LILLIAN MAGALHÃES AZEVEDO

PLANT GROWTH REGULATORS AND ENVIRONMENTAL SIGNALLING REGULATING METABOLIC AND MOLECULAR PATHWAYS INVOLVED ON *Coffea arabica* REPRODUCTIVE DEVELOPMENT

LAVRAS-MG
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Orientador
Prof. Dr. Antonio Chalfun Junior

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REGULADORES DE CRESCIMENTO DE PLANTAS E SINALIZAÇÃO AMBIENTAL REGULANDO VIAS METABÓLICAS E MOLECULARES ENVOLVIDAS NO DESENVOLVIMENTO REPRODUTIVO DE *Coffea arabica*

Tese apresentada à Universidade Federal de Lavras como parte das exigências do Programa de Pós-Graduação em Fisiologia Vegetal, área de concentração em Fisiologia Vegetal, para a obtenção do título de Doutora.

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2024

Aos meus pais, Rodrigo e Marlene, cuja força e amor incondicionais me inspiraram a cada passo desta jornada.

Dedico

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RESUMO

O florescimento do cafeeiro é um processo que impacta significativamente a produtividade da cultura, sendo de extrema importância para a indústria cafeeira. Um dos principais desafios enfrentados pelos cafeicultores é a irregularidade e a assincronia na floração, que podem comprometer a qualidade e a quantidade da produção de café. Esta tese visa aprofundar o entendimento do papel dos hormônios vegetais no desenvolvimento reprodutivo do cafeeiro, com o objetivo de fornecer soluções práticas para melhorar a gestão desse processo. A tese está estruturada em duas partes principais. Na primeira parte, é apresentada a introdução e o referencial teórico, que fornecem a base necessária para as investigações subsequentes (segunda parte). Esta seção inclui um capítulo de livro que revisa o papel dos reguladores de crescimento de plantas (PGRs) em relação ao desenvolvimento reprodutivo de plantas relacionando-os com o cafeeiro, propondo sugestões para a aplicação desses reguladores no campo. O primeiro artigo desta seção é uma revisão que explora o papel do *FT* e *TFL1*, genes chave do florescimento, e sua interação com os hormônios vegetais, com um foco especial em plantas perenes. O segundo artigo apresenta os resultados de experimentos de campo realizados para entender como a aplicação de PGRs, especificamente giberelina (GA) e ácido abscísico (ABA), influenciam o florescimento do cafeeiro em diferentes fases do desenvolvimento reprodutivo, desde a indução até a formação dos botões florais, considerando também os fatores ambientais. Este estudo revelou como esses PGRs afetam as vias moleculares envolvidas no florescimento do cafeeiro e identificou pela primeira vez os genes relacionados às vias de biossíntese, sinalização e degradação de GA e ABA. Além disso, a pesquisa destacou a complexa interação entre giberelina, ácido abscísico e etileno durante o desenvolvimento dos botões florais. Com esta tese, esperamos proporcionar um entendimento mais profundo de como os hormônios vegetais regulam o florescimento do cafeeiro, propondo métodos para a regulação desses processos no campo. Nossa intenção é oferecer tecnologias inovadoras que possam melhorar as práticas agrícolas, enfrentando os desafios relacionados ao desenvolvimento reprodutivo em plantas perenes, como o cafeeiro, e contribuindo para a sustentabilidade e a produtividade da cultura.

Palavras-chave: *Coffea arabica*; florescimento; hormônios vegetais; expressão gênica.

ABSTRACT

The flowering of the coffee plant is a process that significantly impacts crop productivity, being of utmost importance to the coffee industry. One of the main challenges coffee growers face is the irregularity and asynchrony of flowering, which can compromise the quality and quantity of coffee produced. This thesis aims to deepen our understanding of the role of plant hormones in the reproductive development of coffee plants and to provide practical solutions to improve the management of this process. This thesis is divided into two main sections. The first section presents the introduction and theoretical framework, providing the necessary foundation for subsequent investigations (second section). This section includes a book chapter that reviews the role of plant growth regulators (PGRs) in reproductive development, linking them to coffee plants, and proposing suggestions for applying these regulators in the field. The first article in this section is a review exploring the role of *FT* and *TFL1*, key flowering genes, and their interactions with plant hormones, with a special focus on perennial plants. The second article presents the results of field experiments conducted to understand how the application of PGRs, specifically gibberellin (GA) and abscisic acid (ABA), influences the flowering of coffee plants at different stages of reproductive development, from induction to floral bud formation, as well as environmental factors. This study revealed how these PGRs affect the molecular pathways involved in coffee plant flowering and identified, for the first time, genes related to the biosynthesis, signaling, and degradation pathways of GA and ABA. Furthermore, this study highlighted the complex interactions between gibberellin, abscisic acid, and ethylene during floral bud development. With this thesis, we hope to provide a deeper understanding of how plant hormones regulate the flowering of coffee plants and propose methods to regulate these processes in the field. We aim to offer innovative technologies that can improve agricultural practices, address challenges related to reproductive development in perennial plants such as coffee, and contribute to the sustainability and productivity of the crop.

Keywords: *Coffea arabica*; flowering; plant hormones; gene expression.

INDICADORES DE IMPACTO

Os resultados desta tese sobre a regulação hormonal no desenvolvimento reprodutivo do cafeeiro possuem impactos significativos em várias áreas. Socialmente, a implementação das práticas sugeridas pode aumentar a produtividade e a qualidade da produção de café, beneficiando pequenos e grandes produtores, e garantindo a segurança alimentar. Tecnologicamente, a aplicação de reguladores de crescimento vegetal demonstrou ser uma ferramenta potencial na sincronização do florescimento e no aumento da produção, mitigando os efeitos adversos das mudanças climáticas, o que representa um avanço na tecnologia agrícola. Economicamente, ao melhorar a produtividade e a qualidade do café, espera-se um aumento na rentabilidade dos produtores e uma maior competitividade no mercado global. Culturalmente, a valorização do café, um produto de grande importância para a economia e identidade brasileira, é reforçada através da aplicação das práticas recomendadas. Ao promover técnicas que melhoram a sustentabilidade da produção de café, a tese contribui para a preservação e o reconhecimento das tradições culturais associadas ao cultivo e consumo do café. A pesquisa tem um caráter extensionista, pois envolve a colaboração de produtores de café e técnicos agrícolas, além de potencializar programas de extensão da UFLA para a disseminação das práticas recomendadas. Os territórios impactados abrangem regiões produtoras de café, particularmente no Brasil, afetando diretamente os produtores dessas áreas. O público diretamente beneficiado inclui produtores, pesquisadores, técnicos agrícolas, e a comunidade acadêmica. Os impactos se enquadram nas áreas temáticas de Meio Ambiente (5), Tecnologia e Produção (7), e Trabalho (8), conforme a Política Nacional de Extensão. Os resultados também estão alinhados com vários Objetivos de Desenvolvimento Sustentável (ODS) da ONU, incluindo ODS 2 (Fome Zero e Agricultura Sustentável), ODS 12 (Consumo e Produção Responsáveis), e ODS 13 (Ação contra a Mudança Global do Clima). Esta tese, ao proporcionar o entendimento da regulação hormonal no desenvolvimento reprodutivo do cafeeiro, bem como tecnologias inovadoras e práticas agrícolas, contribui para a sustentabilidade e a produtividade da cultura do café, assegurando benefícios econômicos, sociais e ambientais.

IMPACT INDICATORS

The results of this thesis on hormonal regulation in the reproductive development of coffee plants have significant effects in several areas. Socially, the implementation of these practices can increase the productivity and quality of coffee production, benefit small and large producers, and ensure food security. Technologically, the application of plant growth regulators (PGRs) has proven to be a potential tool for synchronizing flowering and increasing production, mitigating the adverse effects of climate change, and represents an advancement in agricultural technology. Economically, by improving the productivity and quality of coffee, an increase in producers' profitability and competitiveness in the global market is expected. Culturally, the appreciation for coffee, a product of great importance for the Brazilian economy and identity, is reinforced through the application of recommended practices. By promoting techniques that improve the sustainability of coffee production, this thesis contributes to the preservation and recognition of the cultural traditions associated with the cultivation and consumption of coffee. The research has an extensionist character, as it involves collaboration with coffee producers and agricultural technicians, in addition to enhancing UFLA's extension programs for the dissemination of recommended practices. The affected territories encompass coffee-producing regions, particularly in Brazil, directly affecting producers in these areas. The directly benefiting public includes producers, researchers, agricultural technicians, and the academic community. The impacts fall into the thematic areas of Environment (5), Technology and Production (7), and Work (8), according to the National Extension Policy. The results also align with several United Nations Sustainable Development Goals (SDGs), including SDG 2 (Zero Hunger and Sustainable Agriculture), SDG 12 (Responsible Consumption and Production), and SDG 13 (Climate Action). This thesis, by providing an understanding of hormonal regulation in the reproductive development of coffee plants, as well as innovative technologies and agricultural practices, contributes to the sustainability and productivity of coffee cultivation, ensuring economic, social, cultural, and environmental benefits.

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO

O cafeeiro é uma planta perene que possui comportamento policárpico, podendo apresentar mais de um evento de floração no ano, dependendo das condições ambientais de cada região (CAMARGO, 1985; BARROS et al., 1978; RENA; BARROS, 2004). Diferente de outras angiospermas, o ciclo fenológico do cafeeiro ocorre em dois anos, sendo que o primeiro ano é caracterizado por um período vegetativo e, o segundo ano, um período reprodutivo (CAMARGO; CAMARGO, 2001; LÓPEZ et al., 2021). Dessa forma, é necessário que haja um equilíbrio entre o desenvolvimento vegetativo e reprodutivo, o que torna mais complexo entender os mecanismos envolvidos no controle dessas fases em plantas perenes.

O desenvolvimento reprodutivo do cafeeiro é conhecido por ser afetado por diversas condições ambientais, tais como chuvas, temperatura e fotoperíodo (ALVIM, 1960; DRINNAN; MENZEL, 1995; QUIÑONES et al., 2011; SCHUCH et al., 1990a), além de fatores endógenos como os fitohormônios (LIMA et al., 2021; SCHUCH et al., 1990b, 1994). Contudo, ainda existem poucos estudos sobre os fatores fisiológicos e moleculares que controlam o desenvolvimento, tanto vegetativo quanto reprodutivo, o que limita a compreensão das vias envolvidas na regulação do ciclo fenológico do cafeeiro bem como o controle de processos que visem a melhoria da cultura.

Além disso, o café tem grande importância para o cenário mundial, destacando-se como a segunda commodity mais comercializada no mundo e uma bebida consumida em quase todos os países. Em 2023, as exportações mundiais de café atingiram cerca de 72,19 milhões de sacas, sendo 42,52 milhões de café arábica (58%) e 29,67 milhões de robusta (42%) no período de outubro de 2022 a abril de 2023. Nesse contexto, o Brasil se destaca como sendo o maior produtor e exportador mundial de café, responsável pela produção de 65,49 milhões de sacas, estabelecendo um novo recorde e superando a maior produção anterior de 65,47 milhões de sacas registrada em 2020 (ICO, 2023).

Para sustentar essa produção, é necessário a compreensão do desenvolvimento vegetativo e reprodutivo em culturas de interesse econômico como o cafeeiro, no qual o florescimento é um processo que afeta diretamente a produtividade de frutos, visto que é uma planta que possui um padrão de floração sequencial devido a assincronia no desenvolvimento das gemas antes e após a indução reprodutiva, o que reflete na desuniformidade de amadurecimento dos frutos (CARDON et al., 2022; DE OLIVEIRA et al., 2014;

MAJEROWICZ; SÖNDAHL, 2005), gerando implicações no custo de produção e na qualidade da bebida, uma vez que apresenta frutos em diferentes estádios de maturação no momento da colheita (DAMATTA et al., 2007; RENA; MAESTRI, 1985).

Visando entender o desenvolvimento reprodutivo do cafeeiro, dados de RNAseq foram gerados previamente pelo Laboratório de Fisiologia Molecular de Plantas (LFMP) da Universidade Federal de Lavras (UFLA), e foi verificado que genes da biossíntese e degradação de diversos hormônios, em especial giberelina (GA), ácido abscísico (ABA) e etileno foram diferencialmente expressos em cultivares de *Coffea arabica,* incluindo um mutante natural de florescimento contínuo (*Semperflorens*) (Lopez et al., 2024, em submissão). Dessa forma, esse trabalho propõe que esses hormônios desempenham um papel importante na indução e regulação do desenvolvimento floral do cafeeiro, entretanto, as vias moleculares relacionadas a esse processo ainda não foram exploradas.

2 REFERENCIAL TEÓRICO

2.1 Fenologia do cafeeiro

O ciclo fenológico do cafeeiro apresenta duas fases que ocorrem simultaneamente no período de dois anos: fase vegetativa e reprodutiva (GOUVEIA, 1984). Com a finalidade de facilitar as pesquisas e observações na cafeicultura, Camargo e Camargo (2001) esquematizaram as fases fenológicas de *Coffea arabica* de acordo com as condições tropicais do Brasil, subdividindo-as em seis fases distintas, as quais duas ocorrem durante o período vegetativo (1º ano fenológico), seguido das quatro fases que compreende o período reprodutivo (2º ano fenológico).

No primeiro ano fenológico, a primeira fase é caracterizada pela vegetação e formação das gemas foliares, que ocorre nos meses de setembro a março, quando os dias apresentam fotoperíodo acima de 13 e 14 horas de luz, portanto, nesse período o cafeeiro sofre a influência de dias longos, desenvolvendo ramos plagiotrópicos a partir de ramos ortotrópicos (CAMARGO; FRANCO, 1985). Já na segunda fase, durante os meses de abril a agosto, os dias se tornam mais curtos, favorecendo a indução e maturação das gemas florais a partir das gemas axilares vegetativas formadas na fase anterior (GOUVEIA, 1984). Dessa forma, percebe-se que o desenvolvimento vegetativo característico da primeira fase influencia diretamente as próximas fases, afetando principalmente a quantidade de flores a serem formadas e, consequentemente, a produtividade (CAMARGO; CAMARGO, 2001; LIVRAMENTO, 2010;

THOMAZIELLO et al., 2000; MAJEROWICZ; SONDAHL, 2005). Os dois últimos meses dessa etapa, julho e agosto, é caracterizado por um período frio e seco, típicos do inverno brasileiro. Nesse período é observado que as gemas em estádio G4 cessam seu crescimento, sendo consideradas dormentes por alguns autores (CAMARGO; CAMARGO, 2001; CAMARGO; FRANCO, 1985; WORMER; GITUANJA, 1970).

O segundo ano fenológico e terceira fase inicia-se com as chuvas e aumento de temperatura em setembro a dezembro, quando ocorre um aumento do potencial hídrico nas gemas florais, sendo retomado o crescimento, que culmina na antese dos botões (florada). Após a antese, as flores são fecundadas dando origem aos "chumbinhos", que consistem em frutos em estádio inicial (CAMARGO; CAMARGO, 2001; LIVRAMENTO, 2010). A quarta e quinta fase, que ocorrem geralmente de janeiro até junho do segundo ano, são caracterizadas pela granação e maturação dos frutos, respectivamente. O ciclo fenológico do cafeeiro termina quando há senescência de ramos terciários e quaternários, processo conhecido como autopoda (CAMARGO; CAMARGO 2001; MORAIS et al., 2008).

Recentemente, López et al. (2021) realizaram uma revisão abrangente dos fatores internos e ambientais que influenciam o processo de floração de *Coffea arabica*, propondo um modelo para a indução e desenvolvimento floral dessa espécie em condições ambientais brasileiras (Fig. 1). De acordo com a literatura, a indução floral ocorre entre fevereiro e abril, mas López et al. (2021) sugerem que esse processo pode começar já em janeiro e se estender até julho para diferentes cultivares, tanto as precoces quanto as tardias. O período de desenvolvimento floral ocorre durando aproximadamente 3 meses nas cultivares precoces e até 5 meses nas tardias, destacando que essa fase varia significativamente entre as cultivares. Nas cultivares precoces, por apresentarem uma fase de desenvolvimento floral mais curta a antese é antecipada. Em contrapartida, enquanto as cultivares tardias iniciam com uma floração menos intensa, as cultivares precoces já progridem no crescimento dos frutos. O ciclo de desenvolvimento dos frutos de ambas as cultivares se completa no início do segundo ano fenológico, seguido pelo amadurecimento e colheita. Esta revisão de López et al. (2021) oferece uma visão detalhada e atualizada dos mecanismos de floração e frutificação do cafeeiro, destacando as variações entre diferentes cultivares e proporcionando um modelo útil para melhorar a gestão e a produtividade da cultura em condições brasileiras.

Para facilitar a compreensão sobre o desenvolvimento reprodutivo do cafeeiro, Morais et al. (2008) elaboraram uma escala que representa desde o desenvolvimento das gemas até a maturação dos frutos, de maneira que as gemas são classificadas por tamanho e os frutos pela

cor: desenvolvimento da gema floral (G), floração (FL), frutificação (F) e maturação (M). A fase G foi subdividida da seguinte forma: G1 - gemas indiferenciadas; G2 - gemas intumescidas; G3 - gemas com até 3 mm de comprimento; G4 - gemas medindo 3,1 a 6 mm de comprimento; G5 - gemas de 6,1 a 10 mm (coloração verde claro); G6 - gema maior que 10 mm e (coloração branca). A escala proposta para o desenvolvimento das gemas é ilustrada na Figura 1b.

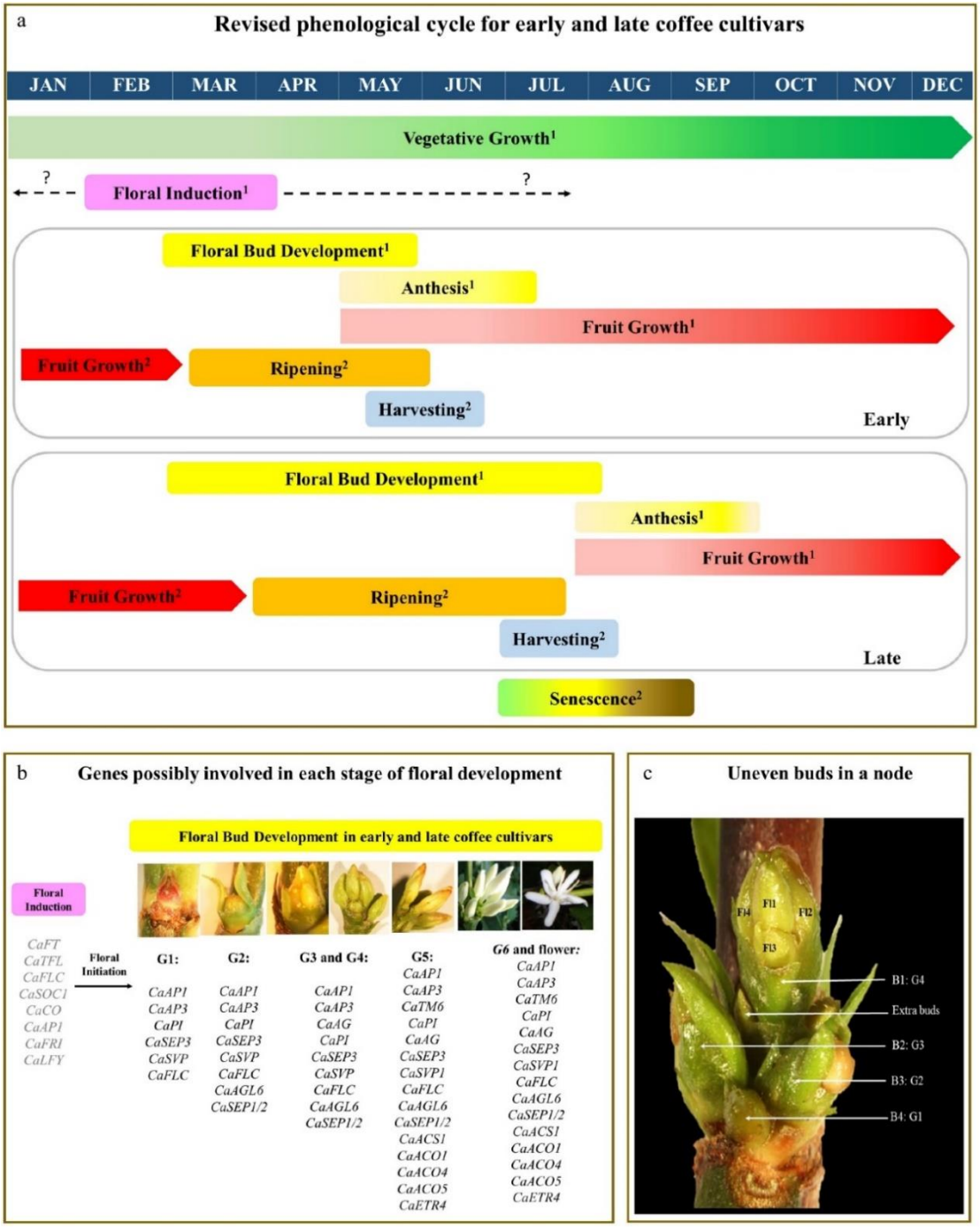

Fig.1 (a) - Modelo proposto para a indução e desenvolvimento floral de *C. arabica* em condições ambientais brasileiras. Modelo revisado para o desenvolvimento vegetativo e reprodutivo de cultivares de *C. arabica* quanto ao tempo de floração para cultivares precoces e tardias. (b) - Genes que estão

possivelmente envolvidos em cada estádio do desenvolvimento floral. (c) - Foto descrevendo o desenvolvimento desigual de botões florais em um nó localizado no ramo de um cafeeiro. Quatro botões florais (B1, B2, B3, B4) são apresentados em ordem crescente de emergência com a respectiva identificação dos estádios de desenvolvimento (G4, G3, G2, G1). No botão floral B1 estão indicadas as quatro flores que terminam seu desenvolvimento com a antese. Assim, cada nó pode potencialmente produzir 16 flores (quatro flores de cada botão), mas B3 e B4 geralmente permanecem latentes e, em alguns casos, botões florais extras podem ser desenvolvidos.

Fonte: LÓPEZ et al., (2021).

2.2 Desenvolvimento reprodutivo do cafeeiro

O cafeeiro é uma planta perene que possui porte arbustivo, com ramos plagiotrópicos (horizontais) se desenvolvendo a partir do ramo ortotrópico (vertical), podendo atingir quatro metros de altura dependendo do manejo da cultura (CASTRO et al., 1987). As inflorescências, denominadas glomérulos, são formadas nos ramos plagiotrópicos nas axilas das folhas, as quais possuem de 4 a 5 gemas, totalizando aproximadamente de 8 a 10 gemas por nó. Cada gema, por sua vez, pode dar origem a até 4 botões florais, ou até mesmo dar origem a um ramo vegetativo (MAJEROWICZ; SONDAHL, 2005).

Todas espécies do gênero *Coffea* são diplóides (2n=2x=22) e apresentam fecundação cruzada com sistema de autoincompatibilidade, com exceção de *Coffea arabica*, originada do cruzamento natural entre *Coffea eugeniodes* e *Coffea canephora* (LASHERMES et al., 1999), que é uma espécie alotetraplóide (2n= 4x = 44) e autógama, ou seja, cerca de 90% da sua reprodução ocorre por autofecundação (CHARRIER; BERTHAUD, 1985; CONAGIN; MENDES, 1961). Com base em seu padrão de floração, o cafeeiro é classificado como uma planta gregária, isto é, plantas localizadas numa certa extensão geográfica florescem simultaneamente, com eventos de floradas variáveis (GUIMARÃES; MENDES, 1996).

O florescimento do cafeeiro é um processo complexo, pois além de ser resultante do comportamento fenológico da cultura, também sofre a influência de fatores exógenos, tais como, fotoperíodo, temperatura, umidade, sombra, altitude e suprimento de água (DAMATTA et al., 2019; MEYLAN et al., 2017; MIRANDA et al; 2020; QUIÑONES et al., 2011; RONCHI; MIRANDA, 2020; VÉLEZ et al., 2000), além de fatores endógenos como hormônios vegetais (MATSUMOTO; LOPEZ, 2016; SCHUCH et al., 1990b, 1992, 1994). Esse processo é dividido em quatro fases: indução floral, diferenciação floral, dormência e antese (PEZZOPANE et al., 2003; RENA; MAESTRI 1985).

Em relação ao estímulo indutivo do comprimento do dia, o cafeeiro é classificado como uma planta de dia curto com fotoperíodo crítico de 13 a 14 horas (CAMARGO, 1985; SCHUCH et al., 1990a). Porém, em condições de 18 horas de luz, diferentes cultivares apresentaram maior

número de ramos e folhas e um florescimento mais intenso, sugerindo que esse florescimento mais intenso aconteceu devido ao maior desenvolvimento vegetativo, e que a idade dos tecidos parece ser elemento determinante na capacidade de indução e diferenciação das gemas florais (MONACO et al., 1978). Além disso, em regiões equatoriais, onde não se observa grandes variações do período diurno, o cafeeiro não apresenta uma estação de floração específica (CAMAYO et al., 2003; WORMER; GITUANJA, 1970). Dessa forma, o principal estímulo indutivo e a necessidade de um fotoperíodo específico para o florescimento do cafeeiro ainda não é claro.

A maioria dos estudos tem focado no período de deficit hídrico moderado combinado ou não com baixas temperaturas durante a fase de dormência das gemas florais (ALVIM, 1960; DAMATTA; RAMALHO, 2006; MIRANDA et al., 2020; RONCHI et al., 2015; RONCHI; MIRANDA, 2020). Diversos autores sugerem que as gemas em estádio G4 tornam-se dormentes (BARROS et al., 1978; GOUVEIA, 1984; MES, 1957), sendo necessário um período de seca para completar eventos fisiológicos e morfológicos, possibilitando que as gemas florais estejam prontas a responder a estímulos externos (BARROS; MAESTRI,1978; RENA et al., 1994 ; SOARES et al., 2005), e que a antese é iniciada logo após as primeiras chuvas ou irrigação, em virtude de uma rápida turgescência dos botões florais, promovendo a quebra de dormência das gemas (ALVIM, 1960; LIVRAMENTO, 2010; MAGALHÃES; ANGELOCCI 1976; MES, 1957; RENA; MAESTRI, 1985). Entretanto, nunca foi demonstrado nenhum mecanismo físico, metabólico ou molecular relacionado à dormência da gema do cafeeiro, sendo este um termo discutível (LÓPEZ et al., 2021).

No Brasil, onde há ocorrência de um período seco bem definido, a florada principal acontece nos meses de setembro/início de outubro, sendo essa responsável por mais de 90% da produção. Já em regiões equatoriais chuvosas, as plantas possuem floração indefinida, havendo vários eventos de florada durante o ano (CARR, 2001; DAMATTA et al., 2007; REIS; CUNHA, 2010). Dessa forma, observou-se que cafeeiros com disponibilidade hídrica constante apresentam diversas floradas com intensidade variável, e que, por outro lado, quando passam por um período seco seguido de reidratação apresentam floradas mais uniformes (CRISOSTO et al. 1992; MASARIRAMBI et al., 2009; MIRANDA et al., 2020; RONCHI et al., 2015). Outros estudos evidenciam que a queda da temperatura pode desempenhar um papel relevante na quebra da dormência dos botões florais do cafeeiro (BROWING et al., 1977; RAMÍREZ et al., 2001; DRINNAN; MENZEL, 1995).

De acordo com essas observações foi sugerido que o estímulo para promover a antese no cafeeiro é desencadeado nas raízes durante o período seco e , após a reidratação, o sinal é transportado pelo xilema até os botões florais, promovendo sua abertura. Nesse contexto, alguns trabalhos tem mostrado o envolvimento dos hormônios vegetais sendo sinais importantes para desencadear a abertura floral no cafeeiro, dentre eles, o etileno tem chamado a atenção dos pesquisadores (LIMA et al., 2021; LÓPEZ et al., 2022 SANTOS et al., 2022). O transporte fisiológico do etileno ocorre através do xilema na forma de seu precursor biossintético, o ácido 1-aminociclopropano carboxílico (ACC), atuando como um sinal que é produzido nas raízes em condições de déficit hídrico e transportado para a parte aérea após a reidratação da planta (LIMA et al., 2021; LIU et al., 2013; MENG et al., 2014). Da mesma forma, os níveis de citocinas (CKs) aumentam no xilema após a reidratação, sugerindo um papel importante desse fitohormônio no desenvolvimento dos botões florais e na antese do cafeeiro (BROWNING, 1973).

Além disso, foi sugerido também que o ACC desempenha um papel crucial na antese do café, independentemente do etileno. Isto é particularmente evidente durante o período de re-irrigação, onde se observa um aumento nos níveis de ACC, levando à antese (LÓPEZ et al., 2021, 2022). O papel do ACC na antese do café é ainda apoiado pela descoberta de que o ACC exógeno promove a abertura dos botões florais (LÓPEZ et al., 2022). Além disso, alterações nos níveis e na sensibilidade do etileno, induzidas pela seca e pela reidratação, estão associadas à antese do café (LIMA et al., 2021).

Por outro lado, a dormência das gemas foi atribuída ao ácido abscísico (ABA), que tem seus níveis aumentados em condições de déficit hídrico (BROWNING; HOAD; GASKIN, 1970; LÓPEZ et al., 2022)**.** Entretanto, existem estudos que demonstram que o conteúdo de ABA nas gemas nem sempre está correlacionado com o grau de dormência (BROWNING, 1973). Essa aparente incoerência pode ser atribuída às interações entre o ABA e outros hormônios, uma vez que o estado de dormência e o crescimento são regulados pelo balanço entre inibidores de crescimento, como o ABA, e substâncias promotoras do crescimento, como as citocininas e as giberelinas (GAs). Dessa forma, a quebra da dormência das gemas pode estar relacionada ao equílibrio entre GA e ABA, que atuam de forma antagônica nesse processo (BROWNING, 1970, 1973).

A concentração de GA aumenta em gemas após a rega, convertendo GAs conjugadas em GAs bioativas (BROWNING, 1973; UNIGARRO et al., 2019). A aplicação de giberelina (GA3) promoveu a antese em botões florais maiores que 4mm independentemente da chuva ou

irrigação, resultando em uma maior sincronização do amadurecimento dos frutos (SCHUCH et al., 1990b). Consistente com isso, o déficit hídrico relativamente leve não foi suficiente para quebrar a dormência das gemas no cafeeiro, mas a aplicação exógena de GA foi crucial para desencadear a antese (SCHUCH et al., 1992). Além disso, a pulverização foliar de GA3 resultou em uma florada mais concentrada e frutos em estádio de maturação mais uniformes no momento da colheita (MATSUMOTO; LOPEZ, 2016). No entanto, os mecanismos moleculares envolvidos na via da GA bem como sua aplicação durante a fase de indução das gemas florais ainda não foram explorados no cafeeiro.

Embora muitos estudos tenham focado em compreender o florescimento em cafeeiro, pouco se sabe sobre a indução e o processo de desenvolvimento de gemas reprodutivas. A maioria das informações disponíveis abrange as fases mais avançadas de desenvolvimento das gemas florais, sendo necessário uma compreensão mais detalhada, principalmente a nível fisiológico e molecular envolvendo todas as fases do processo reprodutivo.

2.3 Vias regulatórias do desenvolvimento floral em plantas

O florescimento é um momento crucial para o sucesso reprodutivo das plantas, influenciando na sobrevivência e perpetuação das espécies. Análises moleculares, principalmente em plantas modelo, têm fornecido informações significativas sobre como as plantas integram sinais endógenos e exógenos para regular o florescimento. As principais vias de regulação estudadas incluem as vias dependentes de fotoperíodo, idade, vernalização, autônoma e giberelinas (AMASINO, 2010; KIM, 2020; LEIJTEN et al., 2018). Recentemente, outras vias adicionais foram exploradas, como aquelas dependentes de açúcares, temperatura ambiente e outros hormônios (BOLOURI MOGHADDAM; VAN DEN ENDE, 2013; IZAWA, 2021; SUSILA et al., 2018). Em uma revisão sobre os fatores que regulam o desenvolvimento reprodutivo do cafeeiro, López et al. (2021) propuseram hipóteses sobre como essas vias poderiam estar envolvidas nesse processo (Fig. 2). Essas vias serão discutidas a seguir, relacionando os mecanismos do cafeeiro com os de outras espécies.

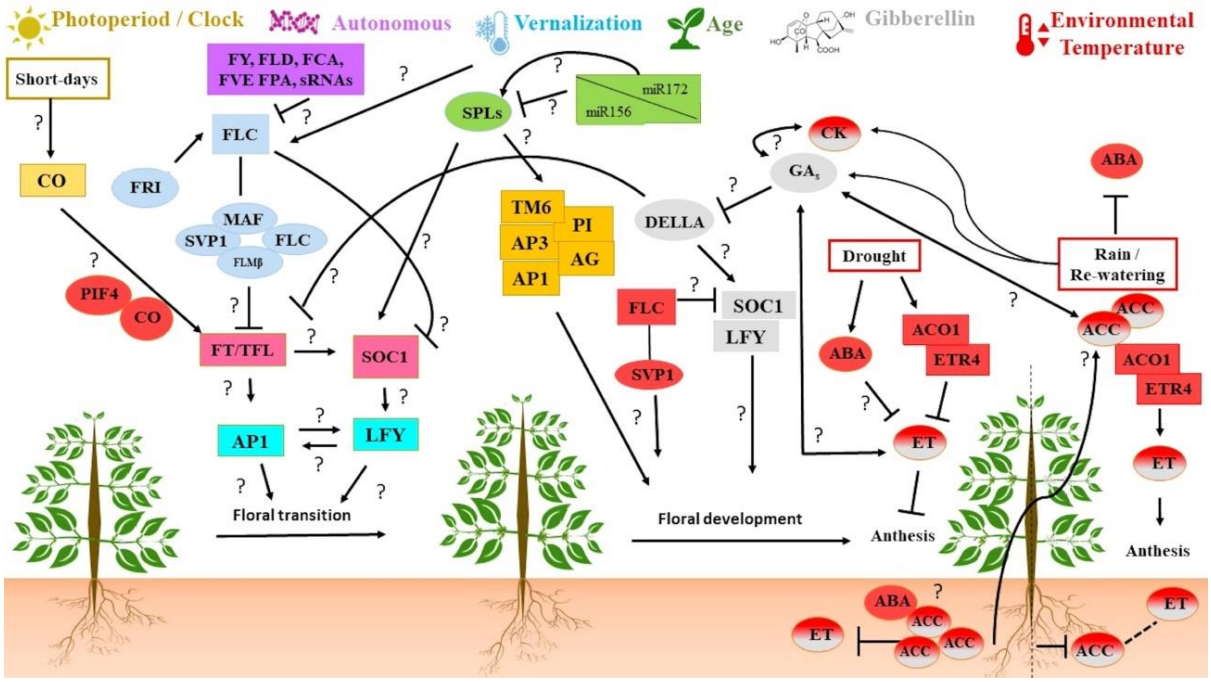

Fig. 2 - Resumo das vias moleculares possivelmente envolvidas no período de floração e processos de desenvolvimento em *C. arabica*: fotoperíodo/relógio (amarelo), autônomo (roxo), vernalização (azul celeste), idade (verde), giberelina (cinza) e temperatura ambiente (vermelho). ABA: ácido abscísico, ACC: ácido 1-aminociclopropano-1-carboxílico, ACO: ácido 1-aminociclopropano-1-carboxílico oxidase AG: AGAMOUS, AP1: APETALA 1, AP3: APETALA 3, CK: CITOCININA, CO: CONSTANS, ET : ETILENO, ETR4: RECEPTOR DE ETILENO 4, FCA: FLOWERING LOCUS CA, FLC: FLOWERING LOCUS C, FLD: FLOWERING LOCUS D, FLM: FLOWERING LOCUS M, FPA: FLOWERING LOCUS PA, FRI: FRIGIDA, FVE: FLOWERING LOCUS VE, FY : FLOWERING LOCUS Y, GA: GIBBERELINA, LFY: FOLHADO, MAF: MADS AFETANDO FLOR PI: PISTILLATA, PIF4: FATOR DE INTERAÇÃO DE FITOCROMO 4, SOC1: SUPRESSOR DE SUPEREXPRESSÃO DE CONSTANS 1, SPL: PROTEÍNA DE LIGAÇÃO AO PROMOTOR SQUAMOSA, sRNAs: RNAs PEQUENOS , SVP: FASE VEGETATIVA CURTA, TFL: FLOR TERMINAL, TM6: TOMATO MADS BOX GENE 6.

Fonte: LÓPEZ et al., (2021).

FLOWERING LOCUS T (FT) é um fator de transcrição bem caracterizado, atuando como chave para a indução do florescimento. Através da indução por sinais internos e externos o *FT* é expresso nas folhas e a respectiva proteína é transportada pelo floema para chegar no meristema apical do caule (SAM, do inglês *Shoot Apical Meristem*) (TSUJI, 2017). No SAM, FT se liga ao fator de transcrição bZIP, FD e proteína 14-3-3 para formar um complexo de ativação floral (FAC) e, assim, promover a expressão de *SUPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1)*, resultando na transcrição de *APETALA 1 (AP1)* e *LEAFY (LFY)*, responsáveis pela identidade do meristema floral e formação dos órgãos florais (FERNÁNDEZ et al., 2016; TSUJI, 2017).

Muitas plantas utilizam o fotoperíodo (comprimento do dia), para perceber as mudanças sazonais regulando, assim, o tempo de floração. A indução fotoperiódica da floração ocorre por

meio de um sistema no qual os níveis de expressão de *CONSTANS* (*CO*) são afetados pela duração do dia. Em plantas de dias longos, como *Arabidopsis*, a expressão de *CO* se estende durante o dia e a luz aumenta a estabilidade da proteína CO, que se liga diretamente à região promotora de *FT*, favorecendo sua transcrição em condições indutivas de luz (KIM, 2020; VALVERDE et al., 2004).

Existem poucos estudos que relacionam essas vias no cafeeiro, principalmente a nível molecular. No entanto, um estudo de Cardon et al. (2022) observou que a análise dos padrões de expressão gênica ao longo de um ano em diversos genótipos de *Coffea* sp. evidenciou uma transcrição contínua do gene *CaFT1* de fevereiro a outubro, indicando uma janela potencial para a indução floral. Em contraste, embora a expressão do gene *CaCO* variasse diariamente e mensalmente em resposta à duração do dia, os genes sensíveis à temperatura, *CaFLC* e *CaPIF4*, não apresentaram correlação significativa com as variações de temperatura. Estes achados sugerem a existência de um continuum na indução floral do cafeeiro, permitindo múltiplos pontos de início para a ativação floral, o que pode explicar a assincronia e a ocorrência prolongada de eventos de floração em espécies perenes tropicais.

Além dos estímulos ambientais, a idade das plantas também influencia na competência para florescer, portanto, foi proposto que microRNAs (pequenos RNAs não codificantes) estão envolvidos na transição floral, fazendo com que a planta transite da fase juvenil vegetativa para adulta reprodutiva (HUIJSER; SCHMID, 2011; YAMAGUCHI; ABE, 2012). Enquanto miR156 é altamente expresso durante a fase vegetativa e diminui com o aumento da idade da planta, miR172 se acumula nas folhas e botões florais ao longo do tempo, quando a planta possui maior aporte vegetativo e maior concentração de açúcares. Já o miR156 regula negativamente a transcrição de 11 dos 17 fatores de transcrição *SQUAMOSA PROMOTER BINDING-LIKE (SPL)* relacionados ao controle da floração. A diminuição de miR156 é acompanhada por um aumento na expressão de *SPL*, resultando na expressão de genes integradores da via floral *FT* e *SOC1*, bem como na ativação de genes de identidade de meristema floral *AP1, LFY* e *FUL* (SPANUDAKIS; JACKSON, 2014). No cafeeiro, alguns miRNAs já foram identificados, demonstrando envolvimento na microsporogênese, na regulação de respostas hormonais e na modulação da expressão de fatores de transcrição associados ao florescimento (DE OLIVEIRA et al., 2024; RIBEIRO et al., 2024).

Além das vias descritas anteriormente, os fitohormônios também têm sido amplamente estudados quanto ao seu envolvimento na transição floral. Dentre eles, as GAs apresentam um papel importante na transição da fase juvenil para fase adulta e iniciação floral, além de regular

muitos outros aspectos do crescimento e desenvolvimento de plantas, incluindo germinação de sementes, expansão foliar, biossíntese de clorofila e alongamento do hipocótilo (DAVIES, 2012). A biossíntese das GAs ocorre a partir do seu precursor ent-caureno, e envolve a atividade da GA20-oxidase e GA3-oxidase que convertem GAs inativas em formas bioativas (GA1, GA3, GA4, GA7), enquanto as GA2-oxidase estão envolvidas na desativação das GAs (HEDDEN; THOMAS, 2016). Uma vez formadas, as GAs bioativas se ligam ao seu receptor GIBBERELLIN INSENSITIVE DWARF1 (GID1) para promover a degradação das proteínas DELLA que atuam como inibidores das respostas de GA (SUN, 2010).

As GAs induzem o florescimento em plantas de dias longos, incluindo *Arabidopsis*, ao promover a expressão dos integradores florais, como *LFY* e *SOC1*, por meio de um mecanismo dependente de DELLA (BAO et al., 2020). As proteínas DELLAs estão amplamente envolvidas na promoção da expressão de *FT* mediada por GA, afetando negativamente vários ativadores de *FT*, como *CO* (WANG et al., 2016; XU et al., 2016). Baixos níveis de GA resultam no acúmulo de proteínas DELLA, que atrasam o florescimento independente do fotoperíodo, através da regulação dos genes *SPLs* nas folhas e no meristema caulinar (GALVÃO et al., 2012). Mutantes nos genes envolvidos na biossíntese de GA não florescem em condições de dias curtos e exibem um atraso na floração em condições de dias longos (MUTASA-GÖTTGENS; HEDDEN, 2009; WILSON et al., 1992).

Já foi demonstrado em diversos estudos que em plantas de dia longo, GA promove o florescimento em dias curtos, portanto, GA substitui exigências de fotoperíodo indutivo. Mas em plantas consideradas de dia curto, como o cafeeiro, GA pode induzir o florescimento em dias longos? Visto que há algumas divergências nos resultados dos estudos citados anteriormente que visam entender o fotoperíodo indutivo, o cafeeiro é mesmo uma planta de dia curto? Nesse sentido, o papel das GAs na indução floral foi bem estabelecido em plantas modelo, como *Arabidopsis*, porém muito pouco compreendido em plantas lenhosas perenes como o cafeeiro, principalmente durante as fases que precedem a antese dos botões florais.

2.4 Importância socioeconômica da cafeicultura

O cafeeiro pertence à família *Rubiaceae* e ao gênero *Coffea* e possui 130 espécies identificadas (DAVIS, 2021), porém apenas duas são cultivadas, *Coffea arabica* e *Coffea canephora*, sendo elas responsáveis por aproximadamente 60% e 40% da produção mundial de café, respectivamente. O cultivo dessas duas espécies se estende por mais de 50 países e juntas totalizaram uma produção de 168 milhões de sacas de 60kg beneficiadas no ano-safra 2022/23

(ICO, 2023). Nesse contexto, o Brasil é o país que mais se destaca em termos de produção e exportação, além de ser o segundo maior consumidor do produto, atrás apenas dos Estados Unidos (ICO, 2023).

O cafeeiro é uma cultura originária da Etiópia e foi introduzido no norte do Brasil no século XVIII, onde se adaptou bem às variações edafoclimáticas, estabelecendo-se rapidamente ao longo do território nacional (GUERREIRO-FILHO et al., 2008). Atualmente, estima-se que a área destinada à cafeicultura brasileira corresponde a 2,24 milhões de hectares (CONAB, 2023).

De acordo com o terceiro levantamento realizado em setembro pela Companhia Nacional de Abastecimento, o ano de 2021 apresentou uma safra marcada pelo efeito da bienalidade negativa, o que resultou em menores estimativas de produção, indicando um total de 46,87 milhões de sacas beneficiadas. Essa redução de 25,7% comparada ao ano anterior também foi resultado de condições climáticas desfavoráveis, como prolongados períodos secos e temperaturas elevadas durante fases importantes do desenvolvimento do cafeeiro (CONAB, 2021).

Diante dos impactos negativos das mudanças climáticas previstos para agricultura, em especial para a cafeicultura brasileira (BUNN et al., 2015) e considerando a crescente demanda mundial por alimentos, fica evidente a necessidade de estudos detalhados sobre o metabolismo e desenvolvimento de culturas perenes em resposta a estímulos ambientais.

REFERÊNCIAS

ALVIM, P. D. T. Moisture stress as a requirement for flowering of coffee. **Science,** v. 132, n. 3423, p. 354-354, 1960.

AMASINO, R. Seasonal and developmental timing of flowering. **The Plant Journal**, v. 61, n. 6, p. 1001–1013, 2010.

BAO, S. et al. New insights into gibberellin signaling in regulating flowering in *Arabidopsis*. **Journal of Integrative Plant Biology**, v. 62, n. 1, p. 118–131, 2020.

BARROS, R. S.; MAESTRI, M.; COONS, M. P. The physiology of flowering in coffee: a review. **J. Coffee Res.,** v. 8, p. 29-73, 1978.

BOLOURI MOGHADDAM, M. R.; VAN DEN ENDE, W. Sugars, the clock and transition to flowering. **Frontiers in Plant Science**, v. 4, 2013.

BREITLER, J.-C. et al. Full moonlight-induced circadian clock entrainment in *Coffea arabica*. **BMC Plant Biology**, v. 20, n. 1, p. 24, 15 Jan. 2020.

BROWING, G. Environmental control of flower bud development in *Coffea arabica* L. In LANDSBERG, J.J.; CUTTING, C.V. (Ed.). **Environmental effects on crop physiology**. New York: Academic, p. 321- 331, 1977.

BROWING, G. Flower bud dormancy in *Coffea Arabica* L. I. Studies of gibberellin in flower buds and xylem sap and of abscísic acid in flower buds about dormancy release. **Journal of Horticultural Science**, London, v. 48, n. 1, p. 29-41, Jan. 1973.

BROWING, G.; HOAD, G. V.; GASKING, G. Identification of abscisic acid in flower buds of *Coffee arabica* L. **Planta**, Berlin, v. 94, n. 3, p. 213-219, 1970.

BUNN, C. et al. A bitter cup: climate change profile of global production of Arabica and Robusta coffee. **Climatic Change**, v. 129, n. 1, p. 89–101, 2015.

CAMARGO, A. P.; CAMARGO, M. B. P. Definição e esquematização das fases fenológicas do cafeeiro arábica nas condições tropicais do Brasil. **Bragantia**, v. 60, n. 1, p. 65-68, 2001.

CAMARGO, A. P.; FRANCO, C. F. Clima e fenologia do cafeeiro. In: **Cultura de café no Brasil:** manual de recomendaçõe**s**. 5. ed. Rio de Janeiro: Instituto Brasileiro do Café, Ministério da Indústria e Comércio, 1985. p.19-50.

CAMAYO V. et al. Desarrollo floral del cafeto y su relación con las condiciones climaticas de Chinchina Caldas. **Revista Cenicafé**, v. 54, n. 1, p. 35- 49, 2003.

CANNELL M. G. R. Physiology of the coffee crop. In: MN Clifford and KC Willson, **eds. Coffee Botany, Biochemistry and Production of Beans and Beverage**, New York: Croom Helm, v. 134, p. 108-134, 1985.

CARDON, C.H. et al. Expression of coffee florigen CaFT1 reveals a sustained floral induction window associated with asynchronous flowering in tropical perennials. **Plant Science**, v. 325, p. 111479, 2022.

CARR M. K. V. The water relations and irrigation requirements of coffee. **Experimental Agriculture,** v. 37, n. 1, 2001.

CASTRO, P. R. C.; FERREIRA, S. O.; YAMADA, T. **Ecofisiologia da produção agrícola**. Piracicaba: Potafós, p. 249, 1987.

CHARRIER, A.; BERTHAUD, J. Botanical classification of coffee. In: CLIFFORD, M.N.; WILLSON, K.C. (Ed.). **Coffee**: botany, biochemistry, and production of beans and beverages. London: Croom Helms, 1985. p. 167-195.

CONAGIN, C. H. T. M.; MENDES, A. J. T. Pesquisas citológicas e genéticas em três espécies de *Coffea*: auto-incompatibilidade em *Coffea canephora*. **Bragantia**, v. 20, n. 2, p. 787-804, 1961.

CRISOSTO, C. H.; GRANTZ, D. A.; MEINZER, F. C. Effect of water deficit on flower opening in coffee (*Coffea arabica* L.). **Tree Physiol**., v. 10, p. 127-139, 1992.

DaMATTA F. M. et al. Ecophysiology of coffee growth and production. **Brazilian Journal of Plant Physiology**, v. 19, n. 4, p. 485–510, 2007.

DaMATTA F. M. et al. Why could the coffee crop endure climate change and global warming to a greater extent than previously estimated? **Climatic Change**, v. 152, n. 1, p. 167-178, 2019.

DaMATTA, F. M.; RAMALHO, J. D. C. Impactos da seca e estresse de temperatura sobre a fisiologia cofeee e produção: uma revisão. **Revista Brasileira de Fisiologia Vegetal**, v.18, p. 5581, 2006.

DAVIES, P. J. **Plant Hormones and their Role in Plant Growth and Development**. [s.l.] Springer Science & Business Media, 2012.

DAVIS, A. P.; RAKOTONASOLO, F. Six new species of coffee (*Coffea*) from northern Madagascar. **Kew Bulletin**, v. 76, p. 497-511, 2021.

DE OLIVEIRA, K. K. P. et al. 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, v. 8, n. 1, p. e561, 2024.

DE OLIVEIRA, R. R. et al. Flower development in *Coffea arabica* L.: New insights into MADS-box genes. **Plant Reproduction**, v. *27*, n. 2, p. 79–94, 2014.

DRINNAN, J. E.; MENZEL, C. M. Synchronisation of anthesis and enhancement of vegetative growth in coffee (*Coffea arabica* L.) following water stress during flower initiation. **Journal of Horticultural Science**, v. 10, p.841-849, 1995.

FERNÁNDEZ, V. et al. Photoperiodic and thermosensory pathways interact through *CONSTANS* to promote flowering at high temperatures under short days. **The Plant Journal**, v. 86, n. 5, p. 426–440, 2016.

FLORES, C. et al. Gibberellic acid, paclobutrazol, fluoridone, abscisic acid affecting the flowering and fruit set of coffee. **Journal of Agriculture of the University of Puerto Rico**, v. 89, p. 159–168, 1 Jul. 2005.

GALVÃO, V. C. et al. Spatial control of flowering by DELLA proteins in *Arabidopsis thaliana*. **Development**, v. 139, n. 21, p. 4072–4082, 1 Nov. 2012.

GOUVEIA, N. M. **Estudo da diferenciação e crescimento de gemas florais de Coffea arabica L: observações sobre a antese e maturação dos frutos**. 1984. 237 p. Dissertação (Mestrado) - Universidade Estadual de Campinas, Campinas.

GUERREIRO-FILHO, O. et al. Origem e Classificação Botânica do Cafeeiro. In: CARVALHO, C. H. S. (Ed.). **Cultivares de café: origem, características e recomendações.** Brasília: Embrapa Café, 334p, 2008.

GUIMARÃES, R. J.; MENDES, A. N. G. Morfologia e fisiologia do cafeeiro. Lavras: UFLA, 1996. 38p.

HEDDEN, P.; THOMAS, S. G. **Annual Plant Reviews, The Gibberellins**. [s.l.] John Wiley & Sons, 2016.

HUIJSER, P.; SCHMID, M. The control of developmental phase transitions in plants. **Development**, v. 138, n. 19, p. 4117–4129, 1 out. 2011.

IZAWA, T. What is going on with the hormonal control of flowering in plants? **The Plant Journal**, v. 105, n. 2, p. 431–445, 2021.

KIM, D.-H. Current understanding of flowering pathways in plants: focusing on the vernalization pathway in *Arabidopsis* and several vegetable crop plants. **Horticulture, Environment, and Biotechnology**, v. 61, n. 2, p. 209–227, 1 abr. 2020.

LASHERMES, P. et al. Molecular characterization and origin of the *Coffea arabica* L. genome. **Molecular and General Genetics**, v. 261, n. 2, p. 259-266, 1999.

LEIJTEN, W. et al. Translating Flowering Time from *Arabidopsis thaliana* to Brassicaceae and Asteraceae Crop Species. **Plants**, v. 7, n. 4, p. 111, dez. 2018.

LIMA, A. A. et al. Drought and re-watering modify ethylene production and sensitivity, and are associated with coffee anthesis. **Environmental and Experimental Botany**, v. 181, p. 104289, 2021.

LIU, D. et al. An organ-specific role for ethylene in rose petal expansion during dehydration and rehydration. **J Exp Bot.**, p. 2333–2344, 2013.

LIVRAMENTO, D. E. Morfologia e Fisiologia do Cafeeiro. In: REIS, P. R.; CUNHA, R. L. (Ed). **Café Arábica do plantio á colheita**. Lavras: U.R. EPAMIG SM, 896 p., 2010.

LÓPEZ M.E. et al. An overview of the endogenous and environmental factors related to the *Coffea arabica* flowering process. **Beverage Plant Research**, v. 1, p. 1–16, 2021.

LÓPEZ M.E. et al. 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**., v. 13, p. 824948, 2022.

MAGALHÃES, A. C.; ANGELOCCI, L. R. Sudden alterations in water balance associated with flower bud opening in coffee plants**. Journal of Horticultural Science**, v. 51, n. 3, p. 419-421, 1976.

MAJEROWICZ, N.; SÖNDAHL, M. R. Induction and differentiation of reproductive buds in *Coffea arabica* L. **Brazilian Journal of Plant Physiology**, v. 17, n. 2, p. 247-254, 2005.

MASARIRAMBI M. T.; CHINGWARA V.; SHONGWE V. D. The effect of irrigation on synchronization of coffee (*Coffea arabica* L.) flowering and berry ripening at Chipinge, Zimbabwe. **Physics and Chemistry of the Earth**, **Parts A/B/C**, v. 34, p. 786-789, 2009.

MATSUMOTO T. K.; LOPEZ J. Coffee harvest management by manipulation of coffee flowering with plant growth regulators. In **XXIX International Horticultural Congress on Horticulture**: Sustaining Lives, Livelihoods and Landscapes (IHC2014): 1130 p. 219-224, 2016.

MELO, B.; SOUSA, L. B. Biologia da reprodução de *Coffea arabica* L. e Coffea canephora Pierre. **Revista Verde de Agroecologia e Desenvolvimento Sustentável,** v. 6, n. 2, 2011.

MENG, Y. et al. Precise spatio-temporal modulation of ACC synthase by MPK6 cascade mediates the response of rose flowers to rehydration. **Plant J.**, v. 79, n. 6, p. 941-50, 2014.

MES, M. G. Studies on the flowering of *Coffea arabica* L. Various phenomena are associated with the dormancy of the coffee flower buds. **Portugaliae Acta Biologica**, v. 5, n. 1, p. 25-44, 1957.

MEYLAN L. et al. Evaluating the effect of shade trees on the provision of ecosystem services in intensively managed coffee plantations. **Agriculture, ecosystems & environment,** v. 245, p. 32-42, 2017.

MIRANDA F. R.; DRUMOND L. C. D.; RONCHI C. P. Synchronizing coffee blossoming and fruit ripening in irrigated crops of the Brazilian Cerrado Mineiro Region. **Australian Journal of Crop Science,** v. 14, n. 4, p. 605-613, 2020.

MONACO, L. C. et al. Efeito de dias longos no crescimento e florescimento de cultivares de café. **Bragantia**, v. 37, n. 1, p. 25–32, 1978.

MORAIS, H. et al. Escala fenológica detalhada da fase reprodutiva de *Coffea arabica*. **Bragantia,** v. 67, n. 1, p. 257-260, 2008.

MUTASA-GÖTTGENS, E.; HEDDEN, P. Gibberellin as a factor in floral regulatory networks. **Journal of Experimental Botany**, v. 60, n. 7, p. 1979–1989, 1 Maio 2009.

PEZZOPANE, J. R. M. et al. Escala para avaliação de estádios fenológicos do

cafeeiro arábica**. Bragantia**, Campinas, v.62, n.3, p.499-505, 2003.

QUIÑONES, A. J. P. et al. Effects of Daylength and Soil Humidity on the Flowering of Coffee *Coffea arabica* L. in Colombia. **Rev.Fac.Nal.Agr. Medellín**, p. 5745–5754, 1 jun. 2011.

RAMÍREZ, B. et al. **Variabilidad climática y la floración del café en Colombia.** Gerencia Técnica / Programa de Investigación Científica, Fondo Nacional del Café, 2001.

REIS, P. R.; CUNHA, R. L. **Café arábica: do plantio à colheita**. Lavras: EPAMIG, 2010. 896p.

RENA, A. B. et al. **Coffee**. In: Schaffer, B., Andersen, P.C. (eds.). Handbook of environmental physiology of tropical fruit crops: sub-tropical and tropical crops. 1. Ed. Boca Raton: CRC Press, p. 101-122, 1994.

RENA, A. B.; BARROS, R. S. Aspectos críticos no estudo da floração do café. In: Zambolim L (Ed.). **Efeitos da Irrigação Sobre a Qualidade e Produtividade do Café**, Universidade Federal de Viçosa, p.149-172, 2004.

RENA, A. B.; MAESTRI, M. Fisiologia do cafeeiro. **Informe Agropecuário,** Belo Horizonte, v.11, n.126, p.26-40, 1985.

RIBEIRO, T. H. C. et al. The floral development of the allotetraploid *Coffea arabica* L. correlates with a small RNA dynamic reprogramming. **The Plant Journal**, 2024.

RONCHI C. P.; MIRANDA F. R. Flowering percentage in arabica coffee crops depends on the water deficit level applied during the pre-flowering stage1. **Revista Caatinga**, v. 33, n. 1, p. 195-204, 2020.

RONCHI, C. P. et al. Respostas ecofisiológicas de cafeeiros submetidos ao déficit hídrico para concentração da florada no Cerrado de Minas Gerais. **Pesquisa Agropecuária Brasileira**, v. 50, p. 24–32, jan. 2015.

SANTOS, I. S., et al. Multigenic regulation in the ethylene biosynthesis pathway during coffee flowering. **Physiology and Molecular Biology of Plants**, 28, p. 1657–1669, 2022.

SCHUCH U. K.; AZARENKO A. N.; FUCHIGAMI L. H. Endogenous IAA levels and development of coffee flower buds from dormancy to anthesis. **Plant growth regulation**, v. 15, n. 1, p. 33-41, 1994.

SCHUCH U. K.; FUCHIGAMI L. H.; NAGAO M. A. Flowering, ethylene production, and ion leakage of coffee in response to water stress and gibberellic acid**. Journal of the American Society for Horticultural Science,** v. 117, n. 1, p.158-163, 1992.

SCHUCH U. K.; FUCHIGAMI L. H.; NAGZAO M. A. Effect of photoperiod on flower initiation of coffee. **HortScience**, v. 25, n. 9, p. 1071-1071, 1990a.

SCHUCH, U. K.; FUCHIGAMI, L. H.; NAGAO, M. A. Gibberellic acid causes earlier flowering and synchronizes fruit ripening of coffee. **J. Plant Growth Regulat**., v. 9, p. 59-64, 1990b.

SOARES, A. S. et al. Irrigação e fisiologia da floração em cafeeiros adultos na região da zona da mata de Minas Gerais. **Acta Scientiarum Agronomy.** Maringá, v.27, n.1, p.117-125, Jan./Mar. 2005.

SPANUDAKIS, E.; JACKSON, S. The role of microRNAs in the control of flowering time. **Journal of Experimental Botany**, v. 65, n. 2, p. 365–380, 1 Feb. 2014.

SUN, T. Gibberellin-GID1-DELLA: A Pivotal Regulatory Module for Plant Growth and Development. **Plant Physiology**, v. 154, n. 2, p. 567–570, 1 out. 2010.

SUSILA, H.; NASIM, Z.; AHN, J. H. Ambient Temperature-Responsive Mechanisms Coordinate Regulation of Flowering Time. **International Journal of Molecular Sciences**, v. 19, n. 10, p. 3196, out. 2018.

THOMAZIELLO, R. A. et al. **Café Arábica: cultura e técnicas de produção**. Campinas: IAC (Boletim Técnico, 187), 82p., 2000.

TSUJI, H. Molecular function of florigen. **Breeding Science**, v. 67, n. 4, p. 327–332, 2017.

UNIGARRO C. A.; BEJARANO L. M. D.; PINZÓN J. F. T. Efecto de dos inductores florales sobre la floración y producción de café. **Revista Cenicafé**, v. 70, n. 2, p. 19-29, 2019.

VALVERDE, F. et al. Photoreceptor Regulation of *CONSTANS* Protein in Photoperiodic Flowering. **Science**, v. 303, n. 5660, p. 1003–1006, 13 Feb. 2004.

VÉLEZ B. E. et al. Distribución de la floración y la cosecha de café en tres altitudes. **Centro Nacional de Investigaciones de Café (Cenicafé)**, v. 272, 2000.

WANG, H. et al. The DELLA-CONSTANS Transcription Factor Cascade Integrates Gibberellic Acid and Photoperiod Signaling to Regulate Flowering. **Plant Physiology**, v. 172, n. 1, p. 479–488, 1 set. 2016.

WILSON, R. N.; HECKMAN, J. W.; SOMERVILLE, C. R. Gibberellin Is Required for Flowering in *Arabidopsis* thaliana under Short Days 1. **Plant Physiology**, v. 100, n. 1, p. 403–408, 1 set. 1992.

WORMER T. M.; GITUANJA J. Floral initiation and flowering of *Coffea arabica* L. in Kenya**. Experimental Agriculture**, v. 6, n. 2, p. 157-170, 1970.

XU, F. et al. DELLA proteins physically interact with CONSTANS to regulate flowering under long days in *Arabidopsis*. **FEBS Letters**, v. 590, n. 4, p. 541–549, 2016.

YAMAGUCHI, A.; ABE, M. Regulation of reproductive development by non-coding RNA in *Arabidopsis*: to flower or not to flower. **Journal of Plant Research**, v. 125, n. 6, p. 693–704, 1 Nov. 2012.

SEGUNDA PARTE

CAPÍTULO DE LIVRO: Regulation of *Coffea arabica* floral development, flowering and fruit maturation by plant growth regulators

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Contents

Abstract

Plants of the *Coffea* genus are largely known for their fruits, which are used to provide beans and produce the coffee beverage very appreciated all over the world. *Coffea arabica* L. is the most cultivated species and presents unique characteristics once it was originated from the interspecific crossing of *C. canephora* and *C. eugenioides* non-reduced gametes resulting in an allotetraploid and autogamous plant. Coffee reproductive development is a complex process occurring in a two-year period and considered sensitive to environmental changes that can desynchronise flowering and fruit maturation impairing bean quality and production. Despite the recent advances clarifying the coffee reproductive cycle and its associated regulatory pathways, specially related to endogenous and environmental signals, the role of plant growth regulators (PGRs) in mitigating these undesired effects is still poorly understood. This chapter aims to summarise the typical applications and effects of PGRs, which include phytohormones, on *C. arabica* plants to provide a new perspective on their use considering the recent advances in its reproductive cycle. This understanding becomes even more important in the face of climate change and alarming predictions of its impact on coffee production, which on a global scale threatens food production. Therefore, understanding the reproductive regulation of fruit-bearing perennial plants by PGRs will pave the way to altering development cycles and improving agricultural practices and directing breeding programmes to ensure productivity in the near future.

Keywords

Coffea arabica, Fruit maturation, Phytohormones, Plant growth regulators, Reproductive development.

1. Introduction

Among the 130 species identified in the *Coffea* L. genus (Davis & Rakotonasolo 2021), practically only *Coffea arabica* L. and *Coffea canephora* Pierre ex A. Froehner are cropped for bean production, constituting one of the most important agricultural and socioeconomic commodity (Hidalgo et al., 2023; Rhiney et al., 2021). The coffee reproductive cycle is rather complex, being responsive to hormonal and environmental signals (López et al., 2021), whose predicted climate changes can impair bean production and quality (Bilen et al., 2022; DaMatta & Ramalho, 2006; Jawo, Kyereh & Lojka, 2022; Läderach et al., 2017). Plant growth regulators (PGRs) are organic molecules that influences plant growth, development and physiology, which

include synthetic molecules and phytohormones naturally produced by plants (Agudelo-Morales, Lerma, Martínez, Palencia, & Combatt, 2021; Mukherjee et al., 2022; Rademacher, 2015). Therefore, a deep understanding of the coffee reproductive cycle and how it is affected by PGRs is essential to ensure the sustainability of the coffee production chain.

Coffea arabica presents unique characteristics in the plant kingdom starting from its origin, which arose from the interspecific hybridisation of unreduced gametes from the diploid progenitors *C. canephora* and the maternal *C. eugenioides* (Lashermes et al., 1999). This rare and natural event, estimated to have occurred between 10,000 and 665,000 years ago in the Arabian region (currently Ethiopia) (Bawin et al., 2020; Scalabrin, et al., 2020; Yu et al., 2011), originated the allotetraploid *C. arabica* (4n=4x=44), an exception of the *Coffea* genus with diploids species (2n=2x=22) (Charrier & Berthaud, 1985; Davis, Govaerts, Bridson, & Stoffelen, 2006). Interestingly, such polyploidisation events may be related to cyclical increases in global temperature, leading to a greater incidence of abnormalities in gametogenesis including infertility and ploidy variation (Pecrix et al., 2011; Wang et al., 2017). In coffee, these aspects are still poorly understood, despite advances in understanding floral organogenesis (de Oliveira et al., 2014) and coffee microsporogenesis (de Oliveira et al., 2024).

Because of its origin, *C. arabica* is described as a species with a narrow genetic base (Lashermes et al., 1999; Scalabrin, et al., 2020) leading to a low variability that could threatens its adaptability (Davis et al., 2019). In agreement, elevated temperatures impose transcriptional constraints in a genotype-dependent manner impairing energetic metabolism (de Oliveira et al., 2020). This highlights the necessity of alternatives to regulate coffee plant development to deal with climate changes and PGRs can be useful for that purpose. On the other hand, certain phenotypic variability is observed between arabica genotypes (Carvalho, 2008), explained by chromosomal rearrangements from the parental genomes and phenotypic differences (Scalabrin et al., 2024). For example, some *C. arabica* genotypes have a greater homeostasis in response to temperature changes as compared to their parents (Bertrand et al., 2015), besides the known superior quality of beans for beverage production (Ahmed et al., 2021; Lemos et al., 2020). Other studies also show differences between homologous genes of the parental subgenomes and transcriptional patterns supporting variability (Ribeiro et al., 2024; Rume et al., 2023). This phenotypic variability in coffee suggests that, once we understand the underlying molecular machinery controlling development, we can manipulate or direct breeding programmes to obtain more adapted and productive genotypes in certain conditions.

Some of the key genes and regulatory molecular pathways for coffee flower and fruit

development have been unveiled (de Oliveira et al., 2014; Cardon et al., 2022; López et al., 2021; Ribeiro et al., 2024) thus, opening the possibility of regulation via the application of PGRs. However, descriptive studies showing the effects of applying such products and detailing the underlying mechanisms, namely revealing responsive genes and pathways and their impacts, are still scarce. This chapter aims to summarise the typical application and effects of PGRs in coffee plants, in addition to providing a parallel with different species, to propose hypotheses on their use to manipulate coffee reproductive development (Figure 1), and hence improving agronomic practices.

2. Overview and recent advances in the understanding of coffee reproductive development

A noteworthy characteristic of coffee reproductive development is the biennial cycle, which means that it takes two years from the formation and floral induction of coffee buds to the flowering and subsequent fruit ripening, and these processes are overlapped since the first reproductive cycle (Camargo & Camargo, 2001). Several studies have contributed to describe a phenological scale for coffee development (reviewed by López et al., 2021), which in general considered the fruit production (and bean commercialisation) the major focus. Because of this, these studies have proposed the beginning of coffee cycle with the flowering (fertilisation and initiation of fruit formation), generally started in September during the rainy season at the Brazilian conditions (Camargo & Camargo, 2001). However, from a biological point of view, the beginning of the reproductive cycle occurs with the transition from the vegetative to the floral meristem and recent advances considering molecular aspects have better specified the developmental stages and their relationships with environmental cues (Cardon et al., 2021; de Oliveira et al., 2014; de Oliveira et al., 2024; Ribeiro et al., 2024).

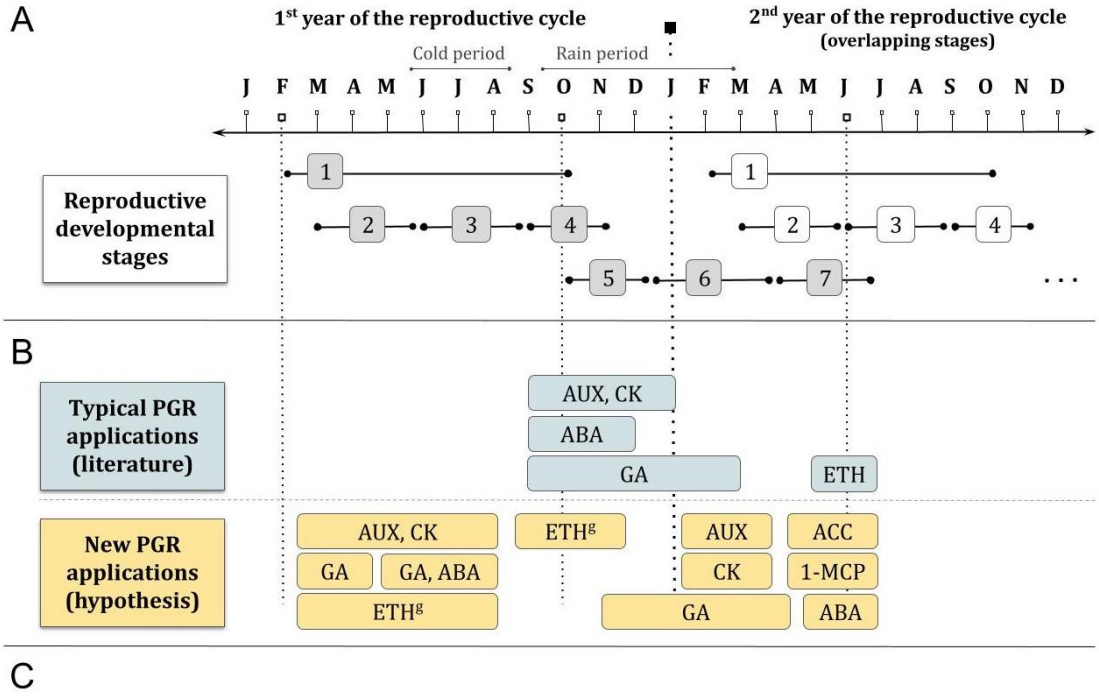
Considering the floral transition of coffee buds as the starting point, here we revised the coffee reproductive development and summarised the main changes in Figure 1A. Importantly, this figure shows a general overview at typical Brazilian conditions, especially in the Minas Gerais state the biggest region of coffee production (WorldAtlas, 2024), to facilitate comprehension of coffee cycle. However, the precise timing of coffee developmental can vary basically according to: i) the specific climate characteristics of a region (microclimates), ii) the coffee genotype (early or late flowering; Carvalho, 2008; López et al., 2022) and iii) the agricultural management practices. Thus, it is important to consider not only the specific stage of coffee development to recommend an application of PGR but also the season and climate

forecast.

In detail, the first stage of reproductive development is marked by the determination of inflorescence meristems and bud swelling (de Oliveira et al., 2014; Morais et al., 2008), an inductive period that begins in February and might extend until October (Stage 1 in Figure 1A) (Cardon et al., 2021). Noteworthy, this extended induction window explains the asynchronous floral bud formation that culminates in several sequential flowering events and, consequently, uneven fruit development (Cardon et al., 2021). Next, stage 2 (Figure 1A) is marked externally by the appearance of mucilage and development of floral meristems that expand until entering a phase without apparent growth (stage 3). The floral buds in this stage are frequently described as dormant (Wormer & Gituanja, 1970) or latent (discussed in López et al., 2021). Nevertheless, there is considerable metabolic and transcriptional activity due to the microsporogenesis (de Oliveira et al., 2024) in accordance with the differentiation of carpels and stamens described at this stage (de Oliveira et al., 2014). Interestingly, this third stage coincides with the Brazilian winter, a period of low temperatures and drought, suggesting sensitivity to increases in temperature that could cause fertility disorders (de Oliveira et al., 2024; Ribeiro et al., 2024). The stage 4 (Figure 1A) is marked by the rapid expansion of floral buds and flowering (anthesis) that occurs with the rising temperatures and beginning of rainfall, pointing to the end of winter from September onwards (Camargo and Camargo, 2001; López et al., 2021). In the following months, the fruit sets, followed by the formation of the pinhead (stage 5), grain filling and an expansion of fruits (stage 6) until the beginning of ripening, which generally occurs between April and August in Brazil, depending on coffee genotype and local temperature (Carvalho, 2008; López et al., 2021), ending with the harvesting (stage 7).

After the first flowering event, the reproductive development of coffee plants takes place biennially, with the flowering and fruiting processes occurring simultaneously from the second reproductive year onwards (Figure 1A). Such process is marked by different points of asynchronous development, from the origin of the vegetative meristems throughout its reproductive transition and floral development (de Oliveira et al., 2014), which results in a sequential flowering and uneven fruit maturation at the end (Cardon et al., 2022). These characteristics are undesirable from an agronomic point of view, as immature green fruits make harvesting difficult and harm the quality of the coffee beverage (DaMatta & Ramalho, 2006; Rena & Maestri, 1986). Depending on the climate characteristics of the site, flowering synchronisation can be successfully achieved by managing irrigation (see Ronchi and DaMatta, 2024, in this book). However, in addition to flowering synchronization, the use of PGR could

be of utmost importance to control and improve various aspects of coffee reproductive development, from the induction of floral buds to fruit maturation (Figure 1), as discussed in the next topics of this chapter.



C

Stages of coffee reproductive development	Typical PGR applications (literature)	New PGR applications (hypothesis)	Physiological effect (hypothesis)
1- Floral induction	None	CK, GA, ETH, ACC, 1-MCP	Stimulate floral transition
2- Floral (uneven) development	None	AUX, CK, GA, ABA	Synchronise development
3- Microsporogenesis	None	AUX, GA	Improve fertilisation
4- Sequential flowering	GA, ABA	ETH, ACC, 1-MCP	Synchronise flowering
5- Fruiting setting	AUX, CK	GA	Improve fruit setting
6- Fruit (uneven) development	GA	AUX, CK, GA	Fruit growth (yield)
7- Fruit maturation/harvesting	ETH	ACC, 1-MCP, ABA	Synchronise ripening

Figure 1. A general overview of the biennial coffee reproductive development at Brazilian conditions and its possible regulation by plant growth regulators (PGRs). A) The figure shows a general view of the coffee reproductive development at Brazilian conditions over two years, starting in January (J), which includes the floral development (stages 1 to 4) and fruiting until harvesting (stages 5 to 7). The period of these developmental stages can vary according to the climate characteristics of a region, the coffee genotype (early or late flowering) and on-farm agricultural management practices. Then, it is important to consider the specific coffee developmental stage to recommend an application of PGR since the pattern presented here is a general overview to facilitate comprehension. B) The typical (blue boxes) and novel (yellow boxes) hypothesis for PGRs applications are indicated chronologically in this figure. C) The figure shows a panel describing the coffee stages (white column), the typical PGRs applied in a certain stage (blue column), the new purposed application (yellow column) and hypothesis for physiological desired effects (pink column). The cold and rainy periods in Brazilian conditions are indicated in the top figure. Legends: the twelve months of each year are

abbreviated starting in January (J) to December (D); Auxin (AUX); Cytokinin (CK); Gibberellin (GA); Abscisic acid (ABA); Ethylene (ETH); 1-Aminocyclopropanecarboxylic acid (ACC); 1-Methylcyclopropene (1-MCP); The ethylene-related compounds, ETH, ACC and 1-MCP, was referred in the boxes as Ethylene-group (ETH^g).

3. Auxins (AUX)

Auxins, particularly indole-3-acetic acid (IAA), are vital for nearly all aspects of plant growth and development, impacting processes such as stress responses and adaptive mechanisms (Bielach, Hrtyan, & Tognetti, 2017; Zhao, 2010). Auxins like IAA are involved in altering the mechanical properties of the cell wall through the modification of pectin components, thereby influencing cell growth and shape acquisition (Jobert, Yadav, & Robert, 2023). Furthermore, this phytohormone promotes apical dominance by inhibiting the formation of lateral branches, delaying leaf abscission, promoting the differentiation of vascular tissues, and stimulating the formation of lateral and adventitious roots, among other effects essential for plant growth (Petrasek et al., 2019)

Considering the fundamental role of auxins in plants, researchers and farmers have explored their applications to enhance various growth and development processes and improve agricultural practices. In coffee, most of the available reports using exogenous auxin demonstrated the enhancement on growth of seedling roots (Andrade et al., 2023) and in vitro culture using both auxins and cytokinins (CK) (Dumaslan, Tad-awan, & Manuel, 2023). The use of auxin-related PGRs in coffee for flowering and fruit development is scarce, being found in old works that both indole-3-acetic acid (IAA) and 2,4- diehlorophenoxyaeetic acid (2,4-D) were suggested to affect floral bud development and flowering (Cueto & Dathe, 1986).

In different species, exogenous auxin can exert both inhibitory and promotive effects on floral initiation and development in a dose-dependent manner (Davis, 2009; Sadka, Walker, Haim & Bennett, 2023; Tanimoto, 2005). Accordingly, recent works showed that in pear buds the NAA inhibiting bud break conflict with results showing no inhibition of bud break after auxin application (Wei et al., 2022). In mango, a gradual increase in auxin-like substances extracted from stems was noticed from September to December, coinciding with the period of floral initiation in the Northern hemisphere (Babu, Srilatha, & Joshi, 2022). The application of naphthalene acetic acid (NAA), a synthetic PGR in the auxin family, is also used to encourage consistent flowering and fruit set by initiating ethylene (ETH) production in pineapple, facilitating its harvesting (Cunha, 2005). Classical studies in fruit crops (cherries, pears, peaches, and plums) long time reported that the application of NAA at 200 to 800 ppm postponed flowering by 1 to 2 weeks (Dennis, Edgerton, & Parker, 1970; Guerriero, Loreti, &

Vltagliano, 1970; Maksymiuk, Grochowska, & Krzewińska, 1987; McLaughlin & Greene, 1991). NAA at 10 to 50 ppm, and 2,4-D at 6 to 10 ppm, promoted early flowering in pineapple. In litchi, NAA is used as an alternative to girdling to enhance flowering (Hajam et al., 2018).

During the transition from flower to fruit, successful pollination triggers metabolic pathways and anatomical transformations, with auxin playing a pivotal role in this developmental shift (An et al., 2020). Synthetic auxins enhance fruit setting in various fruit and vegetable cultivation (Batlang, 2008; Gemici, Bengü, & Kit, 2006; Khan et al., 2006; Serrani et al., 2007), which can be related to its essential function in embryo development (Verma, Attuluri, & Robert, 2021). In Arabica coffee, fruit drop typically occurs within the initial three months following flowering, particularly as the fruits start to enlarge around 8–12 weeks upon blossoming (Aparecido et al., 2022; Gopal, 1971; Montoya & Sylvain, 1962). Low concentrations of 2,4-D reduced the fruit drop when applied at very low concentrations (10 mg per plant) (Gopal, 1971). In contrast, a later study found no effect of 2,4-D application at 2.5 to 15 ppm on fruit set or coffee yield (Miguel et al., 1976). Similarly, application of 2,4-D at 5 ppm just after blossom time showed no effect on coffee fruit drop (Miguel et al., 1981).

Auxin's role in fruit ripening can be indirect, as it can stimulate the synthesis of climacteric ethylene (ETH), a key ripening hormone (Trainotti, Tadiello, & Casadoro, 2007). Studies on ‘Redhaven’ peach fruits demonstrated an increase in IAA content alongside climacteric ETH production in the mesocarp tissues (Miller, Walsh, & Cohen, 1987). This raises questions about whether the rise in auxin content merely amplifies ETH synthesis or if auxin has a direct role in regulating fruit ripening, which needs further investigation in the context of coffee. Exogenous application of synthetic auxins like NAA was shown to promote ETH production and ripening in apples (Ji & Wang, 2021), while suppressing ETH biosynthesis and delaying ripening in tomatoes (Wu et al., 2018). The effects of auxin treatment on ripening vary depending on concentration and plant species, as observed in bananas treated with different concentrations of IAA (Lu et al., 2018). Exogenous NAA enhanced the diameter of tomatoes (Patel et al., 2012), while enhancing fruit setting in the same species in another report (Verma, Meena, & Meena, 2014).

From the above, it is evident that advances in understanding the reproductive development of *C. arabica* (see topic 2 in this chapter) were not accompanied by experiments testing auxin applications at specific developmental stages to improve productivity (Figure 1). For example, the role of auxin-related PGRs at different concentrations may be tested to: 1) stimulate floral transition (around March) and development of latent buds during the cold period

(around May to August) improving and synchronising flowering; 2) promote flowering and fruit setting estimating the quantitative gain in production (from September to January), and; 3) synchronise fruit development and thus improving quality at harvesting (January to April).

4. Cytokinins (CK)

Cytokinins (CK) are plant hormones that primarily promote cell division, or cytokinesis, in both root and shoot organs, while also influencing cell growth, differentiation, apical dominance, axillary bud growth, and leaf senescence (Botha et al., 2013). Concerning plant flowering, exogenous applications of CK seem to both promote and inhibit floral initiation in various species, although promotional effects are more common than inhibitory (Aremu et al., 2020). For some species, such as Passiflora, CK is essential for flowering in explants from adult plants, but does not promote flowering in juvenile explants indicating an associated ageing-dependent effect. In mango, the premature bud break and flowering is anticipated with application of 100 ppm 6-benzylaminopurine (BA) (Chen, 1987), similar to the effect of thidiazuron (Nuñez-Elisea, Caldeira, & Davenport, 1990). More recently, it was demonstrated that CK is primordial for ovule, pollen and seed formation in *Arabidopsis* (Terceros et al., 2020), but also for development of reproductive organs and flowers of strawberry (Pérez-Rojas et al., 2023) and in the perennial woody plant *Jatropha curcas* L. (Ming et al., 2020). Moreover, throughout modern techniques the CK levels have been to target and manipulate to increase seed yield with the concurrent maintenance of quality in different crops (Jameson & Song, 2016). However, very little was explored in the coffee reproductive development and yield in response to CK application (Figure 1).

Fruit setting marks the initial phase of fruit development in angiosperms, dependent on successful pollination and double fertilisation events. These events trigger seed formation, orchestrating synchronised cell division and fruit growth. Current evidence indicates that the combined action of auxin, gibberellins (GA), and CK plays a crucial role in regulating the fruit setting. While each hormone can initiate fruit development individually, their combined application induces normal fruit growth even without fertilisation in both dry and fleshy fruits (Fenn & Giovannoni, 2021).

Thus, similarly to auxins and in the face of recent advances regarding coffee reproductive development (see topic 2 in this chapter), CK could be used to stimulate and synchronise floral and fruiting development, consequently, improving yield and quality (Figure 1). For instance, the following hypothesis are suggested to be tested considering only CK

application or combined to auxin and GA (Figure 1B and C): stimulate floral transition (around March) and development of latent buds during the cold period (around May to August), 2) to accelerate floral buds development and synchronise flowering (in September to October), 3) to promote flower and fruit setting (from November to January), 4) stimulating the fruit growth and development trough carbohydrate transport which could result in gains in production and quality (January to April). Overall, exploring the application of CK in specific developmental stages of coffee and its interactions with other hormones will offer valuable insights for optimising production practices and improving coffee yield and quality.

5. Gibberellins (GA)

Gibberellins (GA), another group of PGRs, is involved in many developmental processes such as plant height, leaf expansion, chlorophyll biosynthesis, hypocotyl elongation, seed germination, and flowering (Castro-Camba et al., 2022; Ritonga et al., 2023). Recent advances in GA metabolism have revealed species-specific modifications acquired during the evolution of flowering plants, highlighting the fundamental importance of this hormone in regulating plant growth and development (He et al., 2020).

In the context of reproductive development GA is a central player in the interconnected pathways that control floral activation integrating many environmental signals (Amasino, 2010; Kim et al., 2020; Leijten et al., 2018). For example, depending on the photoperiod and temperature conditions, GA upregulates the expression of floral integrative genes, such as *LEAFY* (*LFY*), *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*), *FLOWERING LOCUS T* (*FT*), and others, initiating the flowering process (Bao, Hua, Shen, & Yu, 2020). Moreover, GA acts also downstream in this pathway controlling the floral organ development and regulating other genes including miRNAs (Achard et al., 2004; Goto & Pharis, 1999; Leijten et al., 2018).

Although GAs are known to promote flowering in annual and biennial plants, it is also reported that they can inhibit floral initiation in some woody perennial plants (Garmendia et al., 2019; Li et al., 2018; Zhang et al., 2019). For example, gibberellic acid has been found to significantly inhibit flowering in citrus plants (Agustí, Reig, Martínez-Fuentes, & Mesejo, 2022; Muñoz-Fambuena et al., 2012). Therefore, it seems that the effects of GA on flowering are species-dependent, and despite its role being established for floral transition, its direct role in flowering initiation in coffee plants is unclear.

The GA signalling pathway interacts in a complex manner with other floral regulatory

pathways including phytohormones (Bao et al., 2020). For example, previous studies have demonstrated that rehydration of plants resulted in reduced abscisic acid (ABA) levels and increased levels of bioactive GA in coffee trees (Browning, 1973; Browning, Hoad, & Gaskin, 1970), pointing to an antagonistic action of GA and ABA in promoting floral bud development. Additionally, an increase in the levels of CK was observed in both the xylem and floral buds after rehydration, indicating a synergistic interaction with GA in floral development and promotion of anthesis (Browning, 1973; Liu & Sherif, 2019).

As typical of plant hormones, experimental uses of GA should consider the dosage-dependent effects. In *C. arabica*, the application of gibberellin (GA_3) at a concentration of 100 mg L^{-1} promoted anthesis in floral buds larger than 4 mm, regardless of rain or irrigation, resulting in greater synchronisation of fruit ripening (Schuch, Fuchigami, & Nagao, 1990). Consistent with this, relatively mild drought stress is not sufficient to break bud dormancy in coffee plants, but the exogenous application of GA may be crucial in triggering anthesis (Schuch, Fuchigami, & Nagao, 1992). In agreement, more recent studies showed that the application of 200 mg GA L^{-1} triggered a significant increase in flower production as well as in the number and weight of mature fruits in *C. arabica* (Flores, Torre, Monroig, & González, 2005); and 100 ppm GA_3 promotes a more concentrated flowering and greater fruit uniformity at the at harvest (Matsumoto & Lopez, 2016). On the other hand, contradictory findings suggest that exogenous GA does not directly increase the number of floral buds in *C. arabica* (Unigarro et al., 2019). Interestingly, paclobutrazol (PBZ), an inhibitor of GA biosynthesis, induces early and intense flowering, reduces vegetative growth, and increases fruit set in coffee trees (Flores et al. 2005). These results point to GA treatment as a promising strategy for regulating coffee reproductive development (Figure 1), especially considering the recent advances in understanding its phenological cycle (discussed in topic 2 of this chapter) and the predicted impact of climate change (Bilen et al., 2022; de Oliveira et al., 2024; Zapata-Restrepo & Guevara-Ortega, 2020).

In addition to influencing coffee anthesis, a recent study found an increase in the number of internodes in *C. arabic*a with GA application (0.1% and 0.5%, w/v) between August and February, regardless of the concentration used (Zapata-Restrepo et al., 2020). Consistent with this, eight applications of 100 ppm GA_3 increased the length of plagiotropic branches and the number of nodes per branch (Cannell, 1971). This suggests that the longer the branch and the more nodes formed are, the greater will be the production of floral buds and consequently more fruits. These results show that the role of GA is not limited to anthesis induction of floral buds

or increasing the number of flowers, but also influences the vegetative growth of the coffee plant, especially during the period prior to bud formation.

Gibberellin and auxin also influence fruit development by promoting cell division and expansion in many plant species. This effect is especially important during the initial stages of fruit development, contributing to increased fruit size and tissue expansion in arabidopsis, tomato and strawberry (He & Yamamuro, 2022). Furthermore, treatment with GA can influence fruit maturation, increase pulp hardness, decrease respiration intensity, delay fruit softening and ripening, and improve characteristics, such as shape, colour, and sugar content in horticultural crops (Wu et al., 2024; Zhang et al., 2023).

Although the application of GA on fruits has the potential to increase quality and productivity in some species, there is a gap in the scientific literature regarding its application during coffee fruit development. Most existing research has focused on the GA application of pre-flowering to synchronise flowering, which may indirectly influence the uniformisation of fruit maturation. This indirect effect of GA_3 on fruit development was corroborated by Cannell (1971), who used GA_3 to modify the seasonal fruiting patterns of *C. arabica* in Kenya. The application of GA_3 delayed the onset of floral bud development, resulting in a concentrated harvest at the end of the year. Furthermore, Masarirambi, Shongwe, & Chingwara (2010) found that pre-flowering application of 100 ppm GA_3 accelerated coffee bean ripening. The concentrated harvest and accelerated fruit development observed by these authors may be attributed to GA's ability to promote synchronisation of floral bud anthesis, indirectly influencing fruit development.

Overall, there are several studies in coffee plants showing the effects of GA promoting anthesis, increasing the number of flowers and fruits, as well as, stimulating the vegetative growth and synchronising fruit ripening (summarised as “Typical PGR applications” in Figure 1), which seems to interact with other hormones (Liu & Sherif, 2019). Nevertheless, the dose-dependent effects of GA at specific stages of coffee reproductive development remains as yet unexplored in coffee (see “New PGR applications” in Figure 1), especially regarding to: 1) promote floral induction and yield (mainly from February to June); 2) accelerate bud development possible for manipulating the harvesting time (May to August); 3) to promote fruit setting (November to January) for improving yield; and 4) stimulating fruit development and ripening for improving bean quality.

6. Abscisic acid (ABA)

ABA was discovered as a compound that maintained bud dormancy and leads to fruit abscission (Chen, Bressan, Song, Zhu, & Zhao, 2020). Even though it is a plant hormone that also participates in plant growth and development, most frequently it is still known as a plant stress hormone. ABA has important responses to biotic and abiotic environmental changes, including relevant control of stomatal closure, seed dormancy and germination, production of desiccation protectants, osmotic regulation and other functions (Chen et al., 2020; Martignago, Siemiatkowska, Lombardi, & Conti, 2020).

The latent state of floral buds in coffee plants occurs during the dry and cool season of the Brazilian winter that occurs in June to August (see Figure 1A) (de Oliveira et al, 2014; López et al, 2022). Previous studies have indicated an increase in ABA levels in leaves and floral buds of coffee trees during this drought period, showing a direct association with the latent state of floral buds (reviewed by López et al., 2022). Regarding floral bud latency, other studies reported that in coffee GA can be responsible for floral bud latency release, while ABA per se can inhibit anthesis by maintaining floral buds in a latent state (Browning, 1973; Flores et al., 2005). Matsumoto & Lopez (2016) applied GA_3 and *s*-ABA on coffee trees and observed an increase in flowering uniformity and yield (see "Typical PGR applications in Figure 1B). It was suggested that GA and ABA could be working together in holding bud latency and releasing it with a greater uniformity (see May to August in Figure 1B). In agreement, the same pattern of higher ABA levels leading to bud dormancy has been observed in other plant species (Li et al., 2018). Additionally, a reduced expression in *9-CIS-EPOXYCAROTENOID DIOXYGENASE* (*NCED*) was correlated to early bud dormancy release (Gupta et al., 2022).

ABA is involved in different moments in the process of seed development and fruit maturation. The main contributions are during storage accumulation of lipids, proteins and carbohydrates; desiccation tolerance and seed dormancy/germination (Chen et al., 2020). ABA and GA play important roles in the dormancy/germination regulation of seeds, operating in opposite ways (Chen et al., 2020; Gupta et al., 2022). Besides the ABA action on seed dormancy to avoid germination during drought conditions and viviparity (Kermode, 2005; Rodríguez-Gacio, Matilla-Vázquez & Matilla, 2009), it is also reported that ABA has an effect on cold tolerance in different crops (Huang et al., 2017; Venzhik, Talanova & Titov, 2016; Yu et al., 2020). However, this protective effect is poorly explored in *C. arabica*, which could be tested previously to the Brazilian winter (May to June, Figure 1B and C) preparing both floral buds and fruits (concomitantly stages during coffee development; see topic 2 and Figure 1A) to the cold stress and possibly with an impact on yield and bean quality.

In different species such as tomato and strawberry, ABA participates in fruit maturation; indeed, silencing genes involved with ABA biosynthesis (*SlNCED, FaNCED*) has been shown to promote a reduced ABA accumulation and lead to a delay in fruit maturation (Gupta et al., 2022; Jia et al., 2016). The action of ABA seems to have a positive interplay with ETH during the fruit maturation, with ABA involved in the ETH biosynthesis and action in different process, such as the change in colour, softening, accumulation of sugars and others (Kumar, Khurana, & Sharma, 2014; Jia et al., 2015). For example, ABA can upregulate gene expression of ETH biosynthetic enzymes, as ETH can upregulate *NCED* expression (Kumar, Khurana, & Sharma, 2014). Nevertheless, such effects on coffee fruit development are still scarce and are restricted to the effects of ETH during fruit ripening (see next topic). Thus, it is plausible to suggest ABA application to explore its role in coffee fruits to control and synchronise fruit maturation at the harvesting time (May to June, see "New PGR applications" in Figure 1B and C).

7. The group of Ethylene-related compounds (ETH^{g}): Ethylene (ETH), 1-Aminocyclopropanecarboxylic acid (ACC) and 1-Methylcyclopropene (1-MCP)

Ethylene (ETH) is a quite different plant hormone due to its gaseous nature. It is a simple hydrocarbon (C_2H_4) that is involved in many aspects of plant life cycle such as organ abscission, flowering and fruit ripening (Abeles, Morgan, & Saltveit, 1992). ETH production is also regulated in response to biotic and abiotic stress (Cao, Chen, & Zhang, 2008; Paudel & Bede, 2015; Savada et al., 2017; Yu et al., 2019), being altered especially under water deficit conditions (Arraes et al., 2015; Gomez-Cadenas et al., 1996). ETH is produced in plant tissues via the conversion of methionine to S-adenosylmethionine (SAM) (Miyazaki & Yang, 1984), and the rate-limiting steps in EHT biosynthesis include the conversion of SAM to 1-aminocyclopropane-1-carboxylic acid (ACC) by the enzyme 1-aminocyclopropane-1-carboxylate synthase (ACS) and the subsequent metabolism of ACC to ETH by 1-aminocyclopropane-1-carboxylate oxidase (ACO) (Adams & Yang, 1981).

To trigger physiological responses, the binding of ETH to receptors is crucial (Hall, Shakeel, & Schaller, 2007). The ETH signal transduction model comprises a family of receptors (Lacey & Binder, 2014) in which the downstream signal transduction pathway is mediated by members of different gene families (Chang, 2016). The main enzymes involved in ETH biosynthesis, 1-aminocyclopropane-1-carboxylic acid synthase (ACS) and 1-aminocyclopropane-1-carboxylic acid oxidase (ACO), are part of a multigene family in *Coffea* sp. (Ságio et al., 2013; Santos et al., 2022) whose expression of homologous genes can be

modulated in the root and shoot in response to water deficit (Santos et al., 2022). The alternation between dry and wet seasons, during coffee flowering, changes the water content of both the soil and plant and regulates the biosynthesis pathway and the flow of ACC (ETH precursor) between root and shoot. As a result, changes in endogenous ETH levels control the timing of floral bud development and coffee flower opening (Lima et al., 2021; López et al., 2022) which is represented in Figure 1.

Recent studies focused on the ETH regulation of coffee flowering pointed a new function for ETH in the induction of anthesis (Lima et al., 2021; López et al., 2022). The proposed mechanism of action begins during the dry period with an upregulation of genes related to ETH biosynthesis in the root system, increasing ACC production and its accumulation. This molecule is believed to be concentrated in roots during drought and after watering (rain or irrigation) events, after which ACC would be transported to the shoot to induce an ETH burst and trigger anthesis.

To test this ETH action on coffee flowering the growth regulator 1-Methylcyclopropene (1-MCP), an ETH action inhibitor, was used to inhibit the anthesis (Lima et al., 2021). The 1-MCP (C_2H_6) is a potent antagonist of ETH action and is structurally very similar and thus it is able to occupy the same receptor site and compete with ETH (Serek & Sisler, 2001; Sisler & Serek, 1997; Watkins, 2006). Although 1-MCP molecules irreversibly bind to ethylene-receptors binding sites, they are unable to deactivate the downstream component of the metabolic pathway and trigger physiological responses (Blankenship & Dole, 2003; Watkins, 2008). Thus, the signalling pathway remains blocked by 1-MCP and ETH is unable to trigger chemical reactions, inhibiting long-term responses in plants (Sisler & Serek, 1997; Serek & Sisler, 2001). The ETH sensitivity can be reestablished as new receptors are synthesised (Hall, Findell, Schaller, Sisler, & Bleecker, 2000; Sisler, 2006).

The 1-MCP has been used as an active ingredient in different PGRs and extensively used in ornamental and fruit crops to delay senescence and prolong post-harvest viability (Çelikel, Dodge, & Reid, 2002; Galati et al., 2017; Ha et al., 2019). Because of this structural and regulatory relationship, in this chapter the ethylene, ACC (ETH precursor) and 1-MCP (ETH repressor) will be referred to as the Ethylene group or ETH^g in Figure 1. The first report of the 1-MCP application to block the ETH action and control the flowering in woody species occurred in *C. arabica* (Lima et al., 2021). However, the surprisingly and unexpected results showed that the 1-MCP application (50 mg of active ingredient L^{-1}) promoted anthesis in the absence of a rainfall event and without changes in the soil water content (Lima et al., 2021;

López et al., 2022). It was shown that 1-MCP upregulated ETH biosynthesis pathway genes (*CaACS1* and *CaACO1*) in leaves and floral buds and increased endogenous ETH levels (Lima et al., 2021), stimulating metabolic processes similar to those that occur in the ETH pathway after rehydration (Lima et al., 2021; López et al., 2022). Furthermore, 1-MCP modifies the plant's sensitivity to ETH by reducing the gene expression and perception pathway, such as the *CaETR4* receptor (Lima et al., 2021). The mechanism by which this ETH action inhibitor increases ETH levels and induces coffee anthesis has not yet been elucidated. To explain this regulation, a loss of the negative feedback of ETH biosynthesis was suggested, which could induce endogenous ETH production regulating anthesis similar to ETH applications (Lima et al., 2021).

The application of ethylene-based compounds has also adverse effects on flowering and plant development. For example, the use of 2-chloroethyl phosphonic acid (CEPA), commercialised as Ethrel or Ethephon, in different dosages caused floral buds abortion and acceleration of leaf senescence (López et al., 2022). In agreement, excessive doses of CEPA can trigger an imbalance in ETH levels and result in extensive defoliation (Carvalho et al., 2003; Matiello et al., 2010). Because of this aggressive and adverse plant reaction, such PGRs are commonly and strictly recommended for fruit ripening and not to control floral development. However, the results of Lima et al (2021) and López et al (2022) may be associated, since it is described an effect of ETH on floral development of other plant species (Martínez, García & Jamilena, 2022; Mou et al., 2020). In other words, the adverse effects of exogenous ETH could be mitigated if considering: i) a pre-determined and estimated non-toxic dosage; ii) the determination of proper stage and tissue during the coffee reproductive development (see topic 2); and iii) the use of alternative and less aggressive molecules that stimulate the modulatory-pathways of endogenous ETH levels, such as ACC and 1-MCP (the ETH^{g} in Figure 1; López et al., 2022).

Recently, the ETH precursor ACC has been used to induce ETH responses, but recent findings point that ACC is an ETH-independent signalling molecule related to diverse roles of plant development (Li, Mou, Van de Poel & Chang, 2022; Vanderstraeten, Depaepe, Bertrand & Van Der Straeten, 2019) including floral organs (Mou et al., 2020). In coffee plants, ACC promoted anthesis, with more efficiency to synchronise floral buds at the pre-flowering stage (G6 stage in Morais et al. 2008) compared to 1-MCP (López et al., 2022). This result reinforced the relationship between ETH and coffee flowering regulation and the new hypothesis is that ACC can be used as a PGR to synchronise coffee anthesis around September to November (see

"New PGR applications" in Figure 1B and C). These studies expanded the perspective of ETH-related molecules (ETH^{g}) on the control of coffee reproductive development, and a possible effect on bud induction (from February to May in Figure 1B and C) and bud latency break (from May to July in Figure 1B and C). Future research should focus on test this hypothesis in different coffee developmental times and considering the dosage-effects to synchronise bud and flower development.

In contrast to flowering, the involvement of PGRs based on ETH in fruit ripening, including in coffee, has been documented decades ago (Pereira et al., 2005; Ságio et al., 2013, 2014; Salmona et al., 2008). Coffee fruits are classified as climacteric (Ságio et al., 2014), presenting a rapid increase in the ETH biosynthesis and respiration rate, resulting in physiological and biochemical changes necessary for the fruit ripening process (Lelievre et al., 1997; Payasi & Sanwal, 2010). However, *C. arabica* genotypes present differences in their reproductive cycle and, consequently, in the time of harvesting, being classified as early or late cultivars (Carvalho et al., 2008; Carvalho et al., 2022; López et al., 2021. Early coffee cultivars display higher expression levels of genes related to the ETH biosynthesis and signalling pathways than late cultivars, which has been associated with the acceleration of coffee fruit maturation (Ságio et al., 2013). The differences presented between coffee cultivars for fruit maturation and related to the ETH production levels reinforce the potential of regulation through the use of ETH-based PGRs (ETH^{g} in Figure 1B and C).

As previously discussed, the asynchrony of coffee flowering and fruit maturation is a highly undesirable characteristic for coffee producers given that it difficults the harvesting operations, thus impairing the yield, the bean and beverage quality (see topic 2). Unripe fruits are associated with incomplete maturation of the grain, giving the drink high astringency and low quality. In contrast, the beverage made from cherry fruits present greater sweetness and better acidity, obtaining higher scores in the cup quality classification (Pimenta & Vilela, 2003; Siqueira & Abreu, 2006). Thus, to obtain a higher percentage of cherry fruits at harvesting, PGRs based on ETH have been used for a long time to delay or induce fruit maturation (Carvalho et al., 2022; Monaco & Sondahl, 1974).

In several studies, the effect of ETH-based products as a physiological ripener increases the percentage of cherry fruits and anticipates harvest (Carvalho et al., 2003; 2022; Negreiros et al., 2019; Scudeler et al., 2004; Silva, Arré, Tourinho, Gomes, & Alves, 2009). However, despite this ETH ripener effect, in some cases no positive correlation was found between the quantity of cherry fruits and the quality improvement of the coffee beverage (Carvalho et al.,

2003; Silva et al., 2009). The beneficial effect of EHT application is only observed when the coffee beans are completely filled because the early applications may promote maturation of the shell (exocarp) and mucilage (mesocarp), but not affect the seed development (endosperm/endocarp) (Monaco and Sondahl, 1974).

Another beneficial effect of applying ETH close to harvest is that it facilitates fruit fall, increasing yield and avoiding leftovers that cause disease in the next cycle (May to July, see "Typical PGR applications" in Figure 1B). Based on that, the ETH-based regulators are recommended to be applied when plants present more than 75% of cherry fruits (Rena & Maestri, 1986; Benini, 2002) which occur in general close to the harvesting time (May to July in Figure 1B). This recommendation is difficult to follow considering the large extension of farms, the number of plants in the field and the heterogeneity of coffee fruit development observed, even within the same plant (Rena & Barros, 2004; Pereira et al., 2005). Thus, due to the difficulties of analysis, it would be interesting new studies using modern techniques to estimate the percentage of green and cherry fruits, and to provide a more accurate result about the effect of ETH on fruit development (Eron, Noman, de Oliveira & Chalfun-Junior, 2024). By contrast, whenthe objective is to delay the speed of fruit maturation, potassium acetate is used to inhibit the ETH biosynthesis. The mechanism of ETH degradation by potassium ions consists of the oxidation of ETH to form acetaldehyde, which is then oxidised to acetic acid, resulting in the formation of carbon dioxide and water molecules (Sá et al., 2008). The effectiveness of such inhibitors can be measured by the level of delaying ripening, prolonging the fixation of the fruit on the plant (Rodrigues et al., 2019).

Altogether, it should be pointed that ETH-based compounds have been widely used in fruit development and ripening, the results of such application on development are not fully consistent, thus underlining that their use should be done with extra care considering the plant stage and the prevailing environment. In fact, comparisons between coffee plants showed divergent results as delay in maturation and abscission of fruits (Carvalho et al., 2022), induction of maturation with an increase in cherry fruits quantity (Silva et al., 2013), delay in fruit falling without altering the maturation development (Dias et al., 2014), an increase in green colour intensity and a delay in the raisin fruit amount (Silva et al., 2013). In this context, there are still many gaps about the use of ETH-based compounds during the coffee reproductive development (see "New PGR applications" in Figure 1B and C), especially considering the effect of ETH, 1-MCP and ACC (ETH^{g}) on: 1) floral transition (February to April in Figure 1B) and bud breaking latency (May to July), which could impact yield; 2) flowering

synchronisation (September to December in Figure 1B) that could improve fruit uniformity and beverage quality; 3) fruit development exploring the effects of ETH^g application, not only ETH but also 1-MCP and ACC, on seed development and in separated parts of the plant, because the canopy inferior third shows a delayed fruit development compared to the superior one; and 4) to test if the ETH^g application is able to change the harvesting time, which could be useful to manipulate the cycle in response to predictable weather adversities (May to July in Figure 1B). If the link between the effects of ETH^g PGR on floral development, anthesis and fruit ripening could be proven, the impact generated would be very positive to the coffee production chain across the world.

8. Concluding remarks

In this chapter, we initially present the recent advances in understanding the coffee reproductive development, which allowed identification of specific morphological characteristics in each stage until the flowering and, afterwards, fruit ripening. This developmental process is governed by regulatory pathways that interact with environmental and hormonal pathways and, thus, the use of PGRs (including phytohormones) is discussed to regulate coffee cycle towards agricultural improvement. In the various topics of this chapter, we explored the typical uses of PGRs historically reported in coffee and other species and new possibilities for its use at different times of coffee development (Figure 1). New PGR molecules such as 1-MCP and ACC (included in the ETH^g, Figure 1B) were also discussed since they could be successfully employed in manipulating related hormonal levels and coffee development, although additional experiments are needed to validate and define the best dosage action. Here, a special focus was given for all PGRs classes to the regulation of coffee development aiming to manipulate the biennial cycle and, possibly, improving the synchronisation of flowering and fruit development, as well as, the increasing of yield and bean quality.

Despite the research advancements regarding a sustainable PGR use towards an efficient improvement of coffee productivity and quality, there are still several topics that require further research and exploration. Firstly, the precise molecular mechanisms underlying the effects of PGRs on coffee reproductive development need to be deeply elucidated. Understanding these mechanisms will facilitate the development of targeted and effective strategies for optimising flowering and fruit ripening in coffee plants. Secondly, the potential synergistic or antagonistic interactions between different hormones, such as AUX, CK, GAs, ABA and ETH is another

layer of complexity that warrants investigation. Exploring these interactions could provide valuable insights into hormone signalling networks and their regulation during coffee plant development. In addition, there is a need for comprehensive studies assessing the efficacy, safety, and environmental impact of growth regulator applications in coffee cultivation. This includes investigating optimal application methods, dosage, and timing to maximise desired outcomes whereas minimising adverse effects.

Noteworthy, the hypotheses presented here to regulate the coffee cycle by PGRs considered the coffee developmental pattern under the southeastern Brazilian conditions where most coffee is produced. In other words, this overview aims to facilitate the understanding of coffee development stages in a chronological order, knowing that the development cycle is variable depending on the region (environmental effects) and genotype. Thus, for a desirable physiological effect, the hypotheses presented for the moment of PGR applications must follow the developmental stage and not only the suggested periods that were used as a guide (Figure 1). Before any PGR recommendation, we strongly suggest that producers carry out an assessment of the microclimate to which the coffee plants are subject and a complete evaluation of the developmental cycle over two years under such conditions. Therefore, it is possible to have a general overview of development in the given cultivation condition and adapt the moment of PGRs application. In this way, modern techniques based on artificial intelligence have emerged to help producer in identification of coffee developmental stages (Eron, Noman, de Oliveira & Chalfun-Junior, 2024) helping to make decisions to improve the productivity and quality of the fruits.

Finally, future research should also focus on developing novel PGRs strategies tailored specifically for coffee cultivation. These innovations could offer more precise and efficient tools for manipulating the different stages of coffee reproductive development towards sustainability and long-term viability of the coffee industry. Moreover, the singularity of *C. arabica*, due to its developmental complexity and socio-economic importance, make this species a good model for studying the artificial regulation in fruit-bearing perennial crops.

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References:

Abeles, F. B., Morgan, P. W., & Saltveit, M. E. (1992). Ethylene in plant biology, 2nd ed. Academic Press, New York.

Achard, P., Baghour, M., Chapple, A., Hedden, P., Van Der Straeten, D., Genschik, P., et al. (2007). The plant stress hormone ethylene controls floral transition via DELLA-dependent regulation of floral meristem-identity genes. *Proceedings of the National Academy of Sciences*, USA, *104(15)*, 6484–6489.

Adams, D. O., & Yang, S. F. (1981). Ethylene the gaseous plant hormone: mechanism and regulation of biosynthesis. *Trends in Biochemical Sciences*, *6*, 161–164.

Agudelo-Morales, C.E., Lerma, T.A., Martínez, J.M., Palencia, M., & Combatt, E.M. (2021) Phytohormones and plant growth regulators - A review. *Journal of Science with Technological Applications*, *10*, 27-65.

Agustí, M., Reig, C., Martínez-Fuentes, A., & Mesejo, C. (2022). Advances in *Citrus* flowering: A review. *Frontiers in Plant Science*, *13*, 868831.

Ahmed, S., Brinkley, S., Smith, E., Sela, A., Theisen, M., Thibodeau, C., et al. (2021). Climate change and coffee quality: systematic review on the effects of environmental and management variation on secondary metabolites and sensory attributes of *Coffea arabica* and *Coffea canephora*. *Frontiers in Plant Science*, *12*, 708013.

Andrade, F. H. A., Ferreira, A. M. O., Azevedo, L. M., Santos, M. O., Carvalho, G. R., Resende, M. L. V., et al. (2023). IBA and melatonin increase trigonelline and caffeine during the induction and initiation of adventitious roots in *Coffea arabica* L. cuttings. *Science Reports*, *13*, 15151.

Alexander, L., & Grierson, D. (2002). Ethylene biosynthesis and action in tomato: a model for climacteric fruit ripening. *Journal of Experimental Botany, 53*, 2039-2055.

An, J., Almasaud, R. A., Bouzayen, M., Zouine, M., & Chervin, C. (2020). Auxin and ethylene regulation of fruit set. *Plant Science, 292*, 110381.

Amasino, R. (2010). Seasonal and developmental timing of flowering. *Plant Journal*, *61(6)*, 1001–1013.

Aparecido, L.E.O, Lorençone, J.A., Lorençone, P.A. *et al*. (2022). Predicting coffee yield based on agroclimatic data and machine learning. *Theoretical and Applied Climatology*, *148*, 899–914.

Aremu, A. O., Fawole, O. A., Makunga, N. P., Masondo, N. A., Moyo, M., Buthelezi, N. M. D., Amoo, S. O., Spíchal, L., & Doležal, K. (2020). Applications of Cytokinins in Horticultural Fruit Crops: Trends and Future Prospects. Biomolecules, *10(9)*, 1222.

Arraes, F. B. M., Beneventi, M. A., Lisei de Sa, M. E., Paixao, J. F. R., Albuquerque, E. V. S., et al. (2015). Implications of ethylene biosynthesis and signaling in soybean drought stress tolerance. *BMC Plant Biology, 15(1)*, 1–20.

Babu, R. S. H., Srilatha, V., & Joshi, V. (2022). Plant Growth Regulators in Mango (*Mangifera indica* L.). In: *Plant Growth Regulators in Tropical and Sub-Tropical Fruit Crops.* CRC Press, 1st Edition, 154 pages, eBook ISBN 9781003300342.

Bao, S., Hua, C., Shen, L., & Yu, H. (2020). New insights into gibberellin signaling in regulating flowering in Arabidopsis. *Journal of Integrative Plant Biology, 62(1)*, 118–131.

Batlang, U. (2008). Benzyladenine plus gibberellins (GA4+7) increase fruit size and yield in greenhouse-grown hot pepper (*Capsicum annuum* L.). *Journal of Biological Sciences*, *8(3)*, 659–662.

Bawin, Y., Ruttink, T., Staelens, A., Haegeman, A., Stoffelen, P., Mwanga Mwanga, J. C. I., Roldan-Ruiz, I., Honnay, O. & Janssens, S. B. (2020). Phylogenomic analysis clarifies the evolutionary origin of *Coffea arabica*. *Journal of Systematics and Evolution*, *59*, 953-963.

Bertrand, B., Bardil, A., Baraille, H., Dussert, S., Doulbeau, S., Dubois, E., et al. (2015). The greater phenotypic homeostasis of the allopolyploid *Coffea arabica* improved the transcriptional homeostasis over that of both diploid parents. *Plant Cell Physiology*, *56(10)*, 2035–2051.

Bielach, A., Hrtyan, M., & Tognetti, V.B. (2017). Plants under Stress: Involvement of Auxin and Cytokinin. *Journal of Molecular Sciences*, *18*(*7*), 1427.

Bilen, C., El Chami, D., Mereu, V., Trabucco, A., Marras, S., & Spano, D. (2022). A systematic review on the impacts of climate change on coffee agrosystems. *Plants*, *12(1)*, 102.

Blankenship, S. M., & Dole, J. M. (2003). 1-Methylcyclopropene: A review. *Postharvest Biology and Technology*, *28*(1), 1–25.

Botha, F. C., Lakshmanan, P., O'Connell, A., & Moore, P. H. (2013). *Hormones and Growth Regulators. Chapter 14 in Sugarcane: Physiology, Biochemistry, and Functional Biology,* pp. 331–377. Publisher: John Wiley & Sons, Ltd.

Browning, G. (1973). Flower bud dormancy in *Coffea arabica* L. I. Studies of gibberellin in flower buds and xylem sap and of abscisic acid in flower buds in relation to dormancy release. *Journal of Horticultural Science*, *48(1)*, 29–41.

Browning, G., Hoad, G. V., & Gaskin, P. (1970). Identification of abscisic acid in flower buds

of *Coffea arabica* (L.). *Planta*, *94(3)*, 213–219.

Burns, J. K. (2008). 1-Methylcyclopropene applications in preharvest systems: Focus on citrus. *HortScience*, *43(1)*, 112–114.

Cueto, M. A. & Dathe, W. (1986). Effect of auxins on flower formation in coffee (*Coffea arabica* L.). *Biologia Plantarum*, *28(5)*, 355-360.

Camargo A.P. & Camargo M.B.P. (2001). Definição e esquematização das fases fenológicas do cafeeiro arábica nas condições tropicais do Brasil. *Bragantia*, *60*, 65−68.

Cannell, M. G. R. (1971). Use of gibberellic acid to change the seasonal fruiting pattern of Arabica coffee in Kenya. *Journal of Horticultural Science*, *46(3)*, 289–298.

Cao, Y. R., Chen, S. Y., & Zhang, J. S. (2008). Ethylene signaling regulates salt stress response. *Plant Signaling and Behavior*, *3(10)*, 761-763.

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 Science*, *325*, 111479.

Carvalho, C. H. S. (2008) Cultivares de café: Origem, características e recomendações Brasília: Embrapa Café, 334p. ISBN: 9788561519001.

Carvalho, C. H. S., Bartelega, L., Sera, G. H., Matiello, J. B., Almeida, S. R., Santinato, F., Hotz, A. L.(2022). *Catálogo de cultivares de café arábica*. *Documento, 16:* 115 pages. Brasília, DF: Embrapa Café, ISSN: 1678-1694.

Carvalho, D. S., Franco Júnior, K. S., Brigante, G. P., & Dias, M. S. (2022). Effects of coffee maturation regulators. *Revista Brasileira de Engenharia de Biossistemas*, *16*, 1060.

Carvalho, G. R.; Mendes, A. N. G.; Carvalho, L. F., & Bartholo, G. F. (2003). Eficiência do ethephon na uniformização e antecipação da maturação de frutos de cafeeiro (*Coffea arabica* L.) e na qualidade da bebida. *Ciência e Agrotecnologia*, *27*, 98-106.

Castro-Camba, R., Sánchez, C., Vidal, N., & Vielba, J. M. (2022). Plant development and crop yield: The role of gibberellins. *Plants*, *11(19)*, 2650.

Çelikel, F. G., Dodge, L. L., & Reid, M. S. (2002). Efficacy of 1-MCP (1- methylcyclopropene) and Promalin for extending the post-harvest life of Oriental lilies (Lilium × "Mona Lisa" and 'Stargazer'). *Scientia Horticulturae*, *93(2)*, 149–155.

Cenci, A., Combes, M.-C., & Lashermes, P. (2012). Genome evolution in diploid and tetraploid *Coffea* species as revealed by comparative analysis of orthologous genome segments. *Plant Molecular Biology*, *78*, 135–145.

Chang, C. (2016). QandA: How do plants respond to ethylene and what is its importance? *BMC Biology*, *14(1)*, 1-7.

Chang, S.-T., Chen, W.-S., Hsu, C.-Y., Yu, H.-C., Du, B.-S., & Huang, K.-L. (1999). Changes in cytokinin activities before, during and after floral initiation in *Polianthes tuberosa*. *Plant Physiology and Biochemistry*, *37(9)*, 679–684.

Charrier, A., & Berthaud, J. (1985). Botanical classification of coffee. In: Clifford MN and Willson KC (eds.) Coffee, Botany, Biochemistry and Production of beans and beverage. Croom Helm, London, pp 13-47.

Chen, K., Li, G.-J., Bressan, R.A., Song, C.-P., Zhu, J.-K., & Zhao, Y. (2020). Abscisic acid

dynamics, signalling, and functions in plants. *Journal of Integrative Plant Biology*, *62*, 25-54.

Chen, W.-S. (1987). Endogenous growth substances in relation to shoot growth and flower bud development of mango. *Journal of the American Society for Horticultural Science*, *112(2)*, 360–363.

Claeys, H., De Bodt, S., & Inzé, D. (2014). Gibberellins and DELLAs: Central nodes in growth regulatory networks. *Trends in Plant Science*, *19(4)*, 231–239.

Cunha, G. A. P. da. (2005). Applied aspects of pineapple flowering. *Bragantia*, *64*, 499–516.

DaMatta, F. M., & Ramalho, J. D. C. (2006). Impacts of drought and temperature stress on coffee physiology and production: A review. *Brazilian Journal of Plant Physiology*, *18(1)*, 55–81.

Davis, S. J. (2009), Integrating hormones into the floral-transition pathway of *Arabidopsis thaliana*. *Plant, Cell & Environment*, *32*, 1201-1210.

Davis, A. P., & Rakotonasolo, F. (2021). Six new species of coffee (*Coffea*) from northern Madagascar. *Kew Bulletin*, *76(3)*, 497-511.

Davis, A. P., Chadburn, H., Moat, J., O'Sullivan, R., Hargreaves, S., Nic Lughadha, E. (2019). High extinction risk for wild coffee species and implications for coffee sector sustainability. *Science Advances*, *5(1)*, eaav3473.

Davis, A. P., Govaerts, R., Bridson, D. M.,& Stoffelen, P. (2006). An annotated checklist of the genus *Coffea* L. (Rubiaceae). *Botanical Journal of the Linnean Society*, *152(4)*, 465–512.

de Oliveira, K. K. P., de Oliveira, R. R., Rume, G. C., Ribeiro, T. H. C., Fernandes-Brum, C. N., do Amaral, L. R., et al. (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(1)*, e561.

de Oliveira, R. R., Ribeiro, T. H. C., Cardon, C. H., Fedenia, L., Maia, V. A., Barbosa, B. C. F., Caldeira, C. F., Klein, P. E., & Chalfun-Junior, A. (2020). Elevated temperatures impose transcriptional constraints and elicit intraspecific differences between coffee genotypes. *Frontiers in Plant Science*, *11*, 1113.

Dennis, F. G., Edgerton, L. J., & Parker, K. G. (1970). Effects of gibberellin and Alar sprays upon fruit set, seed development, and flowering of 'Bartlett'Pear1. *HortScience*, *5(3)*, 158–160.

Dumaslan, M. R., Tad-awan, B. A., Manuel, S. B. (2023). Direct organogenesis and regeneration of Arabica coffee cultivars. *Mountain Journal of Science and Interdisciplinary Research*, *83(2)*, 43-56.

Eron, F., Noman, M., de Oliveira, R.R., Chalfun-Junior, A. (2024). Computer vision-aided intelligent monitoring of coffee: Towards sustainable coffee production. *Scientia Horticulturae*, *327*, 112847.

El-Sharkawy, I., Kim, W. S., & Jayasankar, S. (2008). Differential regulation of four members of the ACC synthase gene family in plum. *Journal of Experimental Botany*, *59*, 2009–2027.

Felipe, G. M. (1986). Etileno. *Fisiologia vegetal 2. São Paulo, 2. ed*:163-192.

Fenn, M. A., & Giovannoni, J. J. (2021). Phytohormones in fruit development and maturation. *Plant Journal*, *105(2)*, 446–458.

Flores, C. A., Torre, W. D. la, Monroig, M., & González, W. (2005). Ácido giberélico, paclobutrazol, fluoridone, ácido abscísico afectan la sincronización de la floración del cafeto.

The Journal of Agriculture of the University of Puerto Rico, *89(3–4)*, 159-168.

Galati, V. C., Muniz, A. C. C., Guimarães, J. E. R., Inestroza-Lizardo, C. O., Mattiuz, C. M. F., & Mattiuz, B. H. (2017). Conservação pós-colheita de alstroémeria 'ajax' com uso de 1-metilciclopropeno. *Ciencia e Agrotecnologia*, *41(2)*, 181–190.

Garmendia, A., Beltrán, R., Zornoza, C., García-Breijo, F. J., Reig, J., & Merle, H. (2019). Gibberellic acid in *Citrus* spp. flowering and fruiting: A systematic review. *PLOS ONE*, *14(9)*, e0223147.

Gómez-Cadenas, A., Tadeo, F. R., Talon, M., & Primo-Millo, E. (1996). Leaf abscission induced by ethylene in water-stressed intact seedlings of Cleopatra mandarin requires previous abscisic acid accumulation in roots. *Plant Physiology*, *112(1)*, 401–408.

Gopal, N. H. (1971). Preliminary studies on the control of fruit drop in arábica coffee. *Indian Coffee*, *35(10)*, 413–419.

Goto, N., & Pharis, R. P. (1999). Role of gibberellins in the development of floral organs of the gibberellin-deficient mutant, ga1-1, of *Arabidopsis thaliana*. *Canadian Journal of Botany*, *77(7)*, 944–954.

Guerriero, R., Loreti, F., & Vltagliano, C. (1970). The effects of growth regulators on peach flowering date. *Rivista Della Ortoflorofrutticoltura Italiana*, *54*, 580–596.

Gupta K., Wani S.H., Razzaq A., Skalicky M., Samantara K., Gupta S., et al. (2022). Abscisic acid: Role in fruit development and ripening. *Frontiers in Plant Science*, *13*, 817500.

Guyot R., Hamon P., Couturon E., Raharimalala N., Rakotomalala J.J., Lakkanna S., et al. (2020). WCSdb: A database of wild *Coffea* species. Database (Oxford):baaa069. doi: 10.1093/database/baaa069. PMID: 33216899; PMCID: PMC7678786.

Ha, S. T. T., Lim, J. H., & In, B. C. (2019). Simultaneous inhibition of ethylene biosynthesis and binding using AVG and 1-MCP in two rose cultivars with different sensitivities to ethylene. *Journal of Plant Growth Regulation*, *39(2)*, 553-563.

Hajam, M. A., Hassan, G. I., Parray, E. A., Wani, M. A., Shabir, A., Khan, I. F., et al. (2018). Transforming fruit production by plant growth regulators. *Journal of Pharmacognosy and Phytochemistry*, *7(1)*, 1613–1617.

Hall, A. E., Findell, J. L., Schaller, G. E., Sisler, E. C., & Bleecker, A. B. (2000). Ethylene perception by the ERS1 protein in *Arabidopsis*. *Plant Physiology*, *123(4)*, 1449-1458.

Hall, B. P., Shakeel, S. N., & Schaller, G. E. (2007). Ethylene receptors: Ethylene perception and signal transduction. *Journal of Plant Growth Regulation*, *26*, 118–130.

He, H., & Yamamuro, C. (2022). Interplays between auxin and GA signaling coordinate early fruit development. *Horticulture Research*, *9*, uhab078.

He, J., Xin, P., Ma, X., Chu, J., & Wang, G. (2020). Gibberellin metabolism in flowering plants: An update and perspectives. *Frontiers in Plant Science*, *11*, 532.

Hidalgo, F., Quiñones-Ruiz, X.F., Birkenberg, A., Daum, T., Bosch, C., Hirsch, P., Birner, R. (2023). Digitalization, sustainability, and coffee. Opportunities and challenges for agricultural development. *Agricultural Systems*, *208*, 103660.

Huang, X., Shi, H., Hu, Z., Liu, A., Amombo, E., Chen, L., & Fu, J. (2017). ABA Is Involved in Regulation of Cold Stress Response in Bermudagrass. *Frontiers in Plant Science*, *8*, 1613.

Jameson, P. E. & Song, L. (2016). Cytokinin: a key driver of seed yield, *Journal of*

Experimental Botany, *67(3)*, 593–606.

Jawo, T.O., Kyereh, D. & Lojka, B (2022). The impact of climate change on coffee production of small farmers and their adaptation strategies: A review. *Climate and Development*, *15(2)*, 1–17.

Ji, Y., & Wang, A. (2021). Recent advances in phytohormone regulation of apple-fruit ripening. *Plants*, *10(10)*, 2061.

Jia, H., Jiu, S., Zhang, C., Wang, C., Tariq, P., Liu, Z., Wang, B., Cui, L., & Fang, J. (2016).

Jobert, F., Yadav, S., & Robert, S. (2023) Auxin as an architect of the pectin matrix. Journal of Experimental Botany, *74*(*22*), 6933- 6949.

Abscisic acid and sucrose regulate tomato and strawberry fruit ripening through the abscisic acid-stress-ripening transcription factor. *Plant Biotechnology Journal*, *14(10)*, 2045–2065.

Kermode, A (2005). Role of abscisic acid in seed dormancy. *Journal of Plant Growth Regulation*, *24*,319–344.

Khan, M., Masroor, A., Gautam, C., Mohammad, F., Siddiqui, M. H., Naeem, M., et al. (2006). Effect of gibberellic acid spray on performance of tomato. *Turkish Journal of Biology*, *30(1)*, 11–16.

Kim, D.-H. (2020). Current understanding of flowering pathways in plants: Focusing on the vernalization pathway in Arabidopsis and several vegetable crop plants. *Horticulture, Environment, and Biotechnology*, *61(2)*, 209–227.

Lacey, R. F., & Binder, B. M. (2014). How plants sense ethylene GA - The ethylene receptors. *Journal of Inorganic Biochemistry*, *133*, 58–62.

Läderach, P., Ramirez–Villegas, J., Navarro-Racines, C., Zelaya, C., Martinez–Valle, A., & Jarvis, A. (2017). Climate change adaptation of coffee production in space and time. *Climatic Change*, *141(1)*, 47–62.

Lashermes, P., Combes, M. C., Robert, J., Trouslot, P., D'Hont, A., Anthony, F., et al. (1999). Molecular characterisation and origin of the *Coffea arabica* L. *Molecular and General Genetics*, *261(2)*, 259–266.

Lashermes, P., Hueber, Y., Combes, M. C., Severac, D., & Dereeper, A. (2016). Inter-genomic DNA exchanges and homeologous gene silencing shaped the nascent allopolyploid coffee genome (*Coffea arabica* L.). *G3 Genes|Genomes|Genetics*, *6(9)*, 2937–2948.

Leijten, W., Koes, R., Roobeek, I., & Frugis, G. (2018). Translating flowering time from *Arabidopsis thaliana* to *Brassicaceae* and *Asteraceae* crop species. *Plants*, *7*(4), 111.

Lelièvre, J.-M., Latchè, A., Jones, B., Bouzayen, M., & Pech, J.-C. (1997). Ethylene and fruit ripening. *Physiologia Plantarum*, *101(4)*, 727–739.

Lemos, M. F., Perez, C., da Cunha, P. H. P., Filgueiras, P. R., Pereira, L. L., Almeida da Fonseca, A. F., Ifa, D. R., & Scherer, R. (2020). Chemical and sensory profile of new genotypes of Brazilian *Coffea canephora*. *Food Chemistry*, *310*, 125850.

Li, D., Mou, W., Van de Poel, B., & Chang, C. (2022). Something old, something new: Conservation of the ethylene precursor 1-amino-cyclopropane-1-carboxylic acid as a signaling molecule. *Current Opinion in Plant Biology*, *65*, 102116.

Li, J., Pan, B.-Z., Niu, L., Chen, M.-S., Tang, M., & Xu, Z.-F. (2018). Gibberellin inhibits floral initiation in the perennial woody plant *Jatropha curcas*. *Journal of Plant Growth Regulation*,

37(3), 999–1006.

Li, J., Xu, Y., Niu, Q., He, L., Teng, Y., & Bai, S. (2018). Abscisic acid (ABA) promotes the induction and maintenance of pear (*Pyrus pyrifolia* white pear group) flower bud endodormancy. *International Journal of Molecular Science*, *19(1)*, 310.

Lima, A. A., Santos, I. S., Torres, M. E. L., Cardon, C. H., Caldeira, C. F., et al. (2021). Drought and re-watering modify ethylene production and sensitivity, and are associated with coffee anthesis. *Environmental and Experimental Botany*, *181*, 104289.

Liu, X., Shiomi, S., Nakatsuka, A., Kubo, Y., Nakamura, R., & Inaba, A. (1999). Characterization of ethylene biosynthesis associated with ripening in banana fruit. *Plant Physiology*, *121(4)*, 1257-1265.

Liu, J., & Sherif, S. M. (2019). Hormonal Orchestration of Bud Dormancy Cycle in Deciduous Woody Perennials. *Frontiers in Plant Science*, *10*, 1136.

López, M. E, Santos, I. S., Oliveira, R. R., Lima, A. A., Cardon, C. H., et al. (2021). An overview of the endogenous and environmental factors related to the *Coffea arabica* flowering process. *Beverage Plant Research*, *1*, 13.

López, M. E., Santos, I. S., Marquez, R. G., Mesa, A. J., Cardon, C. H., 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. *Frontiers in Plant Science*, *13*, 824948.

Lu, W., Mao, L., Chen, J., Han, X., Ren, X., Ying, T., et al. (2018). Interaction of abscisic acid and auxin on gene expression involved in banana ripening. *Acta Physiologiae Plantarum*, *40*, 1–9.

Majerowicz, N., & Söndahl, M. R. (2005). Induction and differentiation of reproductive buds in *Coffea arabica* L. *Brazilian Journal of Plant Physiology*, *17*, 247-254.

Maksymiuk, J., Grochowska, M. J., & Krzewińska, D. (1987). Effects of successively applied four growth regulators on shoot elongation, flowering and fruiting of apple cultivar Fantazja. *Fruit Science Reports*, *13(4)*, 149-160.

Martínez, C., García, A. and Jamilena, M. (2022). Role of Ethylene in Flower and Fruit Development. In *Ethylene in Plant Biology* (eds S. Singh, T. Husain, V.P. Singh, D.K. Tripathi, S.M. Prasad and N.K. Dubey).

Martignago, D., Siemiatkowska, B., Lombardi, A., & Conti, L. (2020). Abscisic acid and flowering regulation: Many targets, different places. *International Journal of Molecular Sciences*, *21(24)*, 9700.

Masarirambi, M. T., Shongwe, V. D., & Chingwara, V. (2010). The effect of GA3 and ethephon on synchronization of coffee (*Coffea arabica* L.) flowering and berry ripening. *Acta Horticulturae*, *884*, 573–580.

Matiello, J. B., Santinato, R., Garcia, A. W., Almeira, S. R., & Fernades, D. R. (2010). Cultura do café no Brasil manual de recomendações. Varginha: Gráfica Santo Antônio, 542 p.

Matsumoto, T. K., & Lopez, J. (2016). Coffee harvest management by manipulation of coffee flowering with plant growth regulators. *Acta Horticulturae*, *1130*, 219–224.

McLaughlin, J. M., & Greene, D. W. (1991). Fruit and hormones influence flowering of apple. II. Effects of hormones. *Journal of the American Society for Horticultural Science*, *116(3)*, 450–453.

Miguel AE, Matiello JB, Silva JBS, & Alvarenga G. (1976). Estudos preliminares no controle da queda de frutos de cafeeiros através da aplicação de 2,4-D. Congresso Brasileiro Sobre Pesquisas Cafeeiras, 65–67.

Miguel AE, Matiello JB, Franco CM, Ferreira AJ, Silva OA, & Machado JR. (1981). Estudos das causas de queda de frutos do café. Congresso Brasileiro Sobre Pesquisas Cafeeiras, 416–418.

Miller, A. N., Walsh, C. S., & Cohen, J. D. (1987). Measurement of indole-3-acetic acid in peach fruits (*Prunus persica* L. Batsch cv. Redhaven) during development. *Plant Physiology*, *84*(2), 491–494.

Ming, X., Tao, Y. B., Fu, Q., Tang, M., He, H., Chen, M. S., Pan, B. Z., & Xu, Z. F. (2020). Flower-specific overproduction of cytokinins altered flower development and sex expression in the perennial woody plant Jatropha curcas L. *International Journal of Molecular Sciences*, *21(2)*, 640.

Miyazaki, J. H., & Yang, S. F. (1987). The methionine salvage pathway in relation to ethylene and polyamine biosynthesis. *Physiologia Plantarum*, *69*(2), 366–370.

Monaco, L. C., & Söndahl, M. R. (1974). Emprego de etileno na maturação de frutos de café. *Pesquisa Agropecuária Brasileira*, *9*, 135-137.

Morais, H., Caramori, P.H., Koguishi, M.S. & De Arruda Ribeiro, A.M. (2008). Escala fenológica detalhada da fase reprodutiva de *Coffea arabica*. *Agrometeorologia, Bragantia*, *67(1*), 257–260.

Montoya, L. A., & Sylvain, P. G. (1962). Aplicación de soluciones de azúcar en aspersiones folliares, para prevenir la caída prematura de grano verde del café. *Turrialba*, *12(2)*, 100–101.

Mou, W., Kao, Y. T., Michard, E., Simon, A. A., Li, D., Wudick, M. M., Lizzio, M. A., Feijó, J. A., & Chang, C. (2020). Ethylene-independent signaling by the ethylene precursor ACC in Arabidopsis ovular pollen tube attraction. *Nature Communications*, *11(1)*, 4082.

Mukherjee, A., Gaurav, A. K., Singh, S., Yadav, S., Bhowmick, S., Abeysinghe, S., et al. (2022). The bioactive potential of phytohormones: A review. *Biotechnology Reports*, *35*, e00748.

Muñoz-Fambuena, N., Mesejo, C., González-Mas, M. C., Iglesias, D. J., Primo-Millo, E., & Agustí, M. (2012). Gibberellic acid reduces flowering intensity in sweet orange [*Citrus sinensis* (L.) Osbeck] by repressing *CiFT* gene expression. *Journal of Plant Growth Regulation*, *31(4)*, 529–536.

Negreiros, V. M. V., Nascentes, R. F., & Nascentes; M. C. B. (2019). Efeitos da aplicação de regulador vegetal na maturação e na qualidade de bebida dos frutos do café. *Revista Agrofib*, *1*, 13-25.

Nuñez-Elisea, R., Caldeira, M. L., & Davenport, T. L. (1990). Thidiazuron effects on growth initiation and expression in mango (*Mangifera indica* L.). *HortScience*, *25(9)*, 1167f–11168.

Patel, J. S., Sitapara, H. H., & Patel, K. A. (2012). Influence of plant growth regulators on growth, yield and quality of tomato and brinjal. *International Journal of Forestry and Crop Improvement*, *3(2)*, 116–118.

Paudel, J. R., & Bede, J. C. (2015). Ethylene signaling modulates herbivore-induced defense responses in the model legume *Medicago truncatula*. *Molecular Plant-Microbe Interactions*, *28(5)*, 569–579.

Payasi, A., & Sanwal, G. G. (2010). Ripening of climacteric fruits and their control. *Journal of Food Biochemistry*, *34*, 679-710.

Pecrix, Y., Rallo, G., Folzer, H., Cigna, M., Gudin, S., & Le Bris, M. (2011). Polyploidization mechanisms: Temperature environment can induce diploid gamete formation in *Rosa* sp. *Journal of Experimental Botany*, *62(10)*, 3587–3597.

Pereira, L. F. P., Galvão, R. M., Kobayashi, A. K., Cação, S. M. B., & Vieira, L. G. E. (2005). Ethylene production and acc oxidase gene expression during fruit ripening of *Coffea arabica* L. *Brazilian Journal of Plant Physiology*, *17*, 283–289.

Pérez-Rojas, M., Díaz-Ramírez, D., Ortíz-Ramírez, C. I., Galaz-Ávalos, R. M., Loyola-Vargas, V. M., Ferrándiz, C., Abraham-Juárez, M. D. R., & Marsch-Martínez, N. (2023). The role of cytokinins during the development of strawberry flowers and receptacles. *Plants*, *12(21)*, 3672.

Petrasek J., Hoyerova K., Motyka V., Hejatko J., Dobrev P., Kaminek M., & Vankova R. (2018). Auxins and Cytokinins in Plant Development. *International Journal of Molecular Sciences*, 20(4), 909.

Pimenta, C. J., & Vilela, E. R. (2003). Efeito do tipo e época de colheita na qualidade do café (*Coffea arabica* L.). *Acta Scientiarum*, *25(1)*, 131–136.

Rademacher W. (2015). Plant growth regulators: Backgrounds and uses in plant production. *Journal of Plant Growth Regulation*, *34*, 845–72.

Rhiney, K., Guido, Z., Knudson, C., Avelino, J., Bacon, C. M., Leclerc, G., Aime, M. C., & Bebber, D. P. (2021). Epidemics and the future of coffee production. *Proceedings of the National Academy of Sciences of the United States of America*, 118(27), e2023212118.

Ribeiro, T. H. C., Baldrich, P., de Oliveira, R. R., Fernandes-Brum, C. N., Mathioni, S. M., de Sousa Cardoso, T. C., et al. (2024). The floral development of the allotetraploid *Coffea arabica* L. correlates with a small RNA dynamic reprogramming. *Plant Journal*, *118(6)*, 1848-1863.

Ritonga, F. N., Zhou, D., Zhang, Y., Song, R., Li, C., Li, J., et al. (2023). The roles of gibberellins in regulating leaf development. *Plants*, *12(6)*, 1243.

Rodríguez-Gacio, M. del C., Matilla-Vázquez, M. A., & Matilla, A. J. (2009). Seed dormancy and ABA signaling: the breakthrough goes on. *Plant Signaling & Behavior*, *4(11)*, 1035–1049.

Rodrigues, J. P., Salomão, P. E. A., Freitas, S. J., Rodrigues, W. P., Struiving, T. B., Vale, P. (2019). Efeito de reguladores de crescimento na maturação dos frutos e qualidade da bebida de café. *Research, Society and Development*, *8(6)*, 1-19.

Rume, C. G., de Oliveira, R. R., Ribeiro, T. H. C., & Chalfun-Júnior, A. (2023). Genome-wide and expression analyses of MADS-box genes in the tetraploid *Coffea arabica* L. and its diploid parental subgenomes. *Plant Gene*, *34*, 100413.

Sá, C. R. L., Silva, E. O., Terao, D., & Saraiva, A. C. M. (2008). Métodos de controle do etileno na qualidade e conservação pós colheita de frutas. Fortaleza: Embrapa Agroindústria Tropical, 36 p.

Sadka, A., Walker, C. H., Haim, D., & Bennett, T. (2023). Just enough fruit: understanding feedback mechanisms during sexual reproductive development. *Journal of Experimental Botany*, *74(8)*, 2448–2461.

Ságio, S. A., Barreto, H. G., Lima, A. A., Moreira, R. O., Rezende, P. M., Paiva, L. V., et al. (2014). Identification and expression analysis of ethylene biosynthesis and signaling genes provides insights into the early and late coffee cultivars ripening pathway. *Planta*, *239(5)*, 951–

963.

Ságio, S. A., Lima, A. A., Barreto, H. G., Carvalho, C. H. S., Paiva, L. V., & Chalfun-Junior, A. (2013). Physiological and molecular analyses of early and late *Coffea arabica* cultivars at different stages of fruit ripening. *Acta Physiologiae Plantarum*, *35*, 3091-3098.

Salmona, J., Dussert, S., Descroix, F., Kochko, A., Bertand, B., & Joet, T. (2008). Deciphering transcriptional networks that govern *Coffea arabica* seed development using combined cDNA array and real-time RT-PCR approaches. *Plant Molecular Biology*, *66*, 105–124.

Santinato, F., Tavares, T. O., Silva, R. P., Silva, C. D., & Ormond, A. T. S. (2017). Estratégias para uniformização da maturação de frutos do cafeeiro. *Revista Agrarian*, *10*, 321-327.

Santos, I. S., Ribeiro, T. H. C., de Oliveira, K. K. P. Santos, J. O., Moreira, R. O., et al. (2022). Multigenic regulation in the ethylene biosynthesis pathway during coffee flowering. *Physiology and Molecular Biology of Plants*, *28*, 1657–1669.

Sauer, M., Robert, S., & Kleine-Vehn, J. (2013). Auxin: simply complicated. *Journal of Experimental Botany*, *64(9)*, 2565–2577.

Savada, R. P., Ozga, J. A., Jayasinghege, C. P. A., Waduthanthri, K. D., & Reinecke, D. M. (2017). Heat stress differentially modifies ethylene biosynthesis and signaling in pea floral and fruit tissues. *Plant Molecular Biology*, *95(3)*, 313–331.

Scalabrin, S., Magris, G., Liva, M., Vitulo, N., Vidotto, M., Scaglione, D., et al. (2024). A chromosome-scale assembly reveals chromosomal aberrations and exchanges generating genetic diversity in *Coffea arabica* germplasm. *Nature Communications*, *15(1)*, 463.

Scalabrin, S., Toniutti, L., Di Gaspero, G., Scaglione, D., Magris, G., Vidotto, M., et al. (2020). A single polyploidization event at the origin of the tetraploid genome of *Coffea arabica* is responsible for the extremely low genetic variation in wild and cultivated germplasm. *Scientific Reports*, *10(1)*, 4642.

Schuch, U. K., Fuchigami, L. H., & Nagao, M. A. (1990). Gibberellic acid causes earlier flowering and synchronizes fruit ripening of coffee. *Plant Growth Regulation*, *9(1)*, 59–64.

Schuch, U. K., Fuchigami, L. H., & Nagao, M. A. (1992). Flowering, ethylene production, and ion leakage of coffee in response to water stress and gibberellic acid. *Journal of the American Society for Horticultural Science*, *117(1)*, 158–163.

Scudeler, F., Raetano, C. G., Araújo, D., & Bauer, F. C. (2004). Cobertura da pulverização e maturação de frutos do cafeeiro com ethephon em diferentes condições operacionais. *Bragantia*, *63*, 129-139.

Serek, M., & Sisler, E. C. (2001). Efficacy of inhibitors of ethylene binding in improvement of the postharvest characteristics of potted flowering plants. *Postharvest Biology and Technology*, *23(2)*, 161–166.

Serrani, J. C., Fos, M., Atarés, A., & García-Martínez, J. L. (2007). Effect of gibberellin and auxin on parthenocarpic fruit growth induction in the cv Micro-Tom of tomato. *Journal of Plant Growth Regulation*, *26*, 211–221.

Silva, F. M.; Salvador, N.; & Pádua, T. S. (2002). Café: Mecanização da colheita. Sistema Brasileiro de Informações do Café – SBICafé.

Silva, F. M., Arré, T. J., Tourinho, E. S., Gomes, T. S., & Alves, M. C. (2009). Uso de ethrel na colheita mecanizada e seletiva de café arábica (*Coffea arabica* L.). *Coffee Science*, *4*, 178-182.

Silva, R. A., Matsumoto, S. N., Marques, G., Oliveira, P. N., & Oliveira, L. S. (2013). Efeito do Mathury® na maturação de frutos de café variedade Catuaí Vermelho. *Cultivando o Saber*, *6*, 66-74.

Siqueira, H. H. de, & Abreu, C. M. P. de. (2006). Composição físico-química e qualidade do café submetido a dois tipos de torração e com diferentes formas de processamento. *Ciência e Agrotecnologia*, *30(1)*, 112–117.

Sisler, E. C. (2006). The discovery and development of compounds counteracting ethylene at the receptor level. *Biotechnology Advances*, *24*, 357–367.

Sisler, E. C., & Serek, M. (1997). Inhibitors of ethylene responses in plants at the receptor level: Recent developments. *Physiologia Plantarum*, *100(3)*, 577–582.

Sun, T.-P. (2011). The molecular mechanism and evolution of the GA-GID1-DELLA signaling module in plants. *Current Biology*, *21(9)*, R338-R345.

Sylvain, P. G. (1955). Some observations on *Coffea arabica* L. in Ethiopia. *Turrialba*, *5*, 37–53.

Tanimoto, E. (2005). Regulation of root growth by plant hormones—Roles for auxin and gibberellin. *Critical Reviews in Plant Sciences*, *24(4)*, 249–265.

Terceros, G. C., Resentini, F., Cucinotta, M., Manrique, S., Colombo, L., & Mendes, M. A. (2020). The Importance of Cytokinins during Reproductive Development in Arabidopsis and Beyond. *International Journal of Molecular Sciences*, *21(21)*, 8161.

Trainotti, L., Tadiello, A., & Casadoro, G. (2007). The involvement of auxin in the ripening of climacteric fruits comes of age: The hormone plays a role of its own and has an intense interplay with ethylene in ripening peaches. *Journal of Experimental Botany*, *58(12)*, 3299–3308.

Trivellin,i A., Ferrante, A., Vernieri, P., & Serra, G. (2011). Effects of abscisic acid on ethylene biosynthesis and perception in *Hibiscus rosa-sinensis* L. flower development. *Journal of Experimental Botany*, *62*, 5437−5452.

Unigarro, C. A., Bejarano, L. M. D., & Pinzón, J. F. T. (2019). Efecto de dos inductores florales sobre la floración y producción de café. *Revista Cenicafé*, *70(2)*, 19-29.

Vanderstraeten, L., Depaepe, T., Bertrand, S., & Van Der Straeten, D. (2019). The ethylene precursor ACC affects early vegetative development independently of ethylene signaling. *Frontiers in Plant Science*, 10, 1591.

Venzhik, Y., Talanova, V. & Titov, A. (2016). The effect of abscisic acid on cold tolerance and chloroplasts ultrastructure in wheat under optimal and cold stress conditions. *Acta Physiologiae Plantarum*, *38*, 63.

Verma, P. P. S., Meena, M. L., & Meena, S. K. (2014). Influence of plant growth regulators on growth, flowering and quality of tomato (*Lycopersicon esculentum* Mill), cv. H-86. *Indian Journal of Hill Farming*, *27(2)*, 19–22.

Verma, S., Attuluri, V. P. S., & Robert, H. S. (2021). An essential function for auxin in embryo development. *Cold Spring Harbor Perspectives in Biology*, *13(4)*, a039966.

Wang, B., Chu, J., Yu, T., Xu, Q., Sun, X., Yuan, J., et al. (2015). Tryptophan-independent auxin biosynthesis contributes to early embryogenesis in *Arabidopsis*. *Proceedings of the National Academy of Sciences, USA*, *112(15)*, 4821–4826.

Wang, J., Li, D., Shang, F., & Kang, X. (2017). High temperature-induced production of unreduced pollen and its cytological effects in *Populus*. *Scientific Reports*, *7(1)*, 5281.

Watkins, C. B. (2006). The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. *Biotechnology Advances*, *24*, 389–409.

Watkins, C. B. (2008). Postharvest ripening regulation and innovation in storage technology. *International Society for Horticultural Science*, *2*, 51–58.

Wei, J., Yang, Q., Ni, J., Gao, Y., Tang, Y., Bai, S., et al. (2022). Early defoliation induces auxin redistribution, promoting paradormancy release in pear buds. *Plant Physiology*, *190(4)*, 2739–2756.

WorldAtlas (2024). "Top Coffee Producing Countries". https://www.worldatlas.com/articles/top-coffee-producing-countries.html. Retrieved 2024-06-25.

Wormer, T. M., & Gituanja, J. (1970). Floral initiation and flowering of *Coffea arabica* L. in Kenya. *Experimental Agriculture*, *6*, 157−170

Wu, M., Liu, K., Li, H., Li, Y., Zhu, Y., Su, D., et al. (2024). Gibberellins involved in fruit ripening and softening by mediating multiple hormonal signals in tomato. *Horticulture Research*, *11(2)*, uhad275.

Wu, Q., Tao, X., Ai, X., Luo, Z., Mao, L., Ying, T., et al. (2018). Effect of exogenous auxin on aroma volatiles of cherry tomato (*Solanum lycopersicum* L.) fruit during postharvest ripening. *Postharvest Biology and Technology*, *146*, 108–116.

Yu, J., Cang, J., Lu, Q., Fan, B., Xu, Q., Li, W., & Wang, X. (2020). ABA enhanced cold tolerance of wheat 'dn1' via increasing ROS scavenging system. *Plant Signaling & Behavior*, *15(8)*, 1780403.

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.I. & Ming, R. (2011). Micro-collinearity and genome evolution in the vicinity of an ethylene receptor gene of cultivated diploid and allotetraploid coffee species (*Coffea*). *The Plant Journal*, *67*, 305-317.

Yu, W., Sheng, J., Zhao, R., Wang, Q., Ma, P., & Shen, L. (2019). Ethylene biosynthesis is involved in regulating chilling tolerance and SlCBF1 gene expression in tomato fruit. *Postharvest Biology and Technology*, *149*, 139–147.

Zagory, D. (1995). Ethylene-removing packaging In: Rooney, M. L. (ed.) Active food packaging. (pp. 38-54). Chapman e Hall, Glasgow, Scotland.

Zapata-Restrepo, L. N. & Guevara-Ortega, L. M. (2020). Evaluation of the incidence of foliar application of gibberellic acid in flowering trees of *Coffea arabica* L. and its impact on climate change. *DYNA*, *87(215)*, 263–269.

Zhang, C., Jian, M., Li, W., Yao, X., Tan, C., Qian, Q., et al. (2023). Gibberellin signaling modulates flowering via the DELLA–BRAHMA–NF-YC module in Arabidopsis. *Plant Cell*, *35(9)*, 3470–3484.

Zhang, J., Cao, Y., Tang, J., He, X., Li, M., Li, C., et al. (2023). Physiology and application of gibberellins in postharvest horticultural crops. *Horticulturae*, *9(6)*, 625.

Zhang, S., Gottschalk, C., & van Nocker, S. (2019). Genetic mechanisms in the repression of flowering by gibberellins in apple (*Malus* x *domestica* Borkh.). *BMC Genomics*, *20(1)*, 747.

Zhao, Y. (2010). Auxin biosynthesis and its role in plant development. *Annual Review of Plant Biology*, *61*, 49–64.

ARTIGO 1- REVIEW: The role of *FT*/*TFL1* clades and their hormonal interactions to modulate plant architecture and flowering time in annual and perennial crops

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Abstract

Human nutrition is inherently associated with the cultivation of vegetables, grains, and fruits, underscoring the critical need to understand and manipulate the balance between vegetative and reproductive development in plants. Despite the vast diversity within the plant kingdom, these developmental processes share conserved and interconnected pathways among angiosperms, predominantly involving age, vernalization, gibberellin, temperature, photoperiod, and autonomous pathways. These pathways interact with environmental cues and orchestrate the transition from vegetative growth to reproductive stages. Central to this regulation are two key genes, *FLOWERING LOCUS T* (*FT*) and *TERMINAL FLOWER 1* (*TFL1*), which activate and repress the floral initiation, respectively. They compete for transcription factors such as *FLOWERING LOCUS D* (FD) and 14-3-3 to form floral activation complexes (FAC) and floral repression complexes (FRC). The *FT/TFL1* mechanism plays a pivotal role in meristem differentiation, determining developmental outcomes as determinate or indeterminate. This review aims to explore the roles of FT and TFL1 in plant architecture and floral induction in annual and perennial species, in addition to reviewing their interactions with plant hormones. Understanding these mechanisms is crucial to address the challenges of agricultural practices especially in the face of climate change. In this context, we propose that plant development can be regulated by the control of *FT* and *TFL1* in response to plant growth regulators (PGRs), which emerge as potential tools for mitigating the adverse effects of environmental changes on plant reproductive processes.

Keywords: climate change; perennial plants; plant growth regulators; reproductive development; vegetative development.

1. Introduction

Flowering is crucial in the reproductive cycle of plants and exerts a direct influence on their survival and perpetuation. Furthermore, in crops of economic interest, flowering timing plays a fundamental role in determining productivity and harvest yield. Climate change poses a substantial challenge to agricultural production, particularly in terms of affecting the flowering period and subsequent yield [1,2]. Extreme weather events, such as heat waves and droughts, can disrupt the critical flowering stage, leading to reduced production [3]. For example, thermal stress during flowering has a more significant negative effect on maize, an annual crop, than on other stages of the growing season, with temperatures exceeding 30°C causing substantial yield losses [3].

Similarly, coffee plants, an important perennial crop, are highly vulnerable to climate change. Studies indicate that an increase in temperature and changes in precipitation patterns due to climate change can lead to the abortion of coffee flowers and premature ripening of coffee beans, ultimately reducing the area suitable for coffee cultivation [4–6]. Climate models predict a decrease in coffee production by approximately 28% in Latin America and 12% in Africa, highlighting the detrimental effects on production [7,8]. Additionally, the incidence of pests and diseases is expected to increase as a result of climate change, further compromising coffee production and quality [9]. Projections also suggest an increase in temperature variability, precipitation, and soil moisture, which will affect the reliability of the flowering period and complicate management practices such as fertilization and irrigation.[10].

Changes in climate patterns, including rising temperatures and irregular precipitation, necessitate adaptive measures to mitigate adverse impacts on agricultural production and ensure food security [1,2,11–13]. As a result, numerous studies have focused on understanding the factors triggering the transition from the vegetative to the reproductive phase. Molecular analyses have provided significant insights into how plants integrate endogenous and exogenous signals to regulate flowering, with special attention paid to gibberellins (GA), age-dependent factors, temperature, photoperiod, vernalization, and autonomous pathways [14–16].

During floral induction, the expression of many genes is regulated in response to different environmental signals [17]. Recently, considerable attention has been directed to *the FLOWERING LOCUS T* (*FT*) and *TERMINAL FLOWER 1 (TFL1)* genes, which belong to the

phosphatidylethanolamine (PEBP) family of binding proteins and are known for their roles in the induction and repression of flowering [18]. These genes play essential and antagonistic roles in controlling shoot meristem identity, flowering time, and plant architecture [19–22].

Plant architecture is shaped by shoot organs that originate from the shoot apical meristem (SAM), where cells undergo a dynamic balance between cell division and differentiation. However, after the floral transition, SAM takes the form of an inflorescence meristem (IM), which can develop into a floral meristem (MF), thus determining growth as a reproductive phase or maintaining it as a vegetative phase [23]. This process is profoundly influenced by the activities of *FT* and *TFL1* genes, in which *TFL1* maintains the indeterminate meristem, resulting in the formation of leaves and branches, whereas some member of the *FT* family converts the indeterminate meristem into determinate, triggering the formation of flowers [24–26].

These genes, notably, show a high degree of conservation, with the encoded proteins sharing similarities in amino acid sequence [27]. Thus, FT and TFL1 compete for binding with transcription factors such as bZIP, *FLOWERING LOCUS D* (*FD*), and the 14-3-3 protein, respectively forming the floral activation complex (FAC) or the floral repression complex (FRC) in the SAM [28–30]. These molecular interactions play a fundamental role in determining the fate of the meristem and, therefore, the final architecture of the plant, directly connecting gene expression with morphological development [26].

The *FT* has emerged as one of the most widely studied and characterized genes, playing a crucial role in inducing flowering. Its expression is activated in response to a series of internal and external signals, especially in the leaves, and its protein travels through the phloem until it reaches the SAM [17,30]. In SAM, FT interacts with the transcription factor FD, triggering a cascade of events that culminate in the expression of genes such as *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*), resulting in the transcription of *APETALA 1* (*AP1*) and *LEAFY* (*LFY*), fundamental for the identity of the floral meristem and the formation of floral organs [31,32]. In contrast, an antagonistic relationship was observed between *TFL1* and genes that determine floral identity. Overexpression of *TFL1* in MI regulates the expression of *AP1* and *LFY*, whereas *AP1* and *LFY* repress *TFL1* expression in MF [33]. Furthermore, MADS-box transcription factors, such as *SHORT VEGETATIVE PHASE (SVP), SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1), AGAMOUS-LIKE 24 (AGL24),* and *SEPALLATA 4 (SEP4)* also suppress *TFL1* and are regulated by *AP1* in MF. However, it is

important to note that appropriate levels of expression of these genes are necessary for *AP1* to exert its function of suppressing *TFL1* in MF [23,34].

In this context, the coordinated regulation of *FT* and *TFL1* not only directly influences plant architecture throughout its life cycle but also plays a significant role in determining the timing of flowering. Understanding how these genes interact in SAM is fundamental to unravel the mechanisms that govern the transition from the vegetative to the reproductive phase. However, it is important to note that it is not yet known how climate change affects these genes, it is necessary to develop strategies for mitigating the adverse effects in future scenarios. In this regard, the use of plant growth regulators (PGRs) can be a viable strategy, as they can modulate plant growth and development to regulate flowering and the subsequent formation and maturation of fruits, particularly in crops. Additionally, climate change can affect production, and consequently, human nutrition [35]. These strategies can help improve management practices for food production, ensuring better adaptation and resilience in the face of environmental challenges.

2. The *FT/TFL1* developmental regulatory pathways: from the model species to in perennial crops

In the plant kingdom, reproductive patterns vary between two main categories: annual and perennial. Although annual species complete their entire life cycle in a single year, perennial species generally take longer to enter their reproductive state, sometimes requiring several years [36].

In studies focused on annual species, especially *Arabidopsis*, the balance between FT/TFL1 has been the subject of investigation, directly associating it with the fate of the meristem [20,37]. The dynamics of this balance are strongly influenced by the plant's age. In young plants, the concentration of TFL1 at the apex is high relative to that of FT, resulting in a low FT/TFL1 ratio, which maintains the meristem in a vegetative state. However, as the plant grows and more leaves are formed, a larger pool of FT accumulates in the SAM due to the transport of FT from the leaves [29]. This increase in the FT/TFL1 ratio triggers the transition from the vegetative to reproductive meristem, allowing the plant's life cycle to be completed [29]. Thus, the moderate relationship between FT/TFL1 allows balanced development between branches and flowers [22].

Understanding the mechanisms of *FT/TFL1* gene regulation is not only limited to annual plants but also extends to perennial species, where the interaction between these genes plays a

crucial role in controlling the plant life cycle. In contrast to annual plants, perennial plants exhibit high levels of TFL1 in the SAM, maintaining it in a vegetative state, whereas in the axillary meristems, the level of FT is higher, activating genes related to floral meristem identity [29]. Downregulation of *TFL1* accelerates flowering in several perennial species, such as poplar, apple, and pear [38–40], highlighting the importance of TFL1 in these processes. Furthermore, expression of the *FT* and *TFL1* orthologs, *SINGLE FLOWER TRUSS (SFT)* and *AUTO-PODA (SP)*, respectively, directly influence production in cultivated plants such as tomato [22].

More recently, it was discovered that *TFL1* is also expressed in the leaves and mobile in tobacco and tomatoes [41]. Thus, the movement of PEBP mRNA may be a conserved mechanism across different plant species, indicating that both *FT* and *TFL1* can take the same pathway, signaling whether the plant should flower [41]. This recent finding suggests that flowering regulation involves a more complex interaction between *FT* and *TFL1* than previously understood. The presence of *TFL1* in the leaves indicates that both proteins could be simultaneously translocated to the SAM, where their relative concentrations and interactions would influence the decision to activate floral development. In perennial plants, this dual expression could allow for more flexible and responsive control of flowering, enabling plants to adapt to varying environmental conditions and maintain continuous or multiple flowering cycles throughout the year. This finding implies that the mechanisms governing flowering are more nuanced, potentially involving additional regulatory layers and interactions between *FT* and *TFL1*. For example, miRNAs target *FT* and *TFL1* in various species, including perennial plants [42–44]. These non-coding RNAs affect gene expression by influencing the mRNA cleavage, translation, or DNA methylation of target genes [45]. In coffee plants, miRNAs involved in microsporogenesis, the regulation of hormonal responses, and the modulation of transcription factors associated with flowering in response to environmental changes have been identified [43,44]. Additionally, in Dendrobium catenatum, miR156 regulates *FT* and *TFL1* expression during different developmental stages, including the juvenile–adult transition and plant maturation [42]. This highlights the need for further research to understand how the spatial and temporal expression patterns of these proteins affect flowering, which could have significant implications for agricultural practices, especially in the context of climate change and cultivation of perennial crops.

In this context, the role of these genes has been studied in various perennial crops in response to seasonal variations throughout the year to understand how their regulation

influences flowering, and consequently, fruit production [37,46–48]. For example, Cardon et al. [47] demonstrated that *CaFT1* acts as a florigen ortholog, exhibiting active transcription from February to October across various *Coffea* spp. genotypes. This suggests a continuum of floral induction that offers multiple starting points for floral activation, thereby explaining the asynchrony and prolonged flowering events observed in the perennial tropical species. Similarly, it has been demonstrated that hops (*Humulus lupulus* L.) can flower multiple times throughout the year under subtropical conditions in Brazil, regardless of the season, due to consistently inductive photoperiods [48]. Additionally, the study suggests that under these conditions, the expression of *TFL* may not be sufficient to induce branching and inhibit *HlFT* in hops, resulting in multiple flowering events [48].

However, divergences in the literature arise when exploring the FT/TFL1 relationship between annual and perennial plants. Although the antagonistic role of FT and TFL1 can be explained by their competitive interaction with FD, studies with transgenic *Arabidopsis* have revealed a greater affinity of FT with FD to form a floral activation complex (FAC) [37]. This dynamic suggests the possibility of a dominant role for FT in counterbalancing the repressive effect of TFL1 before floral transition [49]. In contrast, in perennial species such as pear (*Pyrus pyrifolia Nakai*), studies indicate that *PpFT1* expression is not upregulated during floral induction, but that a reduction in *PpTFL1* expression is essential to induce flowering [50]. Furthermore, ectopic overexpression of *ScTFL1* in *Arabidopsis* delayed flowering, unexpectedly, overexpression of florigen-like *ScFT1* also caused delayed flowering. This suggests that the roles and expression patterns of *FT* and *TFL1* gene family members in *Saccharum* spp. diverged from those of similar PEBP family members [51].

These discrepancies can be attributed to the inherent differences in the life cycles and environmental responses of annual and perennial plants. Annual plants complete their life cycle in a single growing season, requiring a rapid and decisive transition to flowering, which may necessitate a stronger and more straightforward FT-FD interaction to ensure reproductive success. In contrast, perennial plants, which have lived for several years, have developed more complex regulatory mechanisms to balance vegetative growth and flowering over extended periods. For instance, the expression of *TFL1* in leaves and its mobility can provide perennials with an adaptable system to respond to various environmental conditions, promoting asynchronous and prolonged flowering cycles. This indicates that in perennials, the regulation of flowering is not solely dependent on simple FT/TFL1 antagonism but involves a complex interaction of spatial and temporal expression patterns, as well as additional environmental

factors. Therefore, by manipulating the expression of these genes or using plant growth regulators, it may be possible to optimize flowering time and improve fruit production in perennial crops, thereby enhancing agricultural resilience and food security.

3. Interaction between *TFL1* and plant hormones

Most studies have focused on the interactions between plant hormones and *FT*, a flowering inducer. However, it is essential to understand the balance between *FT* and *TFL1* with these plant hormones, especially in perennial species, where this research is limited. Therefore, this topic aims to review the existing literature regarding the regulation of *TFL1* expression by plant hormones.

In addition to the known roles of FT and TFL1 in determining plant architecture, the interactions between these genes and hormones play a significant role in this process. Auxin, which is produced in the SAM, plays a fundamental role in maintaining apical dominance and stem elongation, inhibiting the growth of lateral branches, whereas cytokinin promotes cell division and stimulates the growth of lateral branches [52]. The balanced regulation of these hormones is essential for the control of vegetative growth and, consequently, reproductive growth [53], which has prompted investigations into the interaction between FT/TFL1 and hormones in shaping plant architecture and promoting flourishing [50,54–57].

In studies carried out in apples (*Malus domestica* Borkh), it was observed that the combination of auxin and cytokinin in SAM increased the expression of *MdTFL1*, maintaining vegetative growth, while during the transition to the reproductive phase, the decrease in auxin levels favors the expression of *MdFT*, determining the fate of the shoot apical meristem [55]. Furthermore, the exogenous application of 6-BA (6-benzylaminopurine), a CK that stimulates floral bud formation in apple trees and is applied before floral induction, has an indirect effect on the endogenous ratio between Zeatin and IAA. This results in a reduction in the expression of *MdTFL1*, consequently increasing the expression of *AFL1* (*APPLE FLORICAULA/LFY*) at the onset of flowering [58,59]. In pear (*Pyrus pyrifolia* Nakai), some genes related to the biosynthesis and signaling of hormones, such as auxin, cytokinin, abscisic acid, and ethylene, showed a synergistic or antagonistic co-expression pattern with *PpTFL1* during floral induction [50]. While the expression of genes related to auxin and abscisic acid tended to decrease, the expression of genes related to cytokinin and ethylene pathways increased during this process [50]. These results suggest that in perennial plants, the hormones auxin, abscisic acid, and

gibberellin can maintain *TFL1* levels higher than *FT*, while ethylene and cytokinin tend to increase *FT* expression (Figure 1).

Consistent with this, it has also been demonstrated that abscisic acid (ABA) plays a role in delaying floral transition in *Arabidopsis*, positively influencing the expression of *FLOWERING LOCUS C* (*FLC*) through the transcription factor ABI5 [60], studies have revealed that ABA functions as a negative regulator of branching, potentially suppressing elements of the cell cycle machinery as well as indole-3-acetic acid (IAA) biosynthesis and transport [61]. These findings suggest a complex interaction among ABA, ABI5, and TFL1, which potentially affects the expression of other genes involved in floral transition and branching. Furthermore, ethylene also influences the expression of *TERMINAL FLOWER 1* (*TFL1*), as demonstrated by studies showing that the ethylene receptor ETR2 can delay floral transition and affect starch accumulation in rice, possibly through downstream gene regulation, such as *TFL1* [62]. These connections reveal an intricate network of molecular regulation between PEBP family members and plant hormones that coordinate floral transition and development in plants.

Some studies have shown that the application of gibberellin (GA) upregulates the *TFL1* homolog and inhibits flower formation in perennial species, such as apples and roses [54,57,63]. In saffron (*Crocus sativus* L.) it was suggested that DELLA negatively regulates *TFL1*, showing its role in the GA signaling pathway [56], the same was reported for grapevine [64]. Therefore, in perennial plants that exhibit repression of flowering by GA, TFL1 may be a key point of this repression and may be a direct target of GA-responsive transcription factors (Figure 1) [57,65]. These studies demonstrate that the use of plant growth regulators (PGRs) can alter the expression of *FT* and *TFL1* genes, potentially influencing the flowering time of these plants and consequently regulating their architecture. Although these studies have expanded our understanding of the interplay between FT/TFL1 and plant hormones in regulating flowering, it is important to recognize that significant gaps remain, and many assumptions remain to be clarified. A schematic representation of floral regulation in perennial plants by PGRs is shown in Figure 1, illustrating a possible pattern of co-expression between plant hormones and *FT* and *TFL1* genes based on the results reviewed in this section. This knowledge can be expanded for the practical applications of these PGRs in the field, enabling the regulation of flowering time and synchronization.

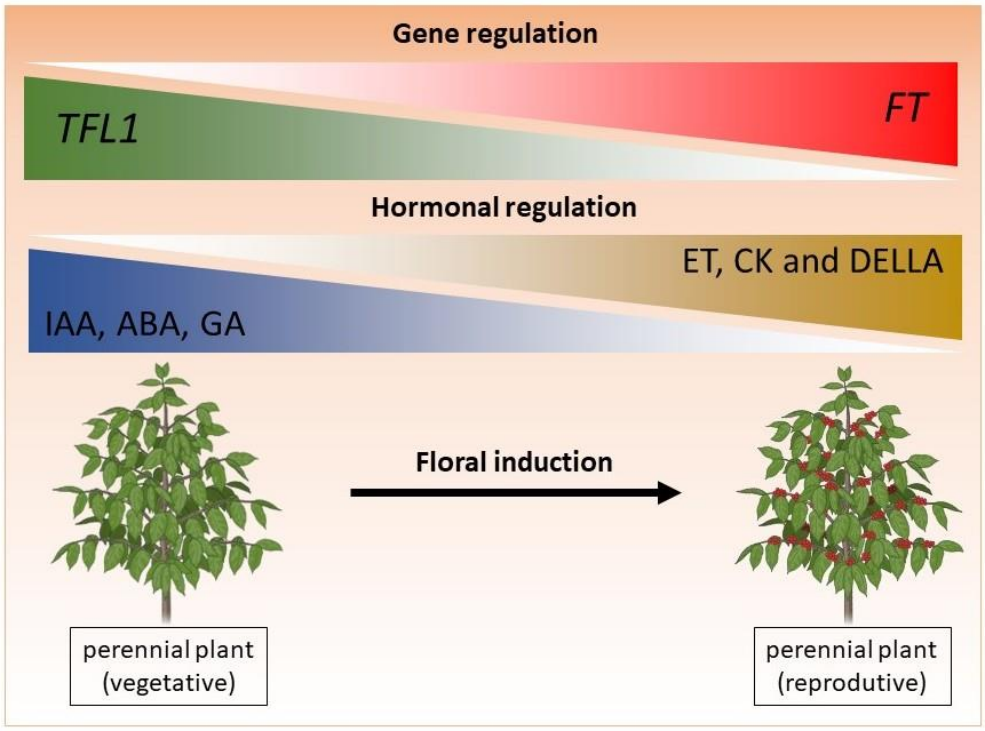

Fig. 1 – Proposed model of co-expression between FT and TFL1 with plant hormones during the transition from the vegetative to reproductive phase in perennial plants. *FT: FLOWERING LOCUS T, TFL1: TERMINAL FLOWER 1*, IAA: auxin, ABA: abscisic acid, GA: Gibberellin, ET: ethylne, CK: citokinin.

4. Conclusion

Flowering regulation in plants, governed by genes such as *FLOWERING LOCUS T* (*FT*) and *TERMINAL FLOWER 1* (*TFL1*), is a pivotal aspect that influences both plant architecture and agricultural productivity. As climate change intensifies, understanding these regulatory mechanisms has become increasingly critical for sustaining global food security. This review underscores the conserved pathways involving *FT* and *TFL1* across annual and perennial species, highlighting their interactions with plant hormones and environmental cues. These interactions not only dictate the transition from vegetative to reproductive phases but also determine the adaptability of plants to changing environmental conditions.

The intricate balance between FT and TFL1, mediated by hormonal signals, such as auxins, cytokinins, gibberellins, ethylene, and abscisic acid, underscores the complexity of flowering regulation. In annuals, the timely expression of *FT* triggers floral transition, whereas in perennials, TFL1 maintains meristematic indeterminacy, enabling continuous or periodic flowering responses to environmental cues. These insights suggest promising avenues for leveraging plant growth regulators (PGRs) to optimize flowering times. Such efforts not only

promise to sustainably improve agricultural practices but also provide strategic tools to mitigate the impacts of climate change on agricultural production and global food security.

Author Contributions

L.M.A, R.R.O and A.C.-J. conceptualized the project; L.M.A wrote the first version of this manuscript; L.M.A and R.R.O. reviewed and wrote the final version of this manuscript; A.C.-J. supported the project. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

Not applicable.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Alotaibi, M. Climate Change, Its Impact on Crop Production, Challenges, and Possible Solutions. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* **2023**, *51*, 13020–13020, doi:10.15835/nbha51113020.
2. Keutgen, A.J. Climate Change: Challenges and Limitations in Agriculture. *IOP Conf. Ser.: Earth Environ. Sci.* **2023**, *1183*, 012069, doi:10.1088/1755-1315/1183/1/012069.

3. Luo, N.; Mueller, N.; Zhang, Y.; Feng, P.; Huang, S.; Liu, D.L.; Yu, Y.; Wang, X.; Wang, P.; Meng, Q. Short-Term Extreme Heat at Flowering Amplifies the Impacts of Climate Change on Maize Production. *Environ. Res. Lett.* **2023**, *18*, 084021, doi:10.1088/1748-9326/ace7e3.

4. Phillip, K.; George, T.E.; Isabirye, M.; Assoua, J.E. Climate Variability and Arabica Coffee Production in Uganda. In *Agricultural Transformation in Africa: Contemporary Issues, Empirics, and Policies*; Odularu, G.O.A., Ed.; Springer International Publishing: Cham, 2023; pp. 87–114 ISBN 978-3-031-19527-3.

5. Venancio, L.P.; Filgueiras, R.; Mantovani, E.C.; do Amaral, C.H.; da Cunha, F.F.; dos Santos Silva, F.C.; Althoff, D.; dos Santos, R.A.; Cavatte, P.C. Impact of Drought Associated with High Temperatures on *Coffea canephora* Plantations: A Case Study in Espírito Santo State, Brazil. *Sci Rep* **2020**, *10*, 19719, doi:10.1038/s41598-020-76713-y.

6. Wagner, S.; Jassogne, L.; Price, E.; Jones, M.; Preziosi, R. Impact of Climate Change on the Production of *Coffea arabica* at Mt. Kilimanjaro, Tanzania. *Agriculture* **2021**, *11*, 53, doi:10.3390/agriculture11010053.

7. Peruta, R.D.; Mereu, V.; Spano, D.; Marras, S.; Trabucco, A. *Coffee Agrosystems and Climate Change*; Copernicus Meetings, 2023;

8. Dinh, T.L.A.; Aires, F.; Rahn, E. Statistical Analysis of the Weather Impact on Robusta Coffee Yield in Vietnam. *Front. Environ. Sci.* **2022**, *10*, doi:10.3389/fenvs.2022.820916.

9. Bracken, P.; Burgess, P.J.; Girkin, N.T. Opportunities for Enhancing the Climate Resilience of Coffee Production through Improved Crop, Soil and Water Management. *Agroecology and Sustainable Food Systems* **2023**, *47*, 1125–1157, doi:10.1080/21683565.2023.2225438.

10. Bathiany, S.; Belleflamme, A.; Zohbi, J.E.; Ney, P.; Goergen, K.; Rechid, D. Increasing Interannual Climate Variability during Crop Flowering in Europe. *Environ. Res. Lett.* **2023**, *18*, 044037, doi:10.1088/1748-9326/acc87e.

11. DaMatta, F.M.; Ramalho, J.D.C. Impacts of Drought and Temperature Stress on Coffee Physiology and Production: A Review. *Braz. J. Plant Physiol.* **2006**, *18*, 55–81, doi:10.1590/S1677-04202006000100006.

12. Bilen, C.; El Chami, D.; Mereu, V.; Trabucco, A.; Marras, S.; Spano, D. A Systematic Review on the Impacts of Climate Change on Coffee Agrosystems. *Plants* **2023**, *12*, 102, doi:10.3390/plants12010102.

13. Jawo, T.O.; Kyereh, D.; Lojka, B. The Impact of Climate Change on Coffee Production of Small Farmers and Their Adaptation Strategies: A Review. *Climate and Development* **2023**, *15*, 93–109, doi:10.1080/17565529.2022.2057906.

14. Leijten, W.; Koes, R.; Roobeek, I.; Frugis, G. Translating Flowering Time from *Arabidopsis thaliana* to Brassicaceae and Asteraceae Crop Species. *Plants* **2018**, *7*, 111, doi:10.3390/plants7040111.

15. Kim, D.-H. Current Understanding of Flowering Pathways in Plants: Focusing on the Vernalization Pathway in *Arabidopsis* and Several Vegetable Crop Plants. *Hortic. Environ. Biotechnol.* **2020**, *61*, 209–227, doi:10.1007/s13580-019-00218-5.

16. Bao, S.; Hua, C.; Shen, L.; Yu, H. New Insights into Gibberellin Signaling in Regulating Flowering in *Arabidopsis*. *Journal of Integrative Plant Biology* **2020**, *62*, 118–131, doi:10.1111/jipb.12892.

17. Khan, M.R.G.; Ai, X.-Y.; Zhang, J.-Z. Genetic Regulation of Flowering Time in Annual and Perennial Plants. *WIREs RNA* **2014**, *5*, 347–359, doi:10.1002/wrna.1215.

18. Kaneko-Suzuki, M.; Kurihara-Ishikawa, R.; Okushita-Terakawa, C.; Kojima, C.; Nagano-Fujiwara, M.; Ohki, I.; Tsuji, H.; Shimamoto, K.; Taoka, K.-I. TFL1-Like Proteins in Rice Antagonize Rice FT-Like Protein in Inflorescence Development by Competition for Complex Formation with 14-3-3 and FD. *Plant and Cell Physiology* **2018**, *59*, 458–468, doi:10.1093/pcp/pcy021.

19. Jin, S.; Nasim, Z.; Susila, H.; Ahn, J.H. Evolution and Functional Diversification of *FLOWERING LOCUS T/TERMINAL FLOWER 1* Family Genes in Plants. *Seminars in Cell & Developmental Biology* **2021**, *109*, 20–30, doi:10.1016/j.semcdb.2020.05.007.

20. Liu, L.; Xuan, L.; Jiang, Y.; Yu, H. Regulation by *FLOWERING LOCUS T* and *TERMINAL FLOWER 1* in Flowering Time and Plant Architecture. *Small Structures* **2021**, *2*, 2000125, doi:10.1002/sstr.202000125.

21. Matsoukas, I.G.; Massiah, A.J.; Thomas, B. Florigenic and Antflorigenic Signaling in Plants. *Plant and Cell Physiology* **2012**, *53*, 1827–1842, doi:10.1093/pcp/pcs130.

22. Moraes, T.S.; Dornelas, M.C.; Martinelli, A.P. FT/TFL1: Calibrating Plant Architecture. *Front Plant Sci* **2019**, *10*, 97, doi:10.3389/fpls.2019.00097.

23. Teo, Z.W.N.; Song, S.; Wang, Y.-Q.; Liu, J.; Yu, H. New Insights into the Regulation of Inflorescence Architecture. *Trends in Plant Science* **2014**, *19*, 158–165, doi:10.1016/j.tplants.2013.11.001.

24. Goretti, D.; Silvestre, M.; Collani, S.; Langenecker, T.; Méndez, C.; Madueño, F.; Schmid, M. *TERMINAL FLOWER1* Functions as a Mobile Transcriptional Cofactor in the Shoot Apical Meristem1 [OPEN]. *Plant Physiology* **2020**, *182*, 2081–2095, doi:10.1104/pp.19.00867.

25. Kim, W.; Park, T.I.; Yoo, S.J.; Jun, A.R.; Ahn, J.H. Generation and Analysis of a Complete Mutant Set for the *Arabidopsis FT/TFL1* Family Shows Specific Effects on Thermo-Sensitive Flowering Regulation. *Journal of Experimental Botany* **2013**, *64*, 1715–1729, doi:10.1093/jxb/ert036.

26. Colleoni, P.E.; van Es, S.W.; Winkelmolen, T.; Immink, R.G.H.; van Esse, G.W. Flowering Time Genes Branching Out. *Journal of Experimental Botany* **2024**, erae112, doi:10.1093/jxb/erae112.

27. Ho, W.W.H.; Weigel, D. Structural Features Determining Flower-Promoting Activity of *Arabidopsis FLOWERING LOCUS T*. *The Plant Cell* **2014**, *26*, 552–564, doi:10.1105/tpc.113.115220.

28. Hanano, S.; Goto, K. *Arabidopsis TERMINAL FLOWER*1 Is Involved in the Regulation of Flowering Time and Inflorescence Development through Transcriptional Repression. *The Plant Cell* **2011**, *23*, 3172–3184, doi:10.1105/tpc.111.088641.

29. McGarry, R.C.; Ayre, B.G. Manipulating Plant Architecture with Members of the CETS Gene Family. *Plant Science* **2012**, *188–189*, 71–81, doi: 10.1016/j.plantsci.2012.03.002.

30. Taoka, K.; Ohki, I.; Tsuji, H.; Furuita, K.; Hayashi, K.; Yanase, T.; Yamaguchi, M.; Nakashima, C.; Purwestri, Y.A.; Tamaki, S.; et al. 14-3-3 Proteins Act as Intracellular Receptors for Rice Hd3a Florigen. *Nature* **2011**, *476*, 332–335, doi:10.1038/nature10272.

31. Fernández, V.; Takahashi, Y.; Le Gourrierec, J.; Coupland, G. Photoperiodic and Thermosensory Pathways Interact through CONSTANS to Promote Flowering at High

Temperature under Short Days. *The Plant Journal* **2016**, *86*, 426–440, doi:10.1111/tpj.13183.

32. Tsuji, H. Molecular Function of Florigen. *Breed Sci* **2017**, *67*, 327–332, doi:10.1270/jsbbs.17026.
33. Ratcliffe, O.J.; Bradley, D.J.; Coen, E.S. Separation of Shoot and Floral Identity in *Arabidopsis*. *Development* **1999**, *126*, 1109–1120, doi:10.1242/dev.126.6.1109.
34. Liu, C.; Teo, Z.W.N.; Bi, Y.; Song, S.; Xi, W.; Yang, X.; Yin, Z.; Yu, H. A Conserved Genetic Pathway Determines Inflorescence Architecture in *Arabidopsis* and Rice. *Developmental Cell* **2013**, *6*, 612–622, doi: 10.1016/j.devcel.2013.02.013.
35. Myers, S.S.; Smith, M.R.; Guth, S.; Golden, C.D.; Vaitla, B.; Mueller, N.D.; Dangour, A.D.; Huybers, P. Climate Change and Global Food Systems: Potential Impacts on Food Security and Undernutrition. *Annual Review of Public Health* **2017**, *38*, 259–277, doi:10.1146/annurev-publhealth-031816-044356.
36. Albani, M.C.; Coupland, G. Chapter Eleven - Comparative Analysis of Flowering in Annual and Perennial Plants. In *Current Topics in Developmental Biology*; Timmermans, M.C.P., Ed.; Plant Development; Academic Press, 2010; Vol. 91, pp. 323–348.
37. Lee, C.; Kim, S.-J.; Jin, S.; Susila, H.; Youn, G.; Nasim, Z.; Alavilli, H.; Chung, K.-S.; Yoo, S.J.; Ahn, J.H. Genetic Interactions Reveal the Antagonistic Roles of FT/TSF and TFL1 in the Determination of Inflorescence Meristem Identity in *Arabidopsis*. *The Plant Journal* **2019**, *99*, 452–464, doi:10.1111/tpj.14335.
38. Freiman, A.; Shlizerman, L.; Golobovitch, S.; Yablovitz, Z.; Korchinsky, R.; Cohen, Y.; Samach, A.; Chevreau, E.; Le Roux, P.-M.; Patocchi, A.; et al. Development of a Transgenic Early Flowering Pear (*Pyrus Communis* L.) Genotype by RNAi Silencing of *PcTFL1-1* and *PcTFL1-2*. *Planta* **2012**, *235*, 1239–1251, doi:10.1007/s00425-011-1571-0.
39. Kotoda, N.; Iwanami, H.; Takahashi, S.; Abe, K. Antisense Expression of *MdTFL1*, a *TFL1-like* Gene, Reduces the Juvenile Phase in Apple. *Journal of the American Society for Horticultural Science* **2006**, *131*, 74–81, doi:10.21273/JASHS.131.1.74.
40. Mohamed, R.; Wang, C.-T.; Ma, C.; Shevchenko, O.; Dye, S.J.; Puzey, J.R.; Etherington, E.; Sheng, X.; Meilan, R.; Strauss, S.H.; et al. *Populus CEN/TFL1* Regulates First Onset of Flowering, Axillary Meristem Identity and Dormancy Release in *Populus*. *The Plant Journal* **2010**, *62*, 674–688, doi:10.1111/j.1365-313X.2010.04185.x.
41. Huang, N.-C.; Luo, K.-R.; Yu, T.-S. Mobility of Antiflorigen and PEBP mRNAs in Tomato-Tobacco Heterografts1[OPEN]. *Plant Physiol* **2018**, *178*, 783–794, doi:10.1104/pp.18.00725.
42. Zheng, J.; Ma, Y.; Zhang, M.; Lyu, M.; Yuan, Y.; Wu, B. Expression Pattern of *FT/TFL1* and miR156-Targeted *SPL* Genes Associated with Developmental Stages in *Dendrobium catenatum*. *International Journal of Molecular Sciences* **2019**, *20*, 2725, doi:10.3390/ijms20112725.
43. 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.; et al. 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* **2024**, *8*, e561, doi:10.1002/pld3.561.

44. Ribeiro, T.H.C.; 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.; et al. The Floral Development of the Allotetraploid *Coffea Arabica* L. Correlates with a Small RNA Dynamic Reprogramming. *The Plant Journal* **2024**, *n/a*, doi:10.1111/tpj.16713.

45. Singh, P.; Dutta, P.; Chakrabarty, D. miRNAs Play Critical Roles in Response to Abiotic Stress by Modulating Cross-Talk of Phytohormone Signaling. *Plant Cell Rep* **2021**, *40*, 1617–1630, doi:10.1007/s00299-021-02736-y.

46. Cardon, C.H. Characterization and Functional Analysis of *FLOWERING LOCUS T* (*FT*) and *TERMINAL FLOWER 1* (*TFL1*) Orthologs in *Coffea arabica* L.: Moving Forward into Understanding Coffee Flowering. **2020**.

47. Cardon, C.H.; de Oliveira, R.R.; Lesy, V.; Ribeiro, T.H.C.; Fust, C.; Pereira, L.P.; Colasanti, J.; Chalfun-Junior, A. Expression of Coffee Florigen *CaFT1* Reveals a Sustained Floral Induction Window Associated with Asynchronous Flowering in Tropical Perennials. *Plant Science* **2022**, *325*, 111479, doi:10.1016/j.plantsci.2022.111479.

48. Gutiérrez, R.M.; Oliveira, R.R. de; Ribeiro, T.H.C.; Oliveira, K.K.P. de; Silva, J.V.N.; Alves, T.C.; Amaral, L.R. do; Gomes, M. de S.; Gomes, M. de S.; Chalfun-Junior, A. Unveiling the Phenology and Associated Floral Regulatory Pathways of *Humulus lupulus* L. in Subtropical Conditions. *Planta* **2024**, *259*, doi:10.1007/s00425-024-04428-9.

49. Jaeger, K.E.; Pullen, N.; Lamzin, S.; Morris, R.J.; Wigge, P.A. Interlocking Feedback Loops Govern the Dynamic Behavior of the Floral Transition in *Arabidopsis*. *The Plant Cell* **2013**, *25*, 820–833, doi:10.1105/tpc.113.109355.

50. Bai, S.; Tuan, P.A.; Saito, T.; Ito, A.; Ubi, B.E.; Ban, Y.; Moriguchi, T. Repression of *TERMINAL FLOWER1* Primarily Mediates Floral Induction in Pear (*Pyrus Pyrifolia* Nakai) Concomitant with Change in Gene Expression of Plant Hormone-Related Genes and Transcription Factors. *Journal of Experimental Botany* **2017**, *68*, 4899–4914, doi:10.1093/jxb/erx296.

51. Coelho, C.P.; Minow, M.A.A.; Chalfun-Júnior, A.; Colasanti, J. Putative Sugarcane *FT*/*TFL1* Genes Delay Flowering Time and Alter Reproductive Architecture in *Arabidopsis*. *Frontiers in Plant Science* **2014**, *5*.

52. Vanstraelen, M.; Benková, E. Hormonal Interactions in the Regulation of Plant Development. *Annual Review of Cell and Developmental Biology* **2012**, *28*, 463–487, doi:10.1146/annurev-cellbio-101011-155741.

53. Yang, M.; Jiao, Y. Regulation of Axillary Meristem Initiation by Transcription Factors and Plant Hormones. *Frontiers in Plant Science* **2016**, *7*.

54. Haberman, A.; Ackerman, M.; Crane, O.; Kelner, J.-J.; Costes, E.; Samach, A. Different Flowering Response to Various Fruit Loads in Apple Cultivars Correlates with Degree of Transcript Reaccumulation of a *TFL1*-Encoding Gene. *The Plant Journal* **2016**, *87*, 161–173, doi:10.1111/tpj.13190.

55. Mimida, N.; Komori, S.; Suzuki, A.; Wada, M. Functions of the Apple *TFL1*/*FT* Orthologs in Phase Transition. *Scientia Horticulturae* **2013**, *156*, 106–112, doi: 10.1016/j.scienta.2013.04.001.

56. Renau-Morata, B.; Nebauer, S.G.; García-Carpintero, V.; Cañizares, J.; Gómez Minguet, E.; de los Mozos, M.; Molina, R.V. Flower Induction and Development in Saffron: Timing

and Hormone Signalling Pathways. *Industrial Crops and Products* **2021**, *164*, 113370, doi: 10.1016/j.indcrop.2021.113370.

57. Zhang, S.; Gottschalk, C.; van Nocker, S. Genetic Mechanisms in the Repression of Flowering by Gibberellins in Apple (*Malus x domestica* Borkh.). *BMC Genomics* **2019**, *20*, 747, doi:10.1186/s12864-019-6090-6.

58. Li, Y.; Zhang, D.; Xing, L.; Zhang, S.; Zhao, C.; Han, M. Effect of Exogenous 6-Benzylaminopurine (6-BA) on Branch Type, Floral Induction and Initiation, and Related Gene Expression in 'Fuji' Apple (*Malus domestica* Borkh). *Plant Growth Regul* **2016**, *79*, 65–70, doi:10.1007/s10725-015-0111-5.

59. Li, Y.; Zhang, D.; Zhang, L.; Zuo, X.; Fan, S.; Zhang, X.; Shalmani, A.; Han, M. Identification and Expression Analysis of Cytokinin Response-Regulator Genes during Floral Induction in Apple (*Malus domestica* Borkh). *Plant Growth Regul* **2017**, *83*, 455–464, doi:10.1007/s10725-017-0311-2.

60. Wang, Y.; Li, L.; Ye, T.; Lu, Y.; Chen, X.; Wu, Y. The Inhibitory Effect of ABA on Floral Transition Is Mediated by ABI5 in *Arabidopsis*. *Journal of Experimental Botany* **2013**, *64*, 675–684, doi:10.1093/jxb/ers361.

61. Yao, C.; Finlayson, S.A. Abscisic Acid Is a General Negative Regulator of Arabidopsis Axillary Bud Growth. *Plant Physiology* **2015**, *169*, 611–626, doi:10.1104/pp.15.00682.

62. Wuriyanghan, H.; Zhang, B.; Cao, W.-H.; Ma, B.; Lei, G.; Liu, Y.-F.; Wei, W.; Wu, H.-J.; Chen, L.-J.; Chen, H.-W.; et al. The Ethylene Receptor ETR2 Delays Floral Transition and Affects Starch Accumulation in Rice. *The Plant Cell* **2009**, *21*, 1473–1494, doi:10.1105/tpc.108.065391.

63. Randoux, M.; Jeauffre, J.; Thouroude, T.; Vasseur, F.; Hamama, L.; Juchaux, M.; Sakr, S.; Foucher, F. Gibberellins Regulate the Transcription of the Continuous Flowering Regulator, *RoKSN*, a Rose *TFL1* Homologue. *Journal of Experimental Botany* **2012**, *63*, 6543–6554, doi:10.1093/jxb/ers310.

64. Arro, J.; Yang, Y.; Song, G.-Q.; Zhong, G.-Y. RNA-Seq Reveals New DELLA Targets and Regulation in Transgenic GA-Insensitive Grapevines. *BMC Plant Biology* **2019**, *19*, 80, doi:10.1186/s12870-019-1675-4.

65. Elsysy, M.A.; Hirst, P.M. Molecular Basis of Flower Formation in Apple Caused by Defoliation and Gibberellins. *Journal of the American Society for Horticultural Science* **2019**, *144*, 414–419, doi:10.21273/JASHS04760-19.

ARTIGO 2: Hormonal crosstalk during the reproductive stage in *Coffea arabica* L.: Interactions between gibberellin, abscisic acid, and ethylene.

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Main conclusion: The application of gibberellin and abscisic acid in coffee plants resulted in increased floral bud formation and fruit production by regulating key genes involved in flowering and hormonal biosynthesis pathways.

ABSTRACT

Despite ongoing efforts, understanding hormonal regulation in perennial and woody species with complex phenological cycles, such as *Coffea arabica* L., remains limited. Given the global importance of coffee and, in general, the fruits for human diet, identifying the main regulators of reproductive development is crucial to guarantee production especially in face of climate change. This study investigated the effects of gibberellin (GA) and abscisic acid (ABA) at different concentrations (5, 25 and 100ppm) on the reproductive development of *C. arabica*. Results showed that GA irrespective of concentration and ABA at 25 ppm applied during floral induction (March) significantly increased the number of floral buds, with ABA also accelerating development. Similarly, applying these regulators in plants with floral buds at more advanced developmental stages (August) increased the number of floral buds and fruit production in the GA (5 and 100 ppm) and ABA (25 and 100 ppm) treatments. Phylogenetic

and molecular analyses identified genes related to GA and ABA biosynthesis, degradation, and signaling in coffee plants. GA and ABA treatments affected the expression of genes related to floral induction and organ formation, such as *CaDELLA* in March, which may relate to the increased number of floral buds. Moreover, in August, treatments of 5 and 100 ppm GA and 100 ppm ABA increased the expression of *CaFT1*, likely due to the downregulation of *CaCO* during this period. These interactions suggest reciprocal regulation of GA-ABA pathways depending on the concentration and tissue, with GA-ABA regulating ethylene biosynthesis in leaves and floral buds. These findings provide new insights into the hormonal regulation of coffee flowering, guiding field practices and breeding programs to maximize coffee production.

Keywords: Floral bud development. Hormones. Induction of flowering. RT-qPCR.

Abbreviations

ACC: 1-aminocyclopropane-1-carboxylic acid

ACO: 1-aminocyclopropane-1-carboxylic acid oxidase

ACS: 1-aminocyclopropane-1-carboxylic acid synthase

ABA: Abscisic Acid

AP1: *APETALA1*

CO: *CONSTANS*

CYP707A: *CYTOCHROME P450, FAMILY 707, SUBFAMILY A*

ET: ethylene

FT: *FLOWERING LOCUS T*

GA: Gibberellin

GA2ox: *GIBBERELLIN 2-OXIDASE*

GA3ox: *GIBBERELLIN 3-OXIDASE*

LFY: *LEAFY*

NCED: *9-CIS-EPOXYCAROTENOID DIOXYGENASE*

PGRs: plant growth regulators

TFL1: *TERMINAL FLOWER 1*

1. INTRODUCTION

Flowering is highly regulated by an intricate network of hormonal and genetic signaling that responds to endogenous and environmental stimuli (Leijten et al. 2018; Kim 2020). *Coffea arabica* L. is a crop of great economic relevance and the success of coffee production is intrinsically linked to the understanding of the mechanisms that regulate its reproductive development, especially the flowering process, which marks the beginning of fruits development (López et al. 2021).

The non-uniformity of flowering and, consequently, fruit maturation in coffee trees is a significant challenge for coffee farming, directly affecting the quality and efficiency of the harvest (Miranda et al. 2020). This disparity can be attributed to asynchronies in floral bud development before and after reproductive induction, which results in prolonged and uneven harvest (de Oliveira et al. 2014; Cardon et al. 2022). This phenomenon has economic and operational implications, as manual harvesting is more expensive and difficult to carry out, in addition to affecting the sensory characteristics and organoleptic properties of the coffee beverage (DaMatta et al. 2007; Melo and Sousa 2011). Therefore, searching for strategies that minimize this non-uniformity in fruit maturation is crucial to optimize production, reduce costs, and promote consistency in the quality of harvested coffee. Achieving this involves regulating the entire reproductive cycle of the coffee plant from flowering induction to fruit maturation.

In this challenging scenario, most investigations have focused on solving the problem after fruit formation (Kazama et al. 2021; Carvalho et al. 2022). Little is known about the potential use of plant growth regulators (PGRs), particularly during the flowering induction phase and subsequent floral bud development in *C. arabica*. This knowledge gap highlights the need to explore innovative strategies for coffee management in the early stages of the reproductive period. Furthermore, several studies have highlighted the complex influence of environmental signals on coffee flowering, such as photoperiod, temperature, water availability, and endogenous signals, such as plant hormones (Lima et al. 2021; López et al. 2021, 2022; Santos et al. 2022; Cardon et al. 2022; de Oliveira et al. 2024).

In this context, Gibberellin (GA) and Abscisic Acid (ABA) are phytohormones that perform essential functions in controlling the reproductive development of plants. GA is traditionally associated with the promotion of growth and development, including the induction of flowering, in long-day plants (Gao and Chu 2020; Castro-Camba et al. 2022; Ritonga et al. 2023). However, recent studies have indicated that GA may have contextual effects that vary depending on the type of plant and environmental conditions (He et al. 2020). ABA, in turn, is

often associated with stress responses and growth inhibition processes (Ali et al. 2020; Nguyen et al. 2023). However, research has revealed its multifunctional role, including modulation of reproductive development (López et al. 2022). Both hormones interact in complex signaling networks (Quesada 2022), and the dynamics of their application can influence floral bud development in coffee plants in different ways.

According to some observations, there is an increase in ethylene levels in the aerial parts of coffee trees during the dry period after rehydration, which is associated with the anthesis of floral buds (Lima et al. 2021). At the same time, ABA increases during the dry period and is directly linked to the latent state of the coffee floral bud, as pointed out by López et al. (2021). Rehydration of plants results in a decrease in ABA levels and an increase in GA levels (Browning 1973). Therefore, understanding the hormonal crosstalk between GA, ABA, and ethylene is essential to unveil the specific responses of coffee plants to these PGRs at different stages of reproductive development. Furthermore, considering the current scenario of climate change, which potentially exacerbates the non-uniformity in coffee plant flowering (Peruta et al., 2023; Venancio et al., 2020), there is a need to explore alternatives that minimize this impact.

Given this panorama, the main objective of this study was to investigate the impact of PGRs GA, ABA and ethylene on the reproductive development of *C. arabica*, and additionally explore the underlying related molecular pathways. Through this approach, we sought to identify the main regulators of coffee flowering and subsequently implement strategies to control these processes in the field. Therefore, this study not only contributes to the understanding of hormonal interactions in the context of coffee reproductive development but also has practical implications, which contributes to the improvement of coffee production.

2. MATERIAL AND METHODS

2.1 Plant material and experimental design

The experiments were conducted in 2022 at the experimental field of the Federal University of Lavras (UFLA), located in the municipality of Lavras, Minas Gerais, Brazil. The field was located at an altitude of 918 m, with geographic coordinates of "21° 14’ 43” S latitude and 44° 59’ 59” W″ longitude. The plant material consisted of six-year-old plants of *C. arabica* cv. Paraíso. The experimental design adopted was randomized blocks (DBC), with seven treatments (control and three concentrations of gibberellin and abscisic acid). Each treatment consisted of four blocks (biological replicates) with each block containing three plants

(experimental unit). Thus, the experiment had 12 plants per treatment, with 84 plants in total. This experimental design was used in both experiments, March (Experiment 1) and August (Experiment 2), to explore different main stages of reproductive development, floral induction and development, respectively. Different plants were used in each experiment to avoid any cumulative effects.

The application took place in March, during the rainy season, and in August, during the dry season, both were carried out at 8 am, using a 12 L knapsack sprayer. To ensure uniformity of application, a fixed volume of 200 ml per plant was established. The application was performed on the entire plant to ensure even distribution. For GA and ABA treatments, specific commercial products were used: Progibb 400® containing 400 g/kg (40% m/m) of GA and Ingrain® with 100 g/L (10% m/v) of ABA, both supplied by Sumitomo do Brasil Ltda. Dilutions were prepared at concentrations of 5, 25, and 100 ppm (or mg/L) for both products. This strategy was adopted to obtain a more precise response in the results, as the response induced in the plant by hormones can vary depending on the concentration, period, and age of the plant. The concentrations were estimated based on previous studies (Flores et al. 2005; Matsumoto and Lopez 2016; Zapata-Restrepo et al. 2020).

Samples intended for gene expression and biochemical analyses were collected 2 hours after application, immediately frozen in liquid nitrogen, and stored in a -80°C freezer until use. In Experiment 1, only leaves were collected as there were no visible signs of floral buds on the plants. In Experiment 2, both leaves and floral buds were collected during the G4 development stage (Morais et al. 2008). Leaves were collected and directly stored in vacuum-sealed glass tubes to be used in ethylene analysis.

2.2 Water potential

To evaluate the water status of plants in the different treatments, the water potential (ψpd) was measured pre-dawn in both experiments, between 3 and 5 am, using a pressure chamber (PMS Instruments-Plant Moisture 1000) according to Scholander et al. (1964). Data were collected from three randomly selected leaves, located in the middle portion of the coffee trees, from each treatment.

2.3 Phenological analyzes

The number of floral buds before and after application was quantified, followed by the classification of their developmental stage according to Morais et al. (2008). In Experiment 1,

the analysis was conducted from March to December. In Experiment 2, analysis was conducted from August to December. For each plant, four plagiotropic branches were selected per biological replicate, in which three nodes were marked (3 plants × 4 plagiotropic branches × 3 nodes = 36 nodes per biological replicate), totaling 144 nodes analyzed for each treatment, considering the four replicates.

At the end of the two experiments, fruit harvesting was carried out in May of 2023. Fruits from each block were placed in bags and weighed using a portable digital scale. Additionally, to classify the fruits within each block, a 1 L sample was taken to ensure uniformity in fruit quantity for each treatment. The fruits were separated and classified according to Morais et al. (2008).

2.4 Molecular analyses

2.4.1 *In silico* analysis of *cis-regulatory* sequences of the *CaFT1* gene

The presence of regions potentially regulated by hormones was investigated in the coffee *FT* gene (*CaFT1*) promoter, previously characterized by Cardon et al. (2022), 2000 bp upstream of transcription start site of *CaFT1* were retrieved using the annotation of the *C. arabica* genome available on the National Center for Biotechnology Information (NCBI, available at https://www.ncbi.nlm.nih.gov/assembly/ GCF_003713225.1/#/def). Identification of *cis-regulatory* DNA sequences in the promoter region of the gene was carried out by searching the plant cis-acting regulatory element (PlantCARE) database (Lescot et al. 2002). For better visualization of the data, the TATA-box and CAAT-box motifs were excluded, and are presented in Suppl. Table S1.

2.4.2 Identification of coffee proteins and Phylogenetic analyses

The predicted protein sequences of *C. arabica* were obtained from the annotation of its genome and proteome available at NCBI page (http://www.ncbi.nlm.nih.gov/assembly/GCF_003713225.1/#/def) and used to create a local database. GIBBERELLIN 3-OXIDASE (GA3ox), GIBBERELLIN 2-OXIDASE (GA2ox), GIBBERELLIN 20-OXIDASE (GA20ox), DELLA protein GAI (DELLA), 9-CIS-EPOXYCAROTENOID DIOXYGENASE (NCED), and CYTOCHROME P450, FAMILY 707, SUBFAMILY A (CYP707A) protein sequences from various monocot and eudicot species were downloaded from GenBank (Benson et al. 2013), UniProt (The UniProt Consortium 2021) and SolGenomics. To identify homologs of GA3ox, GA2ox, GA20ox, DELLA, NCED, and

CYP707A present in coffee, downloaded sequences from each family were used in separate alignments against the *C. arabica* proteome using BLASTp v2.10.1 (Altschul et al. 1990) with an e-value of 10^{-3}. To verify the presence of conserved domains characteristic of each protein family, the candidate sequences were analyzed using PFAM (Mistry et al. 2021) and filtered so that only one protein remained per gene locus.

The classification of GA3ox, GA2ox, GA20ox, DELLA, NCED, and CYP707A proteins was performed using global alignment (Fig. S1 and S2) of the other candidate sequences, together with annotated sequences from multiple species, using MAFFT v7.475 (Rozewicki et al. 2019) through Guidance v2. 02 (Sela et al. 2015), and used in subsequent phylogenetic analyses using the PHYLIP package (Retief 1999). For each protein family, we generated a dataset of 1000 bootstraps using the Seqboot algorithm, and similarity matrices between different sequences were calculated using the ProtDist algorithm. The trees were constructed using the neighbor-joining method with the neighbor algorithm and elucidated using the consensus algorithm. FigTree v1.4.4 program (http://tree.bio.ed.ac.uk/software/figtree/) was used to visualize the trees and generate the figures. The sequence details are presented in Suppl. Table S2.

2.4.3 Transcriptional analysis of coffee GA and ABA related genes in RNAseq libraries from different tissues

To analyze the expression of the identified *GA3ox, GA2ox, GA20ox, DELLA, NCED*, and *CYP707A* genes, we used previously published validated *C. arabica* RNAseq libraries available in the Sequence Read Archive (SRA, https://www.ncbi. nlm.nih.gov/sra). The libraries were subjected to quality analysis using the FastQC v.0.11.9 program (Robinson et al. 2010) and trimmed as necessary with Trimmomatic v0.39 (Bolger et al. 2014). The reads were aligned with the *C. arabica* genome (/GCF_003713225.1/#/def) using STAR v.2.7.8a (Dobin et al. 2013) with default parameters. The accession numbers and additional data for the respective libraries are presented in Suppl. Table S2. The resulting libraries were sorted, duplicate reads were removed using the Picard toolkit (http://broadinstitute.github.io/picard), and mapped fragments were counted using the htseq-count algorithm (Anders et al. 2015). Expression values were calculated in log (FPKM + 1) using the edgeR package (Robinson et al. 2010), from the Bioconductor R v3.13 project (Huber et al. 2015).

2.4.4 Gene expression analysis (RT-qPCR)

Total RNA was isolated from 200 mg of frozen powdered tissue following the protocol described by Oliveira et al. (2015). The samples were subsequently treated with the Turbo DNA-free Kit (Ambion ®) to remove DNA contamination. The RNA quality and quantity were evaluated using a NanoVue® spectrophotometer (NanoVue GE Healthcare). The integrity of the RNA was verified using agarose gel electrophoresis and visualized on a UV-transilluminator photodocumentator (UVITEC FireReader XS D-77Ls-20.M). cDNA was synthesized from treated total RNA using the *High-Capacity cDNA Reverse Transcription Kit* (Applied Biosystems®), according to the manufacturer's recommendations.

The expression of genes involved in flowering induction (*CaFT1*, *CaTFL1* and *CaCO*), GA biosynthesis, signaling and deactivation (*CaGA3ox1*, *CaGA2ox1*, and *CaDELLA3*), ABA biosynthesis and degradation (*CaNCED1* and *CaCYP707A1*), and ethylene biosynthesis (*CaACS3* and *CaACO3*), previously identified in the *C. arabica* genome, were analyzed in leaves and floral buds using the Rotor-Gene Q Real-Time PCR thermocycler (Qiagen) and the SYBR® Green detection system. These analyses were conducted using three biological and two technical replicates for each sample. Each reaction consisted of 1.5μL of cDNA, 7.5 μL of SYBR (QuantiFast SYBR Green PCR Kit – Qiagen), 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.

The relative fold differences were calculated using the $\Delta\Delta^{CT}$ method (Pfaffl 2001), with *Malate Dehydrogenase* (*MDH*) and *Ubiquitin* (*UBQ*) as reference genes for Experiment 1, *MDH* and *Ribosomal Protein L39* (*RPL39*) for Experiment 2 in leaves, and *MDH* and *Actin* (*ACT)* for floral buds (Martins et al. 2017; Fernandes-Brum et al. 2017); and the confidence intervals were calculated according to the method proposed by Steibel et al. (2009). Primer sequences are listed in the Suppl. Table S3. For these experiments, RT-qPCR parameters were followed according to the *Minimum Information for the Publication of Quantitative Real-Time PCR Experiments* (MIQE) provided in the Suppl. Table S4 (Bustin et al. 2009).

2.5 Biochemical analyzes

1-Aminocyclopropane-1-Carboxylate (ACC) quantification, 1-Aminocyclopropane-1-Carboxylate Oxidase (ACO) Enzymatic Activity and Ethylene measurement

ACC concentrations and ACO activity in leaves and floral buds were determined using the Bulens method (Bulens et al. 2011) following the modifications of López et al. (2022). Ethylene was quantified using the method described by López et al. (2022) with some modifications. Leaves and floral buds were immediately incubated in 10 ml glass tubes

containing fresh material placed at the bottom of each vial, sealed with serum caps, and incubated for 24 h. The tubes were filled with ~10 floral buds and six leaves (three leaves on each side of the plant in the middle part), and each treatment consisted of two technical replications. A portable F-900 ethylene partner (Felix Instruments, USA) was used for ACC, ACO and ethylene analyses.

2.6 Statistical analysis

The data were subjected to a normality test (Shapiro-Wilk, $p \geq 0.05$) and homoscedasticity using the Bartlett test ($P \geq 0.05$), then analysis of variance (ANOVA) was performed and the means compared by the Scott Knott test at 5% probability ($P < 0.05$). The expression ratio calculations and confidence intervals were calculated according to the method proposed by Steibel et al. (2009). All analyses were carried out using R software (R CORE TEAM, 2023) and the ExpDes (Ferreira et al. 2021) and Ggplot (Ginestet 2011) packages.

3. RESULTS

3.1 Possible *cis-regulatory* elements present in the *CaFT1* promoter.

Considering that *FLOWERING LOCUS T (CaFT1)* plays a central role in the flowering process (Cardon et al. 2022), a detailed analysis of its promoter was performed aiming at the identification of possible regions related to GA and ABA. Surprisingly, *in silico* analysis of the *CaFT1* promoter revealed a variety *of cis-regulatory* sequences (Fig. S3), providing insights into the molecular control mechanisms associated with the expression of this gene. Motifs related to diverse physiological and environmental responses have been identified, highlighting the adaptability of genes to respond to a variety of stimuli.

The *cis-regulatory* sequences related to the response to drought (MBS) (Conlon et al. 2003), anaerobic induction (ARE), and response to low temperatures (LTR) (Liu et al. 2017), indicate the sensitivity of the *CaFT1* gene to conditions of water stress and the ability to modulate its expression in colder environments. Consistent with this, motifs related to the stress response (STRE) (Conlon et al. 2003), reinforce the regulation of this gene under adverse conditions. Furthermore, the presence of motifs for light response (GATA-motif, GT1-motif, TCT-motif, TCCC-motif, box 4, and G-box) and circadian control (circadian) (Liu et al. 2017) suggests a temporal regulation of the expression of the *CaFT1* gene, aligning with the dynamics of the diurnal and seasonal cycle. Motifs related to meristem expression have also been found in the CAT box (Liu et al. 2017). The analysis also revealed *cis-regulatory* sequences associated

with different hormones–ERE and MYC (Chen et al. 2019), in addition to motifs specific to the gibberellin (GA-motif), abscisic acid (ABRE), and salicylic acid (TCA-element) (Liu et al. 2017), which shows the complexity of the interaction of the *CaFT1* gene with hormonal pathways.

Based on these results, the possibility of the direct regulation of *CaFT1* by GA and ABA was noted. However, it is important to emphasize that identifying these sequences does not confirm these hormones' effective regulation of *CaFT1*. Additional studies are essential to validate this hypothesis and expand the understanding of hormonal regulation of flowering in coffee trees. To explore our understanding of the function of GA and ABA hormones during the coffee reproductive period and their relationship with the *CaFT1* gene as well as to elucidate the main molecular pathways underlying this process, two experiments were conducted to apply these hormones directly to coffee trees in the field during the period of flowering induction (March) and floral bud development (August).

3.2 Application of GA and ABA in March (Experiment 1 - Flowering induction) and August (Experiment 2 - Floral buds development)

3.2.1 Water potential

To ensure that all plants had a homogeneous water status at the beginning of the experiment, leaf water potential (Ψ) was measured (Fig. S4), as this factor interferes with the physiological responses of plants and significantly influences the reproductive development of coffee plants (Alvim et al. 1960; Wu et al. 2022; Chen et al. 2023). In March, in the rainy season, plants were hydrated, with an average water potential of approximately -0.3 Mpa (Fig. S4a). No significant differences were observed in the water potential between the plants subjected to different treatments, indicating homogeneity in the water status of the plants at the time of treatment application. However, in August, during the dry season, plants had a lower water potential, indicating a reduction in hydration compared to March (Fig. S4b). The average water potential of plants in August was approximately -1.5 MPa, confirming the dry season, typical of the Brazilian winter. It is important to highlight that, even with this reduction, these values do not constitute severe water stress for coffee plants (DaMatta et al. 2018; León-Burgos et al. 2022). Furthermore, statistical analysis showed that there were no significant differences in the water potential among plants that received different treatments in August (Fig. S4b).

3.2.2 Phenotypical analysis of the floral buds in March (Floral Induction): Experiment 1

At the beginning of the experiment, before the application of the PGRs, the majority of floral buds were in G1 stage. No significant differences were observed in the number of floral buds per node between treatments (Fig. 1a). One month after application, in April, it was found that GA treatment resulted in a significant increase in the number of floral buds at all concentrations tested (5, 25 and 100 ppm). In contrast, ABA treatment only resulted in a significant increase of floral buds number at the 25 ppm concentration compared to untreated plants (Fig. 1b).

Two months after treatments (in May), only GA 100 ppm resulted in a significant increase in the number of floral buds compared to control. For plants treated with ABA, a concentration of 25 ppm was sufficient to maintain a greater number of floral buds compared to control plants (Fig. 1c). In June, a greater number of floral buds were observed in coffee trees treated with GA at all concentrations and with ABA 25 ppm. Notably, plants treated with 25 ppm ABA showed accelerated floral bud development, reflected in the greater presence of floral buds in more advanced stages, such as G5, flowers, and fruits (Fig. 1d). The greater number of floral buds in the GA and ABA treatments, particularly at the 25 ppm ABA concentration, reflected in a greater quantity of fruits in December (Fig. 1e). Furthermore, even after the induction of floral buds in March, the continued increase in the number of floral buds during the following months confirms the induction window described by Cardon et al. (2022).

The harvest, carried out in May 2023, confirmed the higher fruit production in plants treated with 5 ppm GA and 25 ppm ABA compared to the other treatments (Fig. 1f). Notably, the higher production in plants treated with 5 ppm GA indicates that although all concentrations of GA increased the number of floral buds, this treatment was the most effective in promoting fruit production, this highlights the importance of choosing an appropriate concentration of PGRs to optimize the harvest. However, it is worth remembering that production was carried out based on the weight of the fruits and it is not a metric that represents the quantity of fruits. This may explain why the GA 100 ppm concentration did not result in higher fruit weight, despite promoting the highest number of floral buds.

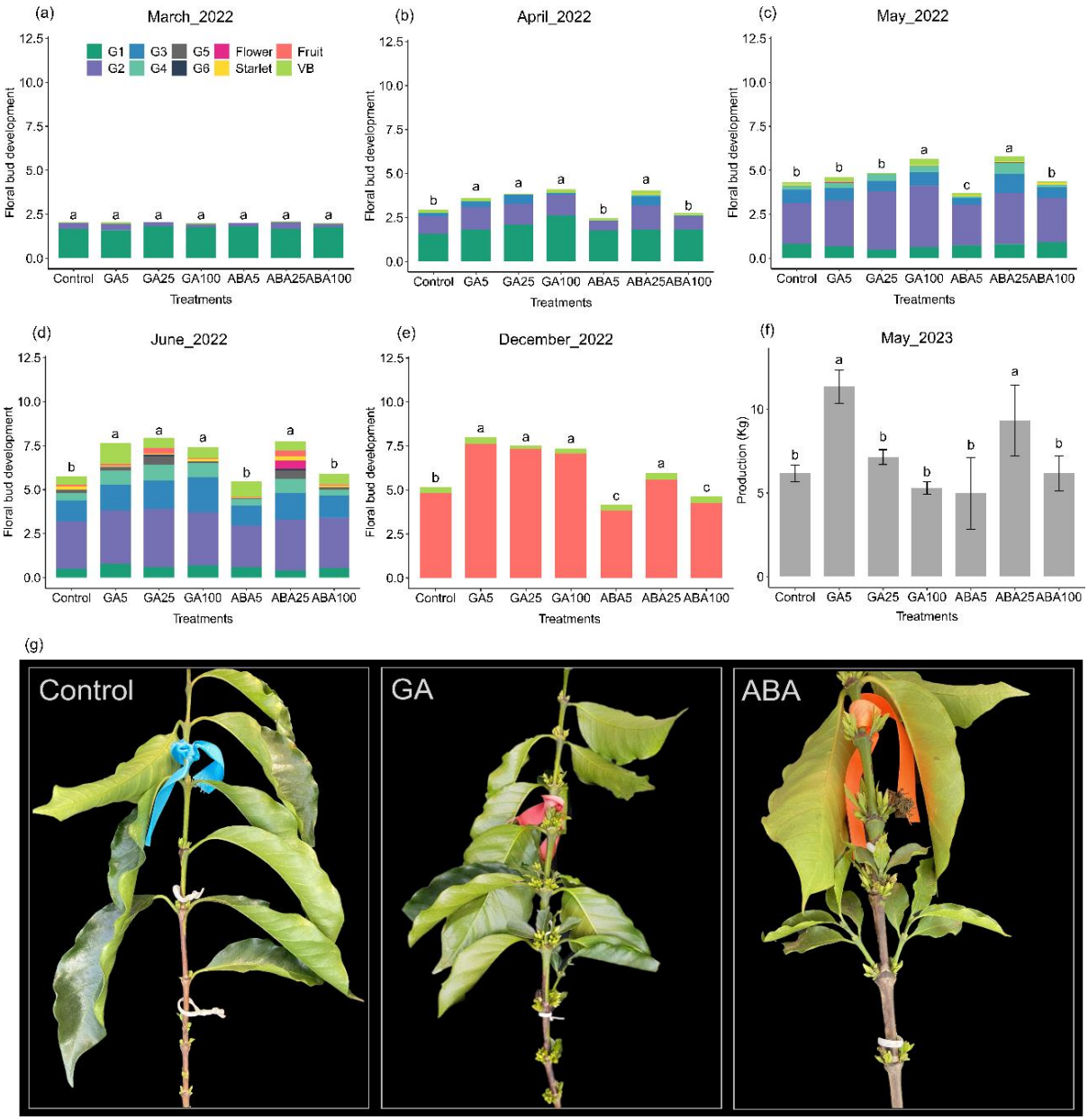

Fig. 1 - Quantification and classification of floral buds over the months in coffee trees subjected to GA and ABA treatment at concentrations of 5, 25, and 100 ppm. Shipments were made in the months of (a) March, (b) April, (c) May, (d) June, and (e) December 2022, (f) and the harvest was carried out in May 2023. (g) The image shows different morphologies of coffee tree branches representing different treatments, with photos captured in June 2022. Averages followed by the same letter do not differ from each other by the Scott Knott test ($P \leq .05$). G1: Undifferentiated buds; G2: Swollen buds; G3: Flower buds up to 3 mm in length; G4: Flower buds from 3.1 to 6 mm in length; G5: Flower buds from 6.1 to 10 mm in length; G6: Flower buds longer than 10 mm; Starlet: Abnormal flowers; Flower: flower bud after anthesis; VB: Vegetative branches.

The results presented are visually portrayed in the photos taken in June, where three distinct branches can be observed (Fig. 1g). The first image represents a control branch without PGRs application. In this case, the floral buds appeared at different stages, predominantly in G2 and G3, representing the normal development of floral buds in June. The second image

shows a branch of a plant treated with 100 ppm GA. In this treatment, a significant increase in the number of floral buds was evident compared to control. The third image represents a branch of a plant treated with 25 ppm ABA. The floral buds in this treatment exhibited advancement in their developmental stages, including G4 stage floral buds and even flower formation. This result indicates not only a quantitative increase but also an accelerated development of floral buds compared to control.

In summary, the application of GA resulted in a significant increase in the number of floral buds, whereas treatment with ABA at 25 ppm demonstrated an evolution in the floral bud development stages, highlighting the positive impacts of these PGRs in the context of the study.

3.2.3 Phenotypical analysis of the floral buds in August (Floral bud development): Experiment 2

In Experiment 2, carried out in August, the application of GA and ABA was done to evaluate their effects on the reproductive development phases of coffee trees (Fig. 2). Before application in August, no significant differences were observed in the total number of floral buds, although small discrepancies in developmental stages were noted (Fig. 2a). In the following month, in September, when it was already possible to differentiate between floral buds and fruits, it was observed that treatments with GA (5 ppm and 100 ppm) resulted in a significant increase in the number of fruits compared to control plants. Treatment with ABA (25 ppm and 100 ppm) also increased fruit production compared to untreated plants (Fig. 2b). In December, despite maintaining the pattern observed in September, a reduction in the number of fruits was noted, indicating a possible natural decline related to fruit set, which is an expected phenomenon in the development of coffee trees (Fig.2c).

Analysis of production in May 2023 revealed that treatments with GA (5 ppm and 100 ppm) and ABA (100 ppm) resulted in significant higher production (Fig. 2d). It is worth mentioning that accelerated floral bud development was not observed in ABA treatments, possibly because most of the floral buds had already formed fruit in September. These results indicate that the application of PGRs in August positively influenced fruit production, with GA (5 ppm and 100 ppm) and ABA (100 ppm) demonstrating the potential to regulate the reproductive development of coffee trees.

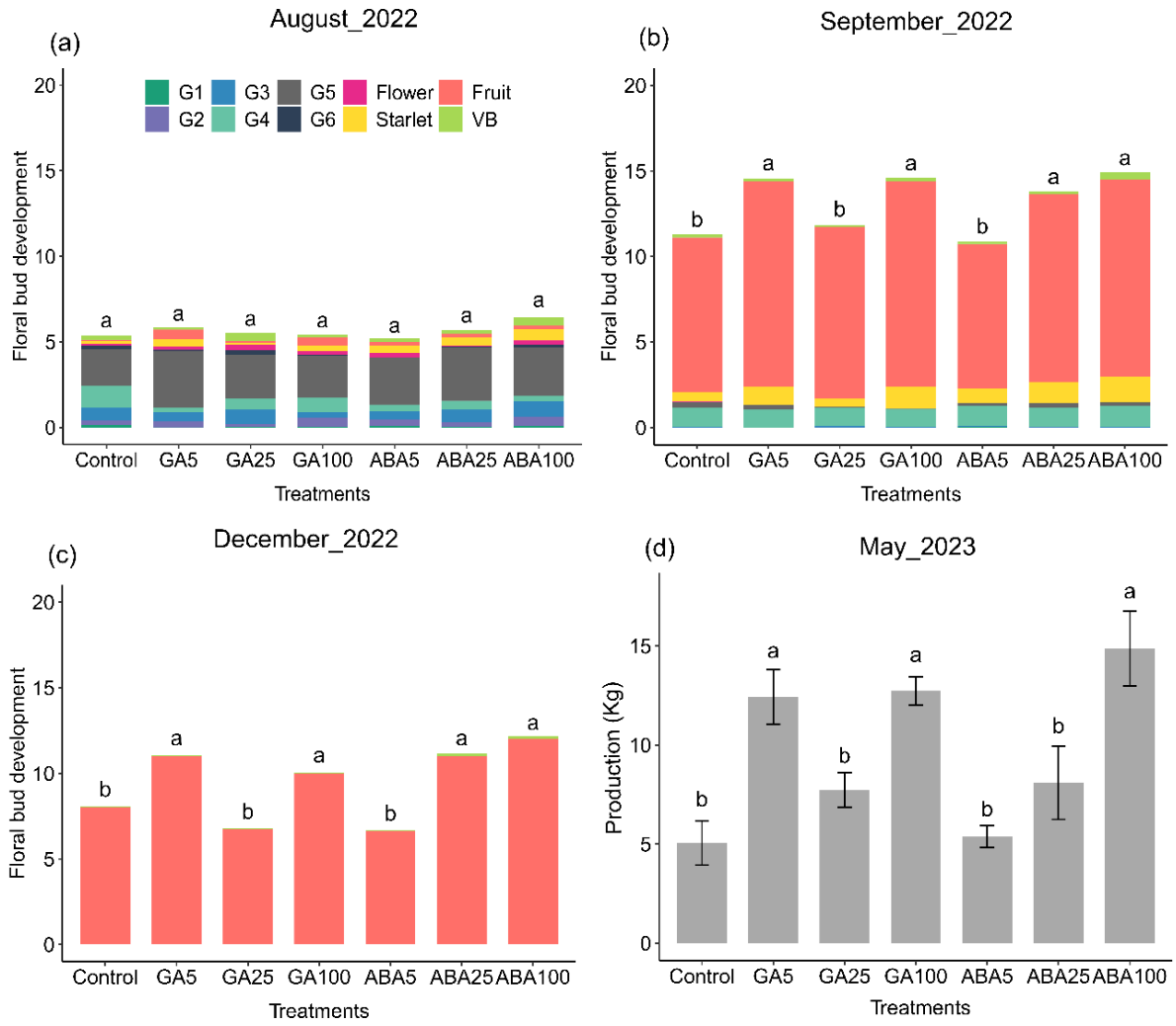

Fig. 2 - Quantification and classification of floral buds and fruits over the months in coffee trees subjected to GA and ABA treatment at concentrations of 5, 25, and 100 ppm. Classification of floral buds was done in August (a), September (b), and December (c), the harvest was carried out in May 2023 (d). Averages followed by the same letter do not differ from each other by the Scott Knott test ($P \leq .05$). G1: Undifferentiated buds; G2: Swollen buds; G3: Flower buds up to 3 mm in length; G4: Flower buds from 3.1 to 6 mm in length; G5: Flower buds from 6.1 to 10 mm in length; G6: Flower buds longer than 10 mm; Starlet: Abnormal flowers; Flower: flower bud after anthesis; VB: Vegetative branches.

3.2.4 Impact of the application of PGRs on fruit development and maturation:

At the end of the experiment, fruits were harvested and separated at different stages of maturation to verify the influence of PGRs on fruit ripening (Fig. 3). The results of Experiment 1 (application in March) indicated that control plants, GA at all concentrations and ABA 100ppm, showed a significant difference in the fresh weight of green and yellow fruits compared with raisin fruits (Fig.3a). While treatments with ABA (5 and 25ppm) maintained the same fresh weight of fruits (Fig. 3a).

The analysis of the photos visually illustrates the predominance of raisin fruits in the treatment with ABA at 25 ppm compared to control and GA treatments (Fig. 3b). This result can be attributed to the fact that ABA treatment accelerated floral bud development, as evidenced by floral bud quantification and classification results (Fig. 1), which is reflected in the early ripening of these fruits. It is important to highlight that although the GA treatment increased the number of floral buds, this fresh weight was standardized to 1L of fruits in each block, ensuring that all treatments had the same quantity of fruits for the specific analysis performed.

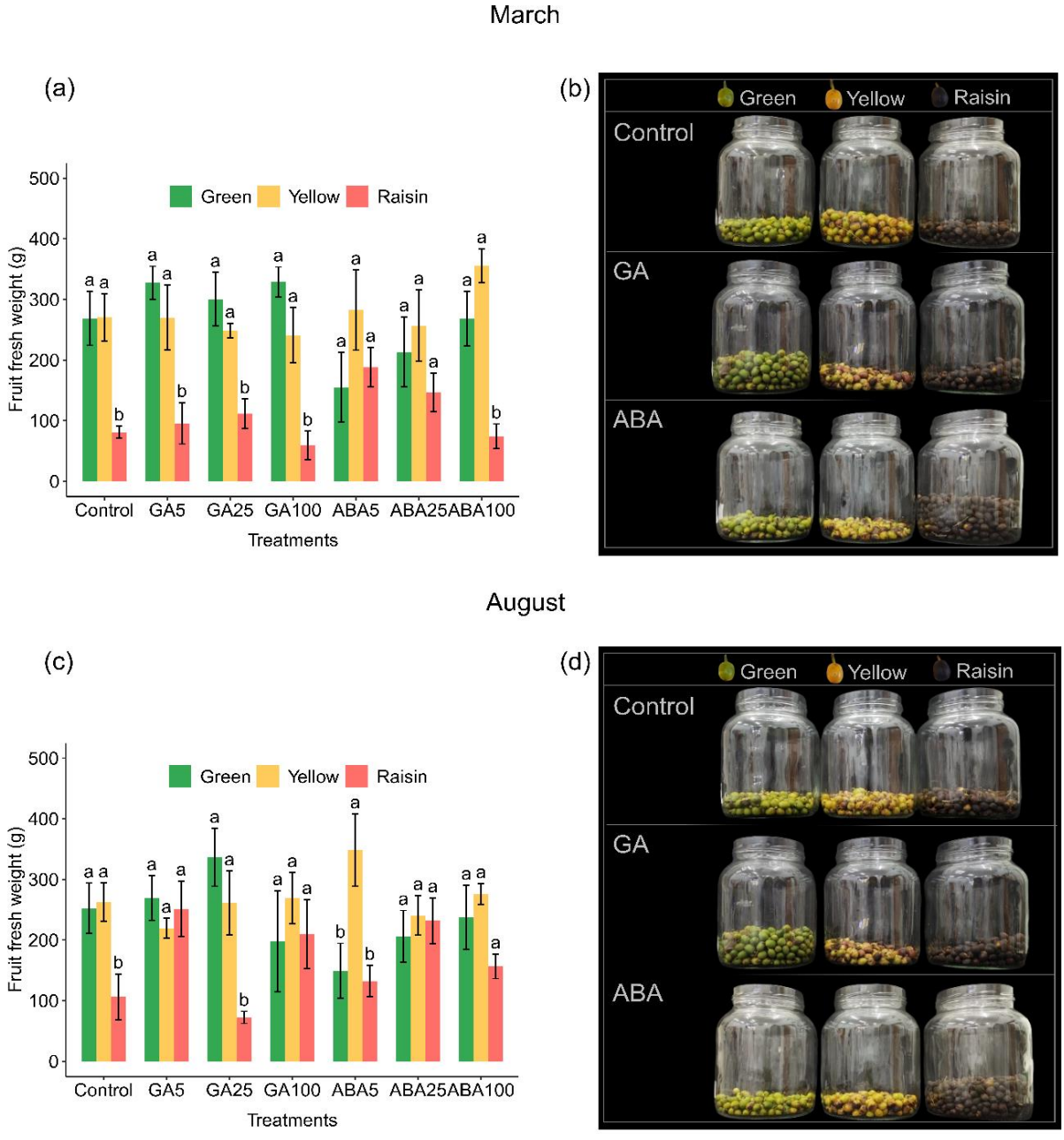

Fig. 3 - Classification and fresh weight of green, yellow, and raisin fruits from coffee trees subjected to treatments with gibberellin (GA) and abscisic acid (ABA) at different concentrations (5, 25, and 100 ppm) in March and August (a) and (c), respectively. Images of the fruits from control, GA 100 ppm, and ABA 25 ppm treatments from March (Experiment 1) and August (Experiment 2) (b) and (d), respectively. Different lowercase letters indicate statistically significant differences between fruit classifications ($P < 0.05$).

The results of Experiment 2 (application in August), revealed distinct patterns in the maturation of coffee fruits. When analyzing the fresh weight of fruits in the different classifications (green, yellow, and raisin), it was observed that the control, GA 25 ppm, and ABA 5 ppm treatments exhibited a greater fresh weight of green and yellow fruits than raisin fruits. In contrast, the GA 5 and 100 ppm treatments, as well as ABA 25 and 100 ppm, showed an increase in the fresh weight of raisin fruits, with no significant difference between the different fruit classifications (Fig. 3c). Interestingly, the treatments that demonstrated an increase in the fresh weight of raisins were the same as those that induced a greater number of floral buds, as shown in Figure 2. This suggests that the application of GA and ABA in August can significantly influence the maturation of coffee fruits, thereby establishing a connection between hormonal regulation, floral bud induction, and fruit development.

After weighing the fruits, it was possible to observe an increase in raisin fruits about green and yellow fruits in the treatment with ABA 25 ppm (Fig. 3d). However, this increase was less evident than in Experiment 1, indicating a possible influence of environmental factors.

3.2.5 Identification of GA and ABA biosynthesis, degradation, and signaling genes in *Coffea arabica*

To identify the key genes involved in the biosynthesis and degradation of GA and ABA in the *C. arabica* genome, we conducted phylogenetic analyses of the genes responsible for the crucial points of these regulatory pathways (Fig. 4). Our results revealed a total of 26 GAox genes, of which 16 were identified as *CaGA20ox*, representing the largest subgroup, indicating that *CaGA20ox* may have undergone a more dynamic evolutionary pathway and, therefore, resulted in more functional redundancy (Fig 4a). GA20ox catalyzes the conversion of GA12 and GA53 to GA9 and GA20, respectively (Lange and Pimenta Lange 2020). Finally, GA3ox is involved in the last step of the GA biosynthesis pathway and catalyzes the 3-beta-hydroxylation of GA20, GA5, GA44, and GA9 into the corresponding bioactive products, GA1, GA3, GA38, and GA4 (Lange and Pimenta Lange 2020). According to phylogenetic analysis, 6 *CaGA3ox* genes were found in the *C. arabica* genome (Fig. 4a). In contrast, GA2ox responsible for the deactivation of bioactive GAs (Lange and Pimenta Lange 2020), presented

4 *CaGA2ox* genes in the *C. arabica* genome (Fig. 4a). In addition, 4 *CaDELLA* genes were found, which act as repressors in the GA signaling pathway (Sun 2011) (Fig. 4b).

In ABA biosynthesis, the enzyme NCED is a key point in this regulation and is responsible for the cleavage of violaxanthin and neoxanthin, resulting in the formation of ABA. Mutants of this enzyme were decisive in proving that xanthophyll cleavage leads to ABA formation (Iuchi et al. 2001). Our genomic analyses revealed the presence of 3 *CaNCEDs* genes, indicating the genetic complexity of this pathway (Fig. 4b). Furthermore, we identified 6 *CaCYP707A* genes (Fig. 4b), which are essential enzymes in the oxidative degradation of ABA (Saito et al. 2004). The presence of these genes in the *C. arabica* genome highlights their importance in hormonal regulation and the progress of plant developmental stages.

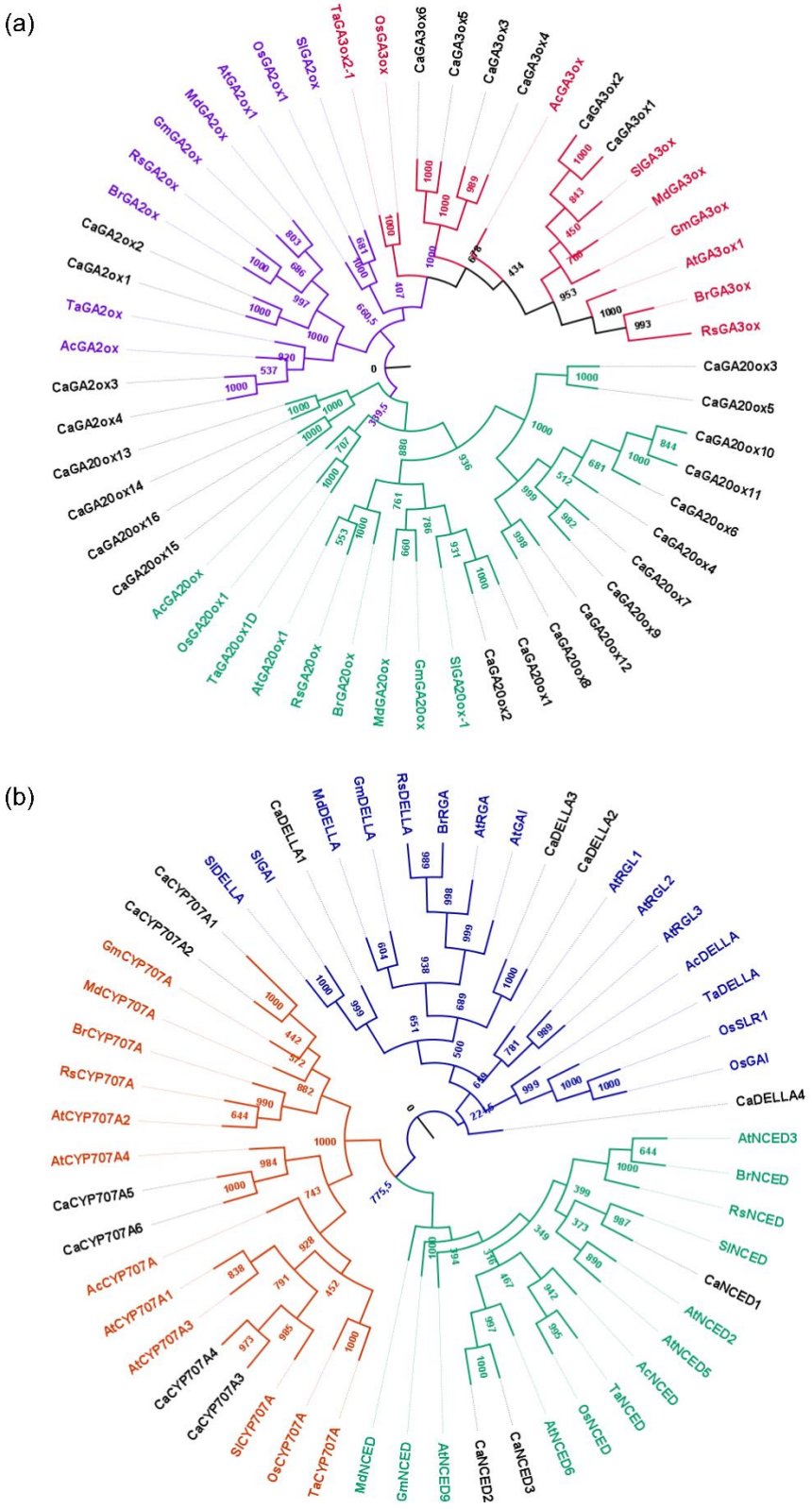

Fig. 4 - Phylogenetic analysis of proteins (a) CaGA3ox, CaGA2ox, CaGA20ox, and (b) CaDELLA, CaNCED, and CaCYP707A from *C. arabica*. The phylogenetic tree was generated using the neighbor-joining method containing all protein sequences identified in *C. arabica* (present study) together with sequences from monocots and eudicots. The sequence details are listed in Suppl. Table S2. The nodes with bootstrap values greater than 70% are shown in the figure. *C. arabica* proteins are marked in black.

3.2.6 Expression analyses of GA and ABA biosynthesis, degradation, and signaling genes in different RNA-seq libraries

To investigate the expression profiles of *CaGA3ox*, *CaGA2ox*, *CaGA20ox*, *CaDELLA*, *CaNCED*, and *CaCYP707A* identified in this study, we aligned these sequences with RNA-seq libraries from different *C. arabica* tissues under different conditions (Fig. 5). The objective of this study was to select genes for subsequent gene expression analysis in experiments with the application of GA and ABA in specific phases of coffee tree reproductive development.

The results highlight the expression of 26 identified *GAox*, of which 14 were expressed in at least one library. Among the six *CaGA3ox* genes identified, only *CaGA3ox1* and *CaGA3ox2* were expressed, which was more evident in embryos and floral buds at the G4 stage (Fig. 5a). These results indicated a positive relationship between these genes and the reproductive stage of coffee plants. Out of the 16 *CaGA20ox* genes identified, only 10 were expressed in the libraries, concentrated in embryos and fruit perisperm 90 days after flowering (Fig. 5a). However, our findings revealed that among the four identified *CaGA2ox* genes, only *CaGA2ox1* and *CaGA2ox2* were expressed, suggesting a relatively low pattern. (Fig. 5a). This suggests a possible endogenous regulation of GA levels during the reproductive period of coffee, especially in the floral buds. In addition, only two *CaDELLA* genes were expressed (*CaDELLA2* and *CaDELLA3*), with *CaDELLA3* showing more prominent expression in young leaves, floral buds, SAM, and fruit perisperms after flowering (Fig. 5b), highlighting the important role of *CaDELLA* across various tissues and stages of development in the coffee tree.

Regarding ABA biosynthetic genes, all three identified *CaNCEDs* were expressed, with *CaNCED1* showing a relatively high expression pattern, particularly in floral buds and fruits (Fig. 5c). Similarly, all identified *CaCYP707A* genes were expressed in the libraries, with *CaCYP707A1* particularly prominent in young leaves, fruits, embryos, and floral buds (Fig. 5d). These gene expression patterns indicate the potential role of ABA in the reproductive development of coffee plants.

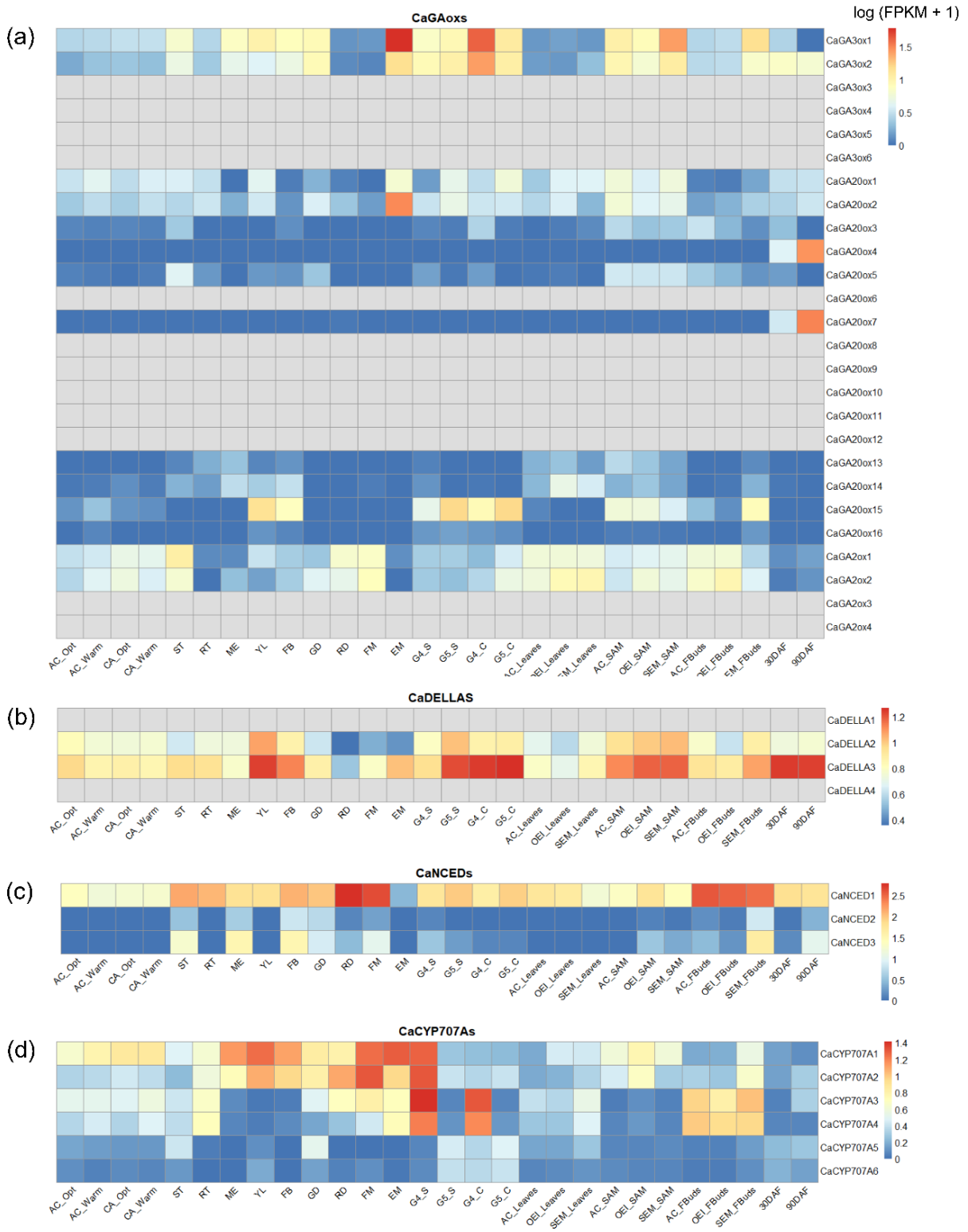

Fig. 5 - Heatmap illustrating the relative transcript levels of (a) *CaGAoxs*, (b) *CaDELLAs*, (c) *CaNCEDs*, and (d) *CaCYP707As* from *C. arabica* (lines) in different RNA-seq libraries (columns). AC_Opt: Leaves cv. Acauã under optimal temperature conditions; AC_Warm: Leaves cv. Acauã in high-temperature conditions; CA_Opt: Leaves cv. Catuaí in optimal temperature conditions; CA_Warm: Leaves cv. Acauã in high-temperature conditions; ST: Stems cv. Bourbon; RT: Roots cv. Bourbon; ME: Meristems cv. Bourbon; YL: Young leaves cv. Bourbon; FB: Floral buds cv. Bourbon; GD: Green fruits cv. Bourbon; RD: Red fruits cv. Bourbon; FM: Fruits at multiple stages of development cv. Bourbon; EM: Embryos; G4_S:

Floral buds G4 cv. Seriema; G5_S: Floral buds; G5 hp. Seriema; G4_C: Floral buds G4 cv. Catuaí; G5_C: Floral buds G5 hp. Catuaí; AC_Leaves: Leaves cv. Acauã; OEI_Leaves: Leaves cv. Oeiras; SEM_Leaves: Leaves cv. Semperflorens; AC_SAM: Apical meristems cv. Acauã; OEI_SAM: Apical meristems cv. Oeiras; SEM_SAM: Apical meristems cv. Semperflorens; AC_FBuds: Floral buds G2 cv. Acauã; OEI_FBuds: Floral buds G2 cv. Oeiras; SEM_FBuds: Floral buds G2 cv. Semperflorens; 30 DAF: Fruit perisperm 30 days after flowering; 90 DAF: Fruit perisperm 90 days after flowering. Colors are coded as a function of logarithmic base 10 of expression values normalized to FPKM +1 (Fragments Per Kilobase of transcript Million mapped reads). More details of the libraries are available in Suppl. Table S2.

3.2.7 Hormonal regulation of gene expression related to flowering induction, biosynthesis, degradation, and signaling pathways of GA, ABA, and ethylene

Experiment 1

The relative expression of the *FLOWERING LOCUS T* (*CaFT1)* and *CONSTANS* (*CaCO)* genes, which are related to flowering induction (Jin et al. 2021; Cardon et al. 2022), did not change after 2h of GA and ABA application, indicating that these genes were not affected in this short time (Fig. 6a and c). However, the expression of *TERMINAL FLOWER 1* (*CaTFL1)*, a repressor of flowering (Cardon et al. 2022; Jin et al. 2021), increased with the GA application at high concentrations (25 and 100 ppm) (Fig. 6b).

In contrast, the *CaDELLA3* expression (Fig. 6d), which acts as a negative regulator of GA signaling (Claeys et al. 2014), increased in GA treatments at all concentrations tested (5, 25, and 100 ppm), as well as in treatment with ABA at 25 and 100 ppm. This suggests a possible correlation between this gene and floral bud induction, since these treatments also resulted in a higher number of floral buds, despite the absence of differences in the expression of *CaFT1* and *CaCO* genes.

The expression of the *CaGA3ox1* gene, which is involved in the GA biosynthesis pathway (Lange and Pimenta Lange 2020), did not differ between the treatments (Fig. 6e). This expression pattern was expected given that GA was applied exogenously. Surprisingly, the *CaGA2ox1* gene, which is related to the deactivation of bioactive GAs (Li et al. 2019), had its expression reduced in all tested concentrations of GA and ABA (Fig. 6f). This indicated that exogenous GA treatment did not induce GA degradation or inactivation through a negative feedback mechanism. Furthermore, this result shows a possible cross-regulation of the GA pathway by ABA application, as ABA treatment reduced the expression of the *CaGA2ox1* gene.

Regarding the *CaNCED1* gene, which is related to ABA biosynthesis (Iuchi et al. 2001), an increased expression was observed in treatments with GA 25 and 100 ppm (Fig. 6g). This indicates a complex correlation between GA and ABA, in which these hormones may interfere with each other's biosynthetic and degradation pathways. The *CaCYP707A1* gene, which is

involved in ABA degradation (Saito et al. 2004), showed a reduction in its expression level under GA treatment at all concentrations (5, 25, and 100 ppm) (Fig. 6h), indicating that GA can interfere not only with biosynthesis but also with the ABA degradation.

The ethylene biosynthetic genes, *ACC SYNTHASE* (*CaACS3*) and *ACC OXIDASE* (*CaACO3*) showed no significant changes in their expression levels in treatments with GA and ABA at all concentrations tested (Fig. 6i and j). This suggests that, at least during the flowering induction period, the exogenous application of GA and ABA does not affect the biosynthesis of ethylene, a hormone associated with later phases of the coffee reproductive development (Lima et al. 2021; López et al. 2022; Santos et al. 2022). These results reinforce the complex interactions between plant hormones during the initial stages of coffee reproductive development, especially gene regulation.

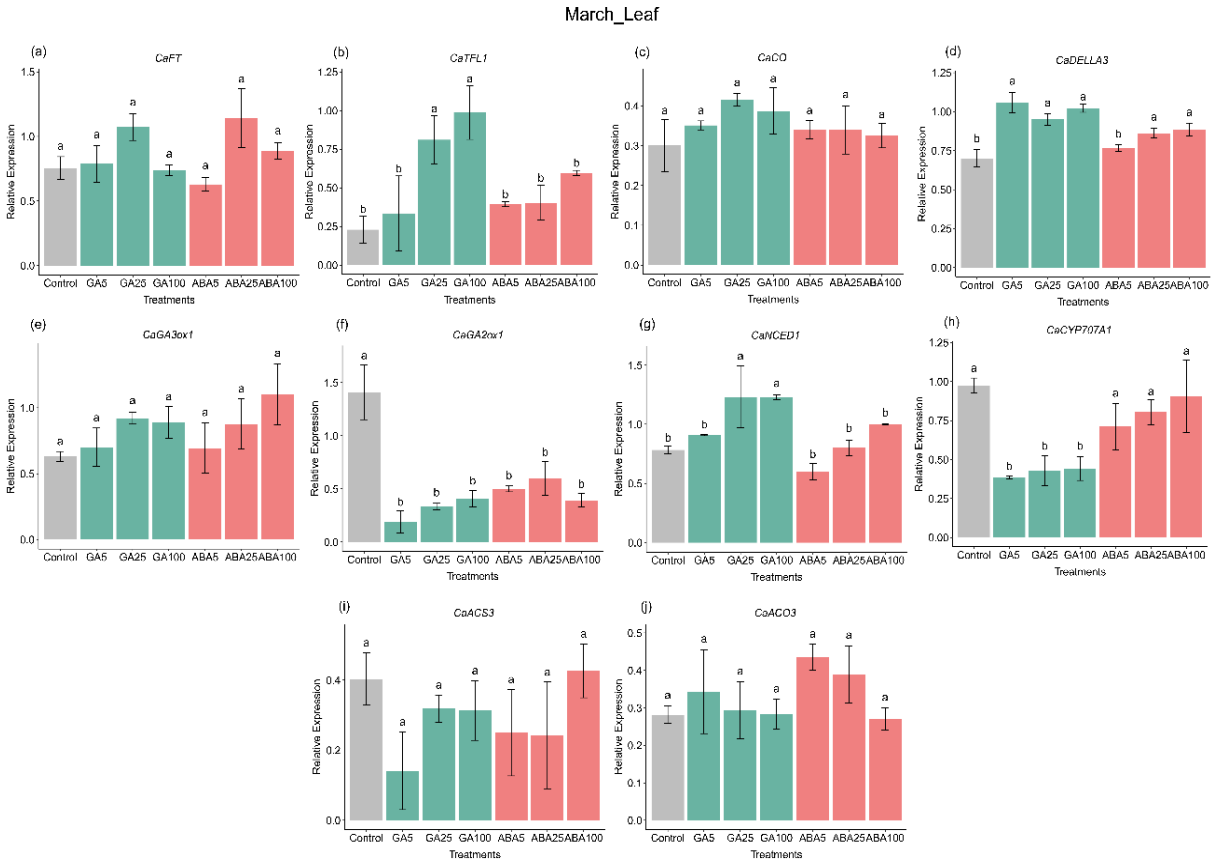

Fig. 6. Gene expression profile in coffee leaves in response to the application of gibberellin (GA) and abscisic acid (ABA) at different concentrations (5, 25, and 100 ppm) in March. (a) *CaFT1*; (b) *CaTFL1*; (c) *CaCO*; (d) *CaDELLA3*; (e) *CaGA3ox1*; (f) *CaGA2ox1*; (g) *CaNCED1*; (h) *CaCYP707A1*; (i) *CaACS3*; (j) *CaACO3*. Different lowercase letters indicate statistically significant differences between treatments ($P < 0.05$).

Experiment 2

The expression level of *CaFT1* in leaves, collected in August 2h after treatments, increased with GA application at concentrations of 5 and 100 ppm, as well as with ABA application at a concentration of 100 ppm compared with control (Fig. 7a). Notably, these same treatments coincided with an increase in floral bud number (Fig. 2). Thus, *CaFT1* appears to exert a direct or indirect influence on floral bud induction in August, going beyond the induction phase described for coffee (López et al. 2021). The morning application of these PGRs led to higher *CaFT1* expression levels, possibly anticipating the concentration of FT protein, which accumulates during the day to induce flowering in long-day plants (Krzymuski et al. 2015). Interestingly, the same treatments that increased *CaFT1* expression also increased *CaTFL1* expression, except ABA at 25 ppm (Fig. 7b). We also observed a reduction in the expression level of *CaCO* at all GA and ABA concentrations (Fig. 7c), indicating a possible inhibition of *CONSTANS* (*CO*) by these hormones in August, in contrast to the results obtained in March.

In contrast, *CaDELLA3* expression (Fig. 7d) increased in the same treatments that induced *CaFT1* (GA 5 and 100 ppm, ABA 100 ppm). As DELLA proteins act as a negative regulator of GA signaling, the reduction in the expression level of *CaDELLA3* may have occurred through a positive feedback mechanism. In other words, when there was a reduction in DELLA proteins, probably caused by the application of GA, it was interpreted as a signal for the plant to express even more *CaDELLA3*. Feedback cycles involving GA-responsive genes such as GID1, GA2ox, and DELLA are important for maintaining the balance of GA levels and regulating plant growth (Gao and Chu 2020).

In this context, the change in *CaFT1* expression exclusively in August raises the possibility that the reduction in *CaCO* expression, associated with DELLA degradation, exerts direct and indirect influences on *CaFT1* expression. This observation justifies the fact that in March, the application of the same hormones did not result in a significant increase in *CaFT1* expression, since there was no change in *CaCO* during this period. However, DELLA reduction may have been the starting point for floral bud induction even in the absence of changes in *CaFT1* expression.

The *CaGA3ox1* expression level was increased in treatments with ABA application at all concentrations, especially at 25 ppm (Fig. 7e). In contrast, the expression level of *CaGA2ox1* did not change (Fig. 7f). These results reinforce the complex interactions between these hormones, and it is possible to note that the expression of *CaNCED1* increased with GA application at low concentrations (5 and 25 ppm), indicating a role of GA in ABA biosynthesis (Fig. 7g). It is important to note that in March, high concentrations of GA were necessary to

increase the expression of *CaNCED1*, whereas, in August, lower concentrations were sufficient, possibly related to endogenous changes in GA during specific phases of coffee reproductive development. The application of these hormones at different concentrations did not result in significant changes in *CaCYP707A1* expression level (Fig. 7h). These results suggest that there is an interaction between GA and ABA during the coffee reproductive period and that ABA may act downstream of GA for floral bud induction.

Regarding the genes involved in the ethylene biosynthesis pathway, only *CaACS3* (Fig. 7i) showed an increase in its expression level with GA application (25 and 100 ppm), whereas *CaACO3* remained unchanged (Fig. 7j), possibly leading to the ACC accumulation in leaves with GA application. These findings show an intricate network of hormonal interactions that modulate gene expression during coffee reproductive development, highlighting the influence of GA and ABA in regulating the processes of inducing flowering and floral bud development.

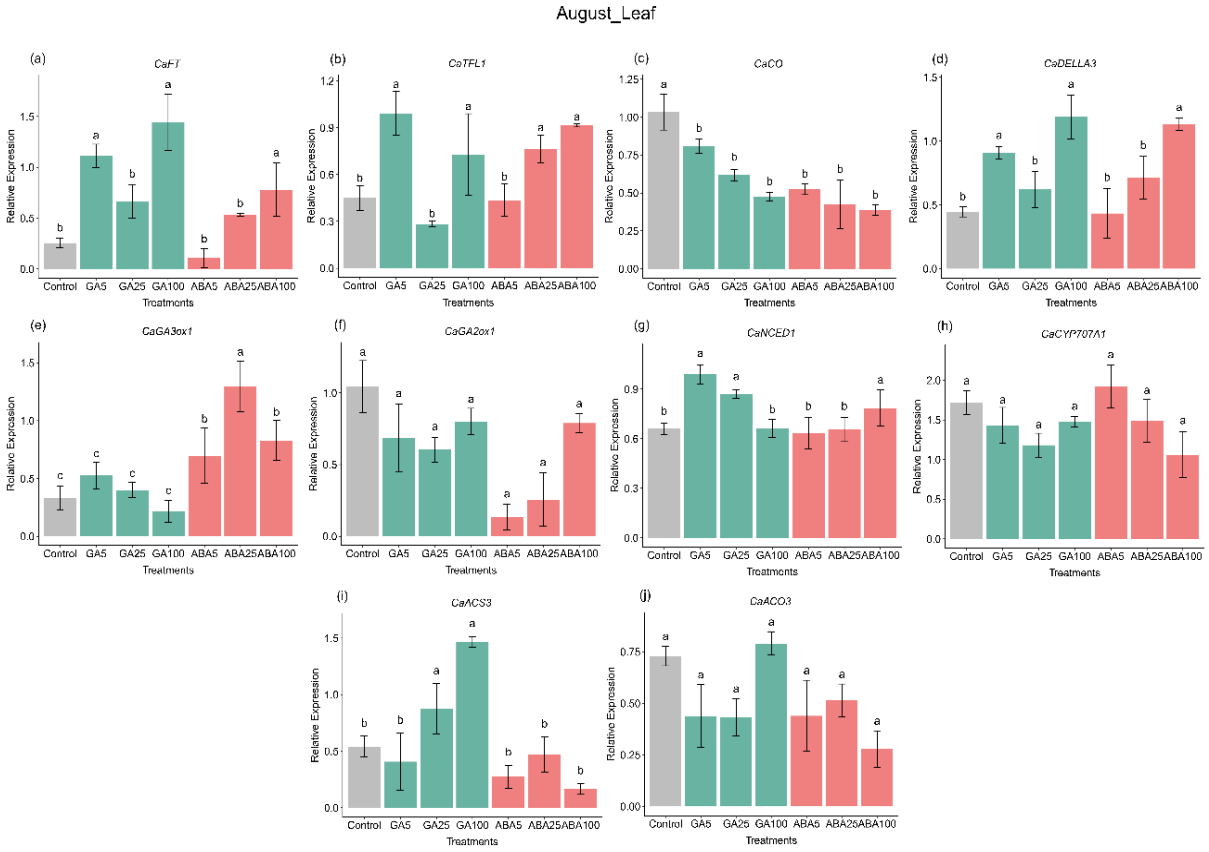

Fig. 7 - Gene expression profile in coffee leaves in response to the application of gibberellin (GA) and abscisic acid (ABA) at different concentrations (5, 25, and 100 ppm) in August. (a) *CaFT1*; (b) *CaTFL1*; (c) *CaCO*; (d) *CaDELLA3*; (e) *CaGA3ox1*; (f) *CaGA2ox1*; (g) *CaNCED1*; (h) *CaCYP707A1*; (i) *CaACS3*; (j) *CaACO3*. Different lowercase letters indicate statistically significant differences between treatments ($P < 0.05$).

In Experiment 2, the expression of genes responsible for the identity of the floral meristem and formation of floral organs, such as *APETALA 1* (*CaAP1*) and *LEAFY* (*CaLFY*), in the floral buds was also analyzed (Fig. 8). The results showed that the application of GA at 5 and 100 ppm, as well as ABA at 25 and 100 ppm, resulted in increased *CaAP1* expression level (Fig. 8a). These concentrations also increased *CaFT1* expression in the leaves (Fig. 7a) and, consequently, the number of floral buds in these treatments (Fig. 2). Furthermore, a significant increase in the expression level of *CaLFY* was observed in GA treatments at all concentrations tested, especially at 100 ppm, and ABA at a concentration of 25 ppm (Fig. 8b). These results indicate that GA and ABA can regulate floral bud development, as expected since *FT* induction results in the expression of *AP1* and *LFY* (Jaeger et al. 2013).

No significant differences were observed in *CaDELLA3* expression level between the treatments (Fig. 8c). Even without significant differences in *CaDELLA3* expression, treatment with 25 and 100 ppm ABA increased *CaGA3ox1* expression (Fig. 8d). Furthermore, the GA application at 100 ppm and ABA at all concentrations reduced the expression of *CaGA2ox1* (Fig. 8e), which is responsible for the degradation of bioactive GAs. This indicates, once again, possible regulation of GA biosynthesis through the application of ABA, which would explain the fact that ABA induces *CaAP1* and *CaLFY*, since this hormone may act downstream of GA to promote the development of the floral bud.

Interestingly, although there was no significant difference in *CaNCED1* (Fig. 8f), treatment with GA at all concentrations increased the expression of *CaCYP707A1* (Fig. 8g), indicating that GA can regulate ABA degradation in floral buds. This result reinforces the previous hypothesis that ABA induces GA and that GA can reduce ABA levels in floral buds.

Regarding the genes of the ethylene biosynthesis pathway, a significant reduction in the expression of *CaACS3* was observed in treatments of GA at 100 ppm and ABA at 25 and 100 ppm (Fig. 8h), indicating that at high concentrations, these hormones can decrease the expression of this gene in the floral buds. In August, there was an increase in *CaACS3* in the leaves with the application of GA (Fig. 7i), possibly due to ACC could be transported to the floral buds as a signal, thus reducing the expression of *CaACS3* in the floral buds to regulate ACC levels in this tissue. In contrast, the expression of *CaACO3*, which encodes the enzyme 1-aminocyclopropane carboxylic oxidase (ACO) responsible to convert ACC to ethylene, increased with GA application at all concentrations (Fig. 8i), suggesting an increase in ethylene in the floral buds in these treatments. These results indicate that, possibly in August, when the

floral buds are already formed and the majority are in the G4 stage, an interaction between GA, ACC, and ethylene may occur to promote floral bud development and, subsequently, anthesis.

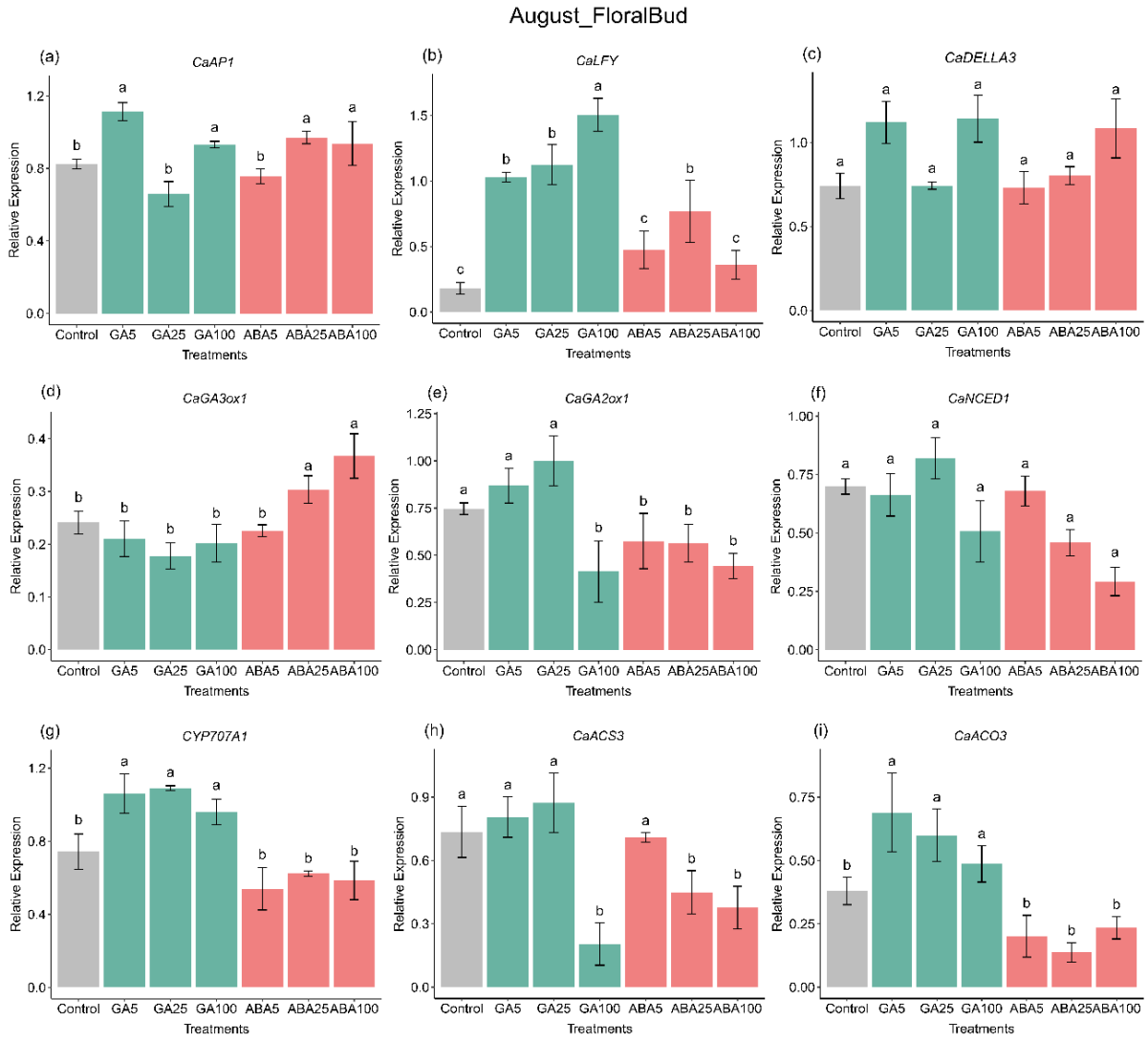

Fig. 8 - Gene expression profile in coffee floral buds in response to the application of gibberellin (GA) and abscisic acid (ABA) at different concentrations (5, 25, and 100 ppm) in August. (a) *CaAP1*; (b) *CaLFY*; (c) *CaDELLA3*; (d) *CaGA3ox1*; (e) *CaGA2ox1*; (f) *CaNCED1*; (g) *CaCYP707A1*; (h) *CaACS3*; (i) *CaACO3*. Different lowercase letters indicate statistically significant differences between treatments ($P < 0.05$).

3.2.8 Influence of Gibberellin (GA) and Abscisic Acid (ABA) application on the content of ACC and ethylene as well as on ACO activity during the reproductive period of coffee trees

The expression level of *CaACS3* and *CaACO3* genes did not show significant changes in response to GA and ABA application in March (Fig. 6), which corresponds to the flowering induction period in coffee trees. These observations were corroborated by the quantitative assessment of ACC, ethylene, and ACO activity in the leaves during this period (Fig. 9),

highlighting the absence of changes in these variables in response to the application of the mentioned PGRs. These data suggest that ethylene may not play a crucial role in the flowering induction phase in coffee plants or that this regulation is not manifested in the leaves. This is supported by the fact that GA and ABA treatments promoted an increase in the number of floral buds (Fig. 1) without influencing the ethylene content in leaves in March.

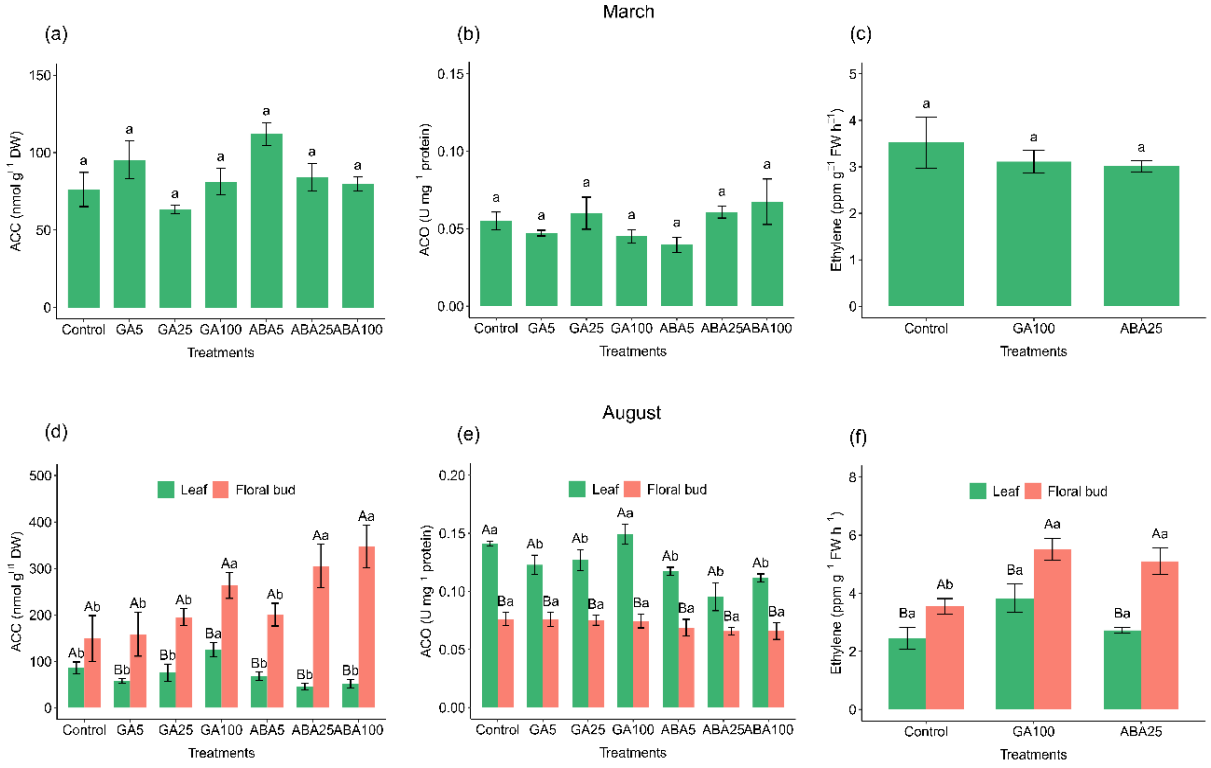

Fig. 9 – Quantification of ACC, ACO, and ethylene in coffee tree leaves after application of Gibberellin (GA) and Abscisic Acid (ABA) in March and August at different concentrations (5, 25, and 100 ppm). (a) and (d) 1-Aminocyclopropane-1-carboxylate (ACC) content; (b) and (e) ACC oxidase [ACO] enzyme activity; (c) and (f) ethylene production. Different lowercase letters indicate statistically significant differences between treatments ($P < 0.05$).

In August, in contrast to March, an increase in the concentration of ACC in leaves was observed only in the treatment with GA at the highest concentration (100 ppm), indicating that high levels of GA are necessary to stimulate the production of this metabolite (Fig. 9d). In parallel, in the floral buds, there was an increase in ACC level with GA application at 100 ppm and ABA at 25 and 100 ppm, suggesting that, in the floral buds, ABA at higher concentration can also induce the accumulation of ACC (Fig. 9d). This regulation may be associated with the potential of ABA at high concentrations to induce genes related to GA biosynthesis, as evidenced by the gene expression results (Fig. 8d). The analysis also revealed that the application of GA and ABA led to the increase of ACC concentration in floral buds compared to its concentration in leaves, regardless of the concentrations tested for these PGRs, which

differed from the control that did not show this change (Fig. 9d). These results support the hypothesis that ACC produced in the leaves can act as a signal to floral buds.

Interestingly, treatment with GA100 ppm maintained ACC oxidase (ACO) enzyme activity similar to the control, whereas plants treated with GA5 and 100 ppm and ABA at all concentrations showed reduced ACO activity in the leaves (Fig. 9e). There was no change in ACO activity in floral buds, but it was possible to observe that, unlike ACC content, there was greater ACO activity in leaves than in floral buds (Fig. 9e). This greater ACO activity in leaves appears to be intrinsic to plants, as even the control plants exhibited this behavior.

Regarding ethylene production, there was no change in the leaves of plants treated with GA 100 ppm and ABA 25 ppm (Fig. 9f). However, in the floral buds, these treatments increased the ethylene content compared with the control (Fig. 9f). Despite the greater production of ACC in the leaves in the GA 100 ppm treatment, the absence of significant changes in the level of ethylene in the leaves suggests that the ACC produced in this context may be transported to the floral buds. Furthermore, even though the GA 100 ppm treatment maintained ACO activity in the leaves, this result did not translate into greater ethylene production compared to ABA treatments. These findings, combined with the gene expression results, corroborate the existence of an interaction between the hormones GA, ABA, and ethylene, playing a joint role in promoting floral bud development during August.

4. DISCUSSION

There are still several gaps in the understanding of the hormonal role in flowering, especially in woody and perennial plants such as coffee. Previous studies have already shown that foliar application of GA3 to coffee trees results in more concentrated flowering, culminating in fruits with more uniform maturation at harvest time (Schuch et al. 1990; Matsumoto and Lopez 2016). It was also observed that GA application promoted a significant increase in flower production, as well as in the number and weight of mature fruits (Flores et al. 2005). In contrast, ABA treatment initially decreased the number of floral buds but resulted in a greater fruit production at the end of the floral bud development period (Flores et al. 2005; Matsumoto and Lopez 2016). It is important to highlight that such studies have addressed more advanced stages of the reproductive development of coffee trees. In turn, our research aimed to understand the role of these hormones during induction in March (rainy period) and bud development in August (dry period), following the phases proposed for floral development in Brazilian environmental conditions (Camargo and Camargo 2001; López et al. 2021).

Our results showed that the application of GA and ABA increased the number of floral buds, both in March and August, with this increase depending on the concentration used (Fig 1 and 2). Additionally, ABA treatment not only increased the number of floral buds but also accelerated their development (Fig. 1 and 3). Considering the importance of water stress for flowering in coffee plants, in which the anthesis begins after the first rain or irrigation and ABA increases under conditions of water deficit (Alvim 1960; López et al. 2022), we can infer that the application of ABA in our study triggered an early stress stimulus, boosting the advancement of floral bud development (Fig. 10a). These findings reinforce the hypothesis that manipulation using PGRs can modulate the reproductive development of coffee plants, with a positive impact on the number of floral buds and, consequently, fruit production.

In addition to influencing floral bud development, ABA application has also been shown to accelerate fruit ripening (Fig. 3). Previous studies have highlighted the ability of ABA to promote fruit ripening, as evidenced by studies involving tomatoes and date palms (Gupta et al. 2022; Elbar et al. 2023; Wu et al. 2023). Despite this, it is important to highlight that in our study, ABA was not applied directly to the fruits, suggesting that the observed response may be a consequence of accelerated floral bud development.

Our results also revealed the molecular pathways that involve these hormones in the different phases of coffee flowering (Fig. 6, 7 and 8). Initially, through *in silico* analysis, we identified *cis-regulatory* regions related to these hormones in the *CaFT1* promoter, an ortholog of florigen in coffee (Cardon et al. 2022). The promoter is the region of DNA located before the beginning of the gene coding sequence, and plays a crucial role in regulating gene transcription (Jores et al. 2021; Villao-Uzho et al. 2023). Considering the sensitivity of *CaFT1* expression to environmental and endogenous stimuli, it plays a central role in coffee flowering, as indicated by Cardon et al. (2022), suggesting that these hormones may directly influence the regulation of this gene. Additionally, Cardon et al. (2022) demonstrated that the highest expression of the *CaFT1* gene occurred in June, a period characterized by cold and dry climatic conditions typical of the Brazilian winter. Based on these findings, we propose that ABA could induce the expression of the *CaFT1* gene during the coffee tree's flowering induction period. In contrast, during the development of floral buds in August, ABA could act as an inhibitor of bud growth and development, keeping them at the G4 (latent) stage. On the other hand, GA could promote the development of buds at the initial stages.

To deepen our understanding, we performed gene expression analyses, which revealed a complex hormonal interaction between the PGRs GA and ABA. It was observed that, in both

March and August, GA induced and repressed genes associated with ABA biosynthesis and degradation in the leaves, respectively (Fig. 6 and 7). Conversely, ABA has demonstrated the ability to induce genes related to GA biosynthesis (Fig. 6 and 7). This complex interaction appears to correlate with the endogenous concentrations of these hormones at different stages of the reproductive development of coffee trees.

In March, the induction period, a low endogenous concentration of ABA in the leaves was found (López et al. 2022), which can infer from our results that the application of GA in high concentrations can stimulate an increase in ABA levels when its endogenous concentration is low. This, in turn, appears to regulate GA levels, especially before flowering. In summary, the pathway by which GA induces ABA, which, in turn, induces more GA, represents a long path, the length of which is conditioned by environmental variables for the full development of the plant and the realization of physiological and morphological events necessary for flowering. Notably, the application of ABA as a stress signal to plants can anticipate this path, leading to an increase in GA concentrations, and resulting in accelerated floral bud development (Fig. 10a).

This regulation between GA and ABA appears to be closely linked to the levels of *DELLA* and *CO*, which orchestrate *FT* (Fig. 6 and 7). DELLAs proteins play a significant role in promoting GA-mediated *FT* expression, exerting a negative influence on several *FT* activators such as *CO* (Xu et al. 2016; Wang et al. 2016). In the present study, the application of GA and ABA did not cause changes in the expression of *CaFT1* in March (Fig. 6). However, the reduction in DELLA levels, possibly resulting from a feedback mechanism, may have initiated the floral bud induction process (Fig. 10a). The expression of *CaFTL1*, a repressor of flowering, increased with GA application at high concentrations (Fig. 6b). However, this regulation did not appear to influence the increase in floral buds in this treatment (Fig. 1). Cardon (2020) demonstrated that the *TFL1* gene of *C. arabica* retains an intron in its mature mRNA, turning the protein unable to interact with the FD/14-3-3 to form the floral repression complex (FRC), which represses floral induction. In this context, even though *CaTFL1* was more highly expressed with GA application, it was likely unable to inhibit floral bud induction. This post-transcriptional regulation may explain the sequential flowering of the coffee plant, as *TFL1* in *C. arabica* is unable to inhibit *FT*, thus allowing multiple flowering events.

In August, when the days became shorter, the decrease in DELLA levels, associated with the reduction in *CaCO* expression, may explain the greater expression of *CaFT1* in treatments with GA and ABA (Fig. 7), also reflecting the increase in the number of floral buds

(Fig. 2). Thus, although there was an increase in *CaTFL1* expression in the same treatments that elevated *CaFT1* (Fig. 7), it appears that *CaCO* may coordinate the regulation of *CaFT1*. These results raise the hypothesis that *CaCO* may act as a repressor of *CaFT1* during shorter days. This is supported by the findings of Cardon et al. (2022), which demonstrated an inverse regulation between *CaFT1* and *CaCO* at different times of the year. The CO protein positively regulates *FT* expression in long-day plants such as *Arabidopsis* (Kim 2020; Zeng et al. 2022). On the other hand, in short-day plants such as rice, *Heading-date1* (*Hd1*) and *Heading-date3a* (*Hd3a*) encode proteins homologous to *CO* and *FT*, respectively, with *Hd1* being a direct repressor of *Hd3a* (Kojima et al. 2002; Ishikawa et al. 2011; Meng et al. 2022).

An intriguing observation is that GA normally inhibits flowering in short-day plants (Li et al. 2018; Garmendia et al. 2019; Zhang et al. 2019)**,** which contrasts with the results obtained in the present study, where the application of GA promoted greater floral bud induction (Fig. 1 and 2). This discrepancy can be explained by the fact that in our research, GA application was carried out in the morning, not interfering with the length of the day and, consequently, with the photoperiodic pathway. Generally, GA application occurs on long-day plants exposed to short-day conditions, to replace the requirement for long days in these plants (Hisamatsu and King 2008). Furthermore, it raises the possibility of questioning whether the coffee tree is a short-day plant, suggesting that the coffee tree could be a day-neutral plant.

In our results, we also showed the interaction between GA and ABA in floral buds in August (Fig. 8). We demonstrated that these hormones can regulate genes associated with floral meristem identity and floral organ formation, including *LFY* and *AP1*. These findings were expected because the application of ABA accelerated floral bud development and ABA can modulate GA levels. These results are in line with previous studies that indicated that GA induces flowering by stimulating the expression of floral integrative genes, such as *LFY* and *SOC1*, and that *LFY* can induce *AP1* (Jaeger et al. 2013; Bao et al. 2020).

The regulation of *LFY* and *AP1* through GA and ABA appears to depend on both the concentrations of these hormones and water availability. During periods of drought, there is a gradual increase in the endogenous concentration of ABA in floral buds, which decreases with the first rain in August, preceding flower anthesis (López et al. 2022). In this context, ABA can be induced by drought, signaling the plant to produce more GA (Fig. 10b). When ABA is leached by rain, GA acts on floral bud development, culminating in anthesis. This is supported by studies that demonstrated a decrease in endogenous GA concentrations in floral buds, associated with an increase in ABA before rain, and that after plant irrigation, there is a

reduction in ABA concentration (Browning et al. 1970; Browning 1973). This complex interaction between GA and ABA demonstrates that there is a balance between these hormones, which act antagonistically to break the floral bud latency described for coffee plants (López et al. 2021).

In addition to the interaction between GA and ABA, our results indicated that these hormones also play a role in regulating ethylene levels in August (Fig. 9). Ethylene, a gaseous hormone, is known to promote the anthesis in coffee plants (Lima et al. 2021; Santos et al. 2022). In contrast, it has been suggested that ACC, the precursor of ethylene, acts as a direct signal to promote anthesis (López et al. 2022). In our study, we observed that the application of GA induced *CaACS3* expression and increased ACC levels in leaves. However, this increases in ACC in leaves did not translate into a corresponding increase in ethylene levels. In contrast, in floral buds, the application of GA and ABA resulted in reduced *CaACS3* expression but contributed to an increase in ACC levels. This observation suggests that ACC in leaves may have been transported to floral buds, playing a crucial role as a signal to trigger anthesis. Interestingly, the increase in ACC in floral buds culminated in greater ethylene production in this tissue, raising the question of which of these signals (ACC or ethylene) plays a central role in promoting anthesis (Fig. 10b).

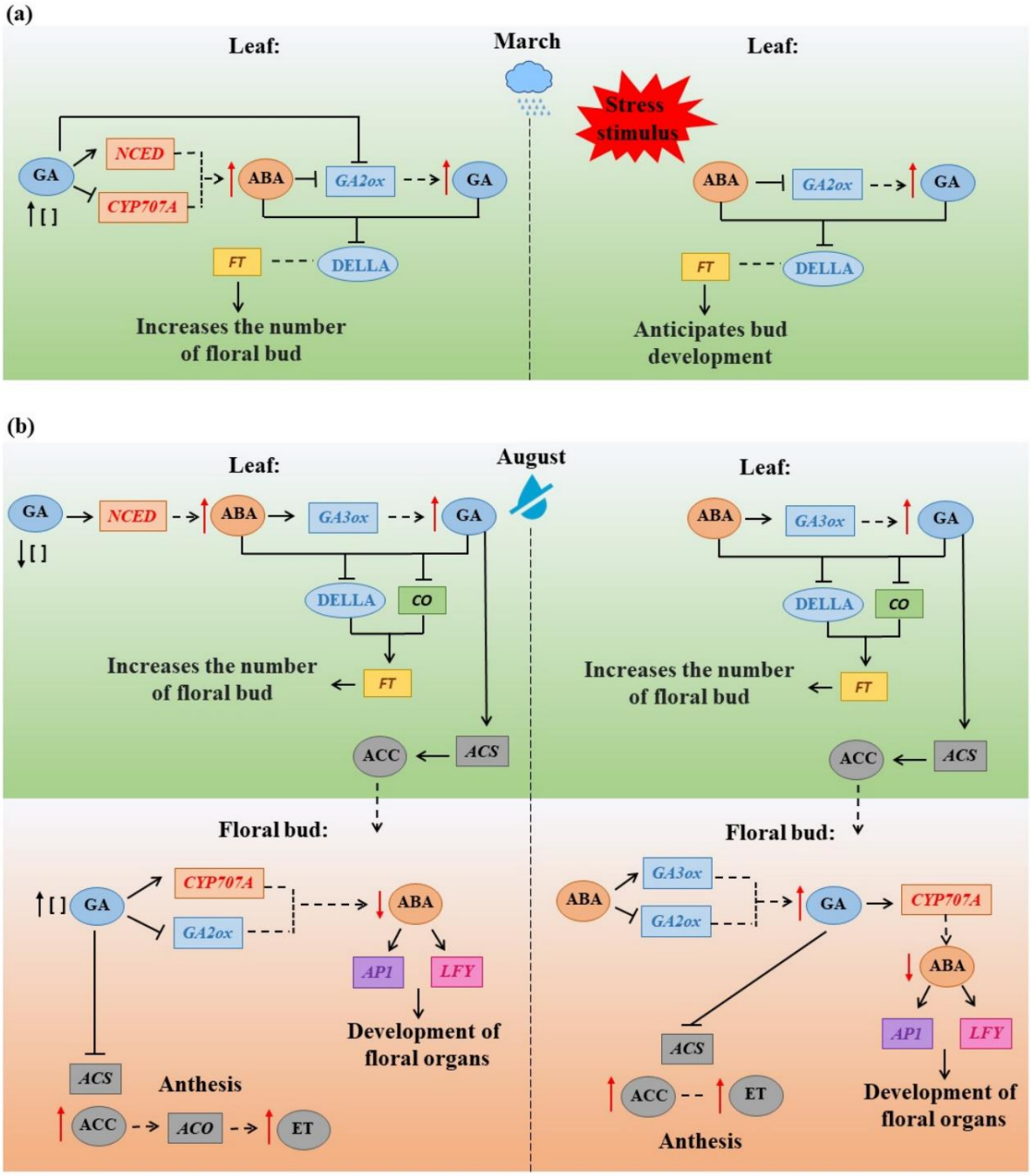

Fig. 10 – A proposed model for regulating the molecular pathways involved during the flowering induction phase in March (a) and in the floral bud development phase in August (b) in *C. arabica* under the application of gibberellin (GA) and abscisic acid (ABA). The green frame represents leaves. The red frame represents floral buds. The square boxes represent genes. The circles represent the hormones, ACC and DELLA proteins. Solid lines indicate induction, dashed lines indicate possible regulation not proven in this study, and the T-end of the arrows indicates inhibition. The red arrows indicate increases or decreases. Where [] is the concentration. The environmental changes associated with water are represented by an image above the tables: March (rainy season) and August (dry season). ABA: Abscisic acid; ACC: 1-aminocyclopropane-1-carboxylic acid; *ACS*: *ACC SYNTHASE*; *ACO*: *ACC OXIDASE*; *AP1*: *APETALA 1*; *CO*: *CONSTANS*; *CYP707A*: *CYTOCHROME P450, FAMILY 707, SUBFAMILY A*; ET: ETHYLENE; *FT*: *FLOWERING LOCUS T*; GA: Gibberellin; *GA2ox*: *GIBBERELLIN*

2-OXIDASE; *GA3ox*: *GIBBERELLIN 3-OXIDASE*; *LFY*: *LEAFY*; *NCED*: *9-CIS-EPOXY CAROTENOID DIOXYGENASE*.

5. CONCLUSION

The application of GA and ABA revealed significant effects, especially considering the induction of floral buds at different periods of the year. The results showed that the application of GA and ABA increased the number of floral buds, depending on the concentration used. Furthermore, ABA treatment not only increased the number of floral buds but also accelerated their development, providing a more comprehensive understanding of the role of this hormone in the context of water stress, which is crucial for coffee flowering.

The complex interaction between GA and ABA, also evidenced in gene expression analyses, highlights the fine regulation of these hormones during different phases of plant reproductive development, highlighting the influence of these hormones on *CaFT1* expression. The influence on ethylene levels, observed in August, adds a layer of complexity to our understanding of these processes, revealing a hormonal crosstalk between these growth regulators throughout coffee tree reproductive development. These results reinforce that precise manipulation of these PGRs can positively affect the reproductive development of coffee plants, directly affecting the number of floral buds and, consequently, fruit production.

This study contributes not only to the fundamental understanding of plant physiology but also has practical implications for improving production under specific conditions of coffee cultivation. Specifically, in the context of climate change, the application of PGRs may emerge as an effective strategy to mitigate these challenges. For example, ABA could be a treatment to prepare plants for cold? GA could manipulate the time of the cycle anticipating harvesting? Could the combined application of GA and ABA both anticipate and accelerate bud development, ensuring greater synchronization of flowering regardless of climatic conditions? Continuing this line of research can uncover even more questions about this hormonal network, offering technologies to improve agricultural practices and facing challenges related to reproductive development in perennial plants such as coffee trees.

Authors' contributions

L.M.A., R.R.O. and A.C-J. conceptualized the project; L.M.A. conducted all experiments and data research; L.M.A., G.L.R., J.C.C, and J.P.A. performed field experiments; G.L.R, R.Y.M.G. and J.P.A supported L.M.A in the gene expression and biochemical analyses; G.C.R supported for bioinformatic analyses; L.M.A. and R.R.O. discussed all data and analysis;

R.R.O. and A.C-J supervised experiments and analyses; L.M.A. wrote the manuscript; all co-authors corrected and contributed to writing of the final manuscript version.

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Data availability statement

All data generated or analyzed during this study are included in this published article [and in its supplementary information file].

Declarations

The authors declare that there are no conflicts of interest.

References

Ali S, Hayat K, Iqbal A, Xie L (2020) Implications of Abscisic Acid in the Drought Stress Tolerance of Plants. Agronomy 10:1323. https://doi.org/10.3390/agronomy10091323

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. Journal of Molecular Biology 215:403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Alvim P de T (1960) Moisture Stress as a Requirement for Flowering of Coffee. Science 132:354. https://doi.org/10.1126/science.132.3423.354

Anders S, Pyl PT, Huber W (2015) HTSeq—a Python framework to work with high-throughput sequencing data. Bioinformatics 31:166–169. https://doi.org/10.1093/bioinformatics/btu638

Bao S, Hua C, Shen L, Yu H (2020) New insights into gibberellin signaling in regulating flowering in *Arabidopsis*. Journal of Integrative Plant Biology 62:118–131. https://doi.org/10.1111/jipb.12892

Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW (2013) GenBank. Nucleic Acids Research 41:D36–D42. https://doi.org/10.1093/nar/gks1195

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Browning G (1973) Flower Bud Dormancy in *Coffea Arabica* L. I. Studies of Gibberellin in Flower Buds and Xylem Sap and of Abscisic Acid in Flower Buds in Relation to Dormancy Release. Journal of Horticultural Science 48:29–41. https://doi.org/10.1080/00221589.1973.11514504

Browning G, Hoad GV, Gaskin P (1970) Identification of abscisic acid in flower buds of *Coffea arabica* (L.). Planta 94:213–219. https://doi.org/10.1007/BF00386131

Bulens I, Van de Poel B, Hertog ML, De Proft MP, Geeraerd AH, Nicolaï BM (2011) Protocol: An updated integrated methodology for analysis of metabolites and enzyme activities of ethylene biosynthesis. Plant Methods 7:17. https://doi.org/10.1186/1746-4811-7-17

Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT (2009) The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. Clinical Chemistry 55:611–622. https://doi.org/10.1373/clinchem.2008.112797

Camargo ÂPD, Camargo MBPD (2001) Definição e esquematização das fases fenológicas do cafeeiro arábica nas condições tropicais do Brasil. Bragantia 60:65–68. https://doi.org/10.1590/S0006-87052001000100008

Cardon CH (2020). Characterization and functional analysis of *FLOWERING LOCUS T* (*FT*) and *TERMINAL FLOWER 1* (*TFL1*) orthologs in *Coffea arabica* L.: Moving forward into understanding Coffee Flowering. Doctoral dissertation, Federal University of Lavras.

Cardon CH, de Oliveira RR, Lesy V, Ribeiro THC, Fust C, Pereira LP, 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 Science 325:111479. https://doi.org/10.1016/j.plantsci.2022.111479

Carvalho D da S, Junior KSF, Brigante GP, Dias M de S (2022) Effects of coffee maturation regulators. Revista Brasileira de Engenharia de Biossistemas 16. https://doi.org/10.18011/bioeng.2022.v16.1060

Castro-Camba R, Sánchez C, Vidal N, Vielba JM (2022) Plant Development and Crop Yield: The Role of Gibberellins. Plants 11:2650. https://doi.org/10.3390/plants11192650

Chen S, ten Tusscher KHWJ, Sasidharan R, Dekker SC, de Boer HJ (2023) Parallels between drought and flooding: An integrated framework for plant eco-physiological responses to water stress. Plant-Environment Interactions 4:175–187. https://doi.org/10.1002/pei3.10117

Chen S, Zhao H, Luo T, Liu Y, Nie X, Li H (2019) Characteristics and Expression Pattern of MYC Genes in Triticum aestivum, *Oryza sativa*, and Brachypodium distachyon. Plants 8:274. https://doi.org/10.3390/plants8080274

Claeys H, De Bodt S, Inzé D (2014) Gibberellins and DELLAs: central nodes in growth regulatory networks. Trends in Plant Science 19:231–239. https://doi.org/10.1016/j.tplants.2013.10.001

Conlon EM, Liu XS, Lieb JD, Liu JS (2003) Integrating regulatory motif discovery and genome-wide expression analysis. Proceedings of the National Academy of Sciences 100:3339–3344. https://doi.org/10.1073/pnas.0630591100

DaMatta FM, Avila RT, Cardoso AA, Martins SCV, Ramalho JC (2018) Physiological and Agronomic Performance of the Coffee Crop in the Context of Climate Change and Global Warming: A Review. J Agric Food Chem 66:5264–5274. https://doi.org/10.1021/acs.jafc.7b04537

DaMatta FM, Ronchi CP, Maestri M, Barros RS (2007) Ecophysiology of coffee growth and production. Braz J Plant Physiol 19:485–510. https://doi.org/10.1590/S1677-04202007000400014

de Oliveira KKP, de Oliveira RR, de Campos Rume G, Ribeiro THC, Fernandes-Brum CN, do Amaral LR, Kakrana A, Mathioni S, Meyers BC, 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 RR, Cesarino I, Mazzafera P, Dornelas MC (2014) Flower development in *Coffea arabica* L.: new insights into MADS-box genes. Plant Reprod 27:79–94. https://doi.org/10.1007/s00497-014-0242-2

Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR (2013) STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29:15–21. https://doi.org/10.1093/bioinformatics/bts635

Elbar S, Maytal Y, David I, Carmeli-Weissberg M, Shaya F, Barnea-Danino Y, Bustan A, Harpaz-Saad S (2023) Abscisic acid plays a key role in the regulation of date palm fruit ripening. Frontiers in Plant Science 13

Fernandes-Brum CN, Garcia B de O, Moreira RO, Ságio SA, Barreto HG, Lima AA, Freitas NC, de Lima RR, de Carvalho CHS, Chalfun-Júnior A (2017) A panel of the most suitable reference genes for RT-qPCR expression studies of coffee: screening their stability under different conditions. Tree Genetics & Genomes 13:131. https://doi.org/10.1007/s11295-017-1213-1

Ferreira EB, Cavalcanti PP, Nogueira DA (2021) ExpDes.pt: Pacote Experimental Designs (Portugues)

Flores CA, Torre WD la, Monroig M, González W (2005) Ácido giberélico, paclobutrazol, fluoridone, ácido abscísico afectan la sincronización de la floración del cafeto. The Journal of Agriculture of the University of Puerto Rico 89:159–168. https://doi.org/10.46429/jaupr.v89i3-4.1037

Gao S, Chu C (2020) Gibberellin Metabolism and Signaling: Targets for Improving Agronomic Performance of Crops. Plant and Cell Physiology 61:1902–1911. https://doi.org/10.1093/pcp/pcaa104

Garmendia A, Beltrán R, Zornoza C, García-Breijo FJ, Reig J, Merle H (2019) Gibberellic acid in *Citrus* spp. flowering and fruiting: A systematic review. PLOS ONE 14: e0223147. https://doi.org/10.1371/journal.pone.0223147

Ginestet C (2011) ggplot2: Elegant Graphics for Data Analysis. Journal of the Royal Statistical Society Series A: Statistics in Society 174:245–246. https://doi.org/10.1111/j.1467-985X.2010.00676_9.x

Gupta K, Wani SH, Razzaq A, Skalicky M, Samantara K, Gupta S, Pandita D, Goel S, Grewal S, Hejnak V, Shiv A, El-Sabrout AM, Elansary HO, Alaklabi A, Brestic M (2022) Abscisic Acid: Role in Fruit Development and Ripening. Frontiers in Plant Science 13:817500. https://doi.org/10.3389/fpls.2022.817500

He J, Xin P, Ma X, Chu J, Wang G (2020) Gibberellin Metabolism in Flowering Plants: An Update and Perspectives. Frontiers in Plant Science 11:532. https://doi.org/10.3389/fpls.2020.00532

Hisamatsu T, King RW (2008) The nature of floral signals in Arabidopsis. II. Roles for *FLOWERING LOCUS T* (*FT*) and gibberellin. Journal of Experimental Botany 59:3821–3829. https://doi.org/10.1093/jxb/ern232

Huber W, Carey VJ, Gentleman R, Anders S, Carlson M, Carvalho BS, Bravo HC, Davis S, Gatto L, Girke T, Gottardo R, Hahne F, Hansen KD, Irizarry RA, Lawrence M, Love MI, MacDonald J, Obenchain V, Oleś AK, Pagès H, Reyes A, Shannon P, Smyth GK, Tenenbaum D, Waldron L, Morgan M (2015) Orchestrating high-throughput genomic analysis with Bioconductor. Nat Methods 12:115–121. https://doi.org/10.1038/nmeth.3252

Ishikawa R, Aoki M, Kurotani K, Yokoi S, Shinomura T, Takano M, Shimamoto K (2011) Phytochrome B regulates Heading date 1 (Hd1)-mediated expression of rice florigen Hd3a and critical day length in rice. Mol Genet Genomics 285:461–470. https://doi.org/10.1007/s00438-011-0621-4

Iuchi S, Kobayashi M, Taji T, Naramoto M, Seki M, Kato T, Tabata S, Kakubari Y, Yamaguchi-Shinozaki K, Shinozaki K (2001) Regulation of drought tolerance by gene manipulation of 9-cis-epoxycarotenoid dioxygenase, a key enzyme in abscisic acid biosynthesis in Arabidopsis. The Plant Journal 27:325–333. https://doi.org/10.1046/j.1365-313x.2001.01096.x

Jaeger KE, Pullen N, Lamzin S, Morris RJ, Wigge PA (2013) Interlocking Feedback Loops Govern the Dynamic Behavior of the Floral Transition in *Arabidopsis*. The Plant Cell 25:820–833. https://doi.org/10.1105/tpc.113.109355

Jin S, Nasim Z, Susila H, Ahn JH (2021) Evolution and functional diversification of *FLOWERING LOCUS T/TERMINAL FLOWER 1* family genes in plants. Seminars in Cell & Developmental Biology 109:20–30. https://doi.org/10.1016/j.semcdb.2020.05.007

Jores T, Tonnies J, Wrightsman T, Buckler ES, Cuperus JT, Fields S, Queitsch C (2021) Synthetic promoter designs enabled by a comprehensive analysis of plant core promoters. Nat Plants 7:842–855. https://doi.org/10.1038/s41477-021-00932-y

Kazama EH, da Silva RP, Tavares T de O, Correa LN, de Lima Estevam FN, Nicolau FE de A, Maldonado Júnior W (2021) Methodology for selective coffee harvesting in management zones of yield and maturation. Precision Agric 22:711–733. https://doi.org/10.1007/s11119-020-09751-1

Kim D-H (2020) Current understanding of flowering pathways in plants: focusing on the vernalization pathway in *Arabidopsis* and several vegetable crop plants. Hortic Environ Biotechnol 61:209–227. https://doi.org/10.1007/s13580-019-00218-5

Kojima S, Takahashi Y, Kobayashi Y, Monna L, Sasaki T, Araki T, Yano M (2002) Hd3a, a Rice Ortholog of the *Arabidopsis* FT Gene, Promotes Transition to Flowering Downstream of Hd1 under Short-Day Conditions. Plant and Cell Physiology 43:1096–1105. https://doi.org/10.1093/pcp/pcf156

Krzymuski M, Andrés F, Cagnola JI, Jang S, Yanovsky MJ, Coupland G, Casal JJ (2015) The dynamics of *FLOWERING LOCUS T* expression encodes long-day information. The Plant Journal 83:952–961. https://doi.org/10.1111/tpj.12938

Lange T, Pimenta Lange MJ (2020) The Multifunctional Dioxygenases of Gibberellin Synthesis. Plant and Cell Physiology 61:1869–1879. https://doi.org/10.1093/pcp/pcaa051

Lescot M, Déhais P, Thijs G, Marchal K, Moreau Y, Van de Peer Y, Rouzé P, Rombauts S (2002) PlantCARE, a database of plant *cis*-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. Nucleic Acids Research 30:325–327. https://doi.org/10.1093/nar/30.1.325.

Leijten W, Koes R, Roobeek I, Frugis G (2018) Translating Flowering Time from Arabidopsis thaliana to Brassicaceae and Asteraceae Crop Species. Plants 7:111. https://doi.org/10.3390/plants7040111

León-Burgos AF, Unigarro C, Balaguera-López HE (2022) Can prolonged conditions of water deficit alter photosynthetic performance and water relations of coffee plants in central-west Colombia? South African Journal of Botany 149:366–375. https://doi.org/10.1016/j.sajb.2022.06.034

Li C, Zheng L, Wang X, Hu Z, Zheng Y, Chen Q, Hao X, Xiao X, Wang X, Wang G, Zhang Y (2019) Comprehensive expression analysis of *Arabidopsis* GA2-oxidase genes and their functional insights. Plant Science 285:1–13. https://doi.org/10.1016/j.plantsci.2019.04.023

Li J, Pan B-Z, Niu L, Chen M-S, Tang M, Xu Z-F (2018) Gibberellin Inhibits Floral Initiation in the Perennial Woody Plant Jatropha curcas. J Plant Growth Regul 37:999–1006. https://doi.org/10.1007/s00344-018-9797-8

Lima AA, Santos IS, Torres MEL, Cardon CH, Caldeira CF, Lima RR, Davies WJ, Dodd IC, Chalfun-Junior A (2021) Drought and re-watering modify ethylene production and

sensitivity, and are associated with coffee anthesis. Environmental and Experimental Botany 181:104289. https://doi.org/10.1016/j.envexpbot.2020.104289

Liu L, Zhang X, Chen F, Mahi AAE, Wu X, Chen Q, Fu Y-F (2017) Analysis of promoter activity reveals that *GmFTL2* expression differs from that of the known Flowering Locus T genes in soybean. The Crop Journal 5:438–448. https://doi.org/10.1016/j.cj.2017.03.004

López ME, Marquez Gutiérrez R, Cardon CH, 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 13:824948. https://doi.org/10.3389/fpls.2022.824948

López ME, Santos IS, Oliveira RR de, Lima AA, Cardon CH, Chalfun-Junior A, López ME, Santos IS, Oliveira RR de, 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 Research 1:1–16. https://doi.org/10.48130/BPR-2021-0013

Martins MQ, Fortunato AS, Rodrigues WP, Partelli FL, Campostrini E, Lidon FC, DaMatta FM, Ramalho JC, Ribeiro-Barros AI (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. Frontiers in Plant Science 8:307. https://doi.org/10.3389/fpls.2017.00307

Matsumoto TK, Lopez J (2016) Coffee harvest management by manipulation of coffee flowering with plant growth regulators. Acta Hortic 1130:219–224. https://doi.org/10.17660/ActaHortic.2016.1130.32

Melo B de, Sousa LB (2011) Biologia da reprodução de *Coffea arabica*. L. e *Coffea canephora* Pierre. Revista Verde de Agroecologia e Desenvolvimento Sustentável 6:1–7

Meng X, Liu S, Guo X, Xing S, Peng Z (2022) Novel Allelic Variation of Hd1 Involves in Photoperiod Sensitivity of Ric

Miranda FR, Drumond LCD, Ronchi CP (2020) Synchronizing coffee blossoming and fruit ripening in irrigated crops of the Brazilian Cerrado Mineiro Region. Australian Journal of Crop Science 14:605–613. https://doi.org/10.3316/informit.261830530746678

Mistry J, Chuguransky S, Williams L, Qureshi M, Salazar GA, Sonnhammer ELL, Tosatto SCE, Paladin L, Raj S, Richardson LJ, Finn RD, Bateman A (2021) Pfam: The protein families database in 2021. Nucleic Acids Research 49:D412–D419. https://doi.org/10.1093/nar/gkaa913

Morais H, Caramori PH, Koguishi MS, Ribeiro AM de A (2008) Escala fenológica detalhada da fase reprodutiva de *Coffea arabica*. Bragantia 67:257–260. https://doi.org/10.1590/S0006-87052008000100031

Nguyen CH, Yan D, Nambara E (2023) Persistence of Abscisic Acid Analogs in Plants: Chemical Control of Plant Growth and Physiology. Genes 14:1078. https://doi.org/10.3390/genes14051078

Oliveira RR, Viana AJC, Reátegui ACE, Vincentz MGA (2015) An efficient method for simultaneous extraction of high-quality RNA and DNA from various plant tissues. Genetics and Molecular Research 14:18828–18838

Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT–PCR. Nucleic Acids Research 29:e45. https://doi.org/10.1093/nar/29.9.e45

Quesada V (2022) Advances in the Molecular Mechanisms of Abscisic Acid and Gibberellins Functions in Plants 2.0. International Journal of Molecular Sciences 23:8524. https://doi.org/10.3390/ijms23158524

Retief JD (1999) Phylogenetic Analysis Using PHYLIP. In: Misener S, Krawetz SA (eds) Bioinformatics Methods and Protocols. Humana Press, Totowa, NJ, pp 243–258

Ritonga FN, Zhou D, Zhang Y, Song R, Li C, Li J, Gao J (2023) The Roles of Gibberellins in Regulating Leaf Development. Plants 12:1243. https://doi.org/10.3390/plants12061243

Robinson MD, McCarthy DJ, Smyth GK (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26:139–140. https://doi.org/10.1093/bioinformatics/btp616

Rozewicki J, Li S, Amada KM, Standley DM, Katoh K (2019) MAFFT-DASH: integrated protein sequence and structural alignment. Nucleic Acids Research 47:W5–W10. https://doi.org/10.1093/nar/gkz342

Saito S, Hirai N, Matsumoto C, Ohigashi H, Ohta D, Sakata K, Mizutani M (2004) Arabidopsis CYP707As Encode (+)-Abscisic Acid 8′-Hydroxylase, a Key Enzyme in the Oxidative Catabolism of Abscisic Acid. Plant Physiology 134:1439–1449. https://doi.org/10.1104/pp.103.037614

Santos IS, Ribeiro THC, de Oliveira KKP, dos Santos JO, Moreira RO, Lima RR, Lima AA, Chalfun-Junior A (2022) Multigenic regulation in the ethylene biosynthesis pathway during coffee flowering. Physiol Mol Biol Plants 28:1657–1669. https://doi.org/10.1007/s12298-022-01235-y

Scholander P, Hammel H, Hemmingsen E, Bradstreet E (1964) Hydrostatic pressure and osmotic potential in leaves of mangroves and some other plants*. Proceedings of the National Academy of Sciences 52:119–125. https://doi.org/10.1073/pnas.52.1.119

Schuch UK, Fuchigami LH, Nagao MA (1990) Gibberellic acid causes earlier flowering and synchronizes fruit ripening of coffee. Plant Growth Regul 9:59–64. https://doi.org/10.1007/BF00025279

Sela I, Ashkenazy H, Katoh K, Pupko T (2015) GUIDANCE2: accurate detection of unreliable alignment regions accounting for the uncertainty of multiple parameters. Nucleic Acids Research 43:W7–W14. https://doi.org/10.1093/nar/gkv318

Steibel JP, Poletto R, Coussens PM, Rosa GJM (2009) A powerful and flexible linear mixed model framework for the analysis of relative quantification RT-PCR data. Genomics 94:146–152. https://doi.org/10.1016/j.ygeno.2009.04.008

Sun T-P (2011) The molecular mechanism and evolution of the GA-GID1-DELLA signaling module in plants. Curr Biol 21:R338-45. https://doi.org/10.1016/j.cub.2011.02.036

Villao-Uzho L, Chávez-Navarrete T, Pacheco-Coello R, Sánchez-Timm E, Santos-Ordóñez E (2023) Plant Promoters: Their Identification, Characterization, and Role in Gene Regulation. Genes 14:1226. https://doi.org/10.3390/genes14061226

Wang H, Pan J, Li Y, Lou D, Hu Y, Yu D (2016) The *DELLA-CONSTANS* Transcription Factor Cascade Integrates Gibberellic Acid and Photoperiod Signaling to Regulate Flowering. Plant Physiology 172:479–488. https://doi.org/10.1104/pp.16.00891

Wu J, Wang J, Hui W, Zhao F, Wang P, Su C, Gong W (2022) Physiology of Plant Responses to Water Stress and Related Genes: A Review. Forests 13:324. https://doi.org/10.3390/f13020324

Wu Q, He Y, Cui C, Tao X, Zhang D, Zhang Y, Ying T, Li L (2023) Quantitative proteomic analysis of tomato fruit ripening behavior in response to exogenous abscisic acid. Journal of the Science of Food and Agriculture 103:7469–7483. https://doi.org/10.1002/jsfa.12838

Xu F, Li T, Xu P-B, Li L, Du S-S, Lian H-L, Yang H-Q (2016) DELLA proteins physically interact with *CONSTANS* to regulate flowering under long days in Arabidopsis. FEBS Letters 590:541–549. https://doi.org/10.1002/1873-3468.12076

Zapata-Restrepo LN, Guevara-Ortega LM, Zapata-Restrepo LN, Guevara-Ortega LM (2020) Evaluation of the incidence of foliar application of gibberellic acid in flowering trees of *Coffea arabica* L. and its impact on climate change. DYNA 87:263–269. https://doi.org/10.15446/dyna.v87n215.85392

Zeng X, Lv X, Liu R, He H, Liang S, Chen L, Zhang F, Chen L, He Y, Du J (2022) Molecular basis of *CONSTANS* oligomerization in *FLOWERING LOCUS T* activation. Journal of Integrative Plant Biology 64:731–740. https://doi.org/10.1111/jipb.13223

Zhang S, Gottschalk C, van Nocker S (2019) Genetic mechanisms in the repression of flowering by gibberellins in apple (*Malus* x *domestica* Borkh.). BMC Genomics 20:747. https://doi.org/10.1186/s12864-019-6090-6

Supplementary material

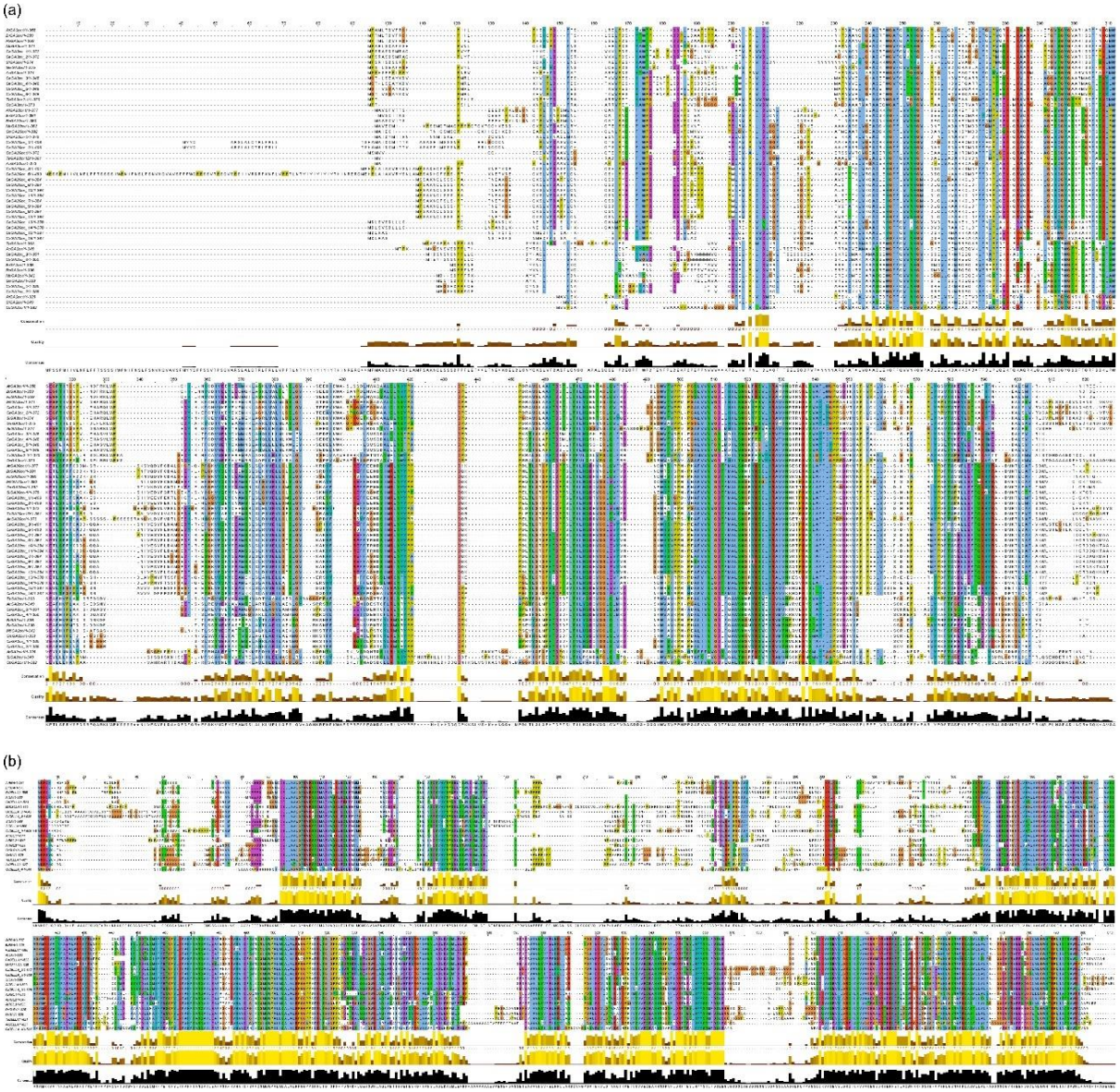

Fig. S1 - Alignments of predicted amino acid sequences belonging to the *GAoxes* (a) and *DELLA* (b) gene family of *Coffea arabica* (Ca), *Arabidopsis thaliana* (At), *Triticum aestivum* (Ta), *Brassica rapa* (Br), *Raphanus sativus* (Rs), *Malus domestica* (Md), *Glycine max* (Gm), *Ananas comosus* (Ac), *Solanum lycopersicum* (Sl), *Oryza sativa* (Os). An HTML file was generated using the GUIDANCE algorithm. Sequence names, amino acid positions, and alignment scores are indicated in the figure, showing conserved and non-conserved motifs, corroborating phylogenetic analysis and sequence classification.

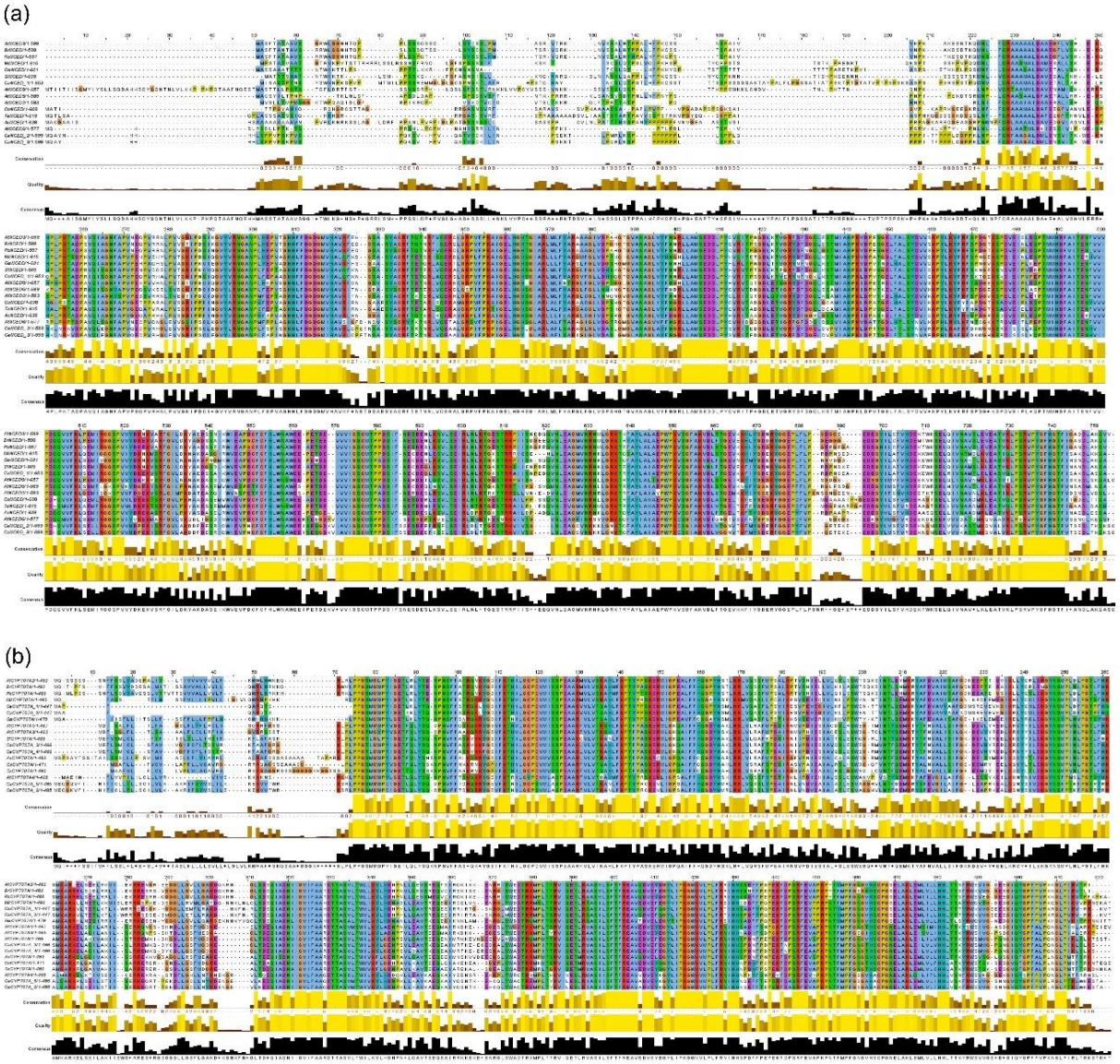

Fig. S2 - Alignments of predicted amino acid sequences belonging to the *NCED* (a) e *CYP707A* (b) gene family of *Coffea arabica* (Ca), *Arabidopsis thaliana* (At), *Triticum aestivum* (Ta), *Brassica rapa* (Br), *Raphanus sativus* (Rs), *Malus domestica* (Md), *Glycine max* (Gm), *Ananas comosus* (Ac), *Solanum lycopersicum* (Sl), *Oryza sativa* (Os). An HTML file was generated using the GUIDANCE algorithm. Sequence names, amino acid positions, and alignment scores are indicated in the figure, showing conserved and non-conserved motifs, corroborating phylogenetic analysis and sequence classification.

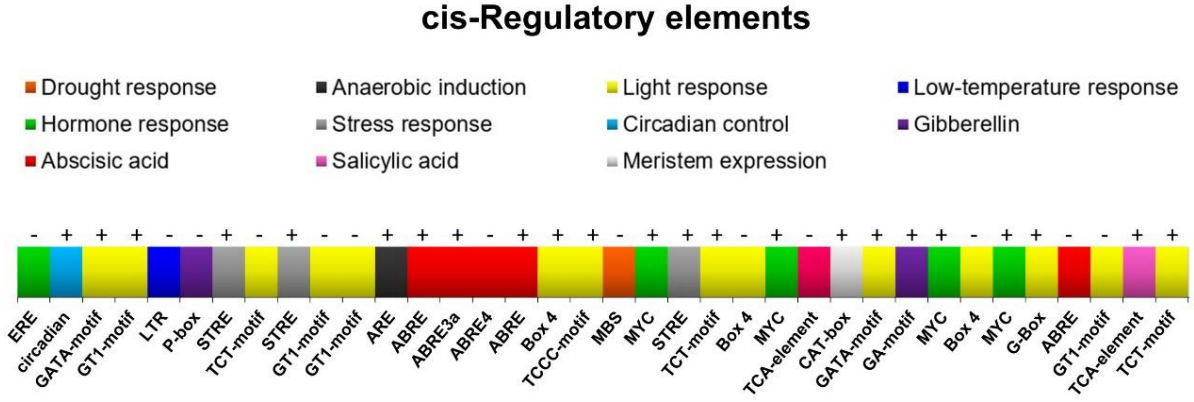

Fig. S3 - *Cis-regulatory* regions found in the promoter sequence of the *CaFT1* gene in the *C. arabica* genome through a search in the PlantCARE database. Motifs related to the same function are represented by the same color. The direction of the sequence is represented by the signs (+) and (−). The sequence size was -2000 bp.

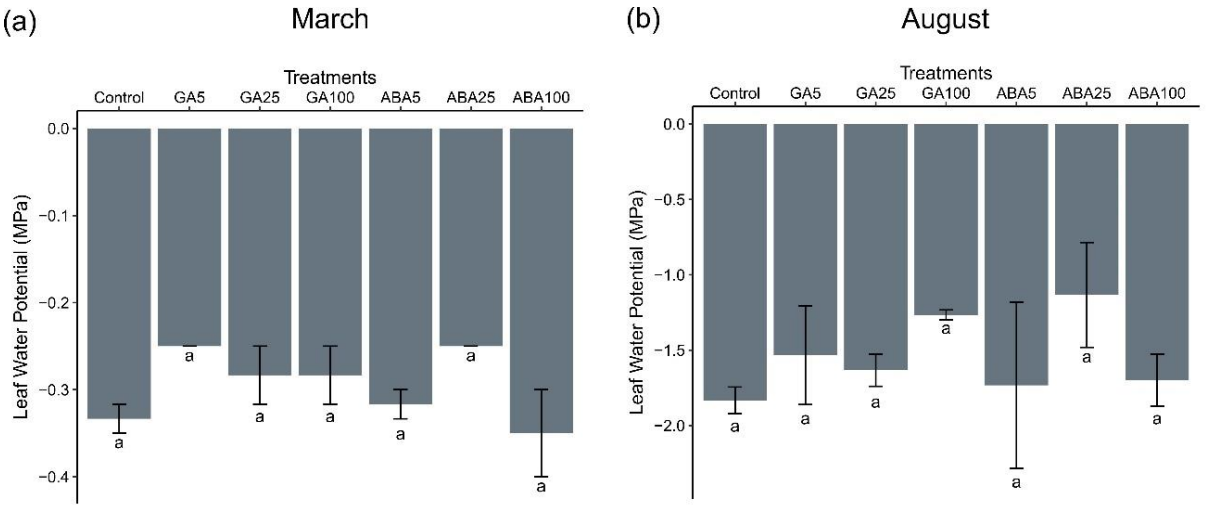

Fig. S4 - Leaf water potential (MPa) in coffee trees subjected to phytoregulator and control treatments. Gibberellin (GA) and abscisic acid (ABA) were applied at different concentrations (5, 25, and 100ppm), in the months of (a) March and (b) August 2022. Lowercase letters indicate statistically significant differences between the treatments (P<0 .05) significance.

Table S1 - Motifs were found in the promoter sequence of the *CaFT1* gene in the *C. arabica* genome through a search of the PlantCARE database.

PlantCARE_28785	motif	sequences	location	size	position	species
PlantCARE_28785	CAAT-box	CAAT	42	4	+	Nicotiana glutinosa
PlantCARE_28785	ERE	ATTTTAAA	76	8	-	Nicotiana glutinos
PlantCARE_28785	CAAT-box	CAAT	88	4	-	Nicotiana glutinosa
PlantCARE_28785	circadian	CAAAGATATC	113	9	+	Lycopersicon esculentum
PlantCARE_28785	GATA-motif	AAGATAAGATT	115	10	+	Arabidopsis thaliana
PlantCARE_28785	TATA-box	taTATAAAtc	122	9	-	Arabidopsis thaliana
PlantCARE_28785	TATA-box	TATAAAA	125	7	-	Pisum sativum
PlantCARE_28785	TATA-box	TATAAA	126	6	-	Helianthus annuus
PlantCARE_28785	TATA-box	TATAA	127	5	-	Arabidopsis thaliana
PlantCARE_28785	TATA	TATAAAAT	128	8	+	Arabidopsis thaliana
PlantCARE_28785	TATA-box	TATA	128	4	+	Arabidopsis thaliana
PlantCARE_28785	CAAT-box	CAAT	167	4	-	Nicotiana glutinosa
PlantCARE_28785	GT1-motif	GGTTAAT	177	7	+	Avena sativa
PlantCARE_28785	CAAT-box	CAAT	223	4	+	Nicotiana glutinosa
PlantCARE_28785	LTR	CCGAAA	235	6	-	Hordeum vulgare
PlantCARE_28785	P-box	CAACAAACCCCTT	245	12	-	Petroselinum crispum
PlantCARE_28785	STRE	AGGGG	246	5	+	Arabidopsis thaliana

PlantCARE_28785	TCT-motif	TCTTAC	281	6	-	Arabidopsis thaliana
PlantCARE_28785	CAAT-box	CAAAT	292	5	-	Pisum sativum
PlantCARE_28785	CAAT-box	CAAAT	299	5	+	Pisum sativum
PlantCARE_28785	CAAT-box	CAAT	311	4	-	Nicotiana glutinosa
PlantCARE_28785	CAAT-box	CCAAT	315	5	-	Arabidopsis thaliana
PlantCARE_28785	STRE	AGGGG	337	5	+	Arabidopsis thaliana
PlantCARE_28785	GT1-motif	GGTTAAT	353	7	-	Avena sativa
PlantCARE_28785	GT1-motif	GGTTAA	354	6	-	Arabidopsis thaliana
PlantCARE_28785	CAAT-box	CAAAT	364	5	+	Pisum sativum
PlantCARE_28785	ARE	AAACCA	373	6	+	Zea mays
PlantCARE_28785	CAAT-box	CAAAT	413	5	+	Pisum sativum
PlantCARE_28785	CAAT-box	CAAAT	419	5	+	Pisum sativum
PlantCARE_28785	ABRE	TACGTGTC	435	8	+	Oryza sativa/Arabidopsis thaliana
PlantCARE_28785	ABRE3a	TACGTG	435	6	+	Zea mays
PlantCARE_28785	ABRE4	CACGTA	435	6	-	Zea mays
PlantCARE_28785	ABRE	ACGTG	436	5	+	Arabidopsis thaliana
PlantCARE_28785	CARE	CAACTCCC	442	8	+	Oryza sativa
PlantCARE_28785	Unnamed__4	CTCC	445	4	+	Petroselinum hortense
PlantCARE_28785	CAAT-box	CAAAT	457	5	+	Pisum sativum
PlantCARE_28785	CAAT-box	CAAT	470	4	+	Nicotiana glutinosa
PlantCARE_28785	TATA-box	ATTATA	472	6	+	Brassica napus
PlantCARE_28785	TATA-box	TATAA	473	5	-	Arabidopsis thaliana
PlantCARE_28785	TATA-box	TATA	474	4	+	Arabidopsis thaliana
PlantCARE_28785	Box 4	ATTAAT	486	6	+	Petroselinum crispum
PlantCARE_28785	W box	TTGACC	513	6	+	Arabidopsis thaliana
PlantCARE_28785	CAAT-box	CAAAT	530	5	+	Pisum sativum
PlantCARE_28785	CAAT-box	CAAT	567	4	+	Nicotiana glutinosa
PlantCARE_28785	TCCC-motif	TCTCCCT	602	7	+	Spinacia oleracea
PlantCARE_28785	Unnamed__4	CTCC	603	4	+	Petroselinum hortense
PlantCARE_28785	MBS	CAACTG	625	6	-	Arabidopsis thaliana
PlantCARE_28785	CAAT-box	CCAAT	648	5	+	Arabidopsis thaliana
PlantCARE_28785	CAAT-box	CAAT	649	4	+	Nicotiana glutinosa
PlantCARE_28785	TATA-box	TATATTTATATTT	713	12	-	Avena sativa
PlantCARE_28785	TATA-box	ATATAA	715	6	+	Brassica oleracea
PlantCARE_28785	Unnamed__6	taTAAATATct	716	10	+	Zea mays
PlantCARE_28785	TATA-box	TATA	716	4	+	Arabidopsis thaliana
PlantCARE_28785	CAAT-box	CAAT	727	4	+	Nicotiana glutinosa
PlantCARE_28785	MYC	CAATTG	727	6	+	Arabidopsis thaliana

PlantCARE_28785	CAAT-box	CAAT	729	4	-	Nicotiana glutinosa
PlantCARE_28785	CAAT-box	CAAT	758	4	-	Nicotiana glutinosa
PlantCARE_28785	TATA-box	TATA	774	4	+	Arabidopsis thaliana
PlantCARE_28785	CAAT-box	CAAAT	830	5	+	Pisum sativum
PlantCARE_28785	CAAT-box	CAAT	855	4	+	Nicotiana glutinosa
PlantCARE_28785	Unnamed__4	CTCC	861	4	+	Petroselinum hortense
PlantCARE_28785	Unnamed__4	CTCC	896	4	+	Petroselinum hortense
PlantCARE_28785	CAAT-box	CAAAT	922	5	-	Pisum sativum
PlantCARE_28785	STRE	AGGGG	928	5	+	Arabidopsis thaliana
PlantCARE_28785	CAAT-box	CAAAT	936	5	+	Pisum sativum
PlantCARE_28785	CAAT-box	CAAT	944	4	-	Nicotiana glutinosa
PlantCARE_28785	CAAT-box	CAAT	956	4	-	Nicotiana glutinosa
PlantCARE_28785	CCAAT-box	CAACGG	999	6	+	Hordeum vulgare
PlantCARE_28785	TATA-box	TATACA	1045	6	-	Helianthus annuus
PlantCARE_28785	TATA-box	TATATA	1047	6	-	Arabidopsis thaliana
PlantCARE_28785	TATA-box	ATATAT	1048	6	-	Brassica napus
PlantCARE_28785	TATA-box	TATATA	1049	6	-	Arabidopsis thaliana
PlantCARE_28785	TATA-box	ATATAT	1050	6	-	Brassica napus
PlantCARE_28785	TATA-box	TATATA	1051	6	-	Arabidopsis thaliana
PlantCARE_28785	TATA-box	ATATAT	1052	6	-	Brassica napus
PlantCARE_28785	TATA-box	TATATA	1053	6	-	Arabidopsis thaliana
PlantCARE_28785	TATA-box	ATATAT	1054	6	-	Brassica napus
PlantCARE_28785	TATA-box	TATATA	1055	6	-	Arabidopsis thaliana
PlantCARE_28785	TATA-box	TATA	1057	4	-	Arabidopsis thaliana
PlantCARE_28785	TATA-box	taTATAAAtc	1084	9	-	Arabidopsis thaliana
PlantCARE_28785	TATA-box	TATAAAT	1085	7	-	Brassica juncea
PlantCARE_28785	TATA-box	TATAAA	1086	6	-	Helianthus annuus
PlantCARE_28785	TATA-box	TATATAA	1087	7	-	Arabidopsis thaliana
PlantCARE_28785	TATA-box	TATATA	1088	6	-	Arabidopsis thaliana
PlantCARE_28785	TATA-box	TATA	1090	4	-	Arabidopsis thaliana
PlantCARE_28785	TCT-motif	TCTTAC	1108	6	+	Arabidopsis thaliana
PlantCARE_28785	Unnamed__4	CTCC	1119	4	+	Petroselinum hortense
PlantCARE_28785	Box 4	ATTAAT	1146	6	-	Petroselinum crispum
PlantCARE_28785	TATA-box	ATATAT	1153	6	-	Brassica napus
PlantCARE_28785	TATA-box	TATA	1154	4	-	Arabidopsis thaliana
PlantCARE_28785	MYC	CATTTG	1173	6	+	Arabidopsis thaliana
PlantCARE_28785	CAAT-box	CAAAT	1174	5	-	Pisum sativum
PlantCARE_28785	TATA-box	ATATAT	1181	6	-	Brassica napus

PlantCARE_28785	TATA-box	TATA	1182	4	-	Arabidopsis thaliana
PlantCARE_28785	CAAT-box	CAAAT	1218	5	+	Pisum sativum
PlantCARE_28785	TCA-element	CCATCTTTTT	1232	9	-	Nicotiana tabacum
PlantCARE_28785	CAAT-box	CAAT	1256	4	+	Nicotiana glutinosa
PlantCARE_28785	CAAT-box	CAAAT	1258	5	-	Pisum sativum
PlantCARE_28785	CAT-box	GCCACT	1262	6	+	Arabidopsis thaliana
PlantCARE_28785	GATA-motif	GATAGGG	1301	7	+	Pisum sativum
PlantCARE_28785	GA-motif	ATAGATAA	1361	8	+	Arabidopsis thaliana
PlantCARE_28785	TATA-box	TATACA	1384	6	-	Helianthus annuus
PlantCARE_28785	TATA-box	TATATA	1386	6	-	Arabidopsis thaliana
PlantCARE_28785	TATA-box	ATATAT	1387	6	-	Brassica napus
PlantCARE_28785	TATA-box	TATATA	1388	6	-	Arabidopsis thaliana
PlantCARE_28785	TATA-box	ATATAT	1389	6	-	Brassica napus
PlantCARE_28785	TATA-box	TATA	1390	4	-	Arabidopsis thaliana
PlantCARE_28785	MYC	CATGTG	1428	6	+	Arabidopsis thaliana
PlantCARE_28785	CAAT-box	CAAAT	1441	5	+	Pisum sativum
PlantCARE_28785	CAAT-box	CAAT	1446	4	-	Nicotiana glutinosa
PlantCARE_28785	TATA-box	ATATAT	1481	6	-	Brassica napus
PlantCARE_28785	TATA-box	TATA	1482	4	-	Arabidopsis thaliana
PlantCARE_28785	TATA-box	TATACA	1500	6	-	Helianthus annuus
PlantCARE_28785	TATA-box	TATA	1502	4	-	Arabidopsis thaliana
PlantCARE_28785	CAAT-box	CAAT	1509	4	+	Nicotiana glutinosa
PlantCARE_28785	Box 4	ATTAAT	1522	6	-	Petroselinum crispum
PlantCARE_28785	CAAT-box	CAAT	1537	4	-	Nicotiana glutinosa
PlantCARE_28785	MYC	CATTTG	1561	6	+	Arabidopsis thaliana
PlantCARE_28785	CAAT-box	CAAAT	1562	5	-	Pisum sativum
PlantCARE_28785	CAAT-box	CAAAT	1586	5	+	Pisum sativum
PlantCARE_28785	CAAT-box	CAAAT	1608	5	+	Pisum sativum
PlantCARE_28785	TATA-box	ATATAT	1611	6	-	Brassica napus
PlantCARE_28785	TATA-box	TATA	1612	4	-	Arabidopsis thaliana
PlantCARE_28785	CAAT-box	CAAAT	1657	5	+	Pisum sativum
PlantCARE_28785	TATA-box	ATATAT	1660	6	-	Brassica napus
PlantCARE_28785	TATA-box	TATA	1661	4	-	Arabidopsis thaliana
PlantCARE_28785	CAAT-box	CAAAT	1686	5	+	Pisum sativum
PlantCARE_28785	AAGAA-motif	GAAAGAA	1693	7	-	Avena sativa
PlantCARE_28785	TATA-box	ATATAT	1721	6	-	Brassica napus
PlantCARE_28785	TATA-box	TATA	1722	4	-	Arabidopsis thaliana

PlantCARE_28785	CAAT-box	CAAT	1737	4	-	Nicotiana glutinosa
PlantCARE_28785	TATA-box	TATAA	1776	5	-	Arabidopsis thaliana
PlantCARE_28785	TATA-box	TATA	1777	4	-	Arabidopsis thaliana
PlantCARE_28785	AAGAA-motif	GAAAGAA	1795	7	+	Avena sativa
PlantCARE_28785	CAAT-box	CCAAT	1808	5	-	Arabidopsis thaliana
PlantCARE_28785	TATA-box	TATAA	1813	5	-	Arabidopsis thaliana
PlantCARE_28785	TATA-box	TATA	1814	4	-	Arabidopsis thaliana
PlantCARE_28785	Unnamed__4	CTCC	1829	4	+	Petroselinum hortense
PlantCARE_28785	CAAT-box	TGCCAAC	1895	7	-	Petunia hybrida
PlantCARE_28785	CAAT-box	CAAT	1910	4	+	Nicotiana glutinosa
PlantCARE_28785	G-Box	CACGTT	1923	6	+	Pisum sativum
PlantCARE_28785	ABRE	ACGTG	1923	5	-	Arabidopsis thaliana
PlantCARE_28785	GT1-motif	GGTTAA	1969	6	-	Arabidopsis thaliana
PlantCARE_28785	CAAT-box	CAAT	1982	4	+	Nicotiana glutinosa
PlantCARE_28785	TCA-element	TCAGAAGAGG	1993	9	+	Brassica oleracea
PlantCARE_28785	TCT-motif	TCTTAC	2018	6	+	Arabidopsis thaliana
PlantCARE_28785	CAAT-box	CAAT	2027	4	-	Nicotiana glutinosa

Table S2 - List of *CaGAoxes* and *CaDELLA* genes identified in *Coffea arabica* and other species. The spreadsheet contains the description of each gene, including the nomenclature, GenBank accession number (ID), and data contained in the genome annotation (NCBI Coffea arabica Annotation Release 100).

Coffee sequences							
Gene name	**Genbank ID**	**Parent mRNA**	**Loci (Genbank)**	**Chr_Id**	**Chromosome**	**Start**	**End**
CaGA3ox1	XP_027123267.1	XM_027267466.1	LOC113740004	NC_039904.1	4C	20630172	20632136
CaGA3ox2	XP_027125964.1	XM_027270163.1	LOC113742336	NC_039905.1	4E	14020858	14022665
CaGA3ox3	XP_027079994.1	XM_027224193.1	LOC113702988	NC_039912.1		36024359	36026272
CaGA3ox4	XP_027083066.1	XM_027227265.1	LOC113705413	NC_039913.1	8C	30784058	30785825
CaGA3ox5	XP_027080012.1	XM_027224211.1	LOC113703003	NC_039912.1	8E	35880263	35882044
CaGA3ox6	XP_027080185.1	XM_027224384.1	LOC113703126	NC_039912.1	8E	35965870	35967652
CaGA20ox1	XP_027110417.1	XM_027254616.1	LOC113730125	NC_039901.1	2E	3040312	3043027
CaGA20ox2	XP_027103889.1	XM_027248088.1	LOC113725096	NC_039900.1	2C	2894422	2897211
CaGA20ox3	XP_027115027.1	XM_027259226.1	LOC113733083	NC_039901.1	2E	68797737	68800060
CaGA20ox4	XP_027120911.1	XM_027265110.1	LOC113737979	NC_039903.1	3E	29425074	29426866
CaGA20ox5	XP_027108193.1	XM_027252392.1	LOC113727965	NC_039900.1	2C	63726648	63728962
CaGA20ox6	XP_027115712.1	XM_027259911.1	LOC113733719	NC_039902.1	3C	18709858	18711645
CaGA20ox7	XP_027118817.1	XM_027263016.1	LOC113736071	NC_039902.1	3C	33893166	33895000
CaGA20ox8	XP_027120566.1	XM_027264765.1	LOC113737549	NC_039903.1	3E	25808151	25809880

CaGA20ox9	XP_027076599.1	XM_027220798.1	LOC113700367	NC_039911.1	7E	17887071	17888961
CaGA20ox10	XP_027115699.1	XM_027259898.1	LOC113733708	NC_039902.1	3C	28496749	28498525
CaGA20ox11	XP_027119118.1	XM_027263317.1	LOC113736379	NC_039903.1	3E	23423222	23425518
CaGA20ox12	XP_027118589.1	XM_027262788.1	LOC113735804	NC_039902.1	3C	29580401	29582117
CaGA20ox13	XP_027077182.1	XM_027221381.1	LOC113700953	NC_039911.1	7E	13239406	13244293
CaGA20ox14	XP_027073252.1	XM_027217451.1	LOC113697856	NC_039910.1	7C	15664841	15669765
CaGA20ox15	XP_027091359.1	XM_027235558.1	LOC113712231	NC_039916.1	10E	11719676	11722718
CaGA20ox16	XP_027094047.1	XM_027238246.1	LOC113714423	NC_039917.1	10C	9433406	9436427

CaGA2ox1	XP_027105051.1	XM_027249250.1	LOC113725851	NC_039900.1	2C	9464003	9471913
CaGA2ox2	XP_027111589.1	XM_027255788.1	LOC113730837	NC_039901.1	2E	9463148	9470847
CaGA2ox3	XP_027097490.1	XM_027241689.1	LOC113717041	NC_039918.1	11C	388232	391422
CaGA2ox4	XP_027098866.1	XM_027243065.1	LOC113718146	NC_039919.1	11E	9046768	9049906

CaDELLA1	XP_027074537.1	XM_027218736.1	LOC113698790	NC_039910.1	7C	9830614	9833425
CaDELLA2	XP_027100221.1	XM_027244420.1	LOC113719254	NC_039919.1	11E	36216421	36219205
CaDELLA3	XP_027097334.1	XM_027241533.1	LOC113716962	NC_039918.1	11C	28313945	28316690
CaDELLA4	XP_027065510.1	XM_027209709.1	LOC113691539	NC_039908.1	6C	5260604	5262895

Reference sequences

Id	Species	Class	Uniprot	Genbank	Solgenomics
AtGA3ox1	Arabidopsis thaliana	Eudicot	Q39103		
AtGA20ox1	Arabidopsis thaliana	Eudicot	Q39110		
AtGA2ox1	Arabidopsis thaliana	Eudicot	Q8LEA2		
AtRGA	Arabidopsis thaliana	Eudicot	Q9SLH3		
AtGAI	Arabidopsis thaliana	Eudicot	Q9LQT8		
AtRGL1	Arabidopsis thaliana	Eudicot	Q9C8Y3		
AtRGL2	Arabidopsis thaliana	Eudicot	Q8GXW1		
AtRGL3	Arabidopsis thaliana	Eudicot	Q9LF53		
TaGA3ox2-1	Triticum aestivum	Monocot	Q3I411		
TaGA20ox1D	Triticum aestivum	Monocot	O04705		
TaGA2ox	Triticum aestivum	Monocot		XP_044320198.1	
TaDELLA	Triticum aestivum	Monocot		XP_044368103.1	
BrGA3ox	Brassica rapa	Eudicot		XP_009148952.1	
BrGA20ox	Brassica rapa	Eudicot		XP_009137526.2	
BrGA2ox	Brassica rapa	Eudicot		XP_009137181.2	
BrRGA	Brassica rapa	Eudicot	Q5BN22.1		
RsGA3ox	Raphanus sativus	Eudicot		XP_018479445.1	
RsGA20ox	Raphanus sativus	Eudicot		KAJ4913154.1	
RsGA2ox	Raphanus sativus	Eudicot		XP_018482077.1	
RsDELLA	Raphanus sativus	Eudicot		XP_018456905.1	
MdGA3ox	Malus domestica	Eudicot		XP_008377587.1	

MdGA20ox	Malus domestica	Eudicot		XP_008376762.1	
MdGA2ox	Malus domestica	Eudicot		XP_008384026.1	
MdDELLA	Malus domestica	Eudicot		AAY56752.1	
GmGA3ox	Glycine max	Eudicot		XP_003533057.1	
GmGA20ox	Glycine max	Eudicot		NP_001358565.1	
GmGA2ox	Glycine max	Eudicot		XP_003538628.1	
GmDELLA	Glycine max	Eudicot		NP_001240948.1	
AcGA3ox	Ananas comosus	Monocot		XP_020088773.1	
AcGA20ox	Ananas comosus	Monocot		XP_020085459.1	
AcGA2ox	Ananas comosus	Monocot		XP_020084228.1	
AcDELLA	Ananas comosus	Monocot		XP_020093282.1	
SlGA3ox	Solanum lycopersicum	Eudicot			Solyc06g066820.4.1
SlGA20ox-1	Solanum lycopersicum	Eudicot			Solyc11g072310.2.1
SlGA2ox	Solanum lycopersicum	Eudicot			Solyc05g053340.2.1
SlGAI	Solanum lycopersicum	Eudicot	Q7Y1B6		
SlDELLA	Solanum lycopersicum	Eudicot			Solyc11g011260.1.1
OsGA3ox	Oryza sativa	Monocot		XP_015634638.1	
OsGA20ox1	Oryza sativa	Monocot	P93771		
OsGA2ox6	Oryza sativa	Monocot	Q7XP65		
OsSLR1		Monocot	Q7G7J6		
OsGAI		Monocot	Q2TN88.2		

Table S2- List of genes *CaNCED* and *CaCYP707A* genes identified in Coffea arabica and other species. The spreadsheet contains the description of each gene, including the nomenclature, GenBank accession number (ID), and data contained in the genome annotation (NCBI Coffea arabica Annotation Release 100).

Coffee sequences

Gene name	Genbank ID	Parent mRNA	Loci (Genbank)	Chr_Id	Chromosome	Start	End
CaNCED1	XP_027063218.1	XM_027207417.1	LOC113689681	NC_039907.1	5C	40920856	40923509
CaNCED2	XP_027119139.1	XM_027263338.1	LOC113736397	NC_039903.1	3E	5131694	5133605
CaNCED3	XP_027115718.1	XM_027259917.1	LOC113733730	NC_039902.1	3C	6467132	6469009
CaCYP707A1	XP_027088675.1	XM_027232874.1	LOC113710025	NC_039899.1	1E	41845697	41851486
CaCYP707A2	XP_027109400.1	XM_027253599.1	LOC113729282	NC_039898.1	1C	45163354	45168751
CaCYP707A3	XP_027083247.1	XM_027227446.1	LOC113705553	NC_039913.1	8C	7266258	7268996
CaCYP707A4	XP_027080027.1	XM_027224226.1	LOC113703019	NC_039912.1	8E	8753211	8755992
CaCYP707A5	XP_027093123.1	XM_027237322.1	LOC113713569	NC_039917.1	10C	2252371	2255339
CaCYP707A6	XP_027092110.1	XM_027236309.1	LOC113712755	NC_039916.1	10E	3255946	3259420

Reference sequences

Id	Species	Class	Uniprot	Genbank
AtNCED3	Arabidopsis thaliana	Eudicot	Q9LRR7	
AtNCED6	Arabidopsis thaliana	Eudicot	Q9LRM7	
AtNCED5	Arabidopsis thaliana	Eudicot	Q9C6Z1	
AtNCED9	Arabidopsis thaliana	Eudicot	Q9M9F5	

AtNCED2	Arabidopsis thaliana	Eudicot	O49505	
AtCYP707A2	Arabidopsis thaliana	Eudicot	O81077	
AtCYP707A1	Arabidopsis thaliana	Eudicot	Q949P1	
AtCYP707A3	Arabidopsis thaliana	Eudicot	Q9FH76	
AtCYP707A4	Arabidopsis thaliana	Eudicot	Q9LJK2	
OsNCED	Oryza sativa	Monocot		NP_001405315.1
OsCYP707A	Oryza sativa	Monocot		XP_015627713.1
TaNCED	Triticum aestivum	Monocot		AGA83648.1
TaCYP707A	Triticum aestivum	Monocot		XP_044403980.1
BrNCED	Brassica rapa	Eudicot		XP_009116867.2
BrCYP707A	Brassica rapa	Eudicot		XP_009124395.2
RsNCED	Raphanus sativus	Eudicot		BAF42336.1
RsCYP707A	Raphanus sativus	Eudicot		XP_018435131.1
MdNCED	Malus domestica	Eudicot		XP_008382970.1
MdCYP707A	Malus domestica	Eudicot		XP_008356917.1
GmNCED	Glycine max	Eudicot		XP_014623805.1
GmCYP707A	Glycine max	Eudicot		XP_003534678.1
AcNCED	Ananas comosus	Monocot		OAY73265.1
AcCYP707A	Ananas comosus	Monocot		XP_020112941.1
SlNCED	Solanum lycopersicum	Eudicot	O24023	
SlCYP707A	Solanum lycopersicum	Eudicot		NP_001362844.1

Table S2- Details of *C. arabica* RNA-seq libraries in different tissues, including database numbers and library descriptions.

Libraries				
Accession	**Description**	**Type**	**Tissue**	
SRR11196539	Coffee leaves under optimal and warm temperatures. https://www.ncbi.nlm.nih.gov/Traces/study/?acc=SRP251013&o=acc_s%3Aa	PAIRED	LEAVES OPTIMUM TEMPERATURES cv. ACAUÃ	REP1
SRR11196538		PAIRED		REP2
SRR11196526		PAIRED		REP3
SRR11196530		PAIRED		REP4
SRR11196525		PAIRED		REP5
SRR11196524		PAIRED	LEAVES OPTIMUM TEMPERATURES cv. CATUAÍ	REP1
SRR11196522		PAIRED		REP2
SRR11196521		PAIRED		REP3
SRR11196523		PAIRED		REP4
SRR11196520		PAIRED		REP5
SRR11196527		PAIRED	LEAVES WARM TEMPERATURES cv. CATUAÍ	REP1
SRR11196528		PAIRED		REP2
SRR11196529		PAIRED		REP3
SRR11196531		PAIRED		REP4
SRR11196532		PAIRED		REP5
SRR11196533		PAIRED	LEAVES WARM TEMPERATURES cv. ACAUÃ	REP1
SRR11196534		PAIRED		REP2
SRR11196535		PAIRED		REP3
SRR11196536		PAIRED		REP4

SRR11196537		PAIRED		REP5
-	Coffee buds at G4 and G5 stages of development. (Unpublished)	SINGLE	BUDS AT G4 cv. CATUAÍ	REP1
		SINGLE		REP2
		SINGLE	BUDS AT G5 cv. CATUAÍ	REP1
		SINGLE		REP2
		SINGLE	BUDS AT G4 cv. SERIEMA	REP1
		SINGLE		REP2
		SINGLE	BUDS AT G5 cv. SERIEMA	REP1
		SINGLE		REP2
SRR4046866	Transcriptome Analysis of Leaves, Flowers and Fruits Perisperm of Coffea arabica L. Reveals the Differential Expression of Genes Involved in Raffinose Biosynthesis. https://www.ncbi.nlm.nih.gov/Traces/study/?acc=SRP082511&o=acc_s%3Aa	SINGLE	FRUITS (PERISPERM) 30DAF	-
SRR4046868		SINGLE	FRUITS (PERISPERM) 90DAF	-
SRR9964229	Coffea arabica cultivar:Bourbon Transcriptome or Gene expression. https://www.ncbi.nlm.nih.gov/sra?linkname=bioproject_sra_all&from_uid=554647	SINGLE	MERISTEMS	-
SRR9964230		SINGLE	FLOWER BUDS	-
SRR9964225		SINGLE	ROOTS	REP1
SRR9964226		SINGLE		REP2
SRR9964224		SINGLE	STEMS	REP1
SRR9964223		SINGLE		REP2
SRR9964220		SINGLE	YOUNG LEAVES	REP1
SRR9964219		SINGLE		REP2
SRR9964221		SINGLE	RED DRUPES	REP1
SRR9964228		SINGLE		REP2
SRR9964222		SINGLE	GREEN DRUPES	-
SRR9964227		SINGLE	FRUITS AT MULTIPLE STAGES OF RIPENING	-
SRR3223077	Transcriptome Analysis of Coffee Seed Germination. https://www.ncbi.nlm.nih.gov/bioproject/PRJNA305756	SINGLE	EMBRYOS	-

Table S3 - Primer sequences used for RT-qPCR in this study.

Gene	Sequence of primers (5'-3')	References
CaFT1	F: GTTCACCAGGTCCCTAAGCC	Cardon et al., 2022
	R: ATCGTCACCTCCAATCTCAACC	
CaCO	F: TGCTATTTGGTGGCGAGGTT	Cardon et al., 2022
	R: CGCTGTCTCCTCCGTAACTC	
CaGA3ox1	F: ATCATCAGAGTGTGACTGTG	This study
	R: CCTGCATCTTCTCGTGTTGA	
CaGA2ox1	F: TGGCTTGATTAACTGTGTTGG	This study
	R: CTGCCGCAGAACACATATTG	
CaDELLA3	F: CCCTGTTTCCGTTTCTCCC	This study
	R: GAACAAGCCTAATCCCATTTTCT	

CaNCED1	F: CACTGGACGACGACTTCTAC	This study
	R: TGAATTTGTAGGAGTGGAAGCA	
CaCYP707A1	F: CTGTTCGTTACTTCTGCTAGTAC	This study
	R: TGTCGGGTGGAGAAAAAG	
CaAP1	F: AACCACTCCCTCCTTGCATC	(Unpublished data Ricon et al.)
	R: GCAGGGAAACAGCGAGTCT	
CaLFY	F: GGGAAGAAGAATGGACTGGAT	(Unpublished data Ricon et al.)
	R: GCGTACCTGAACACCTGATT	
CaACS3	F: GCTGCTTCTTCTCTTTTTGCCT	Santos et al., 2022
	R: CTGCCATCCCAGGAAGTACG	
CaACO3	F: CACTCCTGCCAGCTTTCTCC	Santos et al., 2022
	R: AGCCTTTGTGGTGGCTACTTT	
CaMDH	F: CCTGATGTCAACCACGCAACT	Martins et al., 2017
	R: GTGGTTATGAACTCTCCATTCAACC	
CaUBQ2	F: GATGATACTTGGCCCTGCAC	Martins et al., 2017
	R: CCTTCCCAGCTTGTCAATGT	
CaRL39	F: GCGAAGAAGCAGAGGCAGAA	Fernandes-Brum et al., 2017
	R: TTGGCATTGTAGCGGATGGT	
CaACT	F: AAGCTTGCCTATGTGGCTCTTG	Martins et al., 2017
	R: TCACTTGTCCATCTGGCAATTC	

Table S4 - Parameters for Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) according to Bustin et al. (2009)

Experimental design / Sample	
Experimental group	Six-year-old plants of coffee (*Coffea arabica*)
Sample	Leaves and floral buds
Sampling procedure	Immediately frozen in liquid nitrogen
Storage conditions / Time of storage	Freezer -80 °C / one month
RNA extraction	
Processing procedure	Grinding (mortar and pestle) in liquid nitrogen
Method	Organic extraction, according to De Oliveira et al., 2015.
RNA: DNA-free	TURBO DNA-*free*™ Kit (Ambion, Thermo Fisher Scientific) - Catalog number: AM1907
Nucleic acid quantification	Spectroscopy (NanoVue GE Healthcare)
RNA integrity	Agarose gel (1 %)

Reverse transcription	
Kit	High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Thermo Fisher Scientific) - Catalog number: 4368814
Reaction conditions	25 °C 10' / 37 °C 120' / 85 °C 5'
Reverse transcriptase	MultiScrib^{TM} MuLV
Amount of RNA / Reaction volume	1 µg / 20 µL
Priming strategy	Random primers
Storage conditions of cDNA	Freezer -20 °C
RT-qPCR	
Target information	Supplementary Table 5
Reaction conditions	Materials and Methods section
In silico	Primers were blasted using the BLAST tool at https://www.ncbi.nlm.nih.gov/
Empirical	Primer concentration of 1-2 µM (final concentration on the reaction) Annealing temperature: 60 °C
PCR efficiency	5-Fold dilution series of a mixed sample over at least five dilution points and verified to be higher than 85 % (E = 10−1/slope).
Linear dynamic range	Samples are situated within the range of the efficiency curves for each primer
No template control (NTC)	Cq and dissociation curve verification
Data analysis	
Specialist software	Qiagen Rotor Gene-Q Series software (version 1.7)
Normalization	Two reference genes / $\Delta\Delta^{CT}$ method (Pfaffl, 2001)

CONCLUSÃO GERAL E PERSPECTIVAS FUTURAS

Esta tese analisou detalhadamente a interação entre os reguladores de crescimento vegetal (PGRs) e os mecanismos moleculares que regulam o desenvolvimento reprodutivo em plantas perenes, com foco especial no cafeeiro. Os três artigos que compõem esta tese não apenas forneceram uma compreensão abrangente das complexas redes de sinalização envolvendo PGRs, mas também demonstram como esses reguladores podem ser usados para mitigar os efeitos adversos das mudanças climáticas no crescimento e produtividade das plantas. Além disso, propõem a aplicação desses PGRs em fases específicas do desenvolvimento reprodutivo do cafeeiro, desde a indução do florescimento até a maturação dos frutos, oferecendo diretrizes para práticas agrícolas no campo.

O uso de PGRs para induzir ou reprimir genes chave do controle do florescimento mostrou ser uma técnica eficaz para controlar o tempo de floração das plantas, visando um florescimento mais síncrono. Entender como esses genes atuam nas plantas perenes é de extrema importância para determinar quais PGRs usar e em qual momento aplicá-los. Nesta tese, também comprovamos que o uso de PGRs como giberelinas (GA) e ácido abscísico (ABA) em cafeeiro pode aumentar o número de botões florais e acelerar seu desenvolvimento, respectivamente, resultando em uma maior produção. Demonstramos também que as respostas transcricionais variam de acordo com a dosagem aplicada e o tipo de tecido, destacando a importância da escolha precisa da dosagem.

Nesse sentido, é necessário continuar a pesquisa voltada à otimização das doses e métodos de aplicação de todos PGRs. Aprofundar a compreensão das interações entre PGRs e diferentes genótipos de plantas, bem como as respostas específicas a diversas condições ambientais, é de extrema importância. Além disso, o desenvolvimento de novos PGRs, possivelmente baseados em biotecnologia ou compostos naturais, oferece um campo promissor para melhorar a eficiência e sustentabilidade das práticas agrícolas.