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Plant apocarotenoids: from retrograde signaling to interspecific communication

Juan C. Moreno[1,2], Jianing Mi[2], Yagiz Alagoz[2,3] and Salim Al-Babili[2,*]

[1]*Max Planck Institut für Molekulare Pflanzenphysiologie, Am Mühlenberg 1, Potsdam 14476, Germany,*

[2]*Division of Biological and Environmental Sciences and Engineering, Center for Desert Agriculture, the BioActives Lab, King Abdullah University of Science and Technology, Thuwal 23955-6900, Kingdom of Saudi Arabia, and*

[3]*Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797, Penrith, NSW 2751, Australia*

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*For correspondence (e-mail salim.babili@kaust.edu.sa).

SUMMARY

Carotenoids are isoprenoid compounds synthesized by all photosynthetic and some non-photosynthetic organisms. They are essential for photosynthesis and contribute to many other aspects of a plant's life. The oxidative breakdown of carotenoids gives rise to the formation of a diverse family of essential metabolites called apocarotenoids. This metabolic process either takes place spontaneously through reactive oxygen species or is catalyzed by enzymes generally belonging to the CAROTENOID CLEAVAGE DIOXYGENASE family. Apocarotenoids include the phytohormones abscisic acid and strigolactones (SLs), signaling molecules and growth regulators. Abscisic acid and SLs are vital in regulating plant growth, development and stress response. SLs are also an essential component in plants' rhizospheric communication with symbionts and parasites. Other apocarotenoid small molecules, such as blumenols, mycorradicins, zaxinone, anchorene, β-cyclocitral, β-cyclogeranic acid, β-ionone and loliolide, are involved in plant growth and development, and/or contribute to different processes, including arbuscular mycorrhiza symbiosis, abiotic stress response, plant–plant and plant–herbivore interactions and plastid retrograde signaling. There are also indications for the presence of structurally unidentified linear *cis*-carotene-derived apocarotenoids, which are presumed to modulate plastid biogenesis and leaf morphology, among other developmental processes. Here, we provide an overview on the biology of old, recently discovered and supposed plant apocarotenoid signaling molecules, describing their biosynthesis, developmental and physiological functions, and role as a messenger in plant communication.

Keywords: abscisic acid, anchorene, apocarotenoids, β-cyclocitral, β-ionone, LCDAs, carotenoids, strigolactones, volatiles.zaxinone.

INTRODUCTION

Carotenoids are C_{40} isoprenoids with well-described functions in photosynthesis, pollination, photoprotection and hormone biosynthesis (Krinsky, 1989; Hirschberg, 2001; Dall'Osto *et al.*, 2007; Han *et al.*, 2008). They are synthesized in all photosynthetic organisms and several non-photosynthetic fungi and bacteria (Ruiz-Sola and Rodriguez-Concepcion, 2012; Moise *et al.*, 2014; Nisar *et al.*, 2015; Zheng *et al.*, 2020). Plant carotenoid biosynthesis (Figure 1) takes place in plastids and relies on the methylerythritol phosphate pathway that provides the building blocks (isopentenyldiphosphate and dimethylallyl diphosphate) for different isoprenoid pathways, which also include gibberellin, chlorophyll side chain and tocopherol biosynthesis (Rodriguez-Concepcion, 2010). Carotenoids, as well as these isoprenoids, originate from geranylgeranyl diphosphate that is formed by repeated condensation reactions of these building blocks (Figure 1). The initial step in carotenoid biosynthesis is the formation of 15-*cis*-phytoene, which is synthesized by the PHYTOENE SYNTHASE (PSY) that catalyzes the condensation of two geranylgeranyl diphosphate molecules (Dogbo *et al.*, 1988). This is followed by two-desaturation reactions mediated by the PHYTOENE DESATURASE (PDS), which lead to 9,15,9′-tri-*cis*-ζ-carotene via 9,15-di-*cis*-phytofluene (Li *et al.*, 1996). The ζ-CAROTENE ISOMERASE

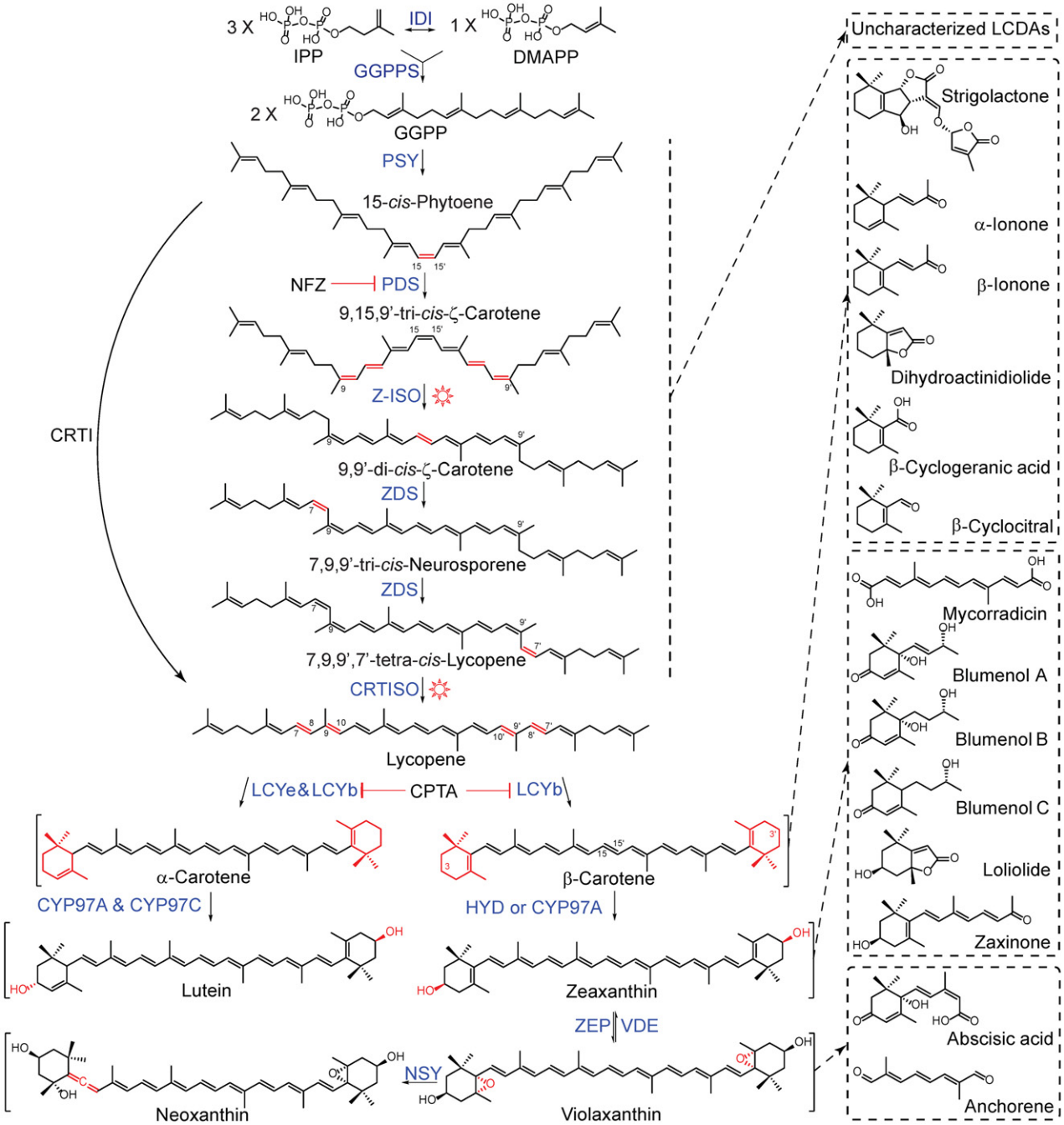

Figure 1. Carotenoid biosynthetic pathway and plant apocarotenoid regulatory metabolites.
Geranylgeranyl pyrophosphate (GGPP) is synthesized by the GGPP SYNTHASE (GGPPS) in plastids through the condensation of three isopentenyl diphosphates (IPP) and one dimethylallyl diphosphate (DMAPP). Sequentially, two GGPP molecules are used by the PHYTOENE SYNTHASE (PSY) to produce 15-*cis*-phytoene, which is an important rate-limiting step in the synthesis of carotenoids. Then 15-*cis*-phytoene is converted into lycopene via multiple steps of desaturation and isomerization catalyzed by enzymes, including PHYTOENE DESATURASE (PDS), ζ-CAROTENE ISOMERASE (Z-ISO), ζ-CAROTENE DESATURASE (ZDS), and CAROTENOID ISOMERASE (CRTISO). These multiple reactions are performed by a single enzyme, CRTI, in non-photosynthetic bacteria. The enzymatic activity of Z-ISO and CRTISO can be partially replaced by photoisomerization in plants. Cyclization of lycopene is catalyzed by LYCOPENE β- and ε-CYCLASE (LCYb and LCYe) that form the β- or ε-ionone rings of carotenes, respectively. β- and α-Carotenes are subsequently converted into their downstream xanthophylls, such as zeaxanthin by NON-HEME DIIRON OXIDASE (HYD)/CYP97A, and lutein by the action of CYP97A and CYP97C, respectively. Then zeaxanthin and violaxanthin can be interconverted in each other by the action of ZEAXANTHIN EPOXIDASE (ZEP) and VIOLAXANTHIN DE-EPOXIDASE (VDE). Afterwards, violaxanthin is transformed into neoxanthin by NEOXANTHIN SYNTHASE (NSY). Modified bonds or moieties are colored red. Norflurazon (NFZ) and 2-(4-chlorophenylthio) ethyl-diethylammonium chloride (CPTA) are inhibitors of carotenoid biosynthesis, affecting the enzymes PDS, LCYb and LCYe. Carotenoids can be metabolized into various apocarotenoids with important biological functions, via different processes. Representative bioactive apocarotenoids are boxed with a rectangular dash box. Sun image represents photoisomerization. CYP97, cytochrome P450 type carotenoid hydroxylase; IDI, isopentenyl diphosphate isomerase; IPP, isopentenyl diphosphate; LCDAs, linear *cis*-carotene-derived apocarotenoids; LCYb, LYCOPENE β-CYCLASE; LCYe, LYCOPENE ε-CYCLASE.

(Z-ISO) converts 9,15,9′-tri-*cis*-ζ-carotene to 9,9′-di-*cis*-ζ-carotene, which can be partially replaced by photoisomerization (Li *et al.*, 2007; Chen *et al.*, 2010). Next, the 9,9′-di-*cis*-ζ-carotene undergoes an additional two-step desaturation catalyzed by the ζ-CAROTENE DESATURASE (ZDS) that yields 7,9,9′-tri-*cis*-neurosporene followed by 7,9,9′,7′-tetra-*cis*-lycopene (pro-lycopene), respectively (Bartley *et al.*, 1999; Matthews *et al.*, 2003). Finally, CRTISO catalyzes the conversion of pro-lycopene to all-*trans*-lycopene. Similar to the tri-*cis* to di-*cis* conversion of ζ-carotene, isomerization of pro-lycopene to its all-*trans* form can be partially complemented by photoisomerization in photosynthetic tissues (Isaacson *et al.*, 2002; Park *et al.*, 2002). In most prokaryotes and fungi, the steps leading from 15-*cis*-phytoene to all-*trans*-lycopene are mediated by a single polypeptide, Crtl, which replaces the four plant enzymes (Schaub *et al.*, 2012). This capability explains the common employment of Crtl in generating transgenic, carotenoid-biofortified crops (Zheng *et al.*, 2020). Following the linear all-*trans*-lycopene, the pathway diverges into two main branches starting with the bicyclic α- or β-carotenes and leading to the corresponding oxygen-containing carotenoids (i.e., xanthophylls; Figure 1). β-Carotene serves as the precursor of strigolactones (SLs), an important phytohormone involved in plant growth and development (Al-Babili and Bouwmeester, 2015; Beltran and Stange, 2016; Shen *et al.*, 2017). On the one hand, LYCOPENE β- and ε-CYCLASES (LCY band LCYe) convert lycopene into α-carotene, which carries an α- and a β-ionone ring, and is subsequently hydroxylated into lutein by the action of cytochrome P450 enzyme CYP97A and CYP97C (Figure 1). On the other hand, LCYb introduces two β-ionone rings into all-*trans*-lycopene, which are hydroxylated by NON-HEME DIIRON OXIDASE (HYD)/CYP97A yielding zeaxanthin. The latter is the substrate of the ZEAXANTHIN EPOXIDASE that produces violaxanthin via antheraxanthin through sequential epoxidation. The reverse reactions back to zeaxanthin are catalyzed by the VIOLAXANTHIN DE-EPOXIDASE (Dall'Osto *et al.*, 2007; Neuman *et al.*, 2014). Violaxanthin is then converted into neoxanthin (Neuman *et al.*, 2014). Both violaxanthin and neoxanthin are precursors of the phytohormone abscisic acid (ABA; Figure 1). Plants utilize only the 9-*cis* isoforms of epoxy-xanthophyll precursors to produce ABA (Tan *et al.*, 2003). The all-*trans*- to 9-*cis*- isomerization of violaxanthin and neoxanthin was poorly understood; however, a recent study suggested the involvement of ABA4 enzyme with the unknown cofactor(s) (Perreau *et al.*, 2020).

Owing to their electron-rich, conjugated double bond system, carotenoids are susceptible to oxidation, causing the breakage of their backbone and leading to diverse carbonyl products generally called apocarotenoids. Initiated by reactive oxygen species (ROS), this process can occur without enzymatic catalysis *in vitro* as well as *in planta*. For instance, *in vitro* treatment of carotenoid solutions with photosensitizers generating singlet oxygen ($^1O_2$) leads to carotenoid degradation and the formation of a wide range of products, such as aldehydes, ketones, endoperoxides, epoxides and lactones (Stratton *et al.*, 1993; Yamauchi *et al.*, 1998; Fiedor *et al.*, 2001; Bando *et al.*, 2004; Fiedor *et al.*, 2005; Ramel *et al.*, 2012a). Interestingly, carotenoid peroxides themselves promote the oxidation of carotenoids as well as of other metabolite species, which makes them suitable for the propagation of oxidative stress signals in cells (Fiedor *et al.*, 2005). Carotenoid backbone cleavage can also be catalyzed enzymatically by CAROTENOID CLEAVAGE DIOXYGENASEs (CCDs) (Al-Babili and Bouwmeester, 2015; Beltran and Stange, 2016). CCDs are a ubiquitous family of non-heme iron enzymes, which convert carotenoids into apocarotenoids acting as signaling molecules or hormone precursors (Giuliano *et al.*, 2003; Hou *et al.*, 2016; Felemban *et al.*, 2019; Fiorilli *et al.*, 2019; Wang *et al.*, 2020b). In Arabidopsis, the CCD family is comprised of nine members, including five 9-*cis*-EPOXYCAROTENOID DIOXYGENASEs (NCED2, NCED3, NCED5, NCED6 and NCED9) and four CCDs (CCD1, CCD4, CCD7 and CCD8) (Tan *et al.*, 2003; Sui *et al.*, 2013). NCEDs are involved in ABA biosynthesis (Schwartz *et al.*, 1997). CCD1 is involved in the cleavage of several carotenoids and apocarotenoids at different positions along their carbon structure (Schwartz *et al.*, 2001; Vogel *et al.*, 2008; Ilg *et al.*, 2009; Ilg *et al.*, 2014) leading to the production of volatiles responsible for flavor and aroma in various species, and dialdehydes with different chain lengths. CCD4 cleaves carotenoids either at the C7–C8 double bond in cryptoxanthin and zeaxanthin or at the C9–C10 double bond in bicyclic carotenoids (Rubio-Moraga *et al.*, 2014; Bruno *et al.*, 2015; Bruno *et al.*, 2016). In citrus, CCD4 is involved in carotenoid turnover in different tissues and in the production of citraurin (Pan *et al.*, 2012). CCD7 and CCD8 are involved in SL biosynthesis by cleaving 9-*cis*-β-carotene and converting 9-*cis*-β-apo-10′-carotenal, respectively (see SL functions and biosynthesis and Figure 3) (Alder *et al.*, 2012; Abe *et al.*, 2014; Bruno *et al.*, 2014; Zhang *et al.*, 2014; Haider *et al.*, 2018). Recently, other types of CCDs were reported, including CCD2 in *Crocus* species, and ZAXINONE SYNTHASE (ZAS), which represents an overlooked CCD clade (Frusciante *et al.*, 2014; Ahrazem *et al.*, 2016; Wang *et al.*, 2019; Zhong *et al.*, 2020).

Both enzymatic and non-enzymatic oxidation processes may contribute to the formation of apocarotenoid signaling (ACS) molecules. For instance, the volatile β-cyclocitral (β-cc) is formed from β-carotene by some CCDs, for example, the citrus fruit CCD4b, lipoxygenases, such as the tomato TomLocX (Gao *et al.*, 2019), and, particularly under high-light conditions, by $^1O_2$ in photosynthetic tissues (Felemban *et al.*, 2019). β-cc is involved in $^1O_2$ signaling, high light, drought and salt tolerance, and acts as a root

growth regulator (Dabbagh *et al.*, 1990; D'Alessandro *et al.*, 2019; Dickinson *et al.*, 2019). Furthermore, the oxidation of β-cc leads to β-cyclogeranic acid (or β-cyclocitric acid [β-ccA]; Figure 1), an apocarotenoid conferring drought tolerance in plants (D'Alessandro *et al.*, 2019). Enzymatic and non-enzymatic oxidation of β-carotene also gives rise to other volatile compounds (Figure 1), including β-ionone and dihydroactinidiolide (dhA) that are involved in a plant–herbivore interaction and $^1O_2$ signaling and photoacclimation, respectively (Wei *et al.*, 2011; Shumbe *et al.*, 2014). The action of other CCDs on zeaxanthin (or lutein) led to the production of apocarotenoids (Figure 1) involved in arbuscular mycorrhiza (AM) symbiosis (i.e., mycorradicin, blumenols) (Lopez-Raez *et al.*, 2015; Wang *et al.*, 2018), plant–plant and plant–herbivore interaction (i.e., loliolide) (Kong *et al.*, 2018; Murata *et al.*, 2019b), and hormone metabolism and growth regulation (i.e., zaxinone) (Wang *et al.*, 2019; Ablazov *et al.*, 2020). In addition, cleavage of violaxanthin (and probably all carotenoids downstream of ζ-carotene) produces anchorene (Figure 1), a diapocarotenoid involved in growth stimulation of anchor roots (ANR; Jia *et al.*, 2019). Here, we summarize important aspects of ABA and SLs metabolism, and highlight recently discovered apocarotenoids and linear *cis*-carotene-derived apocarotenoids (LCDAs) influencing plant growth, development and metabolism.

ABA FUNCTIONS AND BIOSYNTHESIS

ABA has been extensively studied over the last few decades due to its primary importance for fundamental plant science and its essential role in the coordination of agronomically important traits, such as root and shoot development, hypocotyl elongation, and fruit development and ripening (see LCDA-associated phenotypes might be linked with the lack of downstream apocarotenoids) (Galpaz *et al.*, 2008; Felemban *et al.*, 2019). ABA is also necessary to generate and coordinate the plant's response against abiotic and biotic stress factors (i.e., drought, salt and pathogens) through synergistic and antagonistic interactions with other hormones (i.e., gibberellins, ethylene and auxins) (De Vleesschauwer *et al.*, 2010). A recent study showed that enhanced ABA and gibberellin content accumulation through the expression of the *Daucus carota* (carrot) *LYCOPENE* β*-CYCLASE1* (*DcLCYb1*) leads to increased plant yield and enhanced photosynthetic efficiency in tobacco (Moreno *et al.*, 2020). ABA-mediated coordination of the ripening process determines the harvest-time and shelf-life of horticulturally important crop plants. The enhanced ABA accumulation through *LCYb* overexpression leads to an extended shelf-life of the tomato fruit (Diretto *et al.*, 2020). The ABA-mediated mechanism of stomatal closure is essential to control leaf transpiration (Malcheska

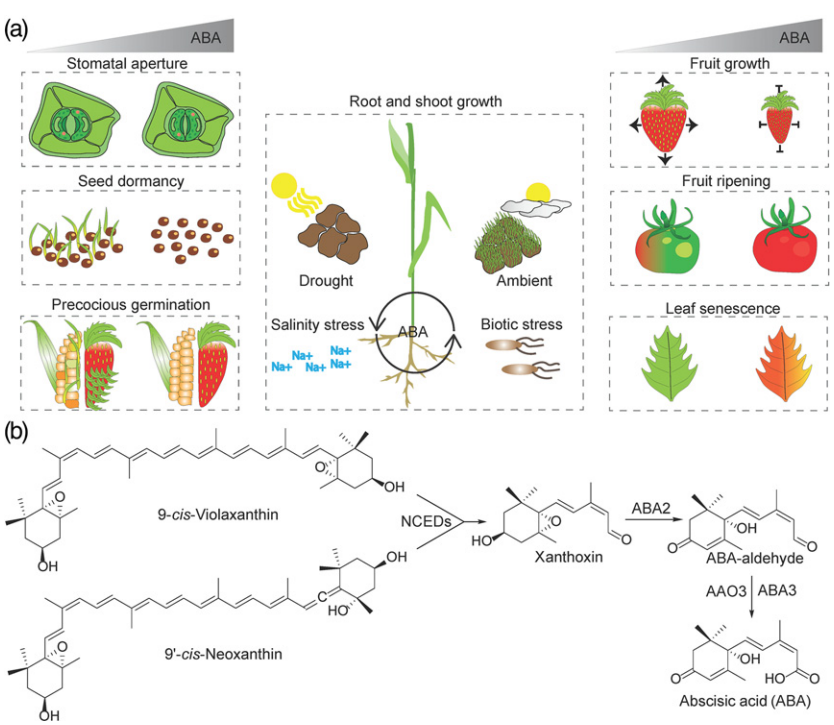

Figure 2. Abscisic acid (ABA) biosynthesis and functions in plants.
(a) ABA modulates plant growth and development. Altered ABA abundance modulates the plant root and shoot growth during biotic and abiotic stress conditions.
(b) ABA is a phytohormone synthesized by 9-*cis*-epoxycarotenoid dioxygenase (NCED)-mediated cleavage of epoxycarotenoid precursors, 9-*cis*-violaxanthin and 9′-*cis*-neoxanthin. AAO3, ABSCISIC ALDEHYDE OXIDASE; ABA2;3, ABA-DEFICIENT 2;3.

et al., 2017) and to generate an immune response upon the perception of pathogen-associated molecular patterns (Lim *et al.*, 2015). High levels of ABA trigger ROS generation, which leads to oxidative stress followed by leaf senescence and cell death (Figure 2a) (An *et al.*, 2019; Kim *et al.*, 2019).

ABA biosynthesis starts with the NCED-mediated enzymatic cleavage of 9-*cis*-violaxanthin or 9′-*cis*-neoxanthin (9-*cis*-epoxy-xanthophylls) into xanthoxin (C_{15}) and the corresponding C_{25}-apocarotenoid in plastids (Figure 2b) (Qin and Zeevaart, 1999; Tan *et al.*, 2003). Xanthoxin is then transported to the cytosol for subsequent modifications to form ABA (Finkelstein, 2013). The short-chain alcohol dehydrogenase, ABA-DEFICIENT 2 (ABA2), converts xanthoxin to abscisic aldehyde, which is oxidized by ABSCISIC ALDEHYDE OXIDASE to ABA (Schwartz *et al.*, 1997). The ABSCISIC ALDEHYDE OXIDASE catalytic activity requires a molybdenum cofactor supplied by sulfurase ABA-DEFICIENT 3 (ABA3) (Seo *et al.*, 2000; Li *et al.*, 2013). ABA3 is also shown to contribute to the tolerance against oxidative stress in Arabidopsis in both an ABA-dependent and an ABA-independent manner (Watanabe *et al.*, 2018). ABA can be further metabolized by some other enzymes to generate a relatively unstable isomer 8′-OH-ABA (Saito *et al.*, 2004), and other modified forms of ABA, including catabolites, such as phaseic acid (PA), dihydrophaseic acid (DPA) and DPA-4-*O*-β-D-glucoside (DPAG) (Cutler and Krochko, 1999). In a recent study, PA is shown to interact with some of the ABA receptors (PYLs), suggesting that PA functions as a phytohormone with relatively lower activity (Weng *et al.*, 2016). Plants can store the esterified form of ABA, ABA glucosyl ester, in vacuoles (Lee *et al.*, 2006; Xu *et al.*, 2012). The ABA-glucose esters can be hydrolyzed by β-glucosidase BGLU18 to liberate ABA in case of immediate demand under drought stress conditions (Wade *et al.*, 1999; Watanabe *et al.*, 2014). ABA is involved, directly or indirectly, in several regulatory processes to modulate development, growth and stress response throughout the life cycle of plants. Here, we briefly explained the biosynthesis and function of ABA in plants. Further information describing the recent advances in ABA research can be found elsewhere (Felemban *et al.*, 2019; Chen *et al.*, 2020; Wang *et al.*, 2020b).

SL FUNCTIONS AND BIOSYNTHESIS

In the last two decades, SLs have increasingly attracted the attention of biologists, in particular plant scientists, due to their versatile functions as rhizospheric, interspecific signaling molecules and plant hormones regulating development and adaptation to environmental changes (Brewer *et al.*, 2013; Zwanenburg and Pospisil, 2013; Seto and Yamaguchi, 2014; Smith and Li, 2014; Waldie *et al.*, 2014; Waters *et al.*, 2017; Jia *et al.*, 2018; Lanfranco *et al.*, 2018; Yao *et al.*, 2018; Burger and Chory, 2020). SLs were discovered in root exudates as germination stimulants of parasitic root weeds, and were shown to induce hyphal branching in symbiotic AM fungi (AMF; Cook *et al.*, 1966; Akiyama *et al.*, 2005). Additionally, SLs determine several aspects of plant physiology and shape plant architecture according to nutrient availability (Figure 3). For instance, Arabidopsis, rice, and pea SL-deficient and -insensitive mutants showed a higher degree of shoot branching/tillering than the wild type (Gomez-Roldan *et al.*, 2008; Umehara *et al.*, 2008). Furthermore, SLs are involved in many other shoot-related developmental processes such as regulation of rice tiller angle by attenuating shoot gravitropism (Sang *et al.*, 2014), promotion of shoot secondary growth, elongation of internodes (Agusti *et al.*, 2011; de Saint *et al.*, 2013), inhibition of hypocotyl and mesocotyl growth (Hu *et al.*, 2010; Tsuchiya *et al.*, 2010; Hu *et al.*, 2014; Jia *et al.*, 2014; Sun *et al.*, 2018; Wang *et al.*, 2020c), induction of leaf senescence (Snowden *et al.*, 2005; Yamada *et al.*, 2014) and decreasing the rice leaf angle in response to nutrient deficiencies (Sun *et al.*, 2014; Shindo *et al.*, 2020). They also play key roles in determining root architecture. For instance, SL-deficient and -insensitive mutants have shorter primary roots and root hairs, shorter crown roots, higher secondary lateral roots (LRs), a higher density of LRs, and more adventitious roots compared with the wild type (Guan *et al.*, 2012; Kohlen *et al.*, 2012; Rasmussen *et al.*, 2012a; Rasmussen *et al.*, 2012b; Sun *et al.*, 2019). SLs are utilized to adapt plants against nutrient deficiency (e.g., shortage in phosphate supply) through modulating root and shoot shape (Sun *et al.*, 2014; Al-Babili and Bouwmeester, 2015; Matthys *et al.*, 2016). In addition, SLs modulate Arabidopsis seed germination (Tsuchiya *et al.*, 2010; Toh *et al.*, 2012) and play essential roles in plant development in the moss *Physcomitrella patens* (Proust *et al.*, 2011; Hoffmann *et al.*, 2014; Decker *et al.*, 2017). Moreover, SLs trigger stomatal closure through an ABA-independent pathway (Lv *et al.*, 2018) and enhance plant resistance to abiotic and biotic stresses (Ha *et al.*, 2014; Torres-Vera *et al.*, 2014; Lopez-Raez *et al.*, 2017; Nasir *et al.*, 2019).

Natural SLs (Figure 4) are characterized by a butenolide ring (D ring) linked through an enol–ether bridge to a second moiety that consists of a tricyclic lactone (ABC ring) in the case of canonical SLs or a less defined structure in non-canonical ones (Al-Babili and Bouwmeester, 2015; Jia *et al.*, 2018). Canonical SLs are further divided intostrigol- and orobanchol-type, according to the stereochemistry of asymmetric carbon atoms at C3a and C8b: C3a *R* and C8b *S* configuration in strigol-type SLs, including 5-deoxystrigol (5DS, **18**) and its derivatives, such as strigol (**19**), strigyl acetate (**20**), strigone (**21**), sorgolactone (**22**), sorgomol (**23**), *ent*-2′-*epi*-orobanchol (**24**) and *ent*-2′-*epi*-orobanchol acetate (**25**), and C3a *S* and C8b *R* configuration in orobanchol-type SLs such as 4-deoxyorobanchol (4DO, **26**) and 4DO derivatives, including orobanchol (**27**), 7-hydroxyorobanchol (**28**), orobanchyl acetate (**29**), 7-OXO-

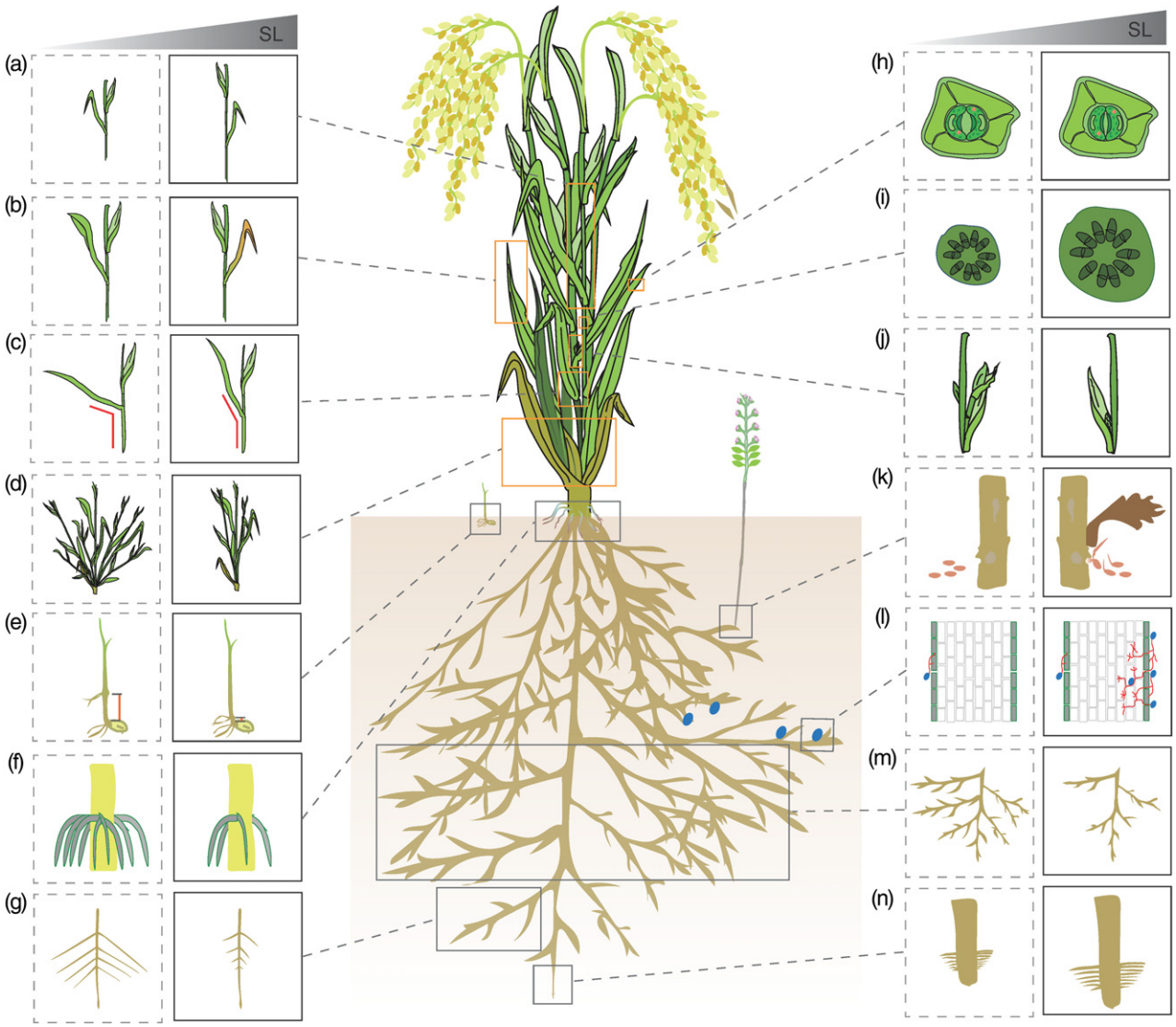

Figure 3. Functions of strigolactones (SLs) in plant development and plant-rhizospheric communication.
SLs modulate many different plant growth and developmental processes, including (a) internode growth, (b) leaf senescence, (c) leaf angle, (d) tillering and tiller angle, (e) mesocotyl elongation, (f) adventitious roots formation, (g) secondary lateral root formation, (h) stomatal closure, (i) stem thickness increase and secondary growth, (j) axillary buds outgrowth, (k) parasitic seeds germination, (l) symbiotic interactions of roots with arbuscular mycorrhiza fungi, (m) lateral roots of formation and (n) root hair elongation and primary root growth.

orobanchol (**30**), 7-hydroxyorobanchyl acetate (**31**), 7-OXO-orobanchyl acetate (**32**), fabacol (**33**), fabacyl acetate (**34**), solanacol (**35**) and solanacyl acetate (**36**) (Yokota *et al.*, 1998; Xie *et al.*, 2008; Ueno *et al.*, 2011; Kohlen *et al.*, 2012; Kisugi *et al.*, 2013; Kohlen *et al.*, 2013; Xie *et al.*, 2013; Xie, 2016). Non-canonical SLs with a β-ionone ring (A ring), including carlactone (CL, **5**), carlactonic acid (CLA, **6**), hydroxyl CLs (i.e., 3-OH-CL [**14**] and 4-OH-CL [**10**]), hydroxyl CLA (i.e., 18-OH-CLA [**11**]), methyl carlactonoate (MeCLA, **7**) and its derivatives (i.e., 18-OH-MeCLA [**12**], 1″-OH-MeCLA [**8**], and heliolactone [**9**]) have been identified in plants (Baz *et al.*, 2018; Iseki *et al.*, 2018; Mori *et al.*, 2020). In addition, plants produce several non-canonical SLs with higher structural complexity, for example, Avenaol (**17**), Lotuslactone (**13**), Zealactone (**15**) and Zeapyranolactone (**16**) (Charnikhova *et al.*, 2017, 2018).

SL biosynthesis starts with the reversible isomerization of all-*trans*-β-carotene to 9-*cis*-β-carotene (**1**) by the carotene isomerase DWARF27 (Alder *et al.*, 2012; Bruno and Al-Babili, 2016; Abuauf *et al.*, 2018). The 9-*cis*-β-carotene (**1**) is then cleaved by CCD7 at the C9′–C10′ double bond to yield 9-*cis*-β-apo-10′-carotenal (**3**) and β-ionone. The 9-*cis*-β-apo-10′-carotenal (**3**) is subsequently converted by CCD8 into CL (**5**), a key center intermediate of SL biosynthesis, and ω-OH-(4-CH_3)-heptanal (Alder *et al.*, 2012; Bruno *et al.*, 2017). The following modifications of CL (**5**), such as

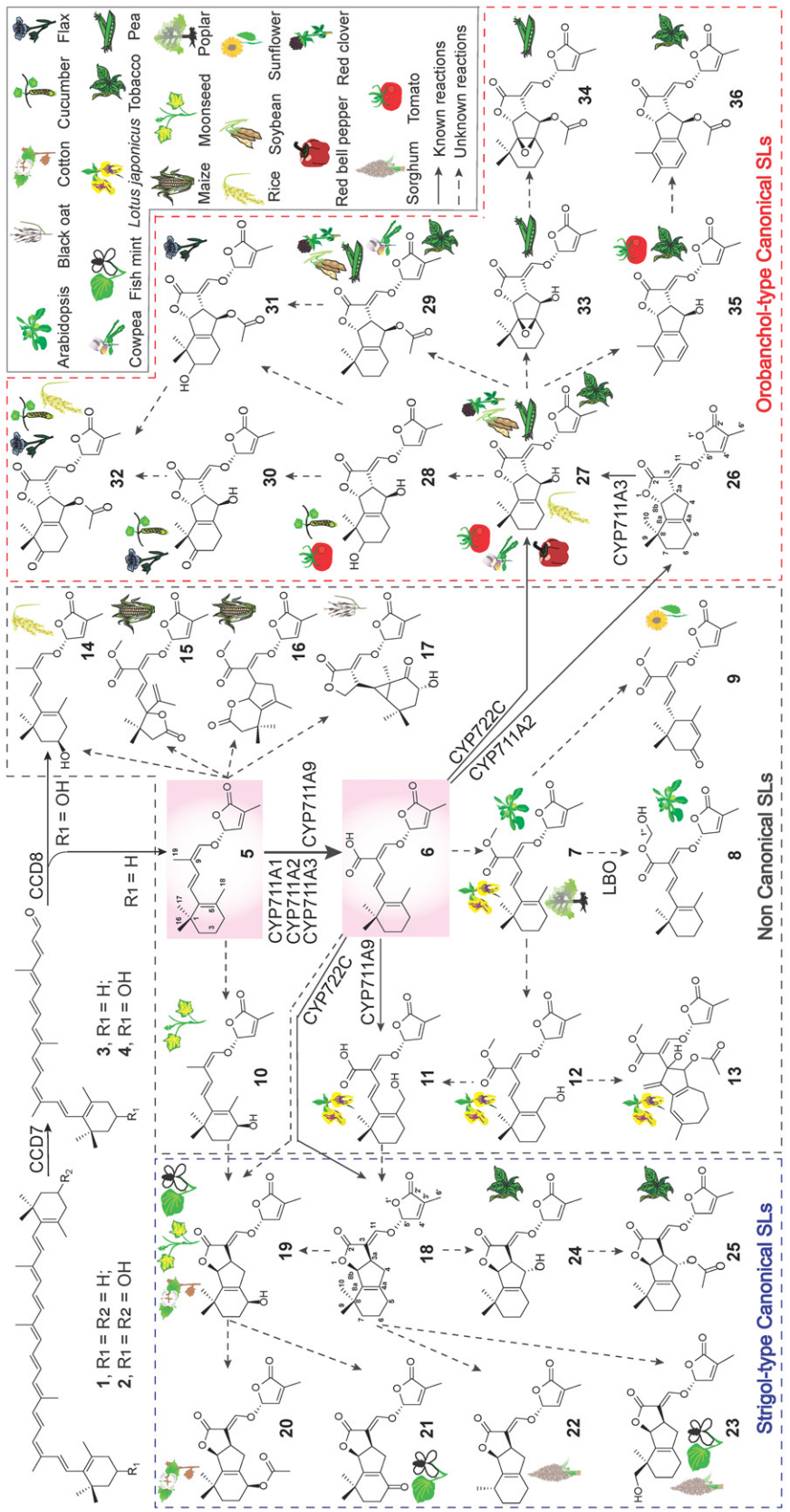

Figure 4. Strigolactone (SL) biosynthesis and structural diversity.
SL biosynthesis starts with isomerization of all-*trans*- into 9-*cis*-β-carotene (**1**) that is cleaved by CCD7 at the C9′–C10′ double bond, yielding 9-*cis*-β-apo-10′-carotenal (**3**) and β-ionone. The former is further converted by CCD8 into carlactone (**5**), a central intermediate of SL biosynthesis (Alder *et al.*, 2012). 3-OH-carlactone (**14**), a presumed precursor of unidentified SLs, is formed from 9-*cis*-zeaxanthin (**2**) also by CCD7 and CCD8 (Bruno *et al.*, 2014; Baz *et al.*, 2018). Other enzymes, such as MAX1 and lateral branching oxidoreductase, further catalyze the post-modification of carlactone (**5**), such as hydroxylation and oxidation, leading to divergent SLs, including canonical and non-canonical SLs, which differ among various species (Jia *et al.*, 2018; Yoneyama *et al.*, 2020). **1**, 9-*cis*-β-Carotene; **2**, 9-*cis*-Zeaxanthin; **3**, 9-*cis*-β-Apo-10′-carotenal; **4**, 3-OH-9-*cis*-β-Apo-10′-carotenal; **5**, Carlactone; **6**, Carlactonic acid; **7**, Methyl carlactonoate; **8**, 1′′-OH-methyl carlactonoate; **9**, Heliolactone; **10**, 4-OH-Carlactone; **11**, 18-OH-Carlactonic acid; **12**, 18-OH-Methyl carlactonoate; **13**, Lotuslactone; **14**, 3-OH-Carlactone; **15**, Zealactone; **16**, Zeapyranolactone; **17**, Avenaol; **18**, 5-Deoxystrigol; **19**, Strigol; **20**, Strigyl acetate; **21**, Strigon; **22**, Sorgolactone; **23**, Sorgomol; **24**, *ent*-2′-*epi*-Orobanchol; **25**, *ent*-2′-*epi*-Orobanchyl acetate; **26**, 4-Deoxyorobanchol; **27**, Orobanchol; **28**, 7-OH-Orobanchol; **29**, Orobanchyl acetate; **30**, 7-OXO-Orobanchol; **31**, 7-OH-Orobanchyl acetate; **32**, 7-OXO-Orobanchyl acetate; **33**, Fabacol; **34**, Fabacyl acetate; **35**, Solanacol; **36**, Solanacyl acetate; CCD7, carotenoid cleavage dioxygenase 7; CCD8, carotenoid cleavage dioxygenase 8; MAX1, more axillary growth 1; LBO, lateral branching oxidoreductase.

oxidation, yield different types of SLs, including canonical and non-canonical ones (Jia *et al.*, 2018). It was also shown that the two CCD enzymes sequentially convert 9-*cis*-zeaxanthin (**2**) into 3-OH-CL (**14**), which might be a precursor of yet unidentified SLs (Bruno *et al.*, 2014; Baz *et al.*, 2018). The oxidation of CL (**5**) is catalyzed by Arabidopsis MORE AXILLARY GROWTH 1 (*At*MAX1)/CYP711A1 to form CLA (**6**) (Abe *et al.*, 2014), which is a common reaction catalyzed by CYP711 enzymes (Iseki *et al.*, 2018). However, the biosynthetic pathway downstream of CLA (**6**) to divergent SLs, differ among various species. In Arabidopsis, CLA (**6**) can be converted by an unidentified methyltransferase into MeCLA (**7**), a substrate of the LATERAL BRANCHING OXIDOREDUCTASE that forms a metabolite with a mass of [MeCLA+ 16 Da] (Brewer *et al.*, 2016). In Arabidopsis, [MeCLA+ 16 Da] was recently identified as hydroxymethyl carlactonoate (1″-OH-MeCLA [**8**]) (Yoneyama *et al.*, 2020). MeCLA (**7**) is also the precursor of heliolactone (**9**), a non-canonical SL formed in sunflower (Iseki *et al.*, 2018). It was shown that *Lotus japonicus* MAX1 (*Lj*MAX1/CYP711A9) expressed in yeast microsomes can convert CL (**5**) into 18-OH-CLA (**11**) via the intermediate CLA (**6**) (Mori *et al.*, 2020). Moreover, feeding experiments indicated that 18-OH-MeCLA (**12**) is transformed to the canonical SL 5DS (**18**), directly or via 18-OH-CLA (**11**) as an intermediate, and to the non-canonical SL lotuslactone (**13**) in *L. japonicus* (Zhang *et al.*, 2014; Yoneyama *et al.*, 2018). In rice, the MAX1 homolog *Os*900 (CYP711A2) catalyzes the repeated oxygenation and ring closures of CL to form 4DO (**26**) via CLA (**6**) (Zhang *et al.*, 2014; Yoneyama *et al.*, 2018). Then *Os*1400 (CYP711A3) catalyzes the subsequent hydroxylation of 4DO (**26**) to produce orobanchol (**27**) (Zhang *et al.*, 2014; Yoneyama *et al.*, 2018). CLA (**6**) is also a precursor of 5DS (**18**), the parent molecule of strigol-type SLs, in sorghum and cotton, where it is converted into sorgomol (**23**), strigol (**19**) and strigyl acetate (**20**) (Iseki *et al.*, 2018). The formation of 5DS (**18**) from CLA (**6**) in cotton is catalyzed by CYP722C, as shown by *in vitro* assays (Wakabayashi *et al.*, 2020). In moonseed, strigol (**19**) can be produced either directly from CLA (**6**) or through a less understood route leading from CL (**5**) to strigol (**19**) via the intermediate 4-OH-CL (**10**), skipping the 5DS (**18**) formation (Iseki *et al.*, 2018). Similarly, in cowpea, tomato, red bell pepper (*Capsicum annuum*), red clover and pea, exogenously applied *rac*-CLA (**6**) is directly metabolized into orobanchol (**27**) without passing through 4DO (**26**) (Iseki *et al.*, 2018; Ueno *et al.*, 2018; Wakabayashi *et al.*, 2019). The direct conversion of CLA (**6**) to orobanchol (**27**) is further illustrated by the functional characterization of cowpea *Vu*CYP722C and tomato *Sl*CYP722C in an *in vitro* assay and by using a tomato *SlCYP722C* mutant (Wakabayashi *et al.*, 2019). These results show that the biosynthetic pathways of monohydroxylated SLs, such as strigol (**19**) and orobanchol (**27**), in some species can bypass the formation of the presumed parent SLs 4DO (**26**) and 5DS (**18**) (Ueno *et al.*, 2018; Wakabayashi *et al.*, 2019).

UNCHARACTERIZED LCDAS

As described above, plant carotenoid biosynthesis starts with the formation of 15-*cis*-phytoene and proceeds via several linear (acyclic) *cis*-carotene intermediates that lead to all-*trans*-lycopene. The linear *cis*-carotenes usually undergo fast conversion but can accumulate if there is a perturbation at the upstream biosynthesis pathway (Alagoz *et al.*, 2018). Previous studies with *cis*-carotene mutants demonstrated the presence of metabolic feedback signals influencing nuclear gene expression and plant phenotype. These metabolic signals were described as putative *cis*-carotene cleavage products referred to as LCDAs. These supposed signaling molecules are among the less-studied metabolites with a carotenoid origin. They have unknown chemical structures and a yet to be uncovered biosynthesis pathway, making them an exciting niche within the carotenoid field.

LCDAs as retrograde signals coordinating metabolic and phenotypic traits

The proposed LCDAs are involved in several metabolic and morphological processes, including the modulation of fruit, leaf and root pigmentation (Kachanovsky *et al.*, 2012; Alvarez *et al.*, 2016), plastid biogenesis at early seedling development (Cazzonelli *et al.*, 2020), and determination of leaf morphology (Avendano-Vazquez *et al.*, 2014). Here, we explain the functions of LCDAs according to the reported metabolic feedback responses described in *cis*-carotene mutants.

PSY is described as the "bottleneck" of the carotenoid biosynthesis pathway, which limits the overall metabolic flux, production and accumulation of downstream carotenoids (Alagoz *et al.*, 2018). Epistatic interaction between the *PSY* and the downstream *CRTISO* genes was shown to influence the fruit pigmentation in tomato fruits (Kachanovsky *et al.*, 2012). A recessive mutation in *yellow-flesh* (r^{2997}) tomato impairs the *PSY1* gene expression and reduces the overall carotenoid accumulation. The tomato loss-of-function *crtiso* mutant, *tangerine* (t^{3002}, t^{3406}), hyper-accumulates poly-*cis*-carotenes and has an enhanced *PSY1* gene expression in fruits (Figure 5a). The fruits of tangerine yellow-flesh (t × r) double mutants have a carotenoid profile similar to that of tangerine but an enhanced expression of *PSY1*. The epistatic interaction between *yellow-flesh* and *tangerine* recovered the *PSY1* expression; however, no epistatic relationship is observed in the *ziso* (*zeta*) *psy* double mutant (r^{2997}/z^{2803}). Thus, the recovery of *PSY1* expression level in *tangerine* is attributed to a feedback response generated by *cis*-carotenes synthesized between ZDS and CRTISO metabolic steps. An LCDA with 7,9,9′-tri-*cis*-neurosporene (pro-neurosporene) or

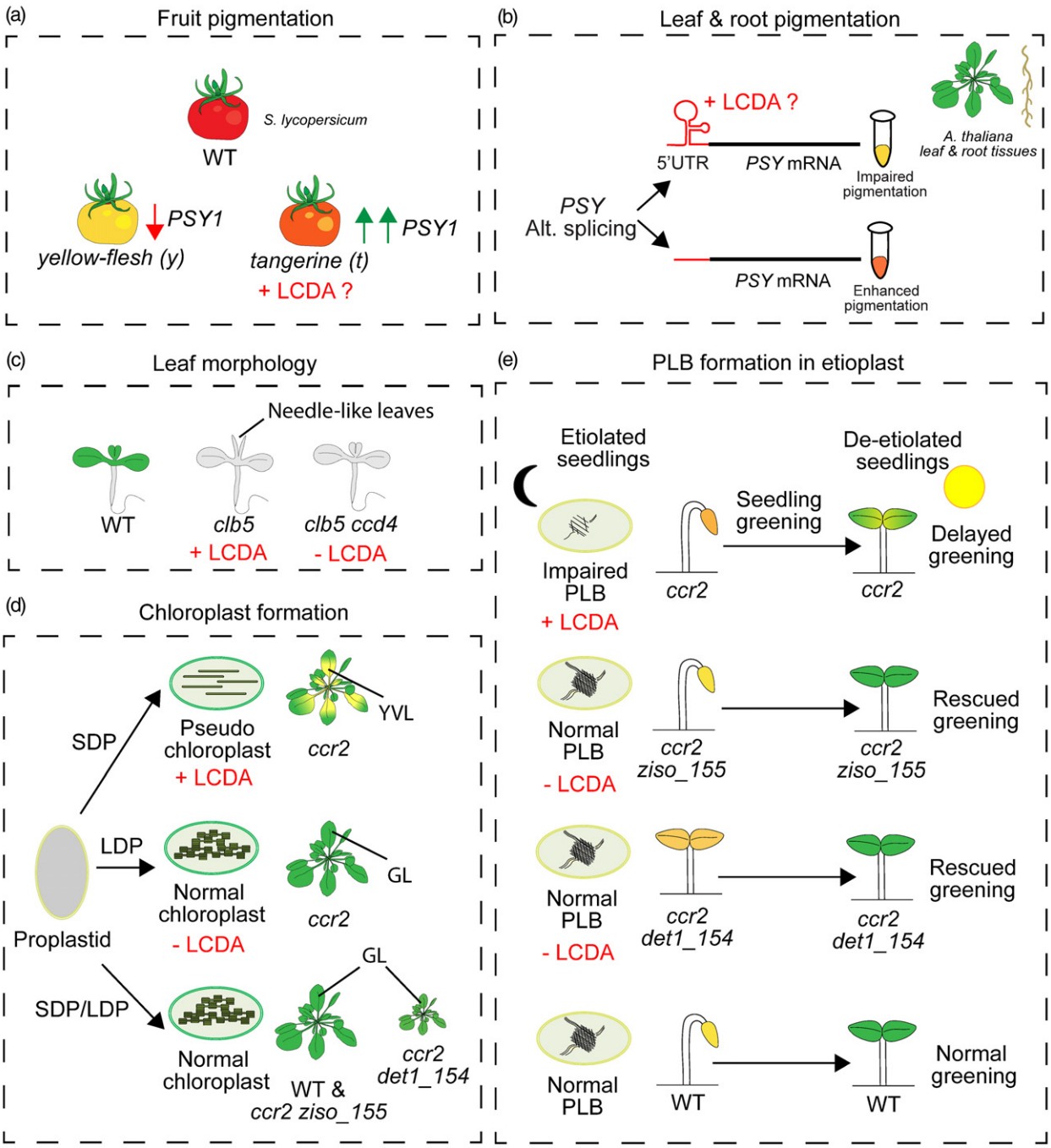

Figure 5. Uncharacterized linear *cis*-carotene-derived apocarotenoids (LCDAs) involved in the regulation of phenotypic processes, including organ pigmentation, leaf morphogenesis and chloroplast development in plants.
(a) An unknown LCDA generates a feedback response to enhance *PHYTOENE SYNTHASE (PSY)1* gene expression in tomato tangerine fruits to modulate pigmentation. The *yellow-flesh* mutant has impaired *PSY1* expression, which results in reduced accumulation of carotenoids in fruits. Red and green arrows represent downregulation and upregulation of gene expression, respectively.
(b) An alternative splicing event of *PSY* modulates the carotenoid accumulation in leaves and roots of Arabidopsis.
(c) Longer *PSY* transcript with a regulatory hairpin motif supposedly interacts with a putative LCDA signal and impairs the carotenogenesis, whereas the shorter transcript variant enhances the carotenogenesis in case of an immediate demand.
(d) The *clb5* mutation in Arabidopsis generates an unknown LCDA signal that induces needle-like leaf development, reversible by the *ccd4*. An unknown LCDA promotes yellow virescent leaf (YVL) development in juvenile leaves of *ccr2* grown under short-day photoperiod (SDP). Long-day photoperiod (LDP) and *ziso_155* eliminate the LCDA and reverse the YVL phenotype in seedlings with *ccr2* background.
(e) The same LCDA signal is found to impair the prolamellar body (PLB) formation, and so the cotyledon greening in dark-grown etiolated *ccr2* seedlings. The *ziso_155* and *det1_154* impair the accumulation of the PLB-regulatory LCDA, thus, rescue the PLB formation and cotyledon greening phenotypes in etiolated seedlings. 5′ UTR, 5′ untranslated region; GL, green leaves; WT, wild type.

7,9,9′,7′-tetra-*cis*-lycopene (pro-lycopene or tetra-*cis*-lycopene) origin is proposed to arise through CCD-mediated cleavage and to act as a feedback signal enhancing *PSY1* gene expression and carotenogenesis in tomato fruits. Whether tomato CCDs can convert pro-neurosporene or pro-lycopene to biosynthesize an LCDA *in vivo* requires further investigation. Nevertheless, it was shown that tomato CCD1A and 1B enzymes cleave pro-lycopene at different positions *in vitro* (Ilg *et al.*, 2014).

Besides transcriptional regulation, the PSY protein level in Arabidopsis post-transcriptionally regulated by an alternative splicing event in the 5′ untranslated region (5′ UTR) of *PSY* transcripts (Alvarez *et al.*, 2016). According to a described model, a hairpin loop motif in the secondary structure of the *PSY* mRNA 5′ UTR acts as a switch that responds to a putative ACS and mediates the PSY translation. The long *PSY* transcript variant that harbors the hairpin loop inhibits the translation and is necessary to maintain the carotenoid homeostasis. The shorter transcript variant missing the hairpin motif does not interact with the ACS and has a translation-permissive secondary structure, thus, enhancing the translation in case of immediate demand for carotenoid production, such as in the dark to light transition (Figure 5b). The *PSY*-regulatory ACS is described as a plastid-derived apocarotenoid that can be produced by the cleavage of any carotenoid downstream of 15-*cis*-phytoene and 9,15-di-*cis*-phytofluene. The identity of the ACS and its interaction with the *PSY* 5′ UTR is yet to be shown.

PDS activity in interaction with the PLASTID TERMINAL OXIDASE modulates the redox state of the thylakoid membranes and modulates tetrapyrrole retrograde signaling to coordinate the expression of *PHOTOSYNTHESIS-ASSOCIATED NUCLEAR GENEs* (*PhANGs*) required in chloroplast biogenesis (Foudree *et al.*, 2010; Wang and Fu, 2016). *PDS* loss-of-function mutations are lethal, and homozygous *pds* mutants have impaired chloroplast biogenesis, which results in an albino phenotype (Qin *et al.*, 2007). PDS enzymatic activity can be impaired by chemical inhibitors, such as the herbicide Norflurazon (NFZ), and give rise to phytoene and phytofluene accumulation. Similar to *pds* mutation, the chemical inhibition of PDS activity by NFZ generates a tetrapyrrole-derived retrograde signal (Mg-Proto IX), which impairs the chlorophyll and carotenoid accumulation, and reduces the expression of *PhANGs* (Song *et al.*, 2018). There are no reports that show the presence or association of a phytoene or phytofluene-derived apocarotenoid in the formation of phenotypes attributed to the impaired PDS activity. Besides, recent research showed that 15-*cis*-phytoene and 9,15-di-*cis*-phytofluene are not targeted by CCD-mediated enzymatic degradation in Arabidopsis (Schaub *et al.*, 2018). However, a member of the CCD family, annotated as *Zm*CCD10a, was recently discovered in maize and shown to cleave the C9–C10 (C9′–C10′) double bond in phytoene to generate geranylacetone (C_{13}) when exogenously expressed in *Escherichia coli* (Zhong *et al.*, 2020). However, this enzyme belongs to the zaxinone synthase clade (Wang *et al.*, 2019), and it remains to be shown whether it catalyzes phytoene cleavage *in planta*. The *in planta* activity of a phytoene and/or phytofluene-derived LCDA, including geranylacetone, remains to be uncovered.

LCDAs are also considered to determine the plant leaf morphology. The Arabidopsis *ZDS*/*CHLOROPLAST BIOGENESIS5* (*CLB5*) mutant *clb5* accumulates putative LCDA signals that are involved in the regulation of plastid biogenesis and cause needle-like leaf formation during early seedling development (Avendano-Vazquez *et al.*, 2014). Molecular and biochemical analyses demonstrated that the change in *PhANG*s expression, as well as the needle-like leaf phenotype, is associated with the accumulation of an LCDA signal proposed to be derived from 9,15-di-*cis*-phytofluene or 9,15,9′-tri-*cis*-ζ-carotene. NFZ treatment and generation of *clb5ccd4* double mutant restored the *PhANG* expression and rescued the leaf phenotype caused by *clb5* mutation (Figure 5c). This indicates that CCD4 might be involved in the formation of the LCDA coordinating *PhANG* expression and leaf morphology. However, both *in vivo* and *in vitro* analysis showed that CCD4 is not capable of cleaving *cis*-ζ-carotene isomers due to their stereochemical configuration not allowing CCD4 to target C9–C10 and/or the C9′–C10′ double bond (Huang *et al.*, 2009; Bruno *et al.*, 2016). It was previously shown that CCD4 interacts with other regulatory proteins to determine plant leaf morphology, plastid development and leaf senescence through interacting with other core regulators located in the plastoglobuli (Naested *et al.*, 2004; Bhuiyan *et al.*, 2016). This shows that the reversion of the needle-like leaf phenotype in the *clb5ccd4* double mutant might be associated with a secondary effect caused by *ccd4* mutation rather than CCD4's direct involvement in the generation of the LCDA signal. The biochemical and molecular mechanisms coordinating the formation of the *clb5* needle-like leaf phenotype require further investigation.

Mutations in *CRTISO* impair plastid development and give rise to the formation of varying degrees of the yellow virescent leaf (YVL) phenotypes in plants, including tomato (*tangerine*) (Isaacson *et al.*, 2002), melon (*yofi*) (Galpaz *et al.*, 2013), rice (*zebra2*) (Chai *et al.*, 2011) and Arabidopsis (*ccr2*) (Park *et al.*, 2002). A previous study also showed that the etiolated cotyledons of *ccr2* seedlings hyper-accumulate *cis*-carotenes and have impaired prolamellar body (PLB) formation, associated with the impaired photomorphogenic development (Park *et al.*, 2002). Recent research with Arabidopsis *ccr2* seedlings demonstrated that the virescence phenotype develops only in the juvenile leaves grown under the short-day photoperiod. The short-day photoperiod limits the photoisomerization and promotes

the accumulation of *cis*-carotenes and an unknown LCDA signal, which coordinates chloroplast formation in photosynthetic tissues of *ccr2* seedlings (Cazzonelli *et al.*, 2020). The YVLs of *ccr2* have pseudo-chloroplast rather than a normal chloroplast, suggesting an impaired photomorphogenic development. The epistatic interaction between *ziso* (*zic*) and *ccr2*, in *ccr2 ziso_155* seedlings, eliminated the unknown LCDA signal and rescued the YVL phenotype (Figure 5d). The *ccr2ziso_155* mutation restored the PLB formation in etiolated seedlings and rescued the cotyledon greening phenotype; thus, it revealed the link between the unknown LCDA and the regulation of PLB formation (Figure 5e). Both etiolated and light-grown leaves of *ccr2 ziso_155* have impaired expression of genes that repress photomorphogenesis (i.e., *CONSTITUTIVE PHOTOMORPHOGENIC 1*/*COP1*, *DE-ETIOLATED 1*/*DET1*) and have enhanced expression of *PhANGs* (i.e., ELONGATED HYPOCOTYL 5/*HY5*, *LIGHT-HARVESTING CHLOROPHYLL A/B-PROTEIN 1.3/LHCB1.3*, *RIBULOSE BISPHOSPHATE CARBOXYLASE SMALL CHAIN 1A/RBCS1a*), suggesting a possible interaction between the unknown LCDA and photomorphogenic development.

Further metabolic analysis with etiolated *ccr2_det1_154* and D15-treated seedlings confirmed that the PLB-regulatory unknown LCDA is possibly derived from 7,9,9′-tri-*cis*-neurosporene or 9,9′-di-*cis*-ζ-carotene. DET1 is a repressor of photomorphogenesis, and its impairment downregulated the *PROTOCHLOROPHYLLIDE OXIDOREDUCTASE* (*POR*) gene expression and decreased the PHYTOCHROME-INTERACTING FACTOR 3 (PIF3) protein abundance while enhancing HY5, thus induced *PhANG* expression and promoted plastid biogenesis in the *ccr2* background. The PLB-regulatory LCDA and *det1* complemented each other by restoring PLB formation and rescued the delayed greening and YVL phenotypes in *ccr2 det_154* seedlings (Figure 5d,e). The characterization of the PLB-regulatory LCDA and the level of its interaction with PIF3 and HY5 transcription factors requires further research.

LCDA-associated phenotypes might be linked with the lack of downstream apocarotenoids

Perturbations at the upstream carotenoid pathway, through genetic mutations or altered gene expression, affect the production of apocarotenoids synthesized at the downstream pathway. For instance, the overexpression of endogenous *PSY* in Arabidopsis enhanced the accumulation of β-apocarotenoids (i.e., β-apo-10′-carotenal, retinal), C_{13} apocarotenoid glycosides (i.e., 3-oxo-α-ionol, 3-hydroxy-5,6-epoxy-β-ionone) (Latari *et al.*, 2015) and short-chain apocarotene-dialdehydes (i.e., glyoxal, methylglyoxal) (Schaub *et al.*, 2018) in leaves and callus tissues. Another study showed that the overexpression of *PSY*-ORFs from maize, rice and Arabidopsis itself did not significantly affect the accumulation of the carotenoid-derived phytohormone ABA in 4-week-old Arabidopsis leaves (Alvarez *et al.*, 2016). However, a *PSY* paralog *PSY3* gene was previously shown to be induced by the abiotic stress conditions (i.e., drought and salinity) and responsible for increased ABA accumulation in rice and maize (Li *et al.*, 2008; Welsch *et al.*, 2008). Besides, expression of a *PSY* gene (*PSY3*) in the dicots *Medicago truncatula* and *Solanum lycopersicum* is highly upregulated during plant root–symbiotic AM interactions and associated with the accumulation of SLs and mycorrhiza-induced α-ionol (C_{13}) and mycorradicin (C_{14}) (Stauder *et al.*, 2018). Similarly, the expression of saffron *PSY3* (a close relative of dicot *PSY3*) was shown to be involved in the production of mycorrhizal-induced apocarotenoids (Ahrazem *et al.*, 2019). The increased abundance of apocarotenoids in plants with induced *PSY* expression or overexpression results from the enhanced metabolic flux and increased precursor availability reinforcing apocarotenoid biosynthesis at the downstream part of the pathway.

Genetic mutation or impaired expression of linear *cis*-carotene biosynthetic genes has a negative impact on ABA biosynthesis and accumulation in plant tissues, giving rise to phenotypes associated with ABA deficiency. For instance, the sunflower *nd1* (*zds*) (Conti *et al.*, 2004), maize *vp5* (*pds*) (Hable *et al.*, 1998), *vp9* (*zds*) (Matthews *et al.*, 2003; Ma *et al.*, 2014; Chen *et al.*, 2017) and *y9* (*ziso*) (Li *et al.*, 2007) and rice *phs-1* (*pds*), *phs-2* (*zds*) and *phs-3* (*crtiso*) (Fang *et al.*, 2008) mutants develop viviparous seeds that lack dormancy. Precocious seed/grain development reduces the overall yield and quality of crops and causes economic loss (Nonogaki and Nonogaki, 2017). Similarly, RNA interference-mediated downregulation of *ZDS* reduces ABA accumulation and causes the development of ABA-specific delayed fruit-ripening phenotype in tomato fruits (McQuinn *et al.*, 2020). The reduction of ABA in plants with impaired linear *cis*-carotene biosynthesis is due to blockage of the metabolic flux at the upstream pathway, limiting the precursor availability to produce ABA.

The linear *cis*-carotene pathway is a bottleneck for the production of downstream apocarotenoids and some phytohormones (i.e., ABA, SLs). Therefore, the LCDA-associated phenotypes defined in *cis*-carotene mutants might be partially linked with the impaired production or accumulation of downstream apocarotenoids functioning as either metabolic signals or phytohormones. The contribution of downstream signaling components to the emergence of the LCDA-associated phenotypes and the feedback interaction between the linear *cis*-carotene biosynthesis pathway and other apocarotenoids require further investigation.

Prospects for future LCDA research

Our current knowledge about the biosynthesis and function of LCDAs is mostly limited to data available on their precursor molecules, the acyclic *cis*-carotenes. Previous

studies defined several functions for putative LCDA signals, but the direct evidence showing the relevance between the described phenotypic and metabolic traits with LCDAs is missing. The molecular structures of LCDAs are unknown, but they might be predictable to a degree as their precursor is well characterized. In fact, some of the theoretically predicted LCDAs and their modified forms (i.e., farnesyl- and geranylacetone, pseudoionone, sulcatone, citral) were previously identified in plants (Shi *et al.*, 2020). A recent study showed that LCDAs accumulate in a tissue-specific manner, suggesting the possibility of having particular functions in plant development or environmental response (Rivers *et al.*, 2019). The question remains unanswered, whether the primary LCDAs or their modified products cause the phenotypes described in the literature, or are they only the metabolic byproducts of the carotenoid biosynthesis pathway? Being specifically biosynthesized by the carotenoid pathway and having a biological function may increase the possibility of an LCDA to be a signaling molecule rather than a byproduct. The LCDAs can be produced by enzymatic cleavage or by ROS-mediated degradation of *cis*-carotenes. There are a few studies that show the possible involvement of CCDs in the generation of LCDAs in plants (Simkin *et al.*, 2004; Yahyaa *et al.*, 2013; Jing *et al.*, 2015). In fact, the capability of CCDs to cleave acyclic *cis*-carotenes to produce LCDAs is very limited (Vogel *et al.*, 2008; Ilg *et al.*, 2014; Bruno *et al.*, 2016). Therefore, the majority of the LCDAs might be generated through the ROS-mediated degradation of *cis*-carotene precursors. Light quality and quantity determine ROS accumulation in plants (El-Esawi *et al.*, 2017; Waszczak *et al.*, 2018). In addition, light- or enzyme-mediated isomerization might be rate-limiting the production of LCDAs during the day/night cycle, which might be a component in a regulatory network influencing metabolic and morphological traits according to light quality. Additional and more profound research is required to identify LCDAs structurally and explain the molecular mechanisms associated with the perception and transduction of these signals, which provoke the generation of responses and influence plant metabolism and phenotype.

CYCLIC AND ACYCLIC APOCAROTENOIDS WITH SIGNALING PROPERTIES

In recent years, various apocarotenoids involved in photoacclimation, drought tolerance, plant growth and development, AM symbiosis and plant defense against herbivores were reported. The still ongoing discovery of apocarotenoids with signaling and regulatory functions highlights the importance of carotenoids in coordinating different processes in the life of plants. For instance, there are cyclic and acyclic apocarotenoids that act as retrograde signals involved in high light and drought tolerance (e.g., β-cc, β-ccA and dhA), plant herbivory tolerance (e.g., β-ionone, loliolide and α-ionone), AM symbiosis (e.g., blumenols, mycorradicins), and plant development and parasitic control (e.g., anchorene and zaxinone). These apocarotenoids may have multiple functions, such as β-ccA, which is involved in growth and development, and zaxinone, which is also shown to be involved in AM symbiosis.

Cyclic apocarotenoids as retrograde signals mediating high light and drought tolerance

High-light stress is accompanied by the generation of $^1O_2$ in the photosystem II, which attacks β-carotene molecule and gives rise to the formation of β-cc that can further be converted into the corresponding acid β-ccA, or to β-ionone, which is the precursor of dhA (Figure 6a). The lipid-soluble nature of β-cc and dhA might allow them to pass through plastid membranes and convey the stress message to the cytosol and the nucleus (D'Alessandro and Havaux, 2019). The retrograde signaling triggered by β-cc is independent of canonical tetrapyrrole signals, but also independent of the EXECUTER 1 and 2 proteins (EX1, EX2), which mediates $^1O_2$-induced cell death (Ramel *et al.*, 2012b; D'Alessandro *et al.*, 2018; Dogra *et al.*, 2018; D'Alessandro and Havaux, 2019). The protein level of 3′(2′),5′-BISPHOSPHATE NUCLEOTIDASE (SAL1) decreased in β-cc-treated plants. SAL1 is responsible for the degradation of the well-known retrograde signal 3′-phosphoadenosine-5′-phosphate (PAP) (Estavillo *et al.*, 2011; Ramel *et al.*, 2012b). In addition, the β-cc application enhanced the protein level of the enzyme SULFOTRANSFERASE (ST2A) that uses PAPS as a sulfate donor generating PAP (D'Alessandro and Havaux, 2019). Thus, β-cc might induce PAP accumulation and trigger PAP retrograde signaling in response to an affected photosynthetic process in plants exposed to high light (Figure 6a). In line with this, transcriptome analysis of β-cc- and dhA-treated plants showed a reprogramming in gene expression, which is usually associated with enhanced photooxidative stress tolerance, for example, higher photosystem II photochemical efficiency and lower lipid peroxidation (Ramel *et al.*, 2012b; Shumbe *et al.*, 2014, 2017). In fact, β-cc-treated plants showed increased high-light tolerance compared with mock-treated or β-ionone-treated plants (Figure 6b).

METHYLENE BLUE SENSITIVE (MBS1) is a small zinc finger protein that was previously identified in a genetic screen of *Chlamydomonas reinhardtii* mutants defective in response to $^1O_2$ (Shao *et al.*, 2013). MBS1 participates in the regulation of $^1O_2$-responsive-genes, which resemble the effect of β-cc/dhA in Arabidopsis. The absence of the MBS1 protein in Arabidopsis resulted in dramatic perturbations of $^1O_2$ marker gene expression (Shao *et al.*, 2013). An Arabidopsis *MBS1* overexpressing line shows increased photoacclimation response and high-light tolerance, while the *mbs1* mutant is insensitive to β-cc treatment (Shumbe *et al.*, 2017). These results demonstrate the involvement of

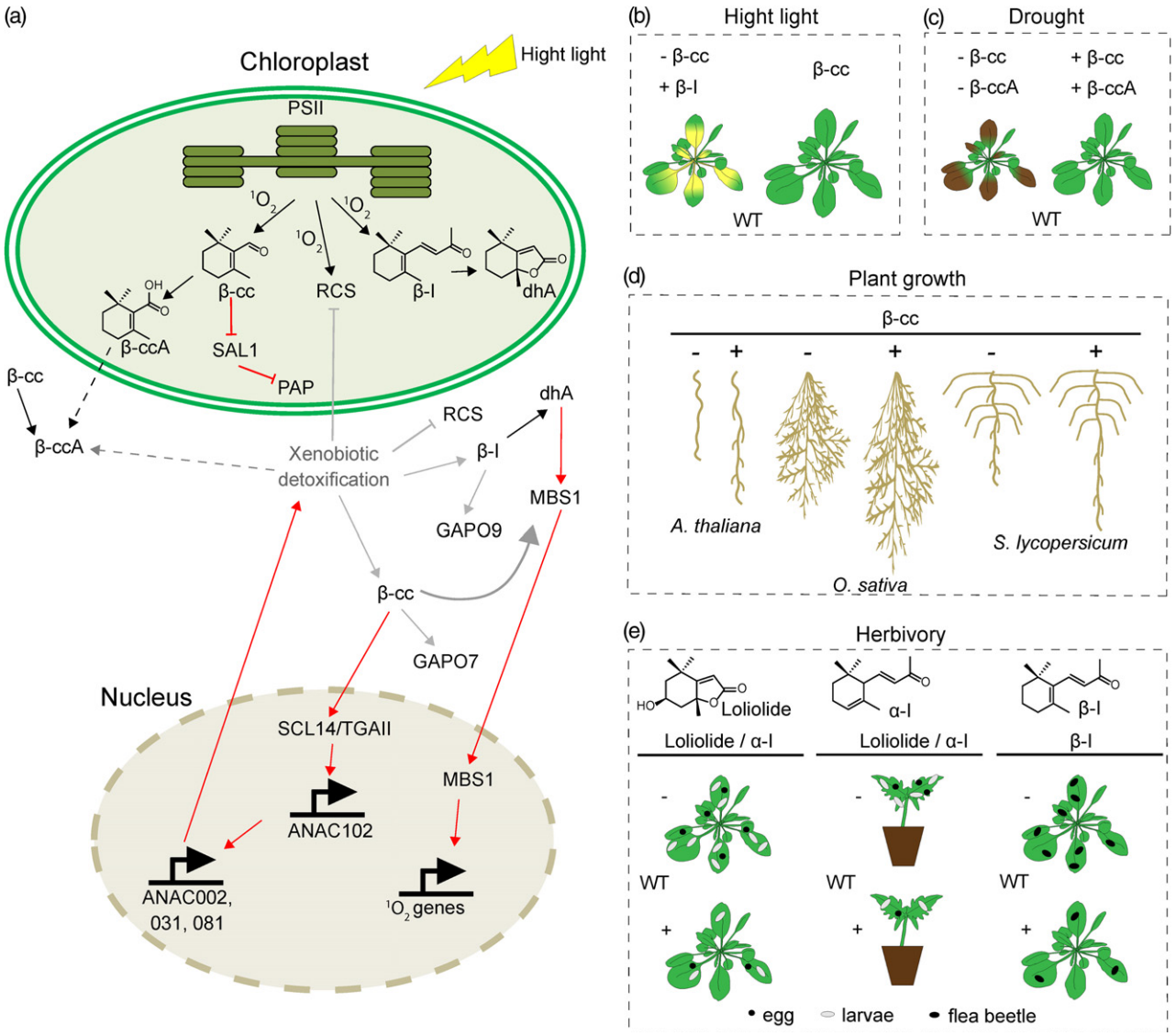

Figure 6. Apocarotenoids involved in high-light and drought stress response, and in herbivore defense.
(a) $^1O_2$ signaling cascade triggered by high-light stress in plant leaves. Apocarotenoids (e.g., β-cc, dhA and β-ionone [β-I]) are produced through the oxidation (black arrows) of β-carotene in photosystem II (PSII). Further oxidation of these compounds (black arrows) forms, for instance, β-cyclocitric acid (β-ccA) and dihydroactinidiolide (dhA). β-ccA might be transported to the cytosol by unknown transporters (dotted black arrow) or can be produced in the cytosol from the volatile β-cc. $^1O_2$ can produce reactive carbonyl species (RCS), which can be detoxified by the xenobiotic detoxification system (shown in gray). The volatile apocarotenoids can passively diffuse to the cytoplasm to activate at least two signaling cascades (red arrows) mediated by MBS1 or by the interaction between the SCL14 and the TGAII transcription factor. These signaling pathways induce $^1O_2$-responsive genes and genes involved in cellular detoxification. Xenobiotic detoxification system targets RCS and might be involved in β-cc, β-I and β-ccA detoxification (gray arrows). β-cc is interconnected with the 3-phosphoadenosine 5-phosphate (PAP)-signaling by inducing PAP accumulation under excess light (red lines inside the chloroplast).
(b) β-cc confers high-light tolerance in Arabidopsis plants subjected to high-light stress. WT, wild type.
(c) β-cc promotes plant growth in Arabidopsis, rice and tomato roots.
(d) β-cc and β-ccA confer drought tolerance to Arabidopsis plants subjected to drought stress.
(e) α- and β-ionone, and loliolide influence plant–herbivore interactions. Exogenous application of these volatiles confers protection against plant herbivores by reducing egg depositions and larvae numbers.

MBS1 in the signaling cascade downstream of β-cc/dhA. The involvement of MBS1 in the $^1O_2$ signaling pathway was further substantiated by a stronger dual localization of the protein in the cytosol and the nucleus after β-cc treatment or high light. Taken together, it can be assumed that the high-light stress signal is transmitted by β-cc/dhA to MBS1, enabling it to enter the nucleus to activate $^1O_2$-responsive genes (Figure 6a).

An MBS-independent activity of β-cc, known as xenobiotic detoxification system, is present in all eukaryotes and comprises a great number of proteins (e.g., transcription factors, transporters and redox enzymes) (Sandermann,

1992). This system is partly coordinated at the transcriptional level by the interaction between the TGAII transcription factors and the GRAS protein SCARECROW-LIKE 14/SCL14 (Fode *et al.*, 2008). D'Alessandro *et al.* (2018) demonstrated that the xenobiotic detoxification coordinated by SCL14 is induced by both β-cc and photooxidation (Figure 6a). This detoxification system is activated by endogenous toxicants (e.g., reactive carbonyl species) that derive from the decomposition of lipid peroxides (Mano, 2012). Upon excessive light exposure and by the action of β-cc, plant cells are prepared for the accumulation of lipid peroxides by increasing the detoxification of reactive carbonyl species (D'Alessandro *et al.*, 2018). As part of the xenobiotic response or by diffusing to the cytosol, β-cc increases the *SCL14* expression level and promotes its interaction with TGAII, a bZIP transcription factor. This interaction modulates expression levels of the chloroplast transcription factor ANAC102 (Inze *et al.*, 2012). ANAC102 induces downstream transcription regulators (ANAC002, ANAC031 and ANAC081), which activate redox enzymes involved in the xenobiotic detoxification process (Figure 6a) (D'Alessandro *et al.*, 2018). Similar to the β-cc application in Arabidopsis, rice overexpressing the SCL14 rice homolog *Os*GRAS23 show increased high light/drought tolerance (Xu *et al.*, 2015). Intriguingly, β-cc and $^1O_2$ also strongly induce the expression of several Arabidopsis glycosyltransferases in leaves (Ramel *et al.*, 2012b, 2013). These enzymes are likely involved in the conversion of hydroxylated apocarotenoids that were shown, together with their glycosylated derivatives (glycosylated apocarotenoids [GAPO]), to increase significantly in Arabidopsis plants subjected to high-light conditions (Mi *et al.*, 2018). Mi *et al.* (2018) showed that β-cc and β-ionone concentrations were similar under control conditions, while the level of glycosylated β-ionone was about 20 times higher than that of glycosylated β-cc. Extremely high β-ionone glycosylation (GAPO9) content could explain that β-ionone shows similar unconjugated levels to those of β-cc. Interestingly, GAPO7 content is just a small fraction of β-cc, while conjugated β-ionone (GAPO9) represents the majority of the β-ionone concentration. Uneven production of the glycosylated β-cc (GAPO7) and β-ionone (GAPO9) forms suggests that β-carotene derivatives have a detoxifying mechanism (D'Alessandro and Havaux, 2019). In addition, hydroxy-β-cc was not detectable (unlike hydroxylated β-ionone), suggesting a quick conversion to its glycosylated form, pointing to an essential role of this process in the regulation of β-cc signaling (Mi *et al.*, 2018). This concludes that β-cc metabolization likely limits its signal role through the aforementioned negative feedback mechanism, thereby returning $^1O_2$-induced signaling to unstressed levels.

Besides its role as a retrograde signal in the network regulating the cellular oxidative stress response, β-cc has recently been shown to be a growth regulator (Dickinson *et al.*, 2019). Dickinson *et al.* used a targeted chemical genetic approach to identify apocarotenoids that enhance root branching in the presence of *N*-(4-fluoro-benzyl)-*N*-hydroxy-3-(4-methoxy-phenyl)-propionamide (D15), a CCD inhibitor that reduces primary root length and inhibits LR capacity by 50% in Arabidopsis. Treatment of β-cc under these conditions increased LR formation by approximately 40% in the presence of D15 in Arabidopsis (Figure 6c, left panel). In the absence of D15, exogenous β-cc application at a concentration <1 μM increased primary root length and LR branching by 30%, suggesting that β-cc acts as a growth promoter in Arabidopsis roots. In addition, experiments at the cellular level revealed that β-cc promotes cell division in LR primordia after initiation and induces root growth by stimulating cell division (Dickinson *et al.*, 2019). Moreover, experiments with mutant lines of pathways related to auxin, brassinosteroids and ROS signaling, which are known to modulate root growth and development, confirm an independent mechanism of action for the β-cc effect. Further experiments with tomato and rice roots (Figure 6c, middle and right panel) confirmed the role of β-cc as a conserved plant growth regulator (Dickinson *et al.*, 2019).

Recently, D'Alessandro *et al.* (2019) showed that β-cc is further metabolized into water-soluble β-ccA in leaves. Indeed, β-ccA was found in Arabidopsis leaves subjected to drought stress or exposed to the β-cc treatment. Moreover, exogenous application of β-ccA was shown to protect plants from drought stress (Figure 6d) and to induce the expression of key water stress-responsive genes (e.g., *ANAC72*, *ATAF1*, *RD29B*) (D'Alessandro *et al.*, 2019). The use of Arabidopsis stomatal regulation (*ost2-2*), ABA (*abi1*) and jasmonate receptor (*coi1*) mutants suggests a different, independent mechanism of action for β-ccA (D'Alessandro *et al.*, 2019). Interestingly, the β-ccA drought-protective effect was observed in pansy flower, pepper and tomato plants, indicating a general, conserved function of β-ccA in drought response. It was also shown that the application of β-cc to Arabidopsis plants grown in the greenhouse increased their drought tolerance (Figure 6d). However, while β-cc treatment mimics the effect of β-ccA, application of β-ccA induced only one branch of the β-cc signaling, suggesting that β-cc exerts a β-ccA-independent function and is perceived by a different signaling pathway.

Role of β-ionone, loliolide and α-ionone in plant defense to herbivores

The sessile lifestyle of plants necessitates a great arsenal of metabolites that protect against herbivores and pathogens. This arsenal includes the so-called plant activators, such as the phytohormone jasmonic acid (JA), which is involved in plant–pathogen protection through the activation of defense mechanisms that do not harm/kill the microbes or insects (Wei *et al.*, 2011; Caceres *et al.*, 2016; Murata *et al.*, 2019a,b; Li *et al.*, 2020). In addition, plants

defend themselves by using metabolites that affect pathogens and exert antimicrobial or insecticidal activity. Interestingly, several cyclic apocarotenoids that induce herbivore resistance in plants without exhibiting insecticidal activity have been reported in the last years (Wei *et al.*, 2011; Caceres *et al.*, 2016; Murata *et al.*, 2019a,b; Li *et al.*, 2020). Examples of such apocarotenoids are α-ionone, β-ionone and loliolide, which arise through the degradation of α- and β-carotene (Schwab *et al.*, 2008).

β-Ionone, a C_{13} β-carotene-derived volatile, is a component of fragrances released from various species of flowering plants (Wei *et al.*, 2011). Plant–insect interaction is frequently characterized by plant volatile emissions that can act as attractants or repellents (Pivnick *et al.*, 1992; Bartlet *et al.*, 1997; Wang *et al.*, 1999; Omura *et al.*, 2000; Gruber *et al.*, 2009). β-Ionone has been reported as one of the components of the volatile emission by *Trifolium stric-tumand* leaves. β-Ionone was previously shown to affect (repellent effect against insects and reduced egg deposition) several insects, such as earth mites, cabbage butterfly and crucifer flea beetle (Wang *et al.*, 1999; Omura *et al.*, 2000; Gruber *et al.*, 2009). Moreover, β-ionone exerted the highest deterrence activity against the redlegged earth mite compared with all the other volatile compounds emitted by the leaves of *T. strictumand* (Wang *et al.*, 1999). β-Ionone was present only in the volatiles emitted by *Trifolium strictum* but not by *T. glanduliferum* and *T. subterraneum*, suggesting specific defense mechanisms in different plant species. Moreover, β-ionone was found in mature leaves and flowers of *Brassica napus* variety *AC Excel* (but not in any other cultivar) and showed one of the most potent inhibitory effects on the crucifer flea beetle (Figure 6e, right panel) (Gruber *et al.*, 2009). The non-repellent effect of β-ionone against the cabbage butterfly (Omura *et al.*, 2000), suggests a certain degree of volatile specificity and raises the possibility of different insects having different volatile receptors. These data point to a specificity on both the plant side and the insect side. Genetic and molecular studies with Arabidopsis plants overexpressing the *AtCCD1* showed that higher transcription of the gene is reflected in higher β-ionone levels and, therefore, in stronger plant defense to herbivores (Wei *et al.*, 2011; Caceres *et al.*, 2016). Leaves from *AtCCD1* overexpressing plants were less eaten by about 50% by the flea beetle and the spider mite and showed reduced oviposition by whiteflies, pointing towards carotenoid pathway engineering as an emergent tool for pest control.

Loliolide is a β-carotene derivative isolated from leaves of mosaic virus-infected tobacco plants showing a hypersensitive response, and shown to induce resistance to herbivores upon exogenous application (Repeta, 1989; Rios *et al.*, 2008; Murata *et al.*, 2019b) (Figure 6e, left and middle panel). For instance, tomato leaves treated with loliolide showed reduced survival rate and egg deposition of the two-spotted spider mite and survival rate of the larvae of the common cutworm (Figure 6e, middle panel) without exhibiting toxicity against these herbivores (Murata *et al.*, 2019b). Interestingly, loliolide cellular content is not only increased after an infestation by the common cutworm larvae but also after the application of their oral secretions in tomato, suggesting that a molecular interaction between a component of the insect secretion and a plant receptor must be occurring. The same interaction is most likely the trigger that activates a signaling cascade resulting in the activation of key defense genes. A combination of microarray analysis and quantitative reverse transcription–polymerase chain reaction revealed the identity of two loliolide-responsive genes, *LIT8* and *LIT13*, involved in plant defense. *LIT8* is a gene-encoding cell wall invertase, while *LIT13* is predicted to encode the WALL-ASSOCIATED RECEPTOR KINASE 2 (Murata *et al.*, 2019b). Both genes were upregulated in response to the exogenous application of loliolide but not in response to JA or salicylic acid (SA). Interestingly, JA- and SA-responsive genes such as *PROTEINASE INHIBITOR II* (*PIN2*; downregulated), *LEUCINE AMINOPEPTIDASE* (*LAPA1*; downregulated), and basic *β-1,3-GLUCANASE* (*GLUB*; unchanged) did not respond to exogenous loliolide application, suggesting a JA-/SA-independent mechanism for gene activation after loliolide application. Furthermore, JA and SA levels remained unchanged after loliolide exogenous application supporting the activation of plant defense genes independently of the canonical JA signaling. (–)-Loliolide was also recently reported as a soil-borne signaling chemical conserved in dozens of plants (Kong *et al.*, 2018). For instance, (–)-loliolide was detected in the barnyard grass–rice allelopathic interaction (Li *et al.*, 2020). The interaction between rice and five biotypes of barnyard grass was characterized by the higher levels of the allelochemicals momilactone B (a diterpenoid) and tricin (a flavonoid), measured in rice, in response to (–)-loliolide, which was detected in the root exudates of all five biotypes of barnyard grass. Interestingly, the exogenous application of loliolide (and JA) elicited momilactone B and tricin production. Moreover, comparative transcriptomic analysis unveils the regulatory activity of (–)-loliolide on the diterpenoid (upregulation of *CPS4*, *KSL4* and *MAS*) and flavonoid (upregulation of *CYP75B3* and *CYP75B4*) pathways. These data suggest that loliolide acts as a soil-borne signal that enhances allelochemical production to coordinate plant–plant interaction.

The volatile apocarotenoid α-ionone was recently reported as a protective molecule against plant herbivores (Murata *et al.*, 2019a). Tomato plants pretreated with α-ionone vapor decreased the survival rate and egg deposition of western flower thrips (Figure 6e, left and middle panel) without exerting insecticidal activity, as was previously reported for β-ionone and loliolide. In addition, Arabidopsis plants pretreated with α-ionone vapor showed a

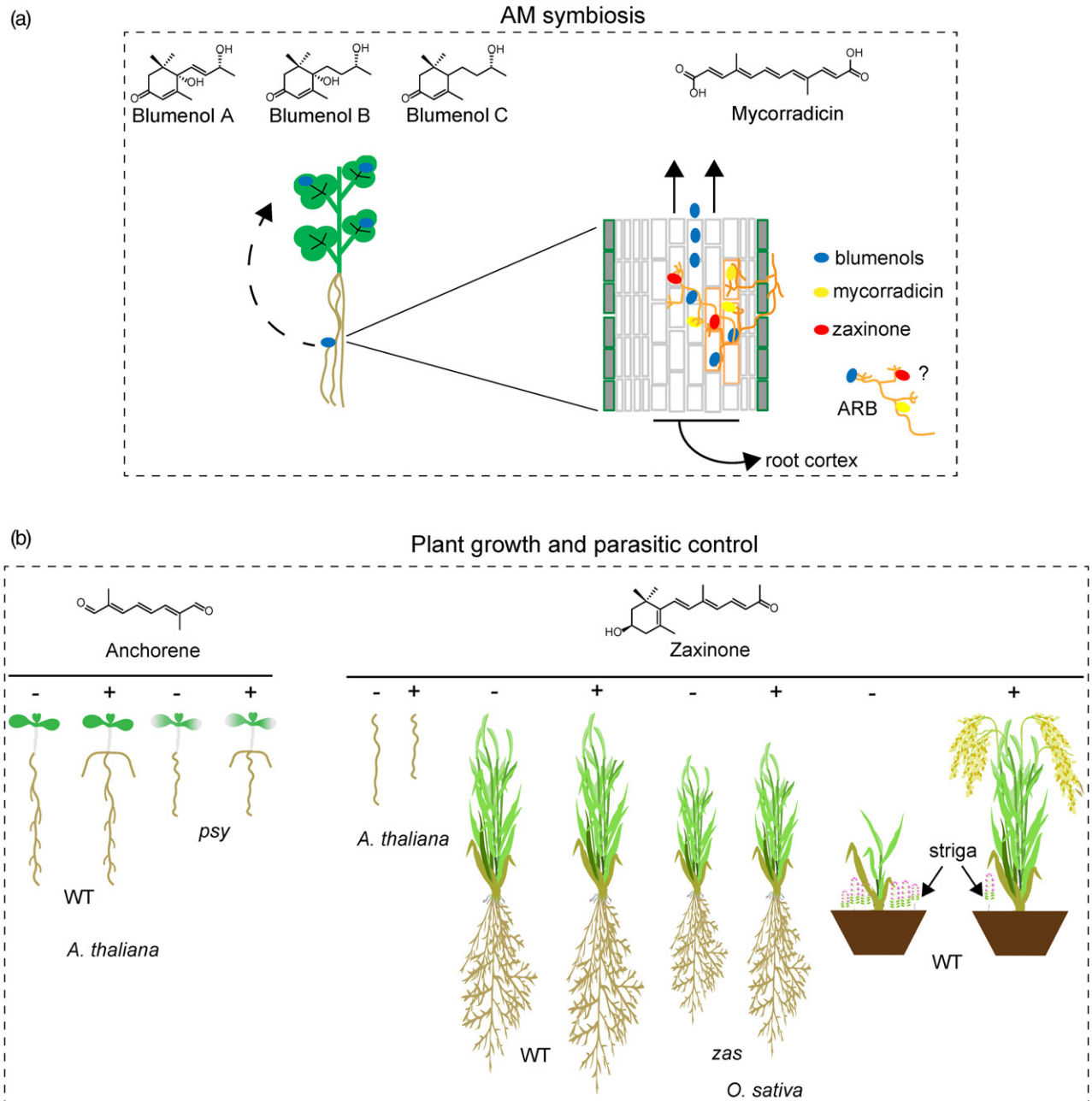

Figure 7. Apocarotenoids are involved in arbuscular mycorrhiza (AM) symbiosis and plant growth and development.
(a) AM symbiosis in the *Medicago truncatula* showing how blumenols produced in the root can be transported to the leaves to serve as an AM fungi colonization marker. Blumenols and mycorradicins are synthesized in the AM and contribute to its establishment and proper function. The involvement of zaxinone in this process has been reported, but its function is not fully characterized. ARB, arbuscule.
(b) Exogenous anchorene application into Arabidopsis roots stimulates the development of anchor roots. Exogenous zaxinone application to Arabidopsis roots reduces hypocotyl growth, while its application in rice promotes root and shoot growth and reduces *Striga* infestation.

decreased survival rate of common cutworm (Figure 6e, left and middle panel). Opposite to JA or loliolide, exogenous application of α-ionone induced the expression of *GLUB* and *BASIC CHITINASE* (*CHI9*) genes, suggesting a different mechanism of action than JA or loliolide.

Interestingly, exogenous β-cc application in African spider plants exhibited protective properties against the two-spotted spider mite, and also anti-pathogenic properties against the oomycete *Plasmopara viticola* in grapevines (Nyalala *et al.*, 2013; Lazazzara *et al.*, 2018). This suggests

that β-apocarotenoid volatiles might have multiple functions affecting plant physiology.

Cyclic and acyclic apocarotenoids involved in AM symbiosis

Approximately 70% of all higher plants establish symbiotic associations with AMF (Fiorilli *et al.*, 2015, 2019; Brundrett and Tedersoo, 2018; Lanfranco *et al.*, 2018). This symbiotic association secures phosphorus and nitrogen for the plant and reduced carbon for the fungus (Helber *et al.*, 2011; Bravo *et al.*, 2017; Jiang *et al.*, 2017; Keymer *et al.*, 2017; Luginbuehl *et al.*, 2017). AM symbiosis influences plant growth (Rooney *et al.*, 2009; Adolfsson *et al.*, 2015) and confers tolerance to abiotic and biotic stresses (Pineda *et al.*, 2010; Vannette *et al.*, 2013; Adolfsson *et al.*, 2015; Sharma *et al.*, 2017). AMF colonization establishment and maintenance have been associated with specific apocarotenoids (Figure 7a) (Walter *et al.*, 2007; Floss *et al.*, 2008a,b; Hill *et al.*, 2018; Wang *et al.*, 2018; Fiorilli *et al.*, 2019), which accumulate after AMF inoculation (Schweiger *et al.*, 2014; Aliferis *et al.*, 2015; Adolfsson *et al.*, 2017). Based on their structure, these apocarotenoids can be divided into two types, i.e., (i) so-called blumenols (C_{13} derivatives), and (ii) so-called mycorradicins (C_{14} derivatives) (Figure 7a) (Hill *et al.*, 2018; Wang *et al.*, 2018). The two types arise through sequential, two-step cleavage, most likely from a C_{40} carotenoid precursor. Studies using *ccd7* mutants in tomato and peas suggested the involvement of CCD7 in cleaving the C_{40} carotenoid precursor to form a C_{27} apocarotenoid and C_{13}cyclohexenone, β- or α-ionone (Vogel *et al.*, 2010; Walter *et al.*, 2010). In the next step, the C_{27} apocarotenoid is cleaved by CCD1, leading to rosafluene-dialdehyde (C_{14}), the mycorradicin precursor, and another cyclohexanone (C_{13}) (Floss *et al.*, 2008b; Walter *et al.*, 2010; Hou *et al.*, 2016). Blumenols are C_{13} cyclohexanone derivatives that accumulate in the roots of AFM-colonized plants in direct correlation with the fungal colonization rate (Maier *et al.*, 1995; Fester *et al.*, 1999; Walter *et al.*, 2000; Strack and Fester, 2006). Although information about blumenols is scarce, recently, a group of blumenols was found in shoots and roots (Figure 7a) of mycorrhizal plants (e.g., tomato, barley and potato) using a combination of targeted and untargeted metabolomics (Wang *et al.*, 2018). This group is composed of five blumenols (11-hydroxyblumenol C-9-*O*-Glc, 11-carboxyblumenol C-9-*O*-Glc, 11-hydroxyblumenol C-9-*O*-Glc-Glc, blumenol C-9-*O*-Glc-Glc and blumenol C-9-*O*-Glc), and their abundance correlates with the AMF colonization rate, as reflected in changes in the transcript profile of canonical mycorrhization marker genes. However, their biological effect is not clear. For the moment, blumenols can be used as foliar markers that allow rapid detection of AM symbiosis and for the screening of functional AMF associations (Wang *et al.*, 2018). Experiments to find their receptors, or generation of mutant lines with reduced/increased blumenol content, or exogenous treatments are required to shed light on the biological effect of these compounds.

Mycorradicins were detected as a mycorrhizal-specific apocarotenoid mixture causing a yellow/orange pigmentation of mycorrhizal roots after AMF colonization (Scannerini and Bonfantefasolo, 1977; Klingner *et al.*, 1995a,b; Floss *et al.*, 2008a). Genetic evidence based on knockdown mutants affected in carotenoid metabolism demonstrated a positive correlation between C_{13} and C_{14} apocarotenoid content and mycorrhizal functionality. These apocarotenoids are synthesized in fungal arbuscules (Figure 7a), which contains the enzymes for their biosynthesis (Fester *et al.*, 2002a,b; Hans *et al.*, 2004; Walter *et al.*, 2007; Walter *et al.*, 2010). In *M. truncatula*, AMF colonization induced the 1-DEOXY-D-XYLULOSE 5-PHOSPHATE SYNTHASE 2 (*Mt*DXS2) expression but not *MtDXS1*, which correlated with the accumulation of C_{13} and C_{14} apocarotenoids. Moreover, transgenic roots with repressed *MtDXS2* expression showed a reduction in the level of both apocarotenoid types as well as in mycorrhization and the expression level of plant AM marker genes (e.g., *phosphate transporter MtPT4*). The reduction in expression of AM-induced markers was accompanied by an increased ratio of degenerating/dead arbuscules to mature arbuscules. Interestingly, the reduction in the *MtCCD1* expression level was reflected in differential reductions of C_{13} (30–47% residual expression) and C_{14} (3–6% residual expression) apocarotenoids. In contrast with the *MtDXS2* mutant, *MtCCD1* reduction did not cause major alteration in AM molecular markers but led to a moderate increase in the relative ratio of degenerated arbuscules. These phenotypes indicate a more prominent role for C_{13} (blumenols) than C_{14} (mycorracidins) derivatives in AM establishment and functioning. Accumulation of blumenols that derive from β- or α-ionone cleavage products was also detected in roots after AMF inoculation (Maier *et al.*, 1995; Walter *et al.*, 2000; Strack and Fester, 2006).

The rice ZAS is a CCD representing an emergent clade in the plant CCD family. *In vitro* studies showed that this enzyme forms the apocarotenoid zaxinone that acts as a growth regulator (s. Zaxinone). The loss-of-function *Oszas* mutant shows a higher SL content and reduced root and shoot growth. Despite the high SL content, this mutant displays a lower level of AM colonization and no changes in arbuscule morphology when compared with the wild type. Moreover, *OsZAS* expression is induced during early (7-day post-inoculation) and during later stages (35-day post-inoculation) of mycorrhizal colonization in rice roots, indicating its involvement in mycorrhization (Fiorilli *et al.*, 2015; Wang *et al.*, 2019) (Figure 7a). The latter assumption is supported by the absence of *ZAS* orthologs in genomes of non-AM plants (e.g., Arabidopsis) (Wang *et al.*, 2019).

However, more evidence is needed to understand the specific function of zaxinone in AM symbiosis.

Anchorene

Anchorene belongs to a group of less-studied carotenoids, the so-called diapocarotenoids. Diapocarotenoids can be found in some bacteria and plants (Giuliano *et al*., 2003; Perez-Fons and Fraser, 2012; Frusciante *et al*., 2014; Demurtas *et al*., 2018). They are formed by the conjugation of two C_{15} molecules (farnesyl diphosphate) and are usually known as 4,4′-diapocarotenoids (e.g., C_{30} diapophytoene and C_{30} diapolycopene) (Perez-Fons and Fraser, 2012). Further oxygenation, glycosylation, methylation or acyl transfer reactions can greatly diversify diapocarotenoid species. The diapocarotenoid anchorene (C_{10}) is found in several plant species (e.g., tomato, carrot, spinach). Although it seems possible that the cleavage of C11–C12 and C11′–C12′ double bonds from carotenoids downstream of ζ-carotene results in the production of anchorene, it is still unclear how it is formed (Jia *et al*., 2019; Mi *et al*., 2019). Anchorene was shown to promote the development of ANR (Figure 7b, left panel) (Jia *et al*., 2019). ANR develop from the collet region situated at the root hypocotyl junction (Lucas *et al*., 2011). The regulation of ANR development by anchorene seems to be structure-dependent as (i) exogenous application of structural isomers showed no effect on ANR development, and (ii) functional group substitutions in the anchorene molecule led to the loss of activity (Jia *et al*., 2019). Interestingly, the carotenoid-deficient Arabidopsis *psy* mutant is deficient in ANR formation; however, this phenotype can be rescued by external anchorene application (Figure 7b, left panel). This result suggests that ANR formation requires a signal derived from carotenoids, which might be the anchorene in this case. Further investigation of anchorene activity demonstrated an effect on auxin homeostasis and indicated its involvement in nitrogen deficiency response (Jia *et al*., 2019).

Zaxinone

Recent phylogenetic analysis of plant CCD orthologs led to the discovery of ZAS, which is present in many plants but not in Arabidopsis or other members of *Brassicaceae* (Wang *et al*., 2019). *In vitro* characterization of this CCD member in rice revealed that ZAS cleaves a molecule of apo-10′-zeaxanthinal (3-OH-β-apo-10′-carotenal, C_{27}) at the C13–C14 double bond, generating a C_{18}-ketone (zaxinone) and an unstable C_9-dialdehyde (Wang *et al*., 2019). Interestingly a *Zea mays* CCD10a was recently shown to cleave phytoene, lycopene, δ-carotene, ε-carotene and β-carotene, producing C_8 (6-methyl-5-hepten-2-one) and C_{13} (geranylacetone, α-ionone and β-ionone) apocarotenoids when expressed in *E. coli* (Zhong *et al*., 2020). However, in that study, the authors did not quantify zaxinone in a catalytic reaction involving the *Zm*CCD10a; thus, ZAS activity remains to be determined.

Zaxinone is produced by various plant species, including rice (Mi *et al*., 2018; Wang *et al*., 2019). Exogenous application of zaxinone to rice increased overall growth and biomass, while the corresponding mutant (*Oszas*) showed growth retardation (Figure 7b, right panel) (Wang *et al*., 2019). Compared with wild type, *Oszas* mutant contains a similar level of zaxinone in leaves but decreased content in roots, confirming the ZAS enzymatic activity *in vivo* but also suggesting an additional zaxinone biosynthetic route (s) (Wang *et al*., 2019). In addition, *Oszas* mutants showed reduced crown root length and number, lower tiller and panicle number (Wang *et al*., 2019). The hormone quantification analysis revealed that *Oszas* roots and root exudates have enhanced SL content compared with the wild type. The exogenous application of zaxinone to the *Oszas* mutant reverted most of the mutant growth phenotypes and reduced SL level and impaired its release. Moreover, zaxinone application resulted in the decreased transcript level of SL biosynthetic genes such as *OsDWARF 27/D27*, *OsCCD8/D10*, *OsCCD7/D17* and *OsCARLACTONE OXIDASE/CO*, confirming its role as a negative regulator of rice SL biosynthesis. In addition, exogenous zaxinone application reduced the *Striga* infestation in a *Striga*-susceptible rice variety by decreasing the amount of released SLs (Figure 7b, right panel). This suggests a potential application in alleviating *Striga* infestation and improving crop growth (Wang *et al*., 2019). In contrast to rice, a very recent publication by Ablazov *et al*. showed that zaxinone is a positive regulator of SL and ABA biosynthesis and does not promote Arabidopsis root growth (Ablazov *et al*., 2020). The same study also showed that exogenous zaxinone application reduces Arabidopsis hypocotyl growth by increasing ABA content (Ablazov *et al*., 2020). The presence of zaxinone in Arabidopsis and its effect on hormone homeostasis shows the occurrence of ZAS-independent routes for zaxinone synthesis and indicates a general growth regulatory activity in AM and non-AM plant species. Interestingly, phenyl-based compounds mimicking zaxinone activity were recently synthesized (Wang *et al*., 2020a). Mimics of zaxinone (MiZax) MiZax3 and MiZax5 showed zaxinone activity. These compounds rescued the root growth phenotype of a (zaxinone-deficient) rice mutant, by promoting growth, and reduced SL accumulation in roots and root exudates of wild-type plants (Wang *et al*., 2020a). MiZax might be a valuable tool to study zaxinone function and its involvement in rice development and growth-related processes further. The use of MiZax in agricultural applications might be an alternative approach in combating *Striga* infestation.

CONCLUDING REMARKS

Carotenoids are isoprenoid compounds that serve as precursors of a wide variety of signaling molecules (e.g.,

apocarotenoids) and hormones involved in almost all aspects of plant physiology and development. Plant apocarotenoids are produced from either spontaneous carotenoid oxidation or enzymatic-assisted cleavage, carried out by the CCDs. Apocarotenoids might act as immediate signaling molecules (β-cc) or growth regulators (zaxinone), while further modifications of some apocarotenoids result in the production of the phytohormones ABA and SLs. However, ACS properties and their ability to modulate plant physiology, architecture and development resemble the functions of phytohormones and raise the question of whether these small molecules are indeed phytohormones. In fact, some of them act independently of canonical hormone signaling pathways (e.g., β-cc and dhA), while others interact with hormones to fulfill their function (e.g., anchorene, zaxinone). Considering these functions, the recent discoveries and further characterization of ACS molecules/hormone candidates may shed light on the mechanistic explanation of the emergence of these previously overlooked phenotypes related to plant development, stress tolerance and biotic interactions. Nevertheless, whether apocarotenoids are hormones or hormone-like molecules, the characterization of the mechanism of action of these molecules will contribute to the understanding of the plant's life and its interaction with the environment.

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AUTHOR CONTRIBUTIONS

JM: wrote the SLs section and designed Figures 1, 3, and 4. YA: wrote LCDAs and ABA sections and designed Figures 2 and 5. JCM: wrote the abstract, introduction (with YA), cyclic apocarotenoids with signaling properties chapter, and designed Figures 6 and 7. JM and YA: prepared the figures with the input of JCM and SAB, JCM, and SAB structured the manuscript and prepared the final version with substantial input from JM and YA

CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

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