On the Biosynthesis and Evolution of Apocarotenoid Plant Growth Regulators

Jian You Wang^{a,1}, Pei-Yu Lin^{a,1} and Salim Al-Babili^{a,*}

Affiliations:

^a The BioActives Lab, Center for Desert Agriculture (CDA), Biological and Environment Science and Engineering (BESE), King Abdullah University of Science and Technology, Thuwal, Saudi Arabia.

¹These authors contributed equally.

* Correspondence to: salim.babili@kaust.edu.sa

Abstract

Carotenoids are an important source of metabolites with regulatory function, which include the plant hormones abscisic acid (ABA) and strigolactones (SLs), and several recently identified growth regulators and signaling molecules. These carotenoid-derivatives originate from oxidative breakdown of double bonds in the carotenoid polyene, a common metabolic process that gives rise to diverse carbonyl cleavage-products known as apocarotenoids. Apocarotenoids exert biologically important functions in all taxa. In plants, they are a major regulator of plant growth, development and response to biotic and abiotic environmental stimuli, and mediate plant's communication with surrounding organisms. In this article, we provide a general overview on the biology of plant apocarotenoids, focusing on ABA, SLs, and recently identified apocarotenoid growth regulators. Following an introduction on carotenoids, we describe plant apocarotenoid biosynthesis, signal transduction, and evolution and summarize their biological functions. Moreover, we discuss the evolution of these intriguing metabolites, which has not been adequately addressed in the literature.

Keywords: carotenoid, apocarotenoid, strigolactone, abscisic acid, zaxinone, β -cyclocitral, anchorene, plant growth regulators, plant hormones

31

32 **1. Introduction**

33 Carotenoids are common lipophilic isoprenoid pigments produced by all photosynthetic organisms
34 and numerous non-photosynthetic microorganisms [1, 2]. They are indispensable components of the
35 photosynthetic apparatus, as they protect it and other cellular components from photo-oxidation,
36 and contribute to the light-harvesting process by absorbing light in the blue-green region [1, 3].
37 Carotenoids are important antioxidants also in non-photosynthetic organisms, including humans. In
38 addition, they stabilize membranes, and are responsible for the bright colors of many fruits, flowers,
39 birds, fishes, and crustaceans [1, 2]. Animals do not synthesize carotenoids *de novo* but obtain them
40 from their diet [1, 3].

41 Generally, carotenoids are defined by a common C₄₀ polyene backbone carrying 3 to 11 conjugated
42 double bonds with different stereo-configurations. Introduction of end-ring structures,
43 hydroxylation, oxygenation, and further derivatization give rise – together with the polyene
44 geometry – to the large structural diversity of the more than 700 known carotenoids [3]. Plants
45 utilize two routes for isopentenyl diphosphate (IPP, C₅) synthesis: the cytosolic mevalonate and the
46 plastid methylerythritol phosphate pathway (MEP) pathway [3], which form the precursor for
47 cytosolic (e.g. sterols), and plastid isoprenoids (e.g. carotenoids), respectively. The colorless
48 carotenoid, 15-*cis*-phytoene (C₄₀), which contains three conjugated double bonds, results from
49 condensation of two molecules of geranylgeranyl diphosphate (GGPP, C₂₀). The latter is formed
50 through condensation of IPP and its isomer dimethylallyl diphosphate (DMAPP) [3, 4].
51 Desaturation and isomerization reactions, catalyzed in plants and cyanobacteria by four different
52 enzymes but only by one in fungi and non-photosynthetic bacteria, transform phytoene into the red
53 all-*trans*-lycopene, a linear C₄₀ polyene with eleven conjugated double bonds. There is some
54 indication that *cis*-configured lycopene precursors, which can accumulate in non-photosynthetic
55 tissues or in leaves of corresponding mutants in prolonged darkness, produce signaling molecules
56 determining different aspects of plant development and regulating the carotenoid biosynthesis

57 pathway itself, which explains why plants produce them and require the activity of four enzymes,
58 instead of one [1, 5, 6, 7].

59 Lycopene is the substrate of cyclases that introduce terminal ring structures, giving rise to β - (Fig.1)
60 and α -carotene and the corresponding two branches in plant carotenogenesis. Hydroxylation of β -
61 and α -carotene leads to zeaxanthin and lutein, respectively. Repeated epoxidation of the two β -rings
62 in zeaxanthin yields violaxanthin via the mono-epoxy carotenoid antheraxanthin and builds together
63 with the reverse, de-epoxidation reactions the xanthophyll cycle, a protection mechanism that
64 regulates the level of the particularly photoprotective zeaxanthin [8]. Finally, violaxanthin is
65 converted into neoxanthin that carries an allenic double bond (Fig.2). The carotenoid pattern in
66 different plant cells and tissues is determined by the type of plastids. Chloroplasts contain high
67 levels of carotenoids composed of around 45% lutein, 25–30% β -carotene, and 10–15% each of
68 neoxanthin and violaxanthin, while root leucoplasts harbor only small amounts with a higher ratio
69 of β -branch carotenoids [8].

70 The polyene structure of carotenoids responsible for their role in photosynthesis, anti-oxidation
71 capacity, and color makes them vulnerable to oxidation that splits double bonds, forming carbonyl
72 products called apocarotenoids (designated based on the C-atom number at the cleavage site, Fig.
73 1). This cleavage can occur at any of the polyene double bonds, which explains, together with
74 subsequent modifications, the structural diversity and different physicochemical properties of
75 natural apocarotenoids ranging from the tomato fragrance 6-methyl-5-hepten-2-one (C_8) to colorful
76 pigments [9], such as β -citraurin in citrus fruits (C_{30}) or neurosporaxanthin (C_{35}) in the fungus
77 *Neurospora crassa* [10]. Apocarotenoids are an important class of compounds [8, 11], which
78 includes retinal, vitamin A, signaling molecules, e.g. the vertebrate morphogen retinoic acid, the
79 fungal pheromone trisporic acid, the plant hormones abscisic acid (ABA) and strigolactones (SLs),
80 and known or postulated plant growth regulators [12, 13, 14]. Examples for the latter are β -
81 cyclocitral [15], zaxinone [16], and anchorene [17].

82 Carotenoid cleavage can be catalyzed enzymatically or by reactive oxygen species (ROS). These
83 pigments are continuously oxidized and cleaved in chloroplasts, particularly under high light that
84 increases the formation of ROS, leading to different products, including the stress signal β -
85 cyclocitral [15]. It may be hypothesized that susceptibility to oxidation makes carotenoids an
86 excellent sensor for oxidative stress, which may have been the reason for recruiting apocarotenoids
87 as signals during evolution. The “spontaneous” formation of apocarotenoid signals may have
88 become tightly regulated by evolving cleavage enzymes, mainly members of the carotenoid
89 cleavage dioxygenase family, which target specific double bonds in defined carotenoid or
90 apocarotenoid substrate [18, 19]. Involving enzyme-catalyzed, regulated cleavage may have
91 increased the sensitivity of the system and allowed using the concept of carotenoid cleavage as a
92 sensor for other stimuli, paving the way for the emergence of apocarotenoid plant hormones.
93 Although there is currently no experimental evidence for this scenario, several studies show an
94 increase in CCD-catalyzed apocarotenoid formation in cyanobacteria under stress conditions, e.g.
95 high-light stress [20, 21]. Apocarotenoid volatiles, including β -ionone, released by cyanobacteria,
96 are also allelopathic agents affecting the growth of several cyanobacterial species [22, 23].
97 In this article, we provide an overview on the function, biosynthesis, and signaling of the known
98 carotenoid-derived hormones ABA and SLs, and describe the role of the recently identified
99 apocarotenoid growth regulators zaxinone, cyclocitral, and anchorene in plant growth and
100 development. In addition, we shed light on the evolution of plant apocarotenoid signaling
101 molecules, which has not been covered in recent literature.

102

103 **2. Absciscic acid**

104 **2.1 Functions of ABA in plants**

105 ABA was discovered in the early 1960s, due to its role in regulating senescence and stress
106 responses in abscising organs. Since then, ABA has been shown to determine many aspects of plant
107 growth and development, which include seed maturation, germination and dormancy, seedling

108 growth, bud dormancy, shoot branching, and leaf senescence [24], and to be key in stress response
109 and regulating water loss through the control of stomatal closure [25]. This regulation was probably
110 a major factor in the evolution of land plants, ensuring the survival under fluctuating water supply
111 [26, 27]. Indeed, ABA is intricately involved in the adaptation of terrestrial plants to abiotic stress
112 conditions, such as water scarcity, increased salinity and extreme temperatures [24, 28], which also
113 includes modulation of plant architecture, e.g. establishing the appropriate root architecture under
114 drought conditions [24] (Fig.1). Moreover, ABA plays a role in the establishment of fungal
115 symbiosis [29, 30] and is a major component in plant-pathogen response [31].

116

117 **2.2 ABA biosynthesis and signaling**

118 ABA is a sesquiterpene (C₁₅) that occurs in a defined stereo-configuration in nature [(+)*cis*, *trans*-
119 ABA] (Fig.1). It can be synthesized either “directly” from farnesyl diphosphate (C₁₅) that arises
120 from the condensation of IPP with geranyldiphosphate (C₁₀), or “indirectly” by cleaving carotenoid
121 precursors [25, 32]. Plants utilize the latter route starting as yet poorly understood isomerization of
122 all-*trans*-violaxanthin/-neoxanthin into the corresponding 9-*cis*-/9'-*cis*-isomer, respectively, which
123 are cleaved by nine-*cis*-epoxycarotenoid cleavage dioxygenases (NCEDs) at the C11'-C12' or C11-
124 C12, respectively, to form the ABA precursor xanthoxin (C₁₅) and the corresponding apo-12'-
125 /apo12-carotenoid (C₂₅) [11, 25]. Xanthoxin is then translocated into the cytoplasm, where it is
126 converted by short-chain dehydrogenase reductase (SDR) into abscisic aldehyde. Oxidation of the
127 latter by abscisic aldehyde oxidase (AAO), an enzyme requiring a molybdenum cofactor provided
128 by the sulfurase ABA3, leads to ABA (Fig.2) [25]. A number of phytopathogenic fungi, e.g.
129 *Botrytis cinerea* [32], synthesize ABA – that might affect the immune response in plants - in the
130 cytosol through the cyclization of farnesyl diphosphate and subsequent oxidation steps in a “direct”
131 pathway [33]. This cytosolic route is likely used by ABA producing animal cells, including human
132 cells, which lack carotenoid biosynthesis (see ABA occurrence and evolution).

Besides biosynthesis, plant ABA levels are determined by conjugation and hydroxylation [28]. The conjugation of ABA by the uridine diphosphate glucosyltransferases (UGTs) leads to inactive ABA-glucose ester (ABA-GE) that can be considered as a storage or long-distance, root-to-shoot transport form [25]. Under stress conditions, ABA-GE can be rapidly hydrolyzed into ABA by β -glucosidase and released from vacuoles, providing a fast response to environment changes [34, 35]. ABA can be deactivated by cytochrome P450 707A enzymes (CYP 707), forming unstable 8'-OH-ABA that spontaneously isomerizes to phaseic acid (PA). PA is further catalyzed to the fully inactive dihydrophaseic acid (DPA) and DPA-4-O- β -D-glucoside (DPAG) by the soluble PA reductase and glucosyltransferase, respectively [36]. Interestingly, PA can still act as a low activity hormone and be recognized by some ABA receptors in seed plants [36].

In angiosperms, the core of ABA perception and signaling pathway comprises three major components: the receptor proteins PYRABACTIN RESISTANCE (PYR)/PYR1-LIKE (PYL)/REGULATORY COMPONENT OF ABA RECEPTOR (RCAR) (hereafter referred to as PYL), TYPE 2C PROTEIN PHOSPHATASE (PP2C), and SNF1-RELATED PROTEIN KINASE 2 (SnRK2) [25, 37, 38]. In the absence of ABA, PP2C prevents the SnRK2 kinase activity by dephosphorylating the kinase-activating loop; while in ABA presence, PYL recruits PP2C to form a complex and release SnRK2 kinase activity that stimulates the transcription factors AREB/ABF [ABA-RESPONSIVE ELEMENT (ABRE)-BINDING PROTEIN/ABRE-BINDING FACTOR], including a basic-domain leucine zipper (bZIP) family and the B3-type transcription factor ABA-INSENSITIVE 3 (ABI3), triggering the expression of ABA-responsive genes [39, 40, 41, 42] (Fig.3). This signaling pathway is highly conserved in plants employing ABA for regulating stress response and development [43]. Stomatal closure is caused by the increase in ABA concentration in guard cells, which leads to the formation of the PYL/PP2C complex and the release of the OPEN STOMATA 1 (OST1; SnRK-III) kinase, resulting in the phosphorylation of several target proteins and the activation of plasma membrane anion channels. The latter triggers membrane depolarization

158 followed by K^+ efflux, which decreases turgor pressure, causing stomatal closure (for review, see
159 [44]).

160 The translocation of ABA between tissues and organs is required for systemic stress response [25].
161 ABA exists as both an anionic ABA^- and a protonated ABAH that can passively diffuse across the
162 cell membrane in a limited amount [45]. Due to a pK_a of 4.7, most ABA in the cytosol ($pH \approx 7.3$)
163 occurs in the non-diffusible ABA^- , which creates a need for active transport. The ATP-BINDING
164 CASSETTE G (ABCG), ABA-IMPORTING TRANSPORTER 1 (AIT1), MULTIDRUG AND
165 TOXIC COMPOUND EXTRUSION (MATE)-type/DTX transporters (DTX50), and AWP1-19
166 family proteins (e.g. the rice OsPM1) transporters [45, 46, 47] can actively deliver ABA to specific
167 tissues in response to developmental and environmental cues (reviewed in [45]). ABA transporters
168 play a key role in plant seed germination, root development, and transpiration [25, 45].

169

170 2.3 Occurrence and evolution of ABA

171 ABA has been found among almost all clades of organisms, including cyanobacteria, algae, lichens,
172 sponges, plants, and mammals [48, 49]. However, our knowledge about the biological function,
173 biosynthesis, and possible signaling pathways in lower photosynthetic and non-photosynthetic
174 organisms is quite limited. In lower photosynthetic organisms, several studies connect ABA to
175 abiotic stress [48, 50]. It was also shown that exogenous ABA modulates *Chlamydomonas*
176 *reinhardtii* HCO_3^- uptake and that ABA concentration determines the position of this aquatic algae
177 in the water column depending on light intensities, which could be one of the original functions of
178 this hormone in regulating tropisms [50].

179 In plant ABA biosynthesis, the most crucial step is the carotenoid cleavage reaction catalyzed by
180 NCEDs, which are absent in green algae, but present together with their epoxy-substrates (Fig. 2) in
181 bryophytes, such as *Physcomitrella patens* [48, 51, 52]. Hence, it has been supposed that the
182 NCED-mediated-“indirect” pathway evolved during land colonization [52]. The comparative

183 genomic analysis and functional divergence analysis of NCEDs revealed that duplication and
184 divergence have occurred at the base of lycophytes to angiosperms, and tissue-specific functional
185 divergence of NCED subfamilies was developed [53]. This evolutionary event was probably
186 followed by the CYP707A-mediated ABA degradation, as no encoded *CYP707A* orthologues have
187 been found in *P. patens* [48, 54].

188 Despite the presence of ABA, plant ABA-mediated PYL receptors are absent in most algae, except
189 *Zygnema circumcarinatum* that contains one *PYL* orthologue named *ZcPYL8* that can inhibit
190 Arabidopsis PP2C independent of ABA [27]. In bryophytes, *PYL* orthologues of liverwort
191 *Marchantia polymorpha* and moss *P. patens* demonstrate both ABA-independent and ABA-induced
192 activities, while in vascular plants, *PYL* orthologues of lycophyte *Selaginella moellendorffii* and
193 Arabidopsis have diverged into two groups, exerting both activities or merely an ABA-induced one
194 [27]. The phylogenetic analysis demonstrated that *PYLs* have diverged into two major clades in
195 vascular plants, suggesting a duplication after the separation from bryophytes [55]. Briefly, ABA
196 receptor *PYL* originated from an ABA-independent algal ancestor diversified and duplicated into
197 two major clades with ABA-independent or ABA-induced activity in the tracheophytes [27].

198 The number of *PP2C* genes increased from one in the green algae *C. reinhardtii* to 80 in
199 Arabidopsis, suggesting repeated gene duplication events that accompanied the emergence of the
200 *PP2C*-SnRK2 regulation during land colonization [56, 57]. Arabidopsis *PP2C* genes are classified
201 into ten subgroups (A–J) with diverse functions [58]. For example, subgroup A contains genes
202 involved in ABA signaling, such as *ABI1*, while subgroup B mediates stress-induced MAPK
203 pathway [58]. Subgroup A is already present in green algae, but not in prokaryotes or non-plant
204 eukaryotes, while subgroup B exists in lycophytes and higher plants, indicating both subgroups are
205 specifically evolved *in planta* [56]. The disruption of both subgroup A *PP2CA* homologs in the
206 moss *P. patens* led to ABA hypersensitivity; however, ABA-dependent kinases were slightly
207 activated, indicating moss *PP2CA* is not the primary factor in releasing their activity, in contrast to
208 the angiosperm *PP2CA*-SnRK2 signaling [59].

209 In angiosperm, SnRK2 proteins are presented by three subfamilies (I–III) that are responsible for
210 ABA signaling pathway. Subfamily III is the ancestral one and exists in early-diverging bryophytes
211 [60, 61]. Interestingly, a single copy gene of subclass III *SnRK2* is already present in the
212 charophyte algae *K. nitens*, indicating that subfamily III SnRK2 signaling might have emerged
213 before ABA response during land colonization [60, 61].

214 Promoters of ABA-inducible genes contain a conserved ABRE motif allowing the binding of ABA-
215 dependent transcription factors, such as bZIP transcription factors [43]. A phylogenetic analysis of
216 bZIPs clustered Arabidopsis ABA-regulated bZIPs together with putative orthologues in the
217 charophyte algae *K. nitens* and the moss *P. patens*, revealing that ABA-regulatory functions
218 appeared early during land colonization that was accompanied by an expansion of bZIP families
219 during terrestrialization [60]. Similarly, B3 family transcription factors, such as ABI3, continuously
220 expanded from 2 members in chlorophyte *C. reinhardtii* to 66 in angiosperm Arabidopsis [60].
221 Although multiple ABI3-like members emerged during this expansion, phylogenetic analysis
222 demonstrates that only a single ABI3 is present in tracheophytes, indicating that a purge of ABI3-
223 like has occurred but a single ancient ABI3 has been maintained during evolution [60].

224

225 3. Strigolactones

226 3.1 Biological functions of SLs

227 SLs were firstly discovered in root exudates as germination stimulants of root parasitic plants of the
228 *Orobanchacea* family, which infest many crops, including cereals and *Solanaceae* species, causing
229 tremendous yield losses in temperate and warm zones [62, 63]. Afterward, SLs were shown to play
230 an important role in establishing arbuscular mycorrhizal (AM) symbiosis, by inducing fungal spore
231 germination, hyphal branching and elongation, and hyphopodia formation [64, 65], and to
232 contribute to further interactions with microorganisms, including symbiotic rhizobia [65]. The role
233 of SLs in establishing AM symbiosis explains their release by plants [65] (Fig.1).

234 Characterization of high-branching/-tillering mutants in different species, including *more axillary*
235 *growth (max)* in Arabidopsis, *ramosus (rms)* in pea, *dwarf (d)/high-tillering dwarf (htd)* in rice, and
236 *decreased apical dominance (dad)* in petunia, unraveled the hormonal function of SLs [4, 66].
237 Indeed, SLs are a major regulator of plant architecture. This hormone inhibits axillary bud
238 outgrowth through either an auxin-dependent or auxin-independent pathway [66, 67, 68], triggers
239 internode elongation through induction of cell division instead of cell elongation [69, 70, 71], and
240 regulates lateral root growth, root hair elongation, and adventitious root formation [24]. Moreover,
241 SLs are a positive regulator of leaf and floral organ senescence and contribute to biotic and abiotic
242 stress response [66] (Fig.1).

243

244 3.2 SL biosynthesis and signaling

245 Natural SLs are characterized by a methylbutenolide ring (D-ring) linked by an enol ether bridge
246 [(*R*)-configuration] to a tricyclic lactone (ABC-ring) in canonical SLs, including orobanchol-type
247 (C-ring in α -orientation/down) and strigol-type SLs (C-ring in β -orientation/up), or to a different
248 structure in non-canonical ones (Fig.4) [4, 66]. The biosynthesis of SLs starts with the D27-
249 catalyzed isomerization of all-*trans*- β -carotene into 9-*cis*- β -carotene that is cleaved by the
250 carotenoid cleavage enzyme CCD7 to produce 9-*cis*- β -apo-10'-carotenal and β -ionone [72, 73].
251 Next, CCD8 converts 9-*cis*- β -apo-10'-carotenal through a combination of reactions into the central
252 intermediate carlactone (CL) (Fig.4) that already contains the D-ring characteristic for SLs [74]. CL
253 biosynthesis is conserved in land plants, as shown for *P. patens* [75]. CCD8 enzymes can also
254 produce a C3-hydroxylated CL, which might be a precursor of yet unidentified SLs, from the
255 corresponding precursor [76]. CL is transported into the cytosol where it is converted by CYP
256 enzymes, in particular the Arabidopsis MORE AXILLARY GROWTH1 (MAX1) and its homologs
257 that belong to the 711 clade [4, 77, 78] (Fig.4). MAX1 homologs can be classified into three types
258 (A1, A2, and A3). Type A1 enzymes, including the Arabidopsis AtMAX1 and its orthologues,
259 convert CL into carlactonoic acid (CLA) [79]. Type A2 enzymes, represented by the rice Os900,

transform CL into the 4-deoxyorobanchol (4DO), which involves the generation of CLA and additional oxygenation followed by B/C lactone ring closure [78]. Enzymes of the CYP711A3 type, represented by the rice Os1400, conduct the hydroxylation of 4DO to produce orobanchol [79] (Fig.4). In Arabidopsis, CLA is converted by an unidentified methyltransferase [4, 77, 80] (Fig.4) into methyl carlactonate (MeCLA) that was shown to be oxygenated *in vitro* by LATERAL BRANCHING OXIDOREDUCTASE (LBO), an enzyme required for normal shoot branching in Arabidopsis and a member of the 2-oxoglutarate and Fe-(II)-dependent dioxygenase family, into unidentified product [81]. Recently, it was shown that members of the CYP722C clade from *Solanum lycopersicum/Vigna unguiculata* and *Gossypium arboreum* mediate the direct conversion of CLA into orobanchol and 5-deoxystigol, respectively [82, 83] (Fig.4).

Despite structural diversity, SLs are perceived by the same receptor, DWARF14 (D14), an α/β -hydrolase containing a conserved Ser-His-Asp catalytic triad [84]. Following binding, D14 hydrolyzes the SL ligand into the D-ring that remains covalently bound to the catalytic His residue, forming a covalently linked receptor molecule (CLIM) and releasing the second moiety [85]. CLIM formation is accompanied by changing D14 conformation, which stimulates its interaction with MAX2/DWARF3 (D3), a component of Skp1/Cullin/F-box (SCF)-type E3 ubiquitin ligase [85]. The D14-SCF^{MAX2/D3} complex recruits a subset of transcription factors, such as the Arabidopsis SUPPRESSOR OF MAX2 1-LIKE (SMXL), and triggers their poly-ubiquitination and proteasomal degradation [86, 87, 88] (Fig.5). Thus, the SL signaling cascade relies on regulated protein turnover through the ubiquitin-proteasome pathway, similar to auxin, jasmonic acid, and gibberellic acid [84, 89].

281

282 3.3. Evolution of SL biosynthesis and perception

During the evolution, an ancestor of the enzyme D27, which is already present in some cyanobacteria, chlorophyte, and charophyte algae, encountered gene duplication and differentiation into three types, i.e. *D27*, *D27-like1* (*D27L1*) and *D27-like2* (*D27L2*) [90, 91, 92]. Phylogenetic

286 analyses of *D27*, *D27L1*, and *D27L2* sequences from major land plant groups, charophyte algae,
 287 and appropriate chlorophyte algae demonstrated that duplication of the ancestral *D27L1* lineage at
 288 the basal land plants gave rise to *D27* that represents a neo-functional lineage in embryophytes [91].
 289 CCD7 homologs are also already present in algae. However, sequence and substrate specificity of
 290 algal and land plants' CCD7 are presumably quite different. Indeed, the essential amino acids for
 291 substrate specificity are absent or inconsistent in algal CCD7s [93]. Nevertheless, phylogenetic
 292 analysis of *CCD7-like* sequences from chlorophytes, charophytes, and major land plants did not
 293 reveal significant gene duplications [91]. Phylogenetic analysis of *CCD8* and *CCD8-like* sequences
 294 from major land plants and chlorophyte algae demonstrated that *CCD8* is ancestral in terrestrial
 295 plants [93]. The moss *P. patens ccd8* mutant, which showed striking phenotypes, such as more
 296 caulonema filament branching, is the first genetic evidence for the production of a strigolactone-like
 297 compound in bryophytes [92, 94]. *Ppccd8* phenotypes were restored by a synthetic SL analog *rac*-
 298 GR24 and by complementation with pea *CCD8*, identifying *PpCCD8* as the functional orthologue
 299 of *CCD8* from the land plants [92, 94]. This conclusion was confirmed by the *in vitro* study of *P.*
 300 *patens* *CCD8* enzymatic activity [75].
 301 Similar to *CCD7* and *CCD8*, *MAX1* is present in a single copy in most of the species, indicating a
 302 low frequency of gene duplication during evolution [92]. While *P. patens* and *M. polymorpha*
 303 genomes do not encode any CYP711 enzyme, most of the other mosses and liverworts possess
 304 *MAX1* mainly as a single copy gene [92, 95]. Unlike the other SL biosynthesis genes, *LBO* may
 305 have originated from gene duplication in the ancestral proto-LBO lineage at the base of
 306 spermatophytes, leading to separation of Related to Strigolactone Synthesis (RSS) and LBO clades,
 307 which appear in both angiosperms and gymnosperms [91]. The phylogenetic studies, which suggest
 308 that SL biosynthesis enzymes are ancestral in land plants, support the hypothesis that SL production
 309 is specific to terrestrial plants [93].
 310 The core SL signaling pathway shares high similarity and common components with that of
 311 karrikins, smoke derived compounds that mimic a yet unidentified plant signaling molecule and

312 trigger seed germination in non-parasitic plants [96]. Indeed, the karrikin receptor, KARRIKIN-
313 INSENSITIVE2 (KAI2)/HYPOSENSITIVE TO LIGHT (HTL)/D14-LIKE (D14L), is a close
314 homolog of D14 [97, 98]. Interestingly, KAI2 is present among all the land plants, including
315 bryophytes, while true D14 is absent in mosses and liverworts [92, 93]. This suggests that the
316 emergence of D14 in seed plants maybe a result of gene duplication of *KAI2* during the evolution of
317 land plants [84, 92]. KAI2 contains the conserved α/β -hydrolase catalytic triad [99] and a lid
318 domain, responsible for its interaction with MAX2 [85]. Interestingly, both *kai2* and *max2* mutants
319 show some identical phenotypes, including increased seed dormancy and lower light sensitivity
320 [100]. Furthermore, it has been reported that KAI2 is essential for the establishment of AM
321 symbiosis in rice [101]. These findings suggest that the D14-dependent signal transduction is likely
322 derived from the karrikin signaling pathway, which is supported by the presence of *KAI2-LIKE*
323 genes in charophytes [93, 102]. Indeed, *MAX2* is present – as a single-copy gene – across more than
324 50 species of land plants and charophyte algae [102]. On the contrary, SMXL proteins, the
325 proteolytic target of SL and karrikin response, show more diversification and high complexity in the
326 seed plant lineage [91]. Most of the non-seed plants, including liverworts, mosses, hornworts,
327 lycophytes, and monilophytes, possess one SMXL clade most closely to SMXL1, while
328 gymnosperms and angiosperms contain two or four distinct clades of SMXL proteins, respectively
329 [91]. The two, distinct gymnosperm SMXL clades show around 50% identity with SMXL proteins
330 from non-seed plants, indicating equal conservation of the ancestral SMXL sequence in both of
331 them [91]. In contrast, the four angiosperm SMXL clades show more diversity among each other
332 [91]. Further duplication at the base of eudicots gave rise to two further sub-clades: SMXL78,
333 including AtSMXL7/OsD53 and SMXL8, and SMXL39, including SMXL3 and SMXL9, leading to
334 the basal set of six SMXL proteins in eudicots [91]. The late occurrence of SMXL7/D53 protein
335 corroborates the late origin of canonical SL signaling, particularly in angiosperms, which supports
336 the independent origin of SL perception in mosses [91].

337

338 4. Novel apocarotenoid signaling molecules

339 4.1 Zaxinone, a candidate for a novel plant hormone

340 The biosynthesis of ABA and SLs demonstrates the importance of CCD enzymes and their role in
341 plant responses to environmental and developmental stimuli. Recently, Wang *et al.* conducted a
342 comprehensive survey of *CCD* genes from 69 plant genomes from mosses, ferns to seed plants,
343 which unraveled a new subfamily common in plant species. In *in vitro* studies, a rice representative
344 of this subfamily produced the C₁₈ ketone 3-OH-β-apo-13-carotenone (apo-13-zeaxanthinal) from
345 3-OH-β-apo-10' -carotenal. The product and the enzyme were designated as zaxinone and
346 ZAXINONE SYNTHASE (ZAS), respectively [16] (Fig.1). ZAS orthologues are widely conserved
347 in land plants, distributed in a taxon-dependent manner. Rice and other cereals contain two sub-
348 clusters, indicating that ZAS may have acquired a new function in *Poaceae* [16, 103]. Interestingly,
349 ZAS orthologues are absent in non-mycorrhizal species, such as *Brassicales* species, including
350 *Arabidopsis* and *Camelina sativa* [16]. Although the loss of AM symbiosis apparently occurred
351 independently in a wide range of plant taxa [16], the disappearance of ZAS from their genomes
352 might be a common feature of this evolutionary event. Indeed, a *Tos17*-insertion *zas* mutant showed
353 decreased mycorrhization, retarded growth, less root zaxinone content, and higher SL content and
354 release [16], which indicates an essential role of zaxinone and ZAS in plant growth, development,
355 biotic interactions and SL homeostasis (Fig.1). Accordingly, exogenous application of zaxinone
356 restored several phenotypes of *zas* mutant, promoted root growth in wild-type seedlings, and
357 decreased SL biosynthesis by reducing transcript levels of SL biosynthetic genes [16]. Zaxinone is a
358 common plant metabolite occurring also in plants lacking ZAS genes [16, 104]. Interestingly,
359 zaxinone is also a regulatory metabolite in *Arabidopsis*; however, it promotes there SL and ABA
360 biosynthesis, in contrast to its activity in rice [105]. Indeed, we are at the very beginning of
361 understanding the biology of zaxinone that may be a new carotenoid-derived plant hormone or a
362 precursor thereof.

363

364 4.2 β -cyclocitral, a novel abiotic stress signal and growth regulator

365 β -cyclocitral (C_{10}) is a volatile originating from β -carotene either through singlet oxygen 1O_2 attack
366 in photosynthetic tissues or by lipoxygenases, which utilize carotenoids as co-substrates during
367 fatty acid oxidation, and some CCD enzymes, such as CCD4b in citrus fruits [106, 107, 108, 109]
368 (Fig.1). The formation of cyclocitral in plastids by 1O_2 makes it a suitable candidate for sensing
369 high-light/oxidative stress and communicating it to the nucleus. Indeed, β -cyclocitral acts in
370 Arabidopsis as a retrograde signal modulating the transcription of 1O_2 -regulated genes, via a small
371 zinc finger protein called METHYLENE BLUE SENSITIVITY protein, which enables acclimation
372 to photo-oxidative stress [110, 111, 112]. Very recently, it has been shown that the conversion of β -
373 cyclocitral into β -cyclocitric acid, a reaction that occurs spontaneously in water, is an initial step in
374 β -cyclocitral signaling and that exogenous application of β -cyclocitric acid triggers the expression
375 of β -cyclocitral induced and 1O_2 -regulated genes [113]. Moreover, β -cyclocitric acid application
376 promoted the expression of several water stress-responsive genes under sufficient water supply and
377 increased Arabidopsis tolerance to drought, indicating a potential for application in agriculture
378 [113]. However, the mechanism underlying this effect is still elusive. Interestingly, β -cyclocitral
379 acts also as a growth regulator, increasing root growth and branching by promoting stem cell
380 divisions. It can be speculated that the growth-regulating activity of β -cyclocitral is linked to its
381 general function in abiotic stress response. Supporting this hypothesis, the exogenous application of
382 β -cyclocitral under high-salinity conditions increased shoot and root growth in plants [15].

383

384 4.3 Anchorene, a representative of overlooked diapocarotenoid signaling molecules?

385 Carotenoids can be repeatedly cleaved at different double bonds, which generates dialdehyde
386 products (diapocarotenoids) [9, 21]. However, instability and low abundance impeded the
387 exploration of possible regulatory functions of diapocarotenoids that have been mainly studied as
388 precursors of natural pigments, such as crocin in saffron stigma [114]. Recently, screening for
389 bioactive, presumably carotenoid-derived dialdehydes, using synthetic compounds and under light

390 conditions that increase diapocarotenoid stability, revealed a C₁₀-dialdehyde, anchorene, as a
391 specific regulator of the growth of anchor roots in Arabidopsis [17]. Anchor roots are a less
392 investigated type of Arabidopsis secondary roots that emerge in the collet region located at the root
393 hypocotyl junction [17] (Fig.1). Confirming its specificity, modifications of anchorene structure
394 significantly diminished the ability to induce anchor root formation. Thus, the application of iso-
395 anchorene, a structural isomer of anchorene, which differs in the position of a methyl group, led to a
396 significant decrease of primary root growth and a slightly increase in anchor root formation
397 (unpublished data). Mutant and chemical inhibitor studies demonstrated that carotenoid
398 biosynthesis is required for anchor root growth and that anchorene can replace this pathway in
399 exerting this function [17]. Although the biosynthesis of anchorene is still elusive, LC-MS analysis
400 confirmed its being a natural metabolite and indicated its carotenogenic origin [17]. Studies
401 employing auxin reporter lines and an auxin transport inhibitor, together with transcriptome
402 analysis, demonstrated that anchorene promotes anchor root formation by altering auxin
403 homeostasis [17]. Interestingly, anchor root formation as well as anchorene content increase under
404 nitrogen deficiency [17], indicating a role in the regulation of nitrogen uptake in Arabidopsis. The
405 discovery of anchorene demonstrates that diapocarotenoids may also have growth regulatory
406 functions, similar to their apocarotenoid counterparts.

407

408 **Conclusion and Future Perspective**

409 The carotenoid biosynthesis pathway is an essential source of signaling molecules and hormones
410 involved in virtually all aspects of the plant's life. These metabolites arise through a common
411 metabolic process, i.e. oxidative cleavage of carotenoids, which yields apocarotenoids. Further
412 conversion of the primary cleavage products increases the structural and functional diversity of this
413 class of compounds. Non-enzymatic formation of apocarotenoids, which accompanies
414 photosynthesis, may explain why they were recruited as signaling molecules and modified them
415 later to hormones with the corresponding perception and signal transduction machinery. Recently

416 discovered apocarotenoid signaling molecules/hormone candidates will unravel overlooked aspects
417 in plant development, resilience, and biotic interactions, emphasizing the importance of
418 apocarotenoids for basic and applied plant science.

419

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423 **Conflict of Interest Statement**

424 The authors declare no any competing financial interests.

425

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691 **Legends to Figures**

692
693 **Figure 1. Biological functions of plant apocarotenoids**

694 The upper structure shows all-*trans*- β -carotene with C-atom numbering. Depicted signaling
695 molecules are formed through oxidative cleavage of double bonds, frequently followed by
696 enzymatic conversions. ABA regulates seed dormancy, stomatal closure, root and shoot
697 development, and biotic/abiotic stress responses. SLs, e.g. orobanchol, determine plant architecture
698 and mediate rhizospheric interactions. Zaxinone, formed by zaxinone synthase (ZAS), is required
699 for normal rice growth and development and is involved in AM symbiosis. β -cyclocital, formed by
700 ROS ($^1\text{O}_2$) attack, CsCCD4 or lipoxygenases, mediates high-light stress response, abiotic stress
701 response and promotes root growth. Anchorene, a diapocarotenoid, stimulates anchor root
702 formation in *Arabidopsis*. Created with ‘Biorender’.

703

704 **Figure 2. ABA biosynthesis**

705 β -hydroxylases convert all-*trans*- β -carotene into all-*trans*-zeaxanthin. Two epoxidation reactions,
 706 catalyzed by ZEP, transform the latter into all-*trans*-violaxanthin. Nine-*cis*-Epoxy-carotenoid
 707 Dioxygenases cleave 9-*cis*-violaxanthin and 9'-*cis*-neoxanthin, which formation is still elusive,
 708 yielding the ABA precursor xanthoxin (C₁₅) and the corresponding apo-12-carotenoid (C₂₅).
 709 Xanthoxin is then converted to ABA by SDR and AAO via ABA-aldehyde in the cytosol.
 710 Abbreviations: β -HYD, β -hydroxylase; ZEP, zeaxanthin epoxidase; SDR: short-chain
 711 dehydrogenase reductase; AAO: Absciscic aldehyde oxidase.

712

713 **Figure 3. Scheme of ABA signaling**

714 (A) PP2C represses SnRK2 activity by dephosphorylating its kinase-activating loop. (B) Binding of
 715 ABA leads to the formation of a complex containing the receptor (PYR/PYL/RCAR) and PP2C,
 716 which inhibits PP2C and releases SnRK2 kinase activity. The latter triggers downstream ABA-
 717 responsive element (ABRE)-binding protein/ABRE-binding factor (AREB/ABF) transcription
 718 factors that induce the ABA response. PP2C, protein phosphatase 2C; SnRK2, Snf1-related protein
 719 kinase 2; PYR, pyrabactin resistance; PYL, PYR1-LIKE; RCAR, regulatory component of aba
 720 receptor. Created with 'Biorender'.

Figure 4. SL biosynthesis

D27 isomerizes all-*trans*- to 9-*cis*-β-carotene that is cleaved by CCD7 to produce 9-*cis*-β-apo-10'-carotenal and β-ionone. Through a combination of reactions, CCD8 transforms 9-*cis*-β-apo-10'-carotenal to carlactone (CL). CL is further modified by cytochrome P450 enzymes to form different types of canonical, e.g. 4-deoxyorobanchol, and non-canonical, e.g. carlactonoic acid, SLs. SL biosynthesis involves further enzymes, such as LBO and a methyl transferase. Abbreviations: MAX: more axillary growth; LBO, lateral branching oxidoreductase; Os, *Oryza sativa*; Sl, *Solanum lycopersicum*; Vu, *Vigna unguiculata*; Ga, *Gossypium arboreum*; At, *Arabidopsis thaliana*.

730

731 **Figure 5. Scheme of SL signaling**

732 The receptor D14 binds and hydrolyzes SLs, forming a covalently linked receptor molecule (CLIM)
 733 that contains the SL D-ring (linked to the catalytic His-residue), and releasing the second SL
 734 moiety. These steps are accompanied by a conformational change of D14, which promotes the
 735 interaction with MAX2 and repressor proteins, e.g. the Arabidopsis SMXL or the rice D53. The
 736 recruitment into the SCF complex leads to polyubiquitination and proteasome-mediated degradation
 737 of repressor proteins. Abbreviations: MAX: more axillary growth. D14, DWARF14; SMXL,
 738 SUPPRESSOR OF MAX2 1-LIKE; D53, DWARF53. Created with 'Biorender'.

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