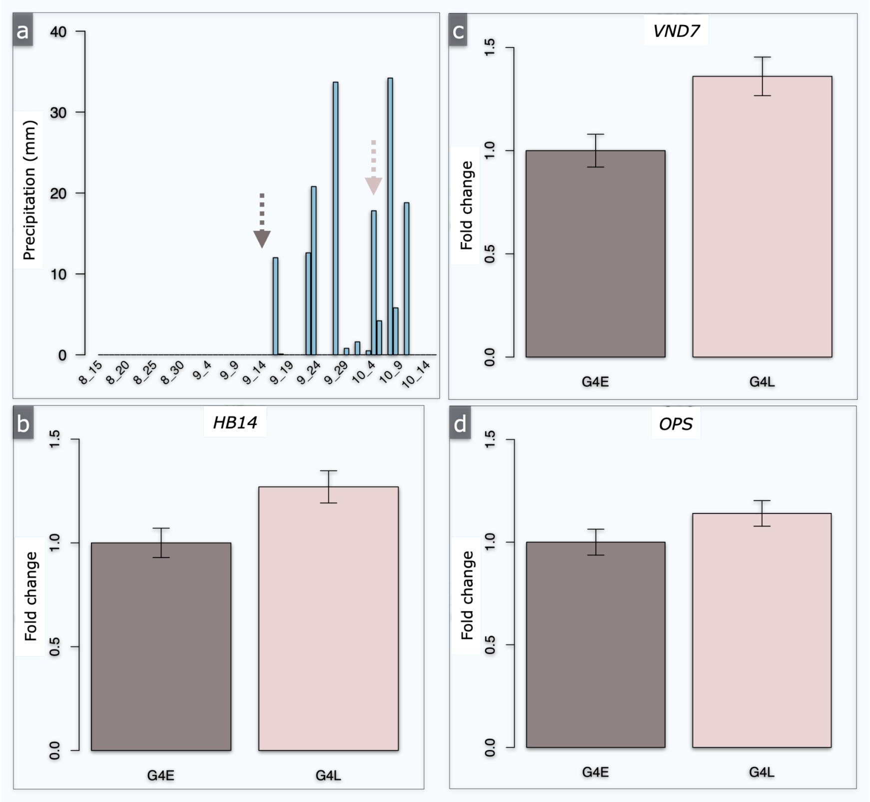

Figure 17. Precipitation histogram (**a**) and RT-qPCR expression analyses of three key candidate genes potentially controlling procambial (*HB14*), xylem (*VND7b*), and phloem (*OPS1*) identity in *C. arabica*, with samples collected before (G4E) and after substantial rainfall (G4L, ~100 mm). (**a**) Arrows indicate the dates when samples were collected, and bars represent the daily rain accumulation, in millimeters, between August 15th and October 15th, 2022. (**b**, **c**, and **d**) Bar plots depicting the expression of *HB14*, *VND7b*, and *OPS1*, respectively, in terms of fold change between both sampled stages (G4E and G4L), with confidence intervals representing the standard error of the means.

2.4 Discussion

The anatomy, including the vascularization, of coffee flowers and fruits has been previously studied and characterized (Dedecca, 1957; Arcila Pulgarín, 2007; de Oliveira et al., 2014). However, no study has recorded the anatomical changes of the coffee flower and fruit peduncle during the different stages of growth, specifically addressing vascular development. Given the crucial role peduncles play in supporting the proper growth of flowers (Zamski et al., 1991; Razeto and Salgado, 2004; Ferrari et al., 2014; Carvalho et al., 2021) and fruits (Setiamihardja and Knavel, 1990; Antognozzi et al., 1991; Bustan et al., 1995; Dražeta et al., 2004; Rančić et al., 2010; Horbens et al., 2015; Knipfer et al., 2015; W. Song et al., 2018; W.-P. Song et al., 2018; Li et al., 2021a; Silveira et al., 2021), our study provides detailed anatomical analyses of vascular development in the peduncles at various stages of the *C. arabica* reproductive organs. Additionally, our computational and molecular analyses have yielded novel insights, particularly regarding three outgroup members that are known to promote vascular development in model plants; *HB14* (HD-ZIP Class III) for procambium (Prigge and Clark, 2006; Côté et al., 2010; Ramachandran et al., 2017), *OCTOPUS* for phloem cell identity (Truernit et al., 2012; Rodriguez-Villalon et al., 2014; Anne et al., 2015a; Ruiz Sola et al., 2017; Greenwood et al., 2023), and *VND7* for protoxylem and metaxylem differentiation (Kubo et al., 2005; Yamaguchi et al., 2008; Ohtani et al., 2011; Endo et al., 2015).

2.4.1 Early vascular differentiation in peduncles of coffee reproductive organs

Although a distinct peduncle is not yet observed in G1 and G2 (Figure 3), during our study, we considered the contact area at the base of the buds in connection with the branch. This contact zone, in the early stages of coffee flower bud development, functions similarly to the peduncle zone that connects flowers and fruits to the rest of the plant. Functionally, the peduncle provides a connection with the entire plant and facilitates the primary transport of water and photo assimilates from source to sink organs (Cannell, 1971; Rančić et al., 2010; Cui et al., 2022; Buthelezi et al., 2023a). Furthermore, Dedecca (1957) describes how the axes that support the flowers of the coffee plant are always very short, giving the inflorescence a compact appearance, botanically known as a glomerulus.

Initially, in G1 and G2, the early stages of floral development, the base of the bud is wider, and the peduncle is still growing, making it indistinguishable from the inflorescence. Additionally, the cell walls of the parenchyma and pith are not yet well defined (Figure 2). Majerowicz and Söndahl (2005) pointed out that in the early stages of coffee floral bud development, specifically when the bud differentiates into a reproductive bud, enhanced cell division occurs in the central meristematic zone. This leads to an increase in bud size and marks an anatomical difference between reproductive and vegetative buds (Majerowicz and Söndahl, 2005). The cells of emerging organs must reorganize to facilitate cell division, expansion, and differentiation of the growing organ (Kutschera, 1989; Bustan et al., 1995; Sachs, 2000). This is in agreement with our anatomical analyses, which showed that in the early stages of coffee flower bud development, the vascular area is still in the process of differentiation.

Nevertheless, we observed that the vascularization is continuous through the node and floral bud, and at the area where the branch and floral bud connect, the vascularization becomes procambial (Figure 2). The presence of procambium in G1 and G2 indicates the potential capacity of the early-established vasculature to transport water and nutrients to the floral bud in latter stages of development, maintaining a continuous vascular connection with the plant. In land plants, both xylem and phloem differentiate from the same precursor cells, procambium and cambium, and remain in close spatial association throughout the plant's life (Scarpella and Helariutta, 2010).

In G3 and G4, flower buds exhibit a general expansion of bud structures, making the inflorescence peduncle more evident and morphologically distinct from the buds (Figure 3). As the flower buds grow from G3 onwards, there is increased development in the vascular zone. From G4 onwards, the vascular zone undergoes extensive cellular differentiation. At this stage, the peduncle shows greater development of the phloem, and after substantial rains, increased differentiation of the xylem was observed (Figure 5). This suggests that water availability significantly influences vascular development, especially during the G4 stage. Additionally, from G4 onwards, we observed greater definition of the cell walls in the parenchyma and pith areas, indicating enhanced cellular development at this stage (Figure 3).

During the reproductive stage of coffee, the buds, measuring between 4 - 6 mm, enter a state of apparent dormancy. This apparent dormancy leads to an accumulation of G4 flower buds that coincide with a stationary dry period. Anthesis then occurs after the first major rains. Thus, the arrival of the rains acts as a signal, causing the dormant flower buds to resume their growth until they bloom (Alvim, 1960; Magalhães and Angelocci, 1976; Crisosto et al., 1992; Drinnan and Menzel, 1994; Wintgens, 2012; Penfield, 2024). Crisosto *et al.* (1992) observed in cross-sections of the peduncles of *C. arabica* flower buds in stage 3 (tight green cluster), similar to our G4, the presence of metaphloem and metaxylem, with vascular bundles separated by interfascicular parenchyma cells and distributed collaterally. In stage 4 (open white cluster), corresponding to our G5, they observed greater differentiation in the vascular area compared to stage 3. They also noted significant anatomical differences in the vascularization of the peduncles of coffee buds in stage 5 (described as the first white candle), our G6, and in the stage 7 or small fruit, where the vascular area showed secondary growth. These findings establish a correlation between the state of the floral bud, vascular development, and flowering, highlighting that increased vascular differentiation in G6 was critical for flowering in response to increased water availability.

Although our anatomical analyses showed greater differentiation in the vascular area from G4 onwards, mainly in the phloem area, we cannot conclusively identify it as metaphloem (Figures 3 and 8). However, after abundant rainfall (100 mm), we observed increased differentiation and development of vascular cells (Figure 5). In this context, we propose that the enhanced vascular development in G4, compared to younger stages, endows it with the necessary vascular characteristics to become more receptive to increased water availability once the dry period ends, thereby promoting the growth of the flower bud. Additionally, in our study, we noted enhanced thickening of the cell walls in the xylem area during late G4 and G5 (Figure 5). This thickening marked a significant degree of development and growth of the xylem during these stages of flower buds development.

Other studies have shown that up to G4, the reproductive organs of the coffee flower develop (De Oliveira et al., 2024). In S5 (corresponding to our G5), there is greater synthesis of compounds necessary for the formation of the cell walls, such as xyloglucans and xylan to support the accelerated growth of flower buds after rains (Cherubino Ribeiro et al., 2024). From the end of G3 onwards, the transport of photoassimilates and other molecules such as phytohormones and miRNAs becomes critical to sustain carbohydrate metabolism and allow the continuing development of flower whorls. Additionally, as coffee flower buds significantly increase in size after rain, our observations indicate that cell walls undergo considerable thickening to support cell division and expansion, facilitating flowering.

From G5 onwards, the peduncles of the inflorescences elongate, clearly separating the flower buds within the inflorescence, with a more developed and well-differentiated vascular area, including both xylem and phloem (Figure 4 and 5). In G6, flower and shedding of floral whorls stages, the vascular area is further expanded, and at the base of the peduncles connecting to the branch, there is a greater number of layers compared to G5 (Figure 4 and 6). This indicates a positive correlation between rainfall, the growth of flower buds, and increased development in the vascular area of the flower peduncles.

On the other hand, based on our anatomical observations, we propose that coffee fruits can influence the development of the vascular bundle of the peduncle as the fruit matures. Although we did not perform a correlation analysis between the diameter of the fruit peduncle and the size or ripening state, we observed a correlation between greater development of the vascular area and the growth and maturation of the coffee fruit, similar to other agronomically important fruits (Nii, 1980; Bustan et al., 1995; Cui et al., 2022; Buthelezi et al., 2023b). Our anatomical analyses indicate that during the fruit-filling stage, vascularization increases in density, and as the coffee fruit matures, the xylem vessels exhibit a larger diameter compared to earlier stages (Figure 7).

Pereira Gardin (2006), observed that coffee fruit peduncles with a higher density of xylem vessels per mm^2 reached their potential growth and maturation faster than those with fewer xylem vessels. Additionally, once the coffee fruit reached maturity and began to dry, further thickening of the xylem cell walls occurred, reducing vessel diameter and limiting water flow, which contributed to fruit senescence (Pereira Gardin, 2006). He also demonstrated that the degree of vascular development in the peduncle can limit fruit growth and ripening over time, contributing to the lack of uniformity in the ripening process of coffee cultivation. These observations indicate that fruit ripening is closely linked to the transport capacity of the peduncle, determined by its structural properties, especially those of the xylem and phloem vessels.

In our anatomical analyses, we observed a greater diameter of the xylem vessels in both the green and red fruit stages (Figure 7 and 8). This suggests that once the fruit reaches its potential size and begins to ripen, the larger diameter of the xylem vessels, along with the phloem, facilitates the greater transport of water and nutrients to support the ripening process (Supplementary material 9) (Bouzayen et al., 2009; Zhang and Keller, 2017; Li et al., 2021b). These observations underline the importance of vascular development in the growth of coffee fruits and highlight how enhanced vascularization impacts maturation processes (Pereira Gardin, 2006).

In general, we observed that the radial patterns of the vascular tissue in the coffee floral and fruit peduncles exhibit a similar arrangement to that found in cylindrical organs, such as stems and pedicels, of dicotyledons. Notably, we identified radial patterns resembling the radial organization of the orthotropic coffee stem as described in the literature (Marchand, 1864; Dedecca, 1957; Jesus et al., 2010; Ferreira et al., 2014; Pereira et al., 2014; Patay et al., 2016; Sakiyama et al., 2017; Carréra et al., 2023). In these structures, the xylem differentiates towards the interior, while the phloem differentiates towards the exterior. Both tissue lineages originate from the procambium during primary growth or from the vascular cambium during secondary growth (Scott, 1897; Philipson and Balfour, 1963; Dengler, 2001; Scarpella and Meijer, 2004; Hardtke, 2023a). To better understand the regulatory networks governing the vascular development processes revealed by our anatomical analyses, we performed bioinformatic analyses to identify and characterize key genes potentially involved in vascular development in *C. arabica*.

2.4.2 Evolutionary history and functional diversification of key vascular development genes

We searched for genes known in model plants to promote vascular development. The identification of orthologs of the *HB15*, *OPS*, and *VND6* genes across 25 plant species reveals their evolutionary history and highlights their functional diversification. Among these three orthogroups, only *HB15* had members in the *Selaginella moellendorffii* genome, suggesting that *OPS* and *VND* correlate with vascular synapomorphies in seed plants. In *Arabidopsis thaliana*, *HB15* is expressed in the procambium and regulates early vascular development (Du et al., 2015) while the expression of *HB8*, also an early marker of procambial cells, correlates with later developmental processes such as the formation of tracheary element precursors (Baima et al., 2001). Surprisingly, we could not characterize *HB15* orthologs in *C. arabica*. Instead, we characterized two orthologs of *HB8*, two orthologs of *HB14*, and a *REV* gene belonging to the same orthogroup. It is possible that any of these *HD-ZIP class III* transcription factors are responsible for procambial cell identity in *C. arabica*.

While orthologs of *Arabidopsis HB15* are likely markers of procambial identity, *OPS* is a strong candidate for phloem identity. In *Arabidopsis*, *OPS* is a genetic determinant of phloem development. It is expressed in provascular cells, and following cell-type-specific differentiation, its expression is restricted to the phloem cell lineage (Bauby et al., 2007; Truernit et al., 2012). The OPS protein is membrane-associated, with polar localization to the apical end of the protophloem precursor cells, opposite to PIN proteins (Truernit et al., 2012). Our phylogenetic analyses indicates that there are two sub-groups of *OPS*, which we termed *OPS1* and *OPS2*. The sub-group *OPS1* was named as such because it contains the only member belonging to *Amborella trichopoda*, suggesting that this is the ancestral lineage. However, it lacked representatives from Poaceae and Asparagales. Meanwhile, the *OPS2* sub-group shows significant expansion, with several members in Asterids, Rosids, and monocots. It is possible that the *OPS1* lineage in the evaluated monocots was lost and functionally replaced by *OPS2*. In *C. arabica*, we identified a balanced proportion of members from both sub-groups, with one copy from each sub-genome suggesting that both sub-genomes contribute to phloem development.

The NAM/ATAF/CUC (NAC) domain protein orthogroup, characterized by homology to *Populus trichocarpa VND6*-A1 (POPTR_0015s14770), represents the largest orthogroup we have characterized. We identified 628 orthologous sequences in addition to 33 paralogs in *Populus trichocarpa*, forming an orthogroup with 662 sequences across all evaluated species, except for *Selaginella moellendorffii*. This subgroup includes crucial transcription factors such as members of *VASCULAR-RELATED NAC-DOMAIN* (*VND*) and *SECONDARY WALL THICKENING PROMOTING FACTOR*s/*SECONDARY WALL-ASSOCIATED NAC DOMAIN* (*NST*s/*SND*s), which are essential regulators of xylem cell differentiation. *VND6* and *VND7* are particularly important for initiating protoxylem and metaxylem vessel formation, respectively. Mutations in these loci can significantly disrupt xylem development, affecting water and mineral transport and overall plant health and productivity (Lin et al., 2017).

In *Coffea arabica*, we found distinct variations in terms of sub-genome distribution of *VND* homoeologs: three *VND6* copies encoded in the *C. canephora* sub-genome, five in the *C. eugenioides* sub-genome, and two *VND7* copies in both the *C. canephora* sub-genome and extant *C. canephora*, but none in the *C. eugenioides* sub-genome. *CaSND1* members were also found in the *VND* orthogroup, but at distinct sub-groups with 2 members in the *C. canephora* sub-genome and three in the *C. eugenioides* sub-genome. To further elucidate the role of these genes in coffee peduncle development, we performed gene expression analyses during key developmental stages.

2.4.3 Molecular and anatomical insights into vascular development and flower bud differentiation in coffee

Organs that are growing and undergoing cell division and cell expansion, such as coffee flower buds, require the continuous self-renewal and proliferation of the procambium cells to ensure the formation of new vascular bundles and provide adequate transport of water, sap, and nutrients for their growth and development (Notaguchi and Okamoto, 2015; Zúñiga-Sánchez et al., 2017; Fukuda and Ohashi-Ito, 2019; Hardtke, 2023b). In this context, we observed that candidate genes related to the maintenance of procambium cells in coffee, such as *CaEHB14*, *CaEHB8*, *CaCHB8*, and *CaEHBREV,* exhibited high abundance in terms of counts per million (CPM) (Figure 12).

CaCHB8 and *CaEHB8* were upregulated at G2 compared to late G4 and G5*,* suggesting that cell lineages destined to differentiate into xylem are established early (Baima et al., 2001; Ohashi-Ito et al., 2005). In the *Arabidopsis* procambium differentiation phase, *ATHB8* and *CNA/ATHB15* transcription factors regulate the expression of downstream genes encoding proteins involved in the formation of distinctive vascular cell features, such as the sieve elements of phloem and the lignified cell wall of xylem cells (Lucas et al., 2013; Ramachandran et al., 2017). In agreement, *CaEHBREV*, along with *PHB/HB14* and *PHV/HB9*, are known to participate in the procambium induction phase, indicating that the meristematic cells should begin differentiating into vascular cells (Lucas et al., 2013; Ramachandran et al., 2017). On the other hand, *CaEHBREV* likelly repress the transcription of *VND6/7*, but was not differentially expressed between any contrast (Talbert et al., 1995; Robischon et al., 2011). Meanwhile, *CaEHB14*, the most abundant *HD-Zip III*, was upregulated in late G4 compared to both G2 and G5, with a fold change increase of 2.5 and 3.5, respectively (Figure 13). This highlights the particular importance of procambial radial pattern maintenance during late G4. In addition, the high abundance of all characterized *HD-Zip III* orthogroup members highlights their importance in maintaining the procambium as a pluripotent stem cell population reservoir (Lucas et al., 2013; Růžička et al., 2015; Campbell and Turner, 2017; Fukuda and Ohashi-Ito, 2019).

In general, *CaOPS* members were upregulated at G2 (Figure 14). In *Arabidopsis*, *OPS* is a polar membrane protein associated with phloem lineage identity (Truernit et al., 2012; Lucas et al., 2013; Anne et al., 2015b; Ruonala et al., 2017). In this context, it is congruent its differential expression during the early stages of coffee flower bud development, such as G2, where the differentiation of functional phloem becomes critical to allow the formation of flower whorls and the subsequent development of the sink organs such as flowers and fruits (Hardtke, 2023b).

No orthologs of *VND6* were DE. However, *VND7*, like the *HB8* orthologs, is upregulated at G2 (Figure 15). Despite this, their normalized expression values in CPM are relatively low. It is important to note that transcription factor genes, such as *VND6* and *VND7*, are typically expressed at low levels in plants, often in a cell-type or tissue-specific manner, and frequently only transiently during development (Czechowski et al., 2004). Furthermore, *VND6* and *VND7*, as master regulators of xylem specification, also directly regulate genes involved in processes such as programmed cell death and secondary cell wall thickening (Kubo et al., 2005; Yamaguchi et al., 2008; Endo et al., 2015; Kuriyama and Fukuda, 2002).

Based on our anatomical analyses, we observed a high degree of secondary cell wall differentiation in coffee flower bud peduncles from stage G5 onwards, a finding supported by a previous report (Cherubino Ribeiro et al., 2024). Our observations confirmed the thickening of cell walls in the xylem area, particularly at G5 (Figure 5). Additionally, RNA-seq analyses revealed differential expression of the transcription factor ortholog *SND1*, which is upregulated at G5 compared to G2 and late G4 (Figure 16). *SND1* is known to positively regulate the coordinated expression of genes involved in the biosynthesis of secondary wall components, such as cellulose, lignin, and hemicelluloses (Zhong et al., 2007b; McCarthy et al., 2009), and plays a role in the development of xylem fibers. Fiber cells, which have thick secondary cell walls, contribute significantly to the mechanical strength of the mature organ (Zhong et al., 2006). Gene enrichment analyses by gene ontology terms has also shown that at G5, expressed genes are primarily involved in developing cell wall structures, including those related to structural carbohydrates such as xyloglucan and xylan (Cherubino Ribeiro et al., 2024) that are known positive signal for tracheary elements (TE) differentiation (Kuriyama and Fukuda, 2001; Turner et al., 2007; Heo et al., 2017).

Although our anatomical findings do not specify the exact developmental stage at which vascular fiber formation occurs, the high expression of transcripts identified as *CaESND1A1*, *CaESND1A2*, and *CaCSND1B2* at G5, compared to G2 and late G4 (Figures 12 and 16), suggests that from this stage onwards, the vascular area of the floral bud peduncle is strengthened due to higher *SND1* expression. These fibers are likely already formed and established in the peduncles from G6 onwards (Crisosto et al., 1992).

It is noteworthy how flower buds, upon perceiving an increase in water availability in the soil and the plant due to rain, quickly grow larger and change color from green to white. This contrasts with the previous stages of development, where apparent dormancy keeps this growth stagnant for months. In contrast, the rapid growth from late G4 to flowering, however, can occur within weeks (Alvim, 1960; Magalhaes and Angelocci, 1976; Crisosto et al., 1992; Drinnan and Menzel, 1994; Lima et al., 2021b; López et al., 2021; Wintgens, 2012; Penfield, 2024). These morphological and anatomical observations prompted us to focus on molecular analyses.

2.4.4 Expression dynamics of vascular identity genes during floral bud reinitiation in response to water availability

The resumption of floral bud development following reproductive arrest due to prolonged water scarcity is initiated by improved water availability (López et al., 2021; López et al., 2022). This reinitiating is associated with increased ethylene production (Lima et al., 2021b), and substantial alterations in the levels of accumulated small RNAs (Cherubino Ribeiro et al., 2024). To test if these modifications also reflect the expression profile of the vasculature identity genes *HB14*, *OPS* and *VND7* orthologs, we performed RT-qPCR analyses of their *Coffea* gene transcripts. Samples were taken before the beginning of the rainy season in southeastern Brazil (15/09/2022, with 0 mm of accumulated rain during the previous 30 days) (INMET, 2024) and during the ongoing rainy season (04/10/2022, with 100 mm of accumulated rain during the previous 30 days) (INMET, 2024).

Phenological analyses determined that on both dates, the majority of buds were at stage G4 following Morais *et al.* (2008) classification. However, they presented clear morphological differences, including variations in color and detachment of inflorescence clusters. These differences led us to sub-classify the buds as G4E (early, 15/09/2022) and G4L (late, 04/10/2022).

Although we detected a trend of upregulation for *HB14*, *OPS* and *VND7* in G4L compared to G4E with confidence intervals of contrasts not containing 0 and significant p-values, no expression change above 2-fold was detected. It is possible that a higher peak of expression for these genes occurred during the interval between both dates, and what we detected is the downward trend of expression. Additional time-course experiments will be required to better resolve the expression trends of these vasculature-related genes during the transition from G4E to G4L in the resumption of floral bud development.

2.4.5 Regulatory network for vascular tissue development and differentiation in coffee flower bud peduncles: insights from anatomical, computational, and molecular analyses

Because the vascular tissues in woody plants are highly radially organized and several pathways regulating the activity of the vascular cambium, phloem, and xylem differentiation are conserved in tree species, particularly in woody plants (Côté et al., 2010; Takata et al., 2019; Haghighat et al., 2024), we propose a regulatory network for the maintenance/renewal of (pro)cambium cells, and the differentiation and development of phloem and xylem cells in the reproductive stage of *C. arabica*, especially in the flower bud peduncles.

Based on our anatomical, computational, and molecular findings, we propose a general model for peduncle vascular development that integrates orthologs of *VND6/7*, *HD-ZIP class III*, and *OPS*, along with downstream genes such as *ALTERED PHLOEM*

DEVELOPMENT (*APL*) (Bonke et al., 2003), and *MYB46/83* (Zhong et al., 2008; McCarthy et al., 2009). We suggest that orthologous genes in coffee plants for these transcription factors hierarchically regulate the expression of genes involved in vascular cell differentiation and formation. This regulation extends to pathways involving genes like *CLE41/CLE44* (*CLAVATA3/EMBRYO SURROUNDING REGION-RELATED*), *TDIF* (*TRACHEARY ELEMENT DIFFERENTIATION INHIBITORY FACTOR*), and *TDR/PXY* (*PHLOEM INTERCALATED WITH XYLEM*)-*WOX4* (*WUSCHEL-RELATED HOMEOBOX 4*)/*WOX14*, which control the activity of the procambium and/or cambium (Hirakawa et al., 2010a; Hirakawa et al., 2010b; Haghighat et al., 2024). We hypothesize that this organization generates the signaling cascade necessary for maintaining (pro)cambium cells and promoting the differentiation of phloem and xylem vessels, thereby supporting the development of flower buds from G2 onwards in the coffee plant (Figure 18).

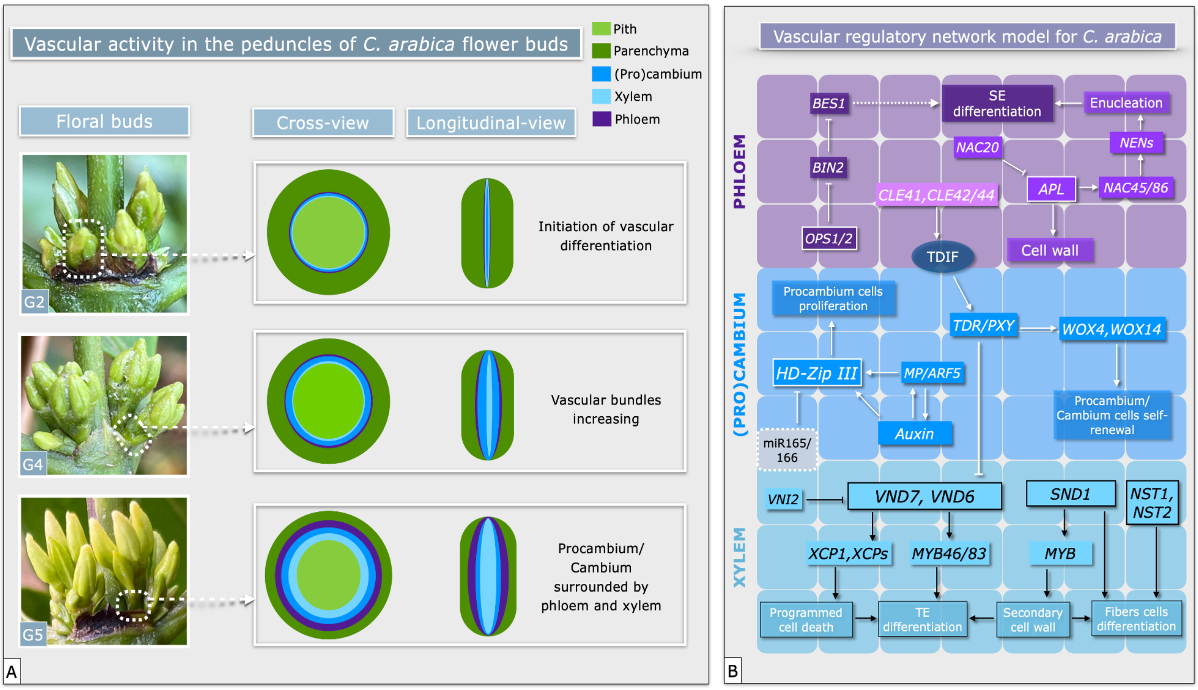

Figure 18. Vascularization network model for vascular development in flower bud peduncles of *C. arabica*. **A:** Floral buds at stages G2, G4, and G5, illustrating the progressive development of the vascular area in peduncles through both cross and longitudinal views. **B**: Proposed regulatory network for cellular maintenance of the (pro)cambium (blue area) and the main genes involved in phloem cell differentiation (purple area) and xylem cell differentiation (light blue area).

This way, The *CLE41/44-TDIF* signaling pathway orchestrates a delicate balance between cell proliferation and differentiation in the vascular (pro)cambium. *CLE41/CLE44-TDIF*, produced in the phloem, act as mobile signals that travel to the (pro)cambium. There, they bind to specific receptor (*TDR/PXY*) on (pro)cambial cells, triggering the expression of

WOX4 and *WOX14* transcription factors. These factors, acting together, promote (pro)cambial cell proliferation while simultaneously suppressing differentiation into xylem cells (Hirakawa et al., 2010b; Haghighat et al., 2024). Furthermore, *CLE*/*TDIF* acts as a negative signal for xylem differentiation. While the *HD-Zip Class III* have been extensively implicated in the regulation of primary and secondary vascular tissue pattern formation (Prigge and Clark, 2006), transcript accumulation in a few *HD-Zip III* sequences is regulated by auxin, specifically *AtHB-8* (Baima et al., 1995), and brassinosteroids (Zhong and Ye, 2001). In addition, miR165/166 binds to *HD-Zip Class III* genes, playing a crucial role in gene silencing, a process highly conserved in plants (Emery et al., 2003).

In the Phloem, *OPS* influences brassinosteroid signaling by directly inhibiting *BIN2*, a *GSK3-like* kinase (*GSK3*) family member (Anne et al., 2015b). *BIN2* is known to suppress *BES1*, a key transcription factor promoting xylem differentiation. This suggests a potential link between brassinosteroid signaling and the balance between phloem and xylem development. *APL*, a protein expressed during late-stage sieve element differentiation, negatively regulates *NAC* transcription factors *NAC20*, *NAC45*, and *NAC86*. These *NAC* factors, in turn, control the NEN proteins involved in nuclear degradation, essential for sieve element formation (Furuta et al., 2014b; Rodriguez-Villalon, 2016). This pathway highlights the complex regulatory network underlying phloem cell differentiation.

Finally, *VND6* and *VND7* trigger xylem specification, regulating genes for programmed cell death and secondary cell wall thickening (Kubo et al., 2005; Ohashi-Ito et al., 2010; Yamaguchi et al., 2008). In addition, the expression of *XCP1*/*XCP2* proteases are also controlled by *VND6*/*7* participates in both vacuolar rupture and subsequent autolysis. On the other hand, *VNI2* interacts with *VND7*, thus repressing its function (Heo et al., 2017). Concomitantly, *SND1* is involved in secondary wall biosynthesis in xylem fibers. In this regard, *SND1* increases the expression of several downstream transcription factors that are highly expressed in fibers during secondary wall synthesis such as *NST1* and *NST2,* that are regulators of secondary wall thickening (Zhong et al., 2006). A deeper understanding of these intricate pathways in *C. arabica* will be crucial for unraveling the mechanisms underlying plant growth and development.

2.5 Concluding remarks and future perspectives

Our work addresses new findings in the vascular development of the peduncles of the reproductive organs in *C. arabica*. Our anatomically analyses shows that the positioning of the phloem and xylem vessels follows the typical pattern of dicotyledons, with the phloem differentiation towards the epidermis and the xylem developing inward, towards the pith. Furthermore, we observed that the vascularization of the branch node is continuous through the emerging floral bud, accompanying the development of the floral organs and coffee fruits.

With our findings on the orthologs for genes known to activate vascular development—*HB* for procambium initiation and formation, *OPS* for phloem identity, and *VND7* for protoxylem and metaxylem differentiation—we also establish a basis for future research on molecular aspects of vascularization, not only in coffee but also in 24 other plant species, many of them with agro-economic interest, such as *Oryza sativa*, *Solanum tuberosum*, *Lactuca sativa*, *Solanum lycopersicum*, and *Cucumis melo* L., among others.

Additionally, through our RNAseq/RT-qPCR analyses we unraveled molecular control mechanisms in coffee vascular development showing different expression behaviors of three important orthogroup members known to promote vascular development in model plants (*HB14*, *OPS*, and *VND7*) during key steps of coffee flower bud differentiation. There are still many uncharacterized genes in the coffee plant related to vascular development, our combined anatomical, computational, and molecular analyses, along with existing knowledge in model plants, allow us to propose a regulatory network for the maintenance of (pro)cambium cells and the differentiation of the phloem and xylem cells during the development of three stages of flower buds, mainly before the rainy period (G2) and during the subsequent incidence of rains (late G4 and G5).

This foundational research enhanced our understanding of peduncle vascular development. Ultimately, this knowledge will be used to improve coffee production by developing management strategies that optimize peduncle vascularity. By enhancing water, sap, and nutrient distribution, we may reduce floral bud non-uniformity and fruit abortion, ultimately increasing yield and providing strategies to deal with an increased unpredictable weather.

REFERENCES

Alvim, P. de T., 1960. Moisture stress as a requirement for flowering of coffee. Science 132, 354–354.

Anne, P., Azzopardi, M., Gissot, L., Beaubiat, S., Hématy, K., Palauqui, J.-C., 2015a. OCTOPUS Negatively Regulates BIN2 to Control Phloem Differentiation in Arabidopsis thaliana. Curr. Biol. 25, 2584–2590. https://doi.org/10.1016/j.cub.2015.08.033

Anne, P., Azzopardi, M., Gissot, L., Beaubiat, S., Hématy, K., Palauqui, J.-C., 2015b. OCTOPUS Negatively Regulates BIN2 to Control Phloem Differentiation in Arabidopsis thaliana. Curr. Biol. 25, 2584–2590. https://doi.org/10.1016/j.cub.2015.08.033

Anthony, F., Diniz, L.E.C., Combes, M.-C., Lashermes, P., 2010. Adaptive radiation in Coffea subgenus Coffea L. (Rubiaceae) in Africa and Madagascar. Plant Syst. Evol. 285, 51–64. https://doi.org/10.1007/s00606-009-0255-8

Antognozzi, E., Tombesi, A., Ferranti, F., Frenguelli, G., 1991. Influence of sink competition on peduncle histogenesis in kiwifruit. N. Z. J. Crop Hortic. Sci. 19, 433–439.

Arcila Pulgarín, J., 2007. Crecimiento y desarrollo de la planta de café. Sist. Prod. Cafe En Colomb. Cenicafe Chinchina Colomb. 21–60.

Arcila-Pulgarin, J., Buhr, L., Bleiholder, H., Hack, H., Wicke, H., 2001. Aplicación de la “Escala BBCH ampliada” para la descripción de las fases fenológicas del desarrollo de la planta de café (Coffea sp.) (No. 23). Federación Nacional de Cafeteros de Colombia, Chinchiná-Caldas-Colombia.

Baima, S., Nobili, F., Sessa, G., Lucchetti, S., Ruberti, I., Morelli, G., 1995. The expression of the Athb-8 homeobox gene is restricted to provascular cells in Arabidopsis thaliana. Development 121, 4171–4182. https://doi.org/10.1242/dev.121.12.4171

Baima, S., Possenti, M., Matteucci, A., Wisman, E., Altamura, M.M., Ruberti, I., Morelli, G., 2001. The Arabidopsis ATHB-8 HD-zip protein acts as a differentiation-promoting transcription factor of the vascular meristems. Plant Physiol. 126, 643–655.

Banks, J.A., Nishiyama, T., Hasebe, M., Bowman, J.L., Gribskov, M., dePamphilis, C., Albert, V.A., Aono, N., Aoyama, T., Ambrose, B.A., Ashton, N.W., Axtell, M.J., Barker, E., Barker, M.S., Bennetzen, J.L., Bonawitz, N.D., Chapple, C., Cheng, C., Correa, L.G.G., Dacre, M., DeBarry, J., Dreyer, I., Elias, M., Engstrom, E.M., Estelle, M., Feng, L., Finet, C., Floyd, S.K., Frommer, W.B., Fujita, T., Gramzow, L., Gutensohn, M., Harholt, J., Hattori, M., Heyl, A., Hirai, T., Hiwatashi, Y., Ishikawa, M., Iwata, M., Karol, K.G., Koehler, B., Kolukisaoglu, U., Kubo, M., Kurata, T., Lalonde, S., Li, K., Li, Y., Litt, A., Lyons, E., Manning, G., Maruyama, T., Michael, T.P., Mikami, K., Miyazaki, S., Morinaga, S., Murata, T., Mueller-Roeber, B., Nelson, D.R., Obara, M., Oguri, Y., Olmstead, R.G., Onodera, N., Petersen, B.L., Pils, B., Prigge, M., Rensing, S.A., Riaño-Pachón, D.M., Roberts, A.W., Sato, Y., Scheller, H.V., Schulz, B., Schulz, C., Shakirov, E.V., Shibagaki, N., Shinohara, N., Shippen, D.E., Sørensen, I., Sotooka, R., Sugimoto, N., Sugita, M., Sumikawa, N., Tanurdzic, M., Theißen, G., Ulvskov, P., Wakazuki, S., Weng, J., Willats, W.W.G.T., Wipf, D., Wolf, P.G., Yang, L., Zimmer, A.D., Zhu, Q., Mitros, T., Hellsten, U., Loqué, D., Otillar, R., Salamov, A., Schmutz, J., Shapiro, H., Lindquist, E., Lucas, S., Rokhsar, D., Grigoriev, I.V., 2011. The Selaginella Genome Identifies Genetic Changes Associated with the Evolution of Vascular Plants. Science 332, 960–963. https://doi.org/10.1126/science.1203810

Bauby, H., Divol, F., Truernit, E., Grandjean, O., Palauqui, J.-C., 2007. Protophloem

Differentiation in Early Arabidopsis thaliana Development. Plant Cell Physiol. 48, 97–109. https://doi.org/10.1093/pcp/pcl045

Beentje, H., Williamson, J., 2010. The Kew Plant glossary: an illustrated dictionary of plant terms. Kew, Richmond, Surrey.

Bonke, M., Thitamadee, S., Mähönen, A.P., Hauser, M.-T., Helariutta, Y., 2003. APL regulates vascular tissue identity in Arabidopsis. Nature 426, 181–186. https://doi.org/10.1038/nature02100

Bouzayen, M., Latché, A., Nath, P., Pech, J.-C., 2009. Mechanism of fruit ripening, in: Plant Developmental Biology-Biotechnological Perspectives: Volume 1. Springer, pp. 319–339.

Brandão, I.R., Silva, D.M.D., Souza, K.R.D.D., Boas, L.V.V., Santos, M.D.O., Silva, F.M.D., Alves, J.D., 2016. Physiological and ultrastructural analysis reveal the absence of a defined abscission zone in coffee fruits. Bragantia 75, 386–395. https://doi.org/10.1590/1678-4499.575

Bustan, A., Erner, Y., Goldschmidt, E.E., 1995. Interactions between Developing Citrus Fruits and their Supportive Vascular System. Ann. Bot. 76, 657–666. https://doi.org/10.1006/anbo.1995.1144

Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M.W., Shipley, G.L., Vandesompele, J., Wittwer, C.T., 2009. The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. Clin. Chem. 55, 611–622. https://doi.org/10.1373/clinchem.2008.112797

Buthelezi, L.G., Mavengahama, S., Sibiya, J., Ntuli, N.R., 2023a. Variation in Shoot, Peduncle and Fruit Growth of Lagenaria siceraria Landraces. Plants 12, 532. https://doi.org/10.3390/plants12030532

Buthelezi, L.G., Mavengahama, S., Sibiya, J., Ntuli, N.R., 2023b. Variation in Shoot, Peduncle and Fruit Growth of Lagenaria siceraria Landraces. Plants 12, 532. https://doi.org/10.3390/plants12030532

Camargo, Â.P. de, Camargo, M.B.P. de, 2001. Definição e esquematização das fases fenológicas do cafeeiro arábica nas condições tropicais do Brasil. Bragantia 60, 65–68.

Campbell, L., Turner, S., 2017. Regulation of vascular cell division. J. Exp. Bot. 68, 27–43. https://doi.org/10.1093/jxb/erw448

Cannell, M., 1971. Production and distribution of dry matter in trees of Coffea arabica L. in Kenya as affected by seasonal climatic differences and the presence of fruits. Ann. Appl. Biol. 67, 99–120.

Cardon, C.H., de Oliveira, R.R., Lesy, V., Ribeiro, T.H.C., Fust, C., Pereira, L.P., Colasanti, J., Chalfun-Junior, A., 2022. Expression of coffee florigen *CaFT1* reveals a sustained floral induction window associated with asynchronous flowering in tropical perennials. Plant Sci. 325, 111479. https://doi.org/10.1016/j.plantsci.2022.111479

Carréra, J.C., Resende, T.B., Vicente Campos, A.A., De Souza, R.R., De Oliveira, I.M.M., Alves Ribeiro, C., Gavilanes, M.L., Guimarães, R.J., Mori, F.A., 2023. Anatomic characteristics of branches related to the vegetative growth of coffee tree (*Coffea arabica* L., Rubiaceae) under nutritional variation. J. Plant Nutr. 46, 4594–4605. https://doi.org/10.1080/01904167.2023.2237524

Carvalho, N., da Mata, L., Ribeiro, P., Moreira, A.D.C., Pinedo, A., Ribeiro, C. da C., Souza, N., Buso, G., 2021. Morphological and anatomical characterization of peduncle, flower and fruit related to easy fruit abscission of Capsicum chinense (Solanaceae) genotypes. Gene Conserve 20.

Cherubino Ribeiro, T.H., Baldrich, P., De Oliveira, R.R., Fernandes-Brum, C.N., Mathioni, S.M., De Sousa Cardoso, T.C., De Souza Gomes, M., Do Amaral, L.R., Pimenta De

Oliveira, K.K., Dos Reis, G.L., Meyers, B.C., Chalfun-Junior, A., 2024. The floral development of the allotetraploid *Coffea arabica* L. correlates with a small RNA dynamic reprogramming. Plant J. tpj.16713. https://doi.org/10.1111/tpj.16713

Cherubino Ribeiro, T.H., Oliveira, R.R. de, Antonio, C.-J., 2023. Differential universal ortholog composition of Coffea arabica L. sub-genomes and its contribution to regulatory networks governing essential biological processes. bioRxiv 2023.03.08.531780. https://doi.org/10.1101/2023.03.08.531780

CONAB, 2024. Conab - Safra de grãos 2023/2024 está estimada em 294,1 milhões de toneladas [WWW Document]. URL https://www.conab.gov.br/ultimas-noticias/5478-safra-de-graos-2023-2024-esta-estimada-em-294-1-milhoes-de-toneladas (accessed 7.15.24).

Côté, C.L., Boileau, F., Roy, V., Ouellet, M., Levasseur, C., Morency, M.-J., Cooke, J.E., Séguin, A., MacKay, J.J., 2010. Gene family structure, expression and functional analysis of HD-Zip III genes in angiosperm and gymnosperm forest trees. BMC Plant Biol. 10, 273. https://doi.org/10.1186/1471-2229-10-273

Crisosto, C.H., Grantz, D.A., Meinzer, F.C., 1992. Effects of water deficit on flower opening in coffee (Coffea arabica L.). Tree Physiol. 10, 127–139.

Cui, Z., Sun, H., Lu, Y., Ren, L., Xu, X., Li, D., Wang, R., Ma, C., 2022. Variations in Pedicel Structural Properties Among Four Pear Species (Pyrus): Insights Into the Relationship Between the Fruit Characteristics and the Pedicel Structure. Front. Plant Sci. 13. https://doi.org/10.3389/fpls.2022.815283

Czechowski, T., Bari, R.P., Stitt, M., Scheible, W.-R., Udvardi, M.K., 2004. Real-time RT-PCR profiling of over 1400 Arabidopsis transcription factors: unprecedented sensitivity reveals novel root- and shoot-specific genes. Plant J. 38, 366–379. https://doi.org/10.1111/j.1365-313X.2004.02051.x

De Block, P., Vrijdaghs, A., 2013. Development of reproductive organs in Canephora madagascariensis (Octotropideae - Rubiaceae). Plant Ecol. Evol. 146, 310–327. https://doi.org/10.5091/plecevo.2013.844

De Melo, W.W.A., Santos, F.L., Scinocca, F., 2023. Modal analysis of the coffee fruit-peduncle-branch system coffee fruit-peduncle-branch system. Smart Agric. Technol. 4, 100217. https://doi.org/10.1016/j.atech.2023.100217

De Oliveira, K.K.P., De Oliveira, R.R., De Campos Rume, G., Ribeiro, T.H.C., Fernandes-Brum, C.N., Do Amaral, L.R., Kakrana, A., Mathioni, S., Meyers, B.C., De Souza Gomes, M., Chalfun-Junior, A., 2024. Microsporogenesis and the biosynthesis of floral small interfering RNAs in coffee have a unique pattern among eudicots, suggesting a sensitivity to climate changes. Plant Direct 8, e561. https://doi.org/10.1002/pld3.561

de Oliveira, R.R., Cesarino, I., Mazzafera, P., Dornelas, M.C., 2014. Flower development in Coffea arabica L.: new insights into MADS-box genes. Plant Reprod. 27, 79–94.

Dedecca, D.M., 1957. Anatomia e desenvolvimento ontogenético de Coffea arabica L. var. typica Cramer. Bragantia 16, 315–366. https://doi.org/10.1590/S0006-87051957000100023

Dengler, N.G., 2001. Regulation of Vascular Development. J. Plant Growth Regul. 20, 1–13. https://doi.org/10.1007/s003440010008

Dražeta, L., Lang, A., Cappellini, C., Hall, A.J., Volz, R.K., Jameson, P.E., 2004. Vessel differentiation in the pedicel of apple and the effects of auxin transport inhibition. Physiol. Plant. 120, 162–170.

Drinnan, J., Menzel, C.M., 1994. Synchronization of anthesis and enhancement of vegetative growth in coffee (Coffea arabica L.) following water stress during floral initiation. J. Hortic. Sci. 69, 841–849.

Donald Alexander Johansen. (McGraw-Hill Publications in the Botanical Sciences.) Pp. xi+ 523. (New York and London: McGraw-Hill Book Co., Inc., 1940.) 30s.

Du, Q., Avci, U., Li, S., Gallego-Giraldo, L., Pattathil, S., Qi, L., Hahn, M.G., Wang, H., 2015. Activation of miR165b represses AtHB15 expression and induces pith secondary wall development in Arabidopsis. Plant J. 83, 388–400. https://doi.org/10.1111/tpj.12897

Emery, J.F., Floyd, S.K., Alvarez, J., Eshed, Y., Hawker, N.P., Izhaki, A., Baum, S.F., Bowman, J.L., 2003. Radial Patterning of Arabidopsis Shoots by Class III HD-ZIP and KANADI Genes. Curr. Biol. 13, 1768–1774. https://doi.org/10.1016/j.cub.2003.09.035

Emms, D.M., Kelly, S., 2019. OrthoFinder: phylogenetic orthology inference for comparative genomics. Genome Biol. 20, 1–14.

Endo, H., Yamaguchi, M., Tamura, T., Nakano, Y., Nishikubo, N., Yoneda, A., Kato, K., Kubo, M., Kajita, S., Katayama, Y., Ohtani, M., Demura, T., 2015. Multiple Classes of Transcription Factors Regulate the Expression of VASCULAR-RELATED NAC-DOMAIN7, a Master Switch of Xylem Vessel Differentiation. Plant Cell Physiol. 56, 242–254. https://doi.org/10.1093/pcp/pcu134

Felsenstein, J., 1993. PHYLIP (phylogeny inference package), version 3.5 c. Joseph Felsenstein.

Ferrari, R.C., Scatena, V.L., Oriani, A., 2014. Leaf and inflorescence peduncle anatomy: a contribution to the taxonomy of Rapateaceae. Plant Syst. Evol. 300, 1579–1590.

Ferreira, I.N.M., dos Santos, C.S., Flho, J.T.C., 2014. Caracterização Anatômica dos Órgãos Vegetativos do Cafeeiro arabica (Coffea arabica L.). Rev. Fragm. Cult.-Rev. Interdiscip. Ciênc. Humanas 24, 153–161.

Ferreira, T., Shuler, J., Guimarães, R., Farah, A., 2019. Introduction to coffee plant and genetics.

Fukuda, H., Ohashi-Ito, K., 2019. Vascular tissue development in plants. Curr. Top. Dev. Biol. 131, 141–160.

Furuta, K.M., Hellmann, E., Helariutta, Y., 2014a. Molecular Control of Cell Specification and Cell Differentiation During Procambial Development. Annu. Rev. Plant Biol. 65, 607–638. https://doi.org/10.1146/annurev-arplant-050213-040306

Furuta, K.M., Yadav, S.R., Lehesranta, S., Belevich, I., Miyashima, S., Heo, J., Vatén, A., Lindgren, O., De Rybel, B., Van Isterdael, G., Somervuo, P., Lichtenberger, R., Rocha, R., Thitamadee, S., Tähtiharju, S., Auvinen, P., Beeckman, T., Jokitalo, E., Helariutta, Y., 2014b. *Arabidopsis* NAC45/86 direct sieve element morphogenesis culminating in enucleation. Science 345, 933–937. https://doi.org/10.1126/science.1253736

Greenwood, K.N., King, C.L., Melena, I., Stegemann, K.A., Donnelly, M., Childers, A., Mozal, R., Collins, C.A., Spears, B.J., 2023. The brassinosteroid-responsive protein OCTOPUS is a novel regulator of Arabidopsis thaliana immune signaling. Plant Direct 7, e524. https://doi.org/10.1002/pld3.524

Haghighat, M., Zhong, R., Ye, Z.-H., 2024. WUSCHEL-RELATED HOMEOBOX genes are crucial for normal vascular organization and wood formation in poplar. Plant Sci. 112138.

Hardtke, C.S., 2023a. Phloem development. New Phytol. 239, 852–867. https://doi.org/10.1111/nph.19003

Hardtke, C.S., 2023b. Phloem development. https://doi.org/10.1111/nph.19003

Harvey, P.H., Pagel, M.D., 1991. The comparative method in evolutionary biology. Oxford university press.

Heo, J., Blob, B., Helariutta, Y., 2017. Differentiation of conductive cells: a matter of life and death. Curr. Opin. Plant Biol. 35, 23–29. https://doi.org/10.1016/j.pbi.2016.10.007

Heo, J., Roszak, P., Furuta, K.M., Helariutta, Y., 2014. Phloem development: Current knowledge and future perspectives. Am. J. Bot. 101, 1393–1402. https://doi.org/10.3732/ajb.1400197

Hirakawa, Y., Kondo, Y., Fukuda, H., 2010a. Regulation of Vascular Development by CLE Peptide-receptor Systems. J. Integr. Plant Biol. 52, 8–16. https://doi.org/10.1111/j.1744-7909.2010.00904.x

Hirakawa, Y., Kondo, Y., Fukuda, H., 2010b. TDIF Peptide Signaling Regulates Vascular Stem Cell Proliferation via the WOX4 Homeobox Gene in Arabidopsis. Plant Cell 22, 2618–2629. https://doi.org/10.1105/tpc.110.076083

Horbens, M., Branke, D., Gärtner, R., Voigt, A., Stenger, F., Neinhuis, C., 2015. Multi-scale simulation of plant stem reinforcement by brachysclereids: A case study in apple fruit peduncles. J. Struct. Biol. 192, 116–126.

INMET, 2024. Instituto Nacional de Meteorologia - INMET [WWW Document]. URL https://portal.inmet.gov.br/ (accessed 7.20.24).

Jesus, A.M.S., Carvalho, S.P.D., Castro, E.M.D., Gomes, C.N., 2010. Observações anatômicas em plantas de Coffea arabica L. obtidas por enraizamento de estacas. Rev. Ceres 57, 175–180. https://doi.org/10.1590/S0034-737X2010000200006

Jones, D.T., Taylor, W.R., Thornton, J.M., 1992. The rapid generation of mutation data matrices from protein sequences. Bioinformatics 8, 275–282.

Katoh, K., Misawa, K., Kuma, K., Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 30, 3059–3066.

Knipfer, T., Fei, J., Gambetta, G.A., McElrone, A.J., Shackel, K.A., Matthews, M.A., 2015. Water transport properties of the grape pedicel during fruit development: insights into xylem anatomy and function using microtomography. Plant Physiol. 168, 1590–1602.

Krishnan, S., 2017. Sustainable Coffee Production, in: Oxford Research Encyclopedia of Environmental Science. Oxford University Press. https://doi.org/10.1093/acrefore/9780199389414.013.224

Kubo, M., Udagawa, M., Nishikubo, N., Horiguchi, G., Yamaguchi, M., Ito, J., Mimura, T., Fukuda, H., Demura, T., 2005. Transcription switches for protoxylem and metaxylem vessel formation. Genes Dev. 19, 1855–1860. https://doi.org/10.1101/gad.1331305

Kuriyama, H., Fukuda, H., 2002. Developmental programmed cell death in plants. Curr. Opin. Plant Biol. 5, 568–573. https://doi.org/10.1016/S1369-5266(02)00305-9

Kuriyama, H., Fukuda, H., 2001. Regulation of Tracheary Element Differentiation. J. Plant Growth Regul. 20, 35–51. https://doi.org/10.1007/s003440010006

Kutschera, U., 1989. Tissue stresses in growing plant organs. Physiol. Plant. 77, 157–163.

Lashermes, P., Combes, M.-C., Robert, J., Trouslot, P., D'Hont, A., Anthony, F., Charrier, A., 1999. Molecular characterisation and origin of the Coffea arabica L. genome. Mol. Gen. Genet. MGG 261, 259–266. https://doi.org/10.1007/s004380050965

Letunic, I., Bork, P., 2021. Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. Nucleic Acids Res. 49, W293–W296.

Li, H., Zhang, X., Hou, X., Du, T., 2021a. Developmental and water deficit-induced changes in hydraulic properties and xylem anatomy of tomato fruit and pedicels. J. Exp. Bot. 72, 2741–2756. https://doi.org/10.1093/jxb/erab001

Li, H., Zhang, X., Hou, X., Du, T., 2021b. Developmental and water deficit-induced changes in hydraulic properties and xylem anatomy of tomato fruit and pedicels. J. Exp. Bot. 72, 2741–2756.

Lima, A.A., Santos, I.S., Torres, M.E.L., Cardon, C.H., Caldeira, C.F., Lima, R.R., Davies, W.J., Dodd, I.C., Chalfun-Junior, A., 2021a. Drought and re-watering modify ethylene production and sensitivity, and are associated with coffee anthesis. Environ. Exp. Bot. 181, 104289.

Lima, A.A., Santos, I.S., Torres, M.E.L., Cardon, C.H., Caldeira, C.F., Lima, R.R., Davies, W.J., Dodd, I.C., Chalfun-Junior, A., 2021b. Drought and re-watering modify ethylene production and sensitivity, and are associated with coffee anthesis. Environ. Exp. Bot. 181, 104289. https://doi.org/10.1016/j.envexpbot.2020.104289

Lin, Y.-C.J., Chen, H., Li, Q., Li, W., Wang, J.P., Shi, R., Tunlaya-Anukit, S., Shuai, P., Wang, Z., Ma, H., Li, H., Sun, Y.-H., Sederoff, R.R., Chiang, V.L., 2017. Reciprocal cross-regulation of VND and SND multigene TF families for wood formation in Populus trichocarpa. Proc. Natl. Acad. Sci. U. S. A. 114, E9722–E9729. https://doi.org/10.1073/pnas.1714422114

López, M.E., Santos, I.S., de Oliveira, R.R., Lima, A.A., Cardon, C.H., Chalfun-Junior, A., 2021. An overview of the endogenous and environmental factors related to the Coffea arabica flowering process. Beverage Plant Res. 1, 1–16.

López, M.E., Silva Santos, I., Marquez Gutiérrez, R., Jaramillo Mesa, A., Cardon, C.H., Espíndola Lima, J.M., Almeida Lima, A., Chalfun-Junior, A., 2022. Crosstalk Between Ethylene and Abscisic Acid During Changes in Soil Water Content Reveals a New Role for 1-Aminocyclopropane-1- Carboxylate in Coffee Anthesis Regulation. Front. Plant Sci. 13. https://doi.org/10.3389/fpls.2022.824948

Lucas, W.J., Groover, A., Lichtenberger, R., Furuta, K., Yadav, S.-R., Helariutta, Y., He, X.-Q., Fukuda, H., Kang, J., Brady, S.M., 2013. The plant vascular system: evolution, development and functions f. J. Integr. Plant Biol. 55, 294–388.

Magalhaes, A., Angelocci, L., 1976. Sudden alterations in water balance associated with flower bud opening in coffee plants. J. Hortic. Sci. 51, 419–423.

Majerowicz, N., Söndahl, M.R., 2005. Induction and differentiation of reproductive buds in Coffea arabica L. Braz. J. Plant Physiol. 17, 247–254.

Malpighi, M., 1968. Anatome plantarum, cui subjungitur appendix, iteratas & auctas ejusdem authoris de ovo incubato observationes continens. No Title.

Marchand, N.L., 1864. Recherches organographiques et organogéniques sur le Coffea arabica L. Baillière.

Martins, M.Q., Fortunato, A.S., Rodrigues, W.P., Partelli, F.L., Campostrini, E., Lidon, F.C., DaMatta, F.M., Ramalho, J.C., Ribeiro-Barros, A.I., 2017. Selection and Validation of Reference Genes for Accurate RT-qPCR Data Normalization in Coffea spp. under a Climate Changes Context of Interacting Elevated [CO2] and Temperature. Front. Plant Sci. 8, 307. https://doi.org/10.3389/fpls.2017.00307

McCarthy, R.L., Zhong, R., Ye, Z.-H., 2009. MYB83 Is a Direct Target of SND1 and Acts Redundantly with MYB46 in the Regulation of Secondary Cell Wall Biosynthesis in Arabidopsis. Plant Cell Physiol. 50, 1950–1964. https://doi.org/10.1093/pcp/pcp139

Nii, N., 1980. Seasonal Changes in Growth and Enlargement of the Japanese Pear Fruit, Pyrus serótina Cv Shinsheiki, in Relation to Vascular Bundle Development in the Pedicel and Flesh. J. Hortic. Sci. 55, 385–396. https://doi.org/10.1080/00221589.1980.11514950

Notaguchi, M., Okamoto, S., 2015. Dynamics of long-distance signaling via plant vascular tissues. Front. Plant Sci. 6, 161.

O'Brien, T.P., Feder, N., McCully, M.E., 1964. Polychromatic staining of plant cell walls by toluidine blue O. Protoplasma 59, 368–373. https://doi.org/10.1007/BF01248568

Ohashi-Ito, K., Kubo, M., Demura, T., Fukuda, H., 2005. Class III Homeodomain Leucine-Zipper Proteins Regulate Xylem Cell Differentiation. Plant Cell Physiol. 46, 1646–1656. https://doi.org/10.1093/pcp/pci180

Ohashi-Ito, K., Oda, Y., Fukuda, H., 2010. Arabidopsis VASCULAR-RELATED NAC-

DOMAIN6 Directly Regulates the Genes That Govern Programmed Cell Death and Secondary Wall Formation during Xylem Differentiation. Plant Cell 22, 3461–3473. https://doi.org/10.1105/tpc.110.075036

Ohtani, M., Nishikubo, N., Xu, B., Yamaguchi, M., Mitsuda, N., Goué, N., Shi, F., Ohme-Takagi, M., Demura, T., 2011. A NAC domain protein family contributing to the regulation of wood formation in poplar. Plant J. 67, 499–512. https://doi.org/10.1111/j.1365-313X.2011.04614.x

Oliveira, R., Viana, A., Reátegui, A., Vincentz, M., 2015. An efficient method for simultaneous extraction of high-quality RNA and DNA from various plant tissues. Genet. Mol. Res. 14, 18828–18838.

Patay, É.B., Németh, T., Németh, T.S., Filep, R., Vlase, L., Papp, N., 2016. HISTOLOGICAL AND PHYTOCHEMICAL STUDIES OF COFFEA BENGHALENSIS B. HEYNE EX SCHULT., COMPARED WITH COFFEA ARABICA L. 64.

Penfield, S., 2024. Beyond floral initiation: the role of flower bud dormancy in flowering time control of annual plants. J. Exp. Bot. erae223. https://doi.org/10.1093/jxb/erae223

Pereira Gardin, J.P., 2006. Desenvolvimento reprodutivo do cafeeiro: anatomia de vasos do xilema e dinâmica de carboidratos (Tesis). UFLA, Lavras, MG.

Pereira, M.A., Silva, J.R.M.D., Rabelo, G.F., Andrade, A.C.D.A., 2014. Propriedades anatômicas, químicas e de densidade da madeira de Coffea arabica L. CERNE 20, 363–367. https://doi.org/10.1590/01047760201420033833

Philipson, W., Balfour, E.E., 1963. Vascular patterns in dicotyledons. Bot. Rev. 382–404.

Prigge, M.J., Clark, S.E., 2006. Evolution of the class III HD-Zip gene family in land plants. Evol. Dev. 8, 350–361. https://doi.org/10.1111/j.1525-142X.2006.00107.x

Qiu, Y.-L., Lee, J., Bernasconi-Quadroni, F., Soltis, D.E., Soltis, P.S., Zanis, M., Zimmer, E.A., Chen, Z., Savolainen, V., Chase, M.W., 1999. The earliest angiosperms: evidence from mitochondrial, plastid and nuclear genomes. Nature 402, 404–407.

Ramachandran, P., Carlsbecker, A., Etchells, J.P., 2017. Class III HD-ZIPs govern vascular cell fate: an HD view on patterning and differentiation. J. Exp. Bot. 68, 55–69. https://doi.org/10.1093/jxb/erw370

Rančić, D., Quarrie, S.P., Radošević, R., Terzić, M., Pećinar, I., Stikić, R., Jansen, S., 2010. The application of various anatomical techniques for studying the hydraulic network in tomato fruit pedicels. Protoplasma 246, 25–31.

Razeto, B., Salgado, J., 2004. The inflorescence and fruit peduncle as indicators of nitrogen status of the avocado tree. HortScience 39, 1173–1174.

Ribeiro, T.H.C., Oliveira, R.R. de, Antonio, C.-J., 2023. Differential universal ortholog composition of Coffea arabica L. sub-genomes and its contribution to regulatory networks governing essential biological processes. https://doi.org/10.1101/2023.03.08.531780

Robinson, M.D., McCarthy, D.J., Smyth, G.K., 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. bioinformatics 26, 139–140.

Robischon, M., Du, J., Miura, E., Groover, A., 2011. The Populus Class III HD ZIP, popREVOLUTA, Influences Cambium Initiation and Patterning of Woody Stems. Plant Physiol. 155, 1214–1225. https://doi.org/10.1104/pp.110.167007

Rodriguez-Villalon, A., 2016. Wiring a plant: genetic networks for phloem formation in Arabidopsis thaliana roots. New Phytol. 210, 45–50. https://doi.org/10.1111/nph.13527

Rodriguez-Villalon, A., Gujas, B., Kang, Y.H., Breda, A.S., Cattaneo, P., Depuydt, S., Hardtke, C.S., 2014. Molecular genetic framework for protophloem formation. Proc.

Natl. Acad. Sci. 111, 11551–11556. https://doi.org/10.1073/pnas.1407337111

Ruiz Sola, M.A., Coiro, M., Crivelli, S., Zeeman, S.C., Schmidt Kjølner Hansen, S., Truernit, E., 2017. OCTOPUS-LIKE 2, a novel player in Arabidopsis root and vascular development, reveals a key role for OCTOPUS family genes in root metaphloem sieve tube differentiation. New Phytol. 216, 1191–1204. https://doi.org/10.1111/nph.14751

Ruonala, R., Ko, D., Helariutta, Y., 2017. Genetic Networks in Plant Vascular Development. Annu. Rev. Genet. 51, 335–359. https://doi.org/10.1146/annurev-genet-120116-024525

Růžička, K., Ursache, R., Hejátko, J., Helariutta, Y., 2015. Xylem development – from the cradle to the grave. New Phytol. 207, 519–535. https://doi.org/10.1111/nph.13383

Sachs, T., 2000. Integrating Cellular and Organismic Aspects of Vascular Differentiation. Plant Cell Physiol. 41, 649–656. https://doi.org/10.1093/pcp/41.6.649

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.

Sakiyama, N.S., Picoli, E.A.D.T., Oliveira, A.C.B.D., Caixeta, E.T., Zambolim, L., Martinez, H.E.P., Pereira, A.A., 2017. Triple plagiotropic branch in coffee: a new promising mutant? Crop Breed. Appl. Biotechnol. 17, 408–411. https://doi.org/10.1590/1984-70332017v17n4n61

Scarpella, E., Helariutta, Y., 2010. Vascular pattern formation in plants. Curr. Top. Dev. Biol. 91, 221–265.

Scarpella, E., Meijer, A.H., 2004. Pattern formation in the vascular system of monocot and dicot plant species. New Phytol. 164, 209–242.

Scott, D.H., 1897. The Anatomical Characters presented by the Peduncle of Cycadaceae. Ann. Bot. os-11, 399–420. https://doi.org/10.1093/oxfordjournals.aob.a088661

Setiamihardja, R., Knavel, D., 1990. Association of pedicel length and diameter with fruit length and diameter and ease of fruit detachment in pepper.

Silveira, D., Pelissoni, M., Buzatto, C., Scheffer-Basso, S., Ebone, L., Machado, J., Lângaro, N., 2021. Anatomical traits and structural components of peduncle associated with lodging in Avena sativa L.

Song, W., Yi, J., Kurniadinata, O.F., Wang, H., Huang, X., 2018. Linking fruit Ca uptake capacity to fruit growth and pedicel anatomy, a cross-species study. Front. Plant Sci. 9, 575.

Song, W.-P., Chen, W., Yi, J.-W., Wang, H.-C., Huang, X.-M., 2018. Ca distribution pattern in litchi fruit and pedicel and impact of Ca channel inhibitor, La3+. Front. Plant Sci. 8, 2228.

Steibel, J.P., Poletto, R., Coussens, P.M., Rosa, G.J., 2009. A powerful and flexible linear mixed model framework for the analysis of relative quantification RT-PCR data. Genomics 94, 146–152.

Stossi, F., Singh, P.K., 2023. Basic image analysis and manipulation in ImageJ/Fiji. Curr. Protoc. 3, e849.

Takata, N., Awano, T., Nakata, M.T., Sano, Y., Sakamoto, S., Mitsuda, N., Taniguchi, T., 2019. Populus NST/SND orthologs are key regulators of secondary cell wall formation in wood fibers, phloem fibers and xylem ray parenchyma cells. Tree Physiol. 39, 514–525. https://doi.org/10.1093/treephys/tpz004

Talbert, P.B., Adler, H.T., Parks, D.W., Comai, L., 1995. The REVOLUTA gene is necessary for apical meristem development and for limiting cell divisions in the leaves and stems of Arabidopsis thaliana. Development 121, 2723–2735. https://doi.org/10.1242/dev.121.9.2723

Truernit, E., Bauby, H., Belcram, K., Barthélémy, J., Palauqui, J.-C., 2012. OCTOPUS, a polarly localised membrane-associated protein, regulates phloem differentiation entry in Arabidopsis thaliana. Development 139, 1306–1315. https://doi.org/10.1242/dev.072629

Turner, S., Gallois, P., Brown, D., 2007. Tracheary element differentiation. Annu Rev Plant Biol 58, 407–433.

Waller, J.M., Bigger, M., Hillocks, R.J., 2007. Coffee pests, diseases and their management. CABI.

Wintgens, J.N., 2012. Coffee - Growing, Processing, Sustainable Production: A Guidebook for Growers, Processors, Traders and Researchers. John Wiley & Sons.

Yamaguchi, M., Kubo, M., Fukuda, H., Demura, T., 2008. VASCULAR-RELATED NAC-DOMAIN7 is involved in the differentiation of all types of xylem vessels in Arabidopsis roots and shoots. Plant J. 55, 652–664. https://doi.org/10.1111/j.1365-313X.2008.03533.x

Zamski, E., Starkman, F., Zieslin, N., 1991. Mechanical strength and anatomical structure of the peduncles of rose (Rosa× hybrida) flowers. Isr. J. Bot. 40, 1–6.

Zhang, Y., Keller, M., 2017. Discharge of surplus phloem water may be required for normal grape ripening. J. Exp. Bot. 68, 585–595. https://doi.org/10.1093/jxb/erw476

Zhong, R., Demura, T., Ye, Z.-H., 2006. SND1, a NAC Domain Transcription Factor, Is a Key Regulator of Secondary Wall Synthesis in Fibers of Arabidopsis. Plant Cell 18, 3158–3170. https://doi.org/10.1105/tpc.106.047399

Zhong, R., Lee, C., Zhou, J., McCarthy, R.L., Ye, Z.-H., 2008. A Battery of Transcription Factors Involved in the Regulation of Secondary Cell Wall Biosynthesis in Arabidopsis. Plant Cell 20, 2763–2782. https://doi.org/10.1105/tpc.108.061325

Zhong, R., Richardson, E.A., Ye, Z.-H., 2007a. Two NAC domain transcription factors, SND1 and NST1, function redundantly in regulation of secondary wall synthesis in fibers of Arabidopsis. Planta 225, 1603–1611.

Zhong, R., Richardson, E.A., Ye, Z.-H., 2007b. Two NAC domain transcription factors, SND1 and NST1, function redundantly in regulation of secondary wall synthesis in fibers of Arabidopsis. Planta 225, 1603–1611. https://doi.org/10.1007/s00425-007-0498-y

Zhong, R., Ye, Z.-H., 2001. Alteration of Auxin Polar Transport in the Arabidopsisifl1 Mutants. Plant Physiol. 126, 549–563. https://doi.org/10.1104/pp.126.2.549

Zúñiga-Sánchez, E., Martínez-Barajas, E., Zavaleta-Mejía, E., Gamboa-de-Buen, A., 2017. El floema y la ruta simplástica durante la formación de órganos de demanda. Rev. Fitotec. Mex. 40, 249–259.

GENERA CONCLUSIONS

Our understanding of plant-environment interactions is largely incomplete and requires further investigation. Given the complexity of plants, we need equally sophisticated tools to fully comprehend these interactions. During our research, we observed significant plasticity in vascular tissues, with their cellular structures changing over time. This plasticity indicates a complex and adaptable architecture within the plant, which is intricately regulated at the transcriptional level by various genetic networks. An example of this complex architecture is the arrangement of vascular bundles extending from the root through the stem. Vascularization adapts, especially in radial patterns, to supply increasingly complex structures such as leaves, flowers, and fruits. This intricate process is regulated by several genes that have been largely conserved across vascular plants.

Therefore, our focus here was on the vascularization of coffee, particularly in the relatively underexplored flower and fruit peduncles, because this critical area directly links the plant with the developing organs. Our anatomical, computational, and molecular approaches allowed us to examine a vascular network that becomes more complex in woody trees, sparking significant research aimed at improving products of interest, such as wood and food.

We have set four objectives to be met in the concluding section of this thesis. The first objective was to "Determine the moment of differentiation of the vascular tissue in the different stages of development of the reproductive organs of *C. arabica* by means of anatomical analyzes in the peduncles of floral buds, flowers and fruits." We found that vascularity was present even at the earliest stages; G1 and G2. At this stage, the peduncle was not yet distinguishable from the rest of the bud, but it was attached to the node with a clearly distinguishable procambial region.

The second objective was to "Correlate the differentiation and development of the vascular tissue (phloem and xylem) with the different morphological changes during the different stages of development of the floral buds and fruits of *C. arabica*." We observed dramatic changes in the vasculature across the different stages of flower and later fruit development. The complexity of the vasculature increased during successive stages of bud and fruit development. Figures 2 to 8 in Chapter 2 provide an in-depth description of this process.

The following objective, "Identify coffee orthologs governing vascular development at the transcriptional level," was addressed through an in-depth analysis of orthologs of *VND*, *OPS*, and *HD-Zip III* orthogroups, not only in coffee but also in a set of 25 plant species, some of economic importance, which may aid future research efforts. In coffee, gene balancing effects were detected in the form of differential composition of orthologs between sub-genomes.

Finally, the fulfillment of objective 4, "Use gene expression analyses to pinpoint the stages where these orthologs are upregulated," revealed that most vascularity-related orthologs were upregulated as early as G2. Notable exceptions included the upregulation of *CaEHB14* during late G4 (a stage triggered by the resumption of rains after water deficit) and the subsequent upregulation of *SND1* genes at G5, indicating enhanced synthesis of cell walls at this stage.

We have also asked, “How could anatomical differences in bud vascularization influence the non-uniformity of flowering?” It turns out that the regulatory networks governing bud vascularization are a complex process. We moved further towards answering this question by identifying many of the actors influencing this development and linking them with their potential phenotypes scrutinized by our anatomical approaches. Nevertheless, our results brought many additional questions that will need to be addressed before this question is fully resolved.

Our research paves the way for future studies on the orthologs of genes involved in vascular development in agriculturally and biologically significant plants. The goal is to improve their production and address global food demands. Additionally, understanding vascularization in economically important crops enhances resource transportation within the plant, which is increasingly vital due to global climate change. Ultimately, our work aims to deepen knowledge about the coffee plant—not only for its economic value but also as a representative example of small, woody trees with complex flowering architecture, contributing to the production of the sought-after coffee fruit.

REFERENCES

Alves, H.M.R., Volpato, M.M.L., Vieira, T.G.C., Borém, F.M., Barbosa, J.N., 2011. **Características ambientais e qualidade da bebida dos cafés do estado de Minas Gerais**. Inf. Agropecuário Belo Horiz. 32, 18–29.

Alvim, P. de T., 1960. **Moisture stress as a requirement for flowering of coffee**. Science 132, 354–354.

Alvim, P. de T., 1958. **Estímulo de la floración y fructificación del cafeto por aspersiones con ácido giberélico**. Turrialba 8, 64–72.

Arcila, J., Buhr, L., Bleiholder, H., Hack, H., Wicke, H., 2001. **Aplicación de la escala BBCH ampliada para la descripción de las fases fenológicas del desarrollo de la planta de café *Coffea* sp.**

Arcila Pulgarín, J., 2007. **Crecimiento y desarrollo de la planta de café**. Sist. Prod. Cafe En Colomb. Cenicafe Chinchina Colomb. 21–60.

Berthaud, J., Charrier, A., n.d. **Genetic resources of *Coffea***.

Camargo, Â.P. de, Camargo, M.B.P. de, 2001. **Definição e esquematização das fases fenológicas do cafeeiro arábica nas condições tropicais do Brasil**. Bragantia 60, 65–68.

Charrier, A., Berthaud, J., 1985. **Botanical Classification of Coffee**, in: Clifford, M.N., Willson, K.C. (Eds.), Coffee. Springer US, Boston, MA, pp. 13–47. https://doi.org/10.1007/978-1-4615-6657-1_2

CONAB, 2024. **Conab - Safra de grãos 2023/2024 está estimada em 294,1 milhões de toneladas** [WWW Document]. URL https://www.conab.gov.br/ultimas-noticias/5478-safra-de-graos-2023-2024-esta-estimada-em-294-1-milhoes-de-toneladas (accessed 7.15.24).

Crisosto, C.H., Grantz, D.A., Meinzer, F.C., 1992. **Effects of water deficit on flower opening in coffee (*Coffea arabica* L.)**. Tree Physiol. 10, 127–139.

DaMatta, F.M., Ronchi, C.P., Maestri, M., Barros, R.S., 2007. **Ecophysiology of coffee growth and production**. Braz. J. Plant Physiol. 19, 485–510.

Davis, A.P., Govaerts, R., Bridson, D.M., Stoffelen, P., 2006. **An annotated taxonomic conspectus of the genus *Coffea* (Rubiaceae)**. Bot. J. Linn. Soc. 152, 465–512.

de Oliveira, R.R., Cesarino, I., Mazzafera, P., Dornelas, M.C., 2014. **Flower development in *Coffea arabica* L.: new insights into MADS-box genes**. Plant Reprod. 27, 79–94.

Dedecca, D.M., 1957. **Anatomia e desenvolvimento ontogenético de *Coffea arabica* L. var. typica Cramer**. Bragantia 16, 315–366. https://doi.org/10.1590/S0006-87051957000100023

Deshmukh, A., 2021. **The World's Top Coffee Producing Countries** [WWW Document]. Vis. Capital. URL https://www.visualcapitalist.com/worlds-top-coffee-producing-countries/ (accessed 2.7.22).

Drinnan, J., Menzel, C.M., 1994. **Synchronization of anthesis and enhancement of vegetative growth in coffee (*Coffea arabica* L.) following water stress during floral initiation**. J. Hortic. Sci. 69, 841–849.

Garcia, A.L.A., Matiello, J.B., 2013. **Índice de abortamento, ao longo do desenvolvimento dos frutos, de flores com antese inibida pelo déficit hídrico em *C. arabica***.

Hamon, P., Hamon, S., Razafinarivo, N.J., Guyot, R., Siljak-Yakovlev, S., Couturon, E., Crouzillat, D., Rigoreau, M., Akaffou, S., Rakotomalala, J.-J., De Kochko, A., 2015. ***Coffea* Genome Organization and Evolution, in: Coffee in Health and Disease Prevention**. Elsevier, pp. 29–37. https://doi.org/10.1016/B978-0-12-409517-5.00004-8

Hoeberichts, F.A., Povero, G., Ibañez, M., Strijker, A., Pezzolato, D., Mills, R., Piaggesi, A., 2017. **Next generation sequencing to characterise the breaking of bud dormancy using a natural biostimulant in kiwifruit (*Actinidia deliciosa*)**. Sci. Hortic. 225, 252–263.

Lashermes, P., Combes, M.-C., Robert, J., Trouslot, P., D'Hont, A., Anthony, F., Charrier, A., 1999. **Molecular characterisation and origin of the *Coffea arabica* L. genome**. Mol. Gen. Genet. MGG 261, 259–266. https://doi.org/10.1007/s004380050965

López, M.E., Santos, I.S., de Oliveira, R.R., Lima, A.A., Cardon, C.H., Chalfun-Junior, A., 2021. **An overview of the endogenous and environmental factors related to the *Coffea arabica* flowering process**. Beverage Plant Res. 1, 1–16.

Magalhaes, A., Angelocci, L., 1976. **Sudden alterations in water balance associated with flower bud opening in coffee plants**. J. Hortic. Sci. 51, 419–423.

Mayer, J.L.S., Carmello-Guerreiro, S.M., Mazzafera, P., 2013. **A functional role for the colleters of coffee flowers**. AoB PLANTS 5, plt029. https://doi.org/10.1093/aobpla/plt029

Meyer, F.G., 1965. **Notes on wild *Coffea arabica* from southwestern Ethiopia, with some historical considerations**. Econ. Bot. 19, 136–151. https://doi.org/10.1007/BF02862825

Morais, H., Caramori, P.H., Koguishi, M.S., Ribeiro, A.M. de A., 2008. **Escala fenológica detalhada da fase reprodutiva de *Coffea arabica***. Bragantia 67, 257–260.

Nascimento, M.N. do, Alves, J.D., Soares, Â.M., Castro, E.M. de, Magalhães, M.M., Alvarenga, A.A. de, Silva, G.H., 2008. **Alterações bioquímicas de plantas e morfológicas de gemas de cafeeiro associadas a eventos do florescimento em resposta a elementos meteorológicos**. Ciênc. Rural 38, 1300–1307.

Nunes, F.L., Camargo, M.B.P. de, Fazuoli, L.C., Rolim, G. de S., Pezzopane, J.R.M., 2010. **Modelos agrometeorológicos de estimativa da duração do estádio floração-maturação para três cultivares de café arábica**. Bragantia 69, 1011–1018.

Penfield, S., 2024. **Beyond floral initiation: the role of flower bud dormancy in flowering time control of annual plants**. J. Exp. Bot. erae223. https://doi.org/10.1093/jxb/erae223

Pereira Gardin, J.P., 2006. **Desenvolvimento reprodutivo do cafeeiro: anatomia de vasos do xilema e dinâmica de carboidratos** (Tesis). UFLA, Lavras, MG.

Pezzopane, J.R.M., Pedro Júnior, M.J., Thomaziello, R.A., Camargo, M.B.P. de, 2003. **Escala para avaliação de estádios fenológicos do cafeeiro arábica**. Bragantia 62, 499–505.

Volsi, B., Telles, T.S., Caldarelli, C.E., Camara, M.R.G. da, 2019. **The dynamics of coffee production in Brazil**. PloS One 14, e0219742.

Voltan, R.B.Q., Fahl, J.I., Carelli, M.L.C., 2011. **Diferenciação de gemas florais em cultivares de cafeeiro**.

Wintgens, J.N., 2012. **Coffee - Growing, Processing, Sustainable Production: A Guidebook for Growers, Processors, Traders and Researchers**. John Wiley & Sons.

Yilma, A., Kufa, T., 2020. **Coffee Peaberry as A Potential Seed Source for Production**. Int. J. Res. Stud. Sci. Eng. Technol. 7, 30–35.

Yu, Q., Guyot, R., de Kochko, A., Byers, A., Navajas-Pérez, R., Langston, B.J., Dubreuil-Tranchant, C., Paterson, A.H., Poncet, V., Nagai, C., Ming, R., 2011. **Micro-collinearity and genome evolution in the vicinity of an ethylene receptor gene of cultivated diploid and allotetraploid coffee species (*Coffea*)**. Plant J. 67, 305–317. https://doi.org/10.1111/j.1365-313X.2011.04590.x

Zanetti, W.A.L., da Silva Marques, M., do Amaral, A.M.S., da Silva, A.B., Putti, F.F., Goes, B.C., 2021. **Analysis of the Technological Evolution of Coffee Production in Brazil**. J. Agric. Stud. 9, 352–362.