

## Carotenoids and their derivatives: A “Swiss Army knife-like” multifunctional tool for fine-tuning plant-environment interactions

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### ABSTRACT

Plants have to cope with different biotic and abiotic stresses, such as the attacks of pathogens and herbivores, high irradiance, droughts, salt stress or nutrient deficiencies throughout their lifetime. These environmental perturbations lead to the regulation of the “primary” and “secondary” carotenoid network. The formers are produced and stored in plastids and they are necessary for photosynthesis and related functions. Otherwise, secondary carotenoids are derivatives of primary carotenoids. They perform other important functions not related to the photosynthetic process but essential for plant survival as regulators of growth and development or as signal molecules at all levels of plant organization. This review provides a complete revision of the status of all these carotenoids, highlighting their newly discovered functions involved in plant-environment interaction. Concurrently, this review covers recent information on how carotenoids perform critical functions for the survival of animals (including humans) and the way they are suitable diagnostic tools for assessing the functioning of terrestrial ecosystems.

### 1. Primary and secondary carotenoids

Carotenoids are a diverse group of isoprenoids found ubiquitously in plants, animals, algae, fungi, and bacteria (Jia et al., 2018; Solovchenko and Neverov, 2017). According to Carotenoid Database (last update in 2020), to date, 1204 carotenoids have been sourced from 722 organisms (Yabuzaki, 2017). Focusing on plants, they are synthesized by eight isoprenes (C<sub>5</sub>) units in plastids, forming a rigid 40-carbon polyene skeleton (typical carotenoids structure) with conjugated double bonds (Ruiz-Sola and Rodríguez-Concepción, 2012). Each carotenoid has a specific absorbance spectrum (natural colour) that depends on the configuration and length of the chromophore of conjugated double bonds (Rodríguez-Concepción et al., 2018). Briefly, carotenoids are mostly derived from the linear tetraterpene phytoene, which is formed after the condensation of two molecules of geranylgeranyl pyrophosphate by the activity of phytoene synthase (PSY) (Fig. 1). This enzyme is considered the most important rate-limiting enzyme in carotenogenesis

(Cazzonelli, 2011). Downstream modifications at one or both ends of the linear backbone by desaturases, isomerases, cyclases, hydroxylases, epoxidases, and cleavage enzymes yield a wide array of primary carotenoids and their derivatives (Fig. 1).

Due to this diversity, carotenoids can be classified in multiple ways (Fig. 1) depending on their (i) structure (cyclic, acyclic), (ii) chemical composition (oxygen-containing, xanthophylls; and oxygen-free, carotenes) (iii) geometrical and optical properties (*cis*, *trans*), (iv) association with other molecules (esterification) and (v) biological functions (essential and specialized) (Rodríguez-Concepción et al., 2018). In this review, we simplify the nomenclature of carotenoids into “primary” and “secondary” (and hereinafter referred to as this classification; Table 1) following these criteria: “Primary carotenoids” are those carotenoids produced and stored in plastids of 40-C without association with other compounds (i.e., fatty acids) and necessary for photosynthesis and related functions (i.e., photoprotection, membrane stabilization): β- and α-carotene –β- and α-Car–, zeaxanthin –Zea–, antheraxanthin –Ant–,

**Abbreviations:** Zea, zeaxanthin; PSY, phytoene synthase; α-Car, α-carotene; β-Car, β-carotene; Ant, antheraxanthin; Vio, violaxanthin; Neo, neoxanthin; Lx, lutein epoxide; Lut, lutein; CCD, carotenoid cleavage dioxygenases; ROS, reactive oxygen species; <sup>1</sup>O<sub>2</sub>, singlet oxygen; ABA, abscisic acid; SLs, strigolactones; β-Cyc, β-cyclocitral; DHA, dihydroactinidiolide; PSII, photosystem II; VAZ-cycle, violaxanthin cycle; VDE, violaxanthin de-epoxidase; NCED, 9-cis-epoxycarotenoid dioxygenase.

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violaxanthin –Vio–, neoxanthin –Neo–, lutein –Lut– and Lutein epoxide-Lx. They are present in leaf tissues of all plants, with some exceptions (Lx and  $\alpha$ -Car; more details below) (Esteban and García-Plazaola, 2019). Meanwhile, “secondary carotenoids”, such as apocarotenoids, retrocarotenoids and xanthophyll esters, are those derivatives of primary carotenoids, which generally fulfill other important functions not related to photosynthetic processes (Fig. 1; Table 1).

Despite this structural diversity, carotenoid composition in plants remains strikingly constant, with a set of six primary carotenoids being invariably present in the leaves of all plant species (Esteban et al., 2015b). In addition, other primary carotenoids complete this basic composition, in response to a phylogenetic trait (e.g. lactucaxanthin in lettuce) or/and to environmental conditions (e.g.,  $\alpha$ -Car and Lx accumulated in shaded leaves) (Esteban and García-Plazaola, 2016). In some instances, xanthophylls may be esterified with fatty acids and accumulate in chromoplasts to form xanthophyll esters (Ariizumi et al., 2014) (Fig. 1). In others, double bonds can be shifted in the polyene chain by one position relative to widely occurring carotenoids, giving rise to red retrocarotenoids (e.g., rhodoxanthin) or they may have one or more ketone groups, either in the linear chain or on the  $\beta$ -ionone ring(s), as is the case of reddish-orange ketocarotenoids (e.g., astaxanthin and canthaxanthin) (Fig. 1). Esterification does not change the spectral

**Table 1**

Classification of carotenoids and their derivatives used in this review. Carotenoids are classified as primary or secondary carotenoids on the basis of their molecular structure and the main functions they perform.

	Primary carotenoids	Secondary carotenoids
<b>Molecule structure</b>	40-C without association with other compounds	Derived from primary carotenoids
<b>Main functions</b>	Functions required for photosynthesis and related functions: -Membrane stabilization (Section 2.1.1) - Light harvesting and energy dissipation (Section 2.1.2) -Antioxidant function (Section 2.1.3)	Functions not related to photosynthetic processes: -Antioxidant function (Section 2.1.3) -Signalling (Section 2.2) -Regulators of plant growth and development (Section 2.3) -Belowground and aboveground communication (Section 2.4)
<b>Which ones?</b>	$\alpha$ - and $\beta$ -Carotenes ( $\alpha$ - and $\beta$ -Car) Zeaxanthin (Zea) Antheraxanthin (Ant) Violaxanthin (Vio) Neoxanthin (Neo) Lutein (Lut) Lutein epoxide (Lx)	Apocarotenoids Retrocarotenoids Ketocarotenoids Xanthophyll esters

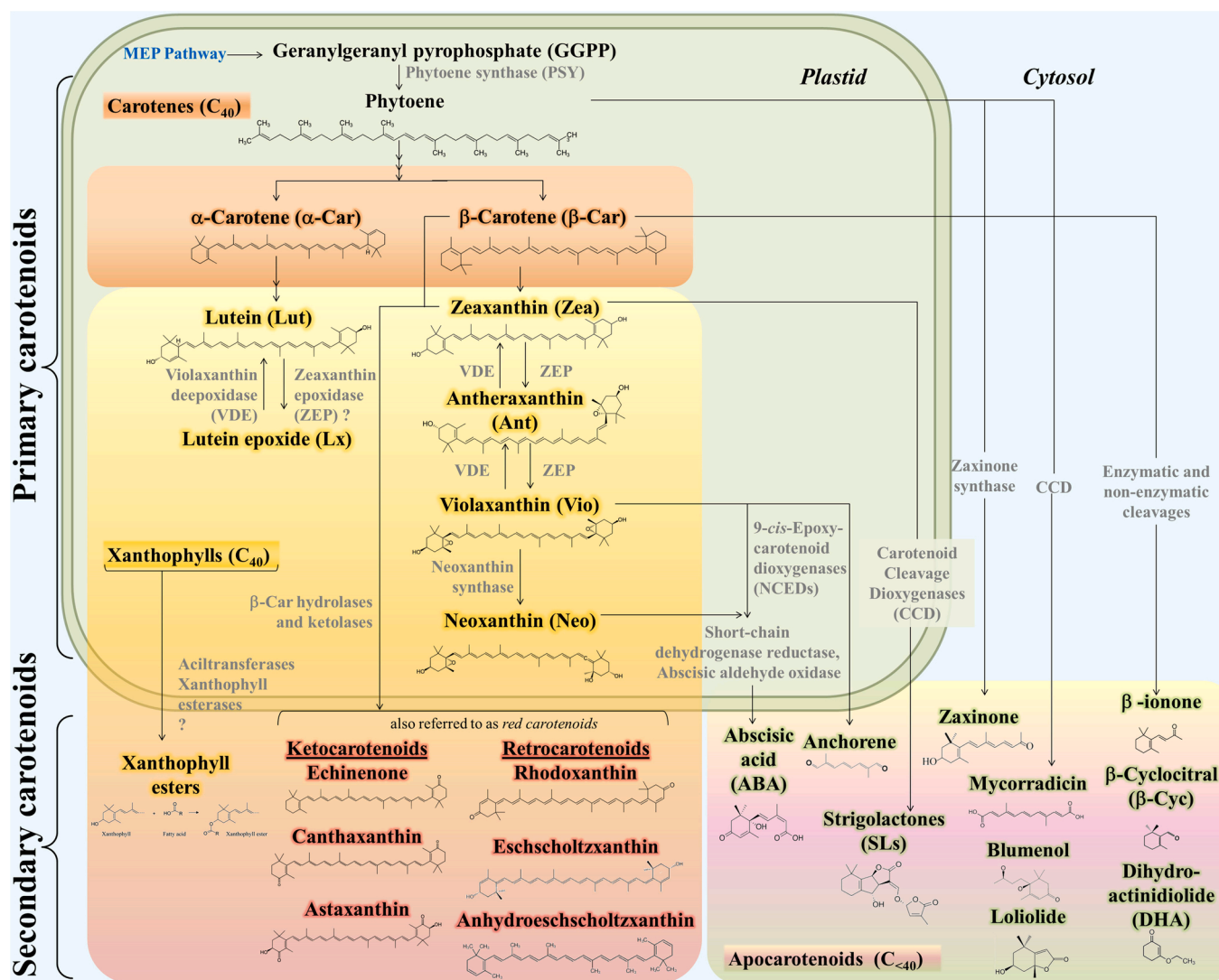


Fig. 1. Simplified biosynthetic pathways in plants for the formation of the primary and secondary carotenoids that are stated in this review. Carotenes, xanthophylls and apocarotenoids are boxed in orange, yellow and pink colour, respectively. Enzymes in the core carotenoid biosynthetic pathway are represented in grey font. Note that only biosynthetic steps from phytoene are shown and the steps that are not stated in the review have been omitted.

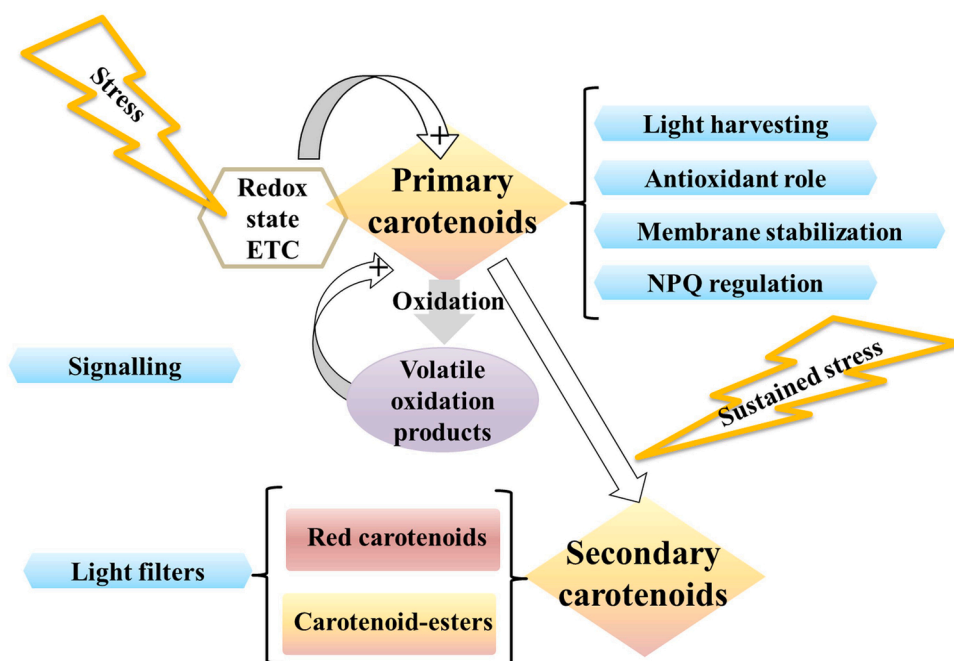
characteristics (Hormaetxe et al., 2004) however retro- and keto-carotenoids typically absorb at longer wavelengths than their primary counterparts. Other secondary carotenoids, so-called apocarotenoids, are also formed after an enzymatic cleavage reaction catalyzed by carotenoid cleavage dioxygenases (CCD) and/or by oxidative cleavage of the double bond of the  $\beta$ -Car by reactive oxygen species (ROS) such as singlet oxygen ( $^1O_2$ ) (Felemban et al., 2019). The enzymatic scission conducting to apocarotenoids formation depends on the CCDs family of enzymes that oxidise carotenoids at specific double bonds resulting in smaller molecules that are used as precursors for the synthesis of bioactive compounds (Rodríguez-Concepcion et al., 2018). The CCD enzymes differ in their specificity for the substrate and the position of the conjugated carotenoid backbone, where oxygen atoms are incorporated resulting in the synthesis of the different apocarotenoids in response to the environmental cues (Rubio-Moraga et al., 2014). This is the case both for CCD enzymes that activate the biosynthetic pathways of both abscisic acid (ABA, C<sub>15</sub>) and strigolactones (SLs, C<sub>19</sub>) and for those that catalyse the formation of other compounds (zaxinone, C<sub>18</sub>; blumenol, C<sub>13</sub>; and mycorradicin, C<sub>14</sub>) from Zea (Liang et al., 2021; as shown in detail in Fig. 1). This enzymatic turnover of C<sub>40</sub> carotenoids by CCDs is critical for maintaining carotenoids at physiological levels (Nisar et al., 2015). Moreover, a series of short-chain, volatile compounds can be produced by non-enzymatic (e.g., photochemical oxidation, auto-oxidation, and thermal degradation) or enzymatic oxidative cleavage of carotenoid precursors. These include  $\beta$ -cyclocitral ( $\beta$ -Cyc, C<sub>10</sub>), dihydroactinidiolide (DHA, C<sub>11</sub>), 3-hydroxy- $\beta$ -ionone (C<sub>13</sub>), loliole (C<sub>11</sub>), and anchorene (C<sub>10</sub>) (Liang et al., 2021) (Fig. 1). This non-enzymatic mechanism is known as chemical quenching, and despite being less common than the physical quenching mentioned above, it is of vital importance for the cellular detoxification of ROS (Havaux, 2014).

As a consequence of a sessile lifestyle, plants are continuously subjected, during their development, to a myriad of environmental stresses (both abiotic and biotic) that inevitably affect their physiology and overall well-being. As a response to these challenges, plants have developed over a long evolutionary period a set of sophisticated metabolic networks (in which primary and secondary carotenoids are integrated) with multiple and diverse functions (a “Swiss army knife”-like) to cope with, acclimate or adapt to these stresses. Therefore, in this

review, we explore the multiple functions of primary and secondary carotenoids in plants for fine-tuning plant-environment interactions (Section 2). We also compile information on the functional parallels of carotenoids that have been described between plants and animals. (Section 3). The usefulness of assessing the correspondence between the carotenoid pool and the primary production/functioning of terrestrial ecosystems in the current scenario of climate change is emphasized at the end of this review (Section 4).

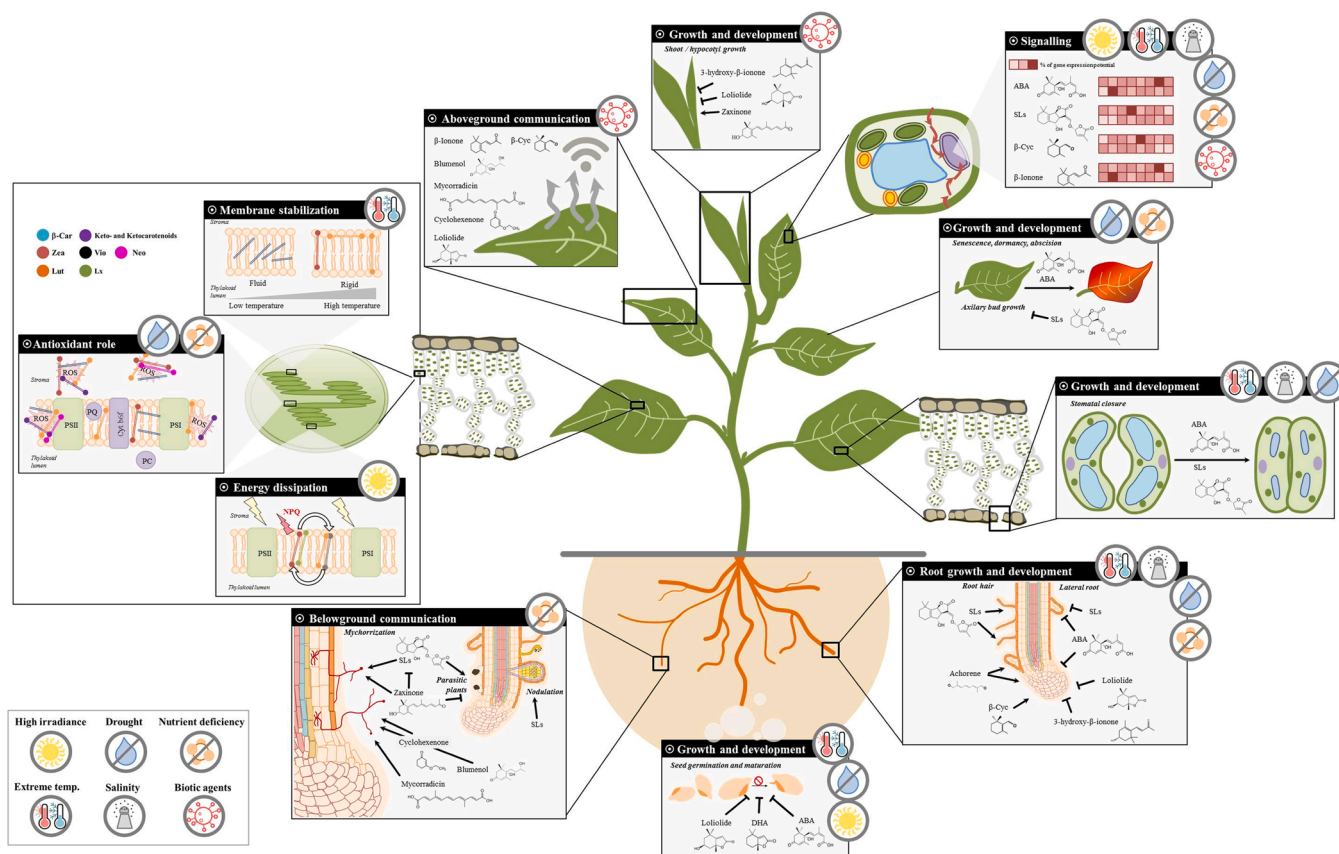
## 2. Functions of carotenoids in plant-environment interactions

Irrespective of their metabolic origin, carotenoids fulfill a variety of critical biological and environmental functions in plants, microorganisms and animals. Focusing on plants, carotenoid functions occur at different spatial scales from the chloroplast, through the whole plant, to the terrestrial ecosystem. Undoubtedly, carotenoids are mainly recognised for their important structural and biochemical roles in chloroplast thylakoids (Section 2.1), which are highly specialized membrane systems within chloroplasts, where the light-dependent reactions of photosynthesis occur (Figs. 2 and 3). As compared to many other biological membranes, the thylakoid membranes have relatively high fluidity, due to the lack of cholesterol (or other membrane-stabilizing sterols) and the high concentration of highly unsaturated fatty acids, which ensures most photosynthetic processes (Bykowski et al., 2021). Each primary carotenoid is specifically bound to a certain site in the thylakoid proteins. The presence and spatial arrangement of primary carotenoids in these membranes influence their thermodynamic and mechanical properties (Section 2.1.1). Located in membranes, carotenoids play a dual role in antennae complexes in the relation to light (Section 2.1.2; Figs. 2 and 3): harvesting and photoprotection (against light excess and oxidative damage). Carotenoids can cope with this oxidative stress through their antioxidant role (see Section 2.1.3; Figs. 2 and 3). Besides, some secondary carotenoids may accumulate outside the thylakoids, in plastoglobules or lipid granules (as is the case of red carotenoids and xanthophyll esters) with a protective role (Section 2.1.4; Figs. 2 and 3). At the plant cell level, carotenoids are pivotal actors in molecular sensing and signalling and play regulatory roles in plants (Section 2.2; Figs. 2 and 3). For instance,  $\beta$ -Car can be oxidized by  $^1O_2$  giving rise to a large variety of oxidized derivatives with signalling and



**Fig. 2.** The holistic view of the interactions between environmental stress and the protective role of carotenoids (Section 2.1). Most physical stresses cause the over-reduction of the photosynthetic electron transport chain (ETC) and are perceived as changes in the redox state of plastoquinone. This upregulates the synthesis of primary carotenoids involved in photoprotection, antioxidant activity or light harvesting. The oxidation of these molecules can result in the production of volatile products that initiate signalling pathways to enhance protection (Section 2.2). When the stress is sustained on time, secondary carotenoids (xanthophyll esters or red carotenoids) accumulate in chloroplasts, acting as passive filters that reduce light energy reaching photosynthetic antennae.





**Fig. 3.** Functions of primary ( $\beta$ -Car, Zea, Vio, Neo, Lut and Lx) and secondary carotenoids (keto- and retro-carotenoids and apocarotenoids) in plant-environment interaction at i) chloroplast level (membrane stabilization, antioxidant role, energy dissipation), ii) cellular level (signalling), iii) the whole-plant level (growth and development), and at iv) ecosystem level (aboveground and belowground communication). Note that only carotenoids stated in the review are presented.

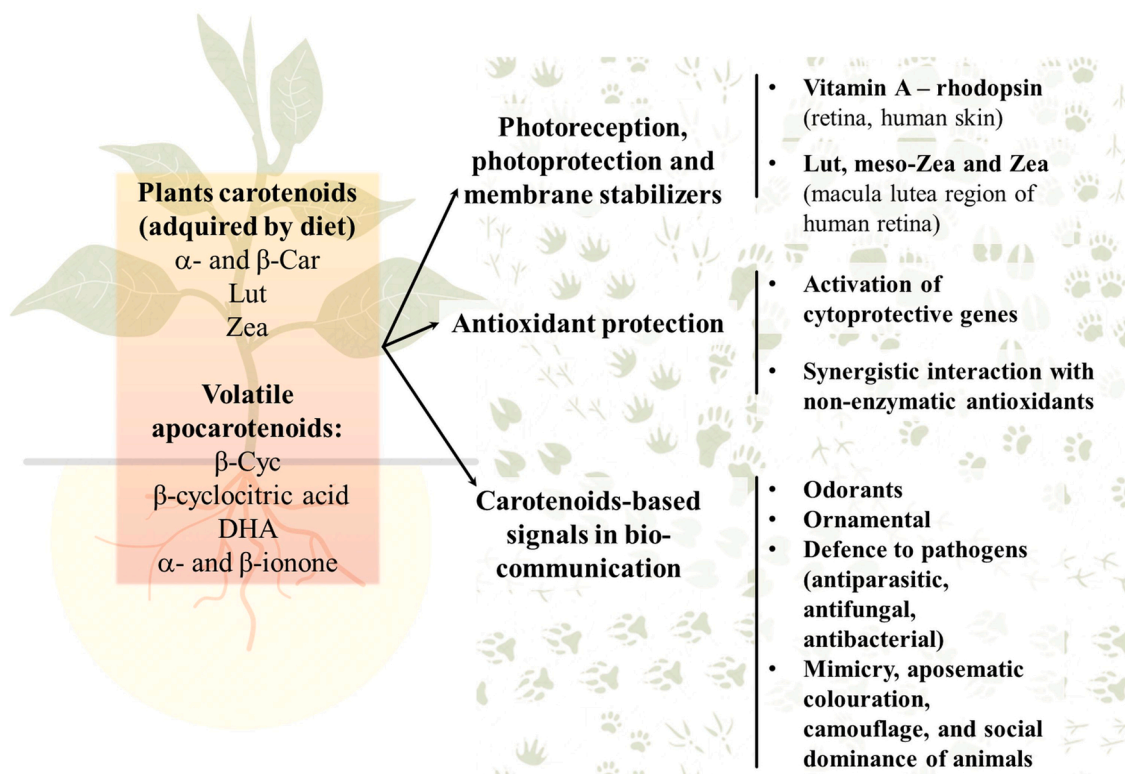
communicating roles (Havaux, 2014; Ramel et al., 2012). Nonetheless, more recent and cutting-edge research into the functions of carotenoids is focusing on their role in growth and development at the whole plant level, as shown in Section 2.3 (Fig. 3). The most prevalent abiotic (i.e., salinity, water scarcity –drought or dehydration–, low or high temperature, high light intensity) or biotic stresses (i.e., plant-pathogen and herbivorous insect attacks) result in both general and specific effects on plant growth and development (Huang et al., 2012), leading to a reduction in vegetative growth, biomass production and plant photosynthetic yield. Carotenoids-derived phytohormones, ABA and SLs, and zaxinone are implicated in conservation resources for plant growth and development under environmental stresses (see Section 2.3.1). Volatile carotenoid derivatives, such as  $\beta$ -Cyc, are also involved in the regulation of developmental functions under stress conditions (Section 2.3.2). From an ecosystem perspective, primary and secondary carotenoids are also part of the complex chemical language between plants and the organisms that inhabit their environment, both belowground and aboveground (e.g., neighbouring plants, animals, and soil microbial communities) (Walter and Strack, 2011) (see Sections 2.4 and 3; Fig. 4).

## 2.1. Functions at chloroplast level: carotenoids as membrane stabilizers, photoprotectors and antioxidants

### 2.1.1. Primary carotenoids with a structural role in membranes

The maintenance of an adequate fluidity of the thylakoid membranes under stresses (i.e., low/high temperatures and light excess) is essential for preserving the mobility of electron and proton carriers and, consequently, for the proper functioning of the photosynthetic apparatus. The physical state of the photosynthetic membrane is also important for the ROS scavenging by lipid-soluble antioxidants, such as plastoquinone,

$\alpha$ -tocopherol quinone and  $\alpha$ -tocopherol (Strzalka et al., 2003). Even if most of the carotenoids are bound to specific sites of photosynthetic complexes, there is an unbound carotenoid pool freely dispersed in the lipid bilayer that may represent about 15% of the total carotenoid content in chloroplasts (Dall'Osto et al., 2010). The degree of integration and the orientation of these free carotenoids into the lipid bilayer, and, consequently, their effect on the thermodynamic and mechanical properties of thylakoid membranes depends on the lipid content of the bilayer and the chemical structure of the carotenoid concerned (Strzalka et al., 2003). Amphiphilic carotenoids, xanthophylls (Lut and Zea), localized in the lipid phase, effectively modulate the fluidity of the lipid bilayer via van der Waals interactions with polar lipid components (Gruszecki and Strzalka, 2005). Due to the specific structure of Lut (its molecules in the all-*trans* conformation), it can adopt two orthogonal orientations in the lipid bilayer (Sujak et al., 1999): parallel orientation in the same polar surface (by the interaction with both hydroxyl groups in the same surface/in the same plane) or it can be oriented perpendicular to the bilayer plane (by the interaction with opposite hydroxyl groups) (Fig. 3), restricting lipid motional freedom and leading to a general rigidifying effect (Popova and Andreeva, 2013). In the case of Zea, the presence of hydroxyl groups on the rings makes the molecule oriented perpendicular to the membrane surface, decreasing its fluidity (Fig. 3). Under conditions of photooxidation, Zea transiently appears within the lipid phase of the thylakoid membrane, functioning as an efficient stabilizer-protectant (Sujak et al., 1999). Contrarily, carotenes that lack polar head groups, such as  $\beta$ -Car, are randomly and homogeneously distributed inside the hydrophobic core of the membrane, increasing its fluidity. Fine-tuning of the ratio of xanthophylls to  $\beta$ -Car converts thylakoid membranes dynamic and flexible to cope with environmental stresses (Mazur et al., 2020).



**Fig. 4.** Parallel functions of carotenoids in plants and animals. Carotenoids support animal health and reproductive success by acting as photoreceptors, photoprotectors, membrane stabilizers, antioxidants or being warnings, mating signals and feeding signals.

### 2.1.2. Dual role of primary carotenoids in light harvesting and energy dissipation

Primary carotenoids are involved in the process of photosynthesis (and above all) as well as in the photoprotection of the photosynthetic machinery. In the photosynthetic process, carotenoids function as accessory light-harvesting pigments (blue-green spectral region; 400–550 nm) that effectively extend the range of absorbed light (Fig. 3). Once solar light energy reaches carotenoids, it is transferred by single-to-singlet excitation mechanism to chlorophylls (Chl) located in the antenna complexes, converting it into energy available for photosynthesis. In low-light environments, such as forest understory, where the fraction of light reaching the plants is scarce and unpredictable, extremely efficient light harvesting photosystems are indispensable. In this regard, Lx, restricted to some groups (see details below), increases light harvesting efficiency in the antennae of photosystem II (PSII) (Esteban et al., 2009; Esteban and García-Plazaola, 2016; García-Plazaola et al., 2007, and references herein).

If sunlight-harvesting systems become overloaded with absorbed solar energy, plants most likely will suffer some form of damage. Based on their specific locations distinct photoprotective roles have been described for each carotenoid species. Among them, the central role in the regulation of photoprotective thermal dissipation relies on three xanthophylls involved in the so-called violaxanthin cycle (VAZ-cycle; Fig. 3). This mechanism has been extensively described in detail in a large collection of reviews (i.e., Demmig-Adams and Adams, 2006; Pinnola and Bassi, 2018; Ruban, 2018). It consists of the light-driven deepoxidation of Vio to form Zea (with Ant as intermediate), and the back reaction in low light or darkness. The enzyme involved in this reaction, violaxanthin de-epoxidase (VDE), is activated at acidic pH (Arnoux et al., 2009) when light induces proton pumping through the thylakoids. Thereby the amount of Zea is usually proportional to the amount of light in excess, which is dissipated as heat in the antenna proteins, protecting the photosynthetic apparatus from the risk of overexcitation (Ruban, 2018). Besides, a distinct role in the regulation of

thermal energy dissipation has been demonstrated for Lut by the use of *Arabidopsis* mutants defective in Zea (Li et al., 2009). The contribution of Lut to the regulation of thermal energy dissipation could be mechanistically linked with the operation of the so-called LxL-cycle (Fig. 3). This cycle, reviewed in García-Plazaola et al. (2007) and Esteban and García-Plazaola (2014), consists of the accumulation of Lut by de-epoxidation of Lx. In contrast with Vio, which is ubiquitous in the Plant Kingdom, Lx is present in less than fifty percent of the species tested so far (Esteban et al., 2009). However, Lx concentration ( $>5 \text{ mmol mol}^{-1} \text{ Chl}$ ) was significant in only 13% of the taxa analysed (Esteban and García-Plazaola, 2014). The reaction is catalysed by the same enzyme (VDE), as in the VAZ-cycle. In contrast with the VAZ-cycle, the relaxation in the darkness of LxL-cycle is much slower, providing larger flexibility of the dynamic responses to light energy excess (Esteban et al., 2009; Matsubara et al., 2011).

### 2.1.3. Primary and secondary carotenoids as antioxidant role

Xanthophylls are not only involved in the photoprotection of PSII, but also in the safety of the photosynthetic membranes against photo-oxidation (Havaux et al., 2000), in which phytohormones are also implicated (Müller and Munné-Bosch, 2021). This photoprotection depends on the form in which carotenoids are found in the thylakoids. They can be in free form, scavenging ROS released from light-harvesting complexes and reaction centers, as Zea, and thus plays an antioxidant role analogous to that of tocopherols (Dall'Osto et al., 2010). Or, conversely, they may appear bound to the proteins (and close to the chlorophyll molecule), as β-Car, almost exclusively located in the core complexes of both photosystems, where it plays an essential antioxidant role (Cazzaniga et al., 2012).

According to Pinnola and Bassi and references within (2018), carotenoids are involved in three photoprotection constitutive mechanisms: (i) the modulation of  $^3\text{Chl}^*$  quenching that prevents  $^1\text{O}_2$  formation. This is the case of Lut and Zea in the antenna complexes and β-Car in the core complexes; (ii) ROS scavenging by excitation energy

transfer from ROS to carotenoids (e.g., Neo, Zea,  $\beta$ -Car) leading to the formation of triplet carotenoids, being the energy converted into heat. And, finally; (iii) the modulation of  $^1\text{Chl}$  yield by energy transfer from  $^1\text{Chl}^*$  to Zea (Fig. 3). Secondary carotenoids, such as astaxanthin (considered the most potent antioxidant), canthaxanthin, and rhodoxanthin, can also exhibit this antioxidant role, both in higher plants and algae (Solovchenko and Neverov, 2017). The increase or decrease in carotenoid content when acting as antioxidants is strongly related to the species studied, exposure time and intensity and type of stress.

#### 2.1.4. Red carotenoids and xanthophyll esters and the carotenogenic response

When present, secondary carotenoids, such as red carotenoids and xanthophyll esters, frequently accumulate massively resulting in a visual change of leaf colour that gives rise to the so-called carotenogenic response (i.e., a massive accumulation of secondary carotenoids) (Solovchenko and Neverov, 2017). Among red carotenoids, the keto-carotenoid astaxanthin and the retro-carotenoids rhodoxanthin, eschscholtzanthin and anhydroeschscholtzanthin stand out. Astaxanthin is common and widespread in several lineages of green algae such as the genus *Haematococcus* (Wang et al., 2003) or *Chlamydomonas nivalis* (Remias et al., 2005). The group of retro-carotenoids is taxonomically restricted to a few plant species. Thus, the presence of rhodoxanthin has been described in *Aloe* (Diaz et al., 1990) and some conifers (Maslova et al., 2009), whereas eschscholtzanthin and anhydroeschscholtzanthin have been reported in the genus *Buxus* (Hormaetxe et al., 2004).

While primary carotenoids are typically bound to photosynthetic proteins (described in the previous Section 2.1.2), red carotenoids accumulate in plastoglobules within chloroplasts, chromoplasts or in extra-plastidial lipid bodies. In algae, their synthesis is triggered in response to desiccation, low and high temperature, nutrient deficiency, high irradiance or salinity (Lemoine and Schoefs, 2010). In the case of the common box (*Buxus sempervirens* L.), the high irradiance and cold that characterize Mediterranean winters produce the accumulation of red carotenoids in leaves (Hormaetxe et al., 2004). This accumulation may be reversible once the environmental conditions return to optimal (Silva-Cancino et al., 2012). Several protective mechanisms have been proposed for the process of leaf reddening, such as photoprotection, biotic interactions, and osmoregulation (Chalker-Scott, 1999), being the photoprotection the most plausible one. This hypothesis postulates that an external layer of red-pigmented cells would increase the absorption of photosynthetically active photons before reaching active chloroplasts, thereby decreasing excitation pressure. Hormaetxe et al. (2005) corroborated, to some extent, this hypothesis by observing how the presence of red carotenoids decreased the photoinhibitory damage in the leaves of the common box (Figs. 2 and 3).

Regarding xanthophyll esters (Figs. 2 and 3), as they are highly hydrophobic are easily (and typically) incorporated into lipid granules and plastoglobules (Ariizumi et al., 2014). This process is particularly noticeable during leaf senescence. At this life stage, xanthophylls are released after protein degradation in chloroplast dismantling, leading to a process of leaf yellowing that is particularly conspicuous in autumn leaves (García-Plazaola et al., 2003). These xanthophyll esters exert some degree of photoprotection to underlying cells by filtering out harmful blue light until the later stages of senescence (Solovchenko and Neverov, 2017).

#### 2.2. Functions at the cellular level: carotenoids as signalling molecules

The main signalling molecules derived from carotenoids in plants include phytohormones (ABA and SLs) and carotenoid oxidation products as  $\beta$ -Cyc and  $\beta$ -ionone, which also serve relevant functions in plant growth and development (Section 2.3) and as messengers in plant-mediated belowground/aboveground interactions (Section 2.4). The mentioned molecules can induce changes in stress-responsive genes at

transcriptional, post-transcriptional, post-translational and epigenetic levels (Sun et al., 2022; and references within), leading to acclimation/adaptation of both plants and animals to stress conditions (Section 3).

The most critical hormone that confers stress tolerance in plants is the isoprenoid ABA (Sah et al., 2016). This phytohormone is a C<sub>15</sub> apocarotenoid commonly recognized as a “stress hormone” because of its rapid biosynthesis and accumulation in response to abiotic stresses (Zhang et al., 2006), especially osmotic stress (elicited by water deficit, high salinity or extreme temperature, both heat and chilling/freezing) or biotic stresses when plants are infected with pathogens, such as *Fusarium* (Boba et al., 2020). The response is usually by inducing short-term responses, such as stomatal closure that blocks most water loss and gains time for long-term responses, including leaf senescence and abscission and dormancy in meristems or buds (Zhao et al., 2016). These stresses entail an increase in *de novo* ABA biosynthesis, due to the activation of ABA biosynthetic genes corresponding to zeaxanthin oxidase, 9-cis-epoxycarotenoid dioxygenase (NCED), ABA-aldehyde oxidase and molybdenum cofactor sulfuryase (Vishwakarma et al., 2017) (Fig. 1). ABA also is involved in the modification of the expression of many genes, which ultimately induce dehydration tolerance in plants (for a review see Vishwakarma et al., 2017) (Fig. 3). Some of those genes are related to the synthesis of osmotic protectants and late embryogenesis abundant class proteins under osmotic stress conditions (Nakashima and Yamaguchi-Shinozaki, 2013). Transcription factors work with their analogous *cis*-regulatory elements to integrate hormonal, environmental, and developmental signals (Section 2.3) in plants (Nakashima and Yamaguchi-Shinozaki, 2013).

Strigolactones constitute a structurally diverse group of apocarotenoids formed mainly in the roots and some parts of the stem. The core of SLs biosynthesis starts with the conversion of all-*trans*- $\beta$ -carotene followed by sequential reactions catalysed by carotenoid cleavage dioxygenases, CCD7 and CCD8 (Fig. 1) (Yoneyama and Brewer, 2021). A working model of the SL signalling cascade has been recently proposed by Faizan et al. (2020). In addition to their important function in plant architecture (Section 2.3), SLs, similarly to ABA, also activate signalling pathways in plants during biotic (Faizan et al., 2020; Section 2.4) and abiotic stresses, such as nitrogen/phosphate starvation (Section 2.4), drought and salinity, dark chilling stress, and light stress (Kaniganti et al., 2022). Thus far, the underlying mechanisms by which SLs enhance plant stress tolerance remain elusive.

There is increasing evidence that SL signalling network exists to face stresses and it involves crosstalk with other phytohormones such as auxins, cytokinins, gibberellins and ABA in several physiological processes. For example, SLs play a mediator role in auxin-induced secondary messengers, moving upward to the buds and retarding their outgrowth, meanwhile auxins regulate the expression of genes responsible for the synthesis of SLs (Faizan et al., 2020). Strigolactones and cytokinins are known to act antagonistically and changes in the level or sensitivity to SLs influence ABA concentrations and ABA responses (Faizan et al., 2020). However, it is unclear whether SLs and ABA are involved in a synergistic effect during stress responses (Ren et al., 2018).

Short-chain apocarotenoids function in the regulation/modulation of plant development (Section 2.3) and/or stress responses, as suggested by genetic and biochemical studies (Havaux, 2020, 2014). The production of  $\beta$ -Cyc and  $\beta$ -ionone is activated under drought (D'Alessandro et al., 2019), dehydration, and also under wounding, heat, cold and osmotic stresses (Rubio-Moraga et al., 2014). In addition,  $\beta$ -ionone,  $\beta$ -Cyc and DHA are stress signals produced under high light. Specifically, they regulate the expression of singlet oxygen-responsive genes, and elicit gene expression patterns that reduce plant growth and enhance photo-oxidative stress tolerance (Ramel et al., 2012; Shumbe et al., 2014). In this sense, it has been described that  $\beta$ -Cyc upregulates salicylic acid concentration under excess light, inhibiting ROS accumulation in the chloroplast (Lv et al., 2015). Moreover, these volatile molecules also mediate in the response to biotic stresses, with further implications in interplant communication, as explained in the following Section 2.4.



$\beta$ -ionone elicits resistance interplant propagation of the barley defence system, acting as priming markers (signals) in the systemic acquired resistance response that allows plants to defend against *Blumeria graminis* (mildew) (Brambilla et al., 2022). Similarly,  $\beta$ -ionone induces resistance to thrips in *Frankliniella occidentalis* (Murata et al., 2020).

According to Havaux (2014), the signalling function of these volatile apocarotenoids relies on their electrophilic reactivity. This is to say, they contain a carbonyl function adjacent to a double bond that can react with an electron donor (nucleophilic) atoms, such as S and N. This makes them susceptible to reaction with nucleophilic groups of macromolecules, such as thiols in proteins. This activates transcription factors, thus inducing gene responses (Farmer and Mueller, 2013). Although environmental stimuli are claimed to promote the synthesis of these specific secondary carotenoids, much research remains to be done on how these molecules are perceived by cells and how they regulate biological processes.

## 2.3. Functions at the whole-plant level: carotenoids as regulators of plant growth and development

### 2.3.1. Secondary carotenoids (non-volatiles) in conservation resources for plant development

Tolerance or susceptibility/sensitivity to environmental stresses is a multifaceted, coordinated and complex phenomenon because they may occur at different stages of plant development and life cycle. To address such stress situations, phytohormones are reported to be the key regulators of plant growth and development. Plant hormones interact among them causing synergistic and/or antagonistic effects; so upon stressful conditions, hormone balance shifts plant cells from active growth to defence mode by reprogramming gene expression (Bari and Jones, 2009).

Abscisic acid is particularly crucial phytohormone in mediating multiple developmental adaptive processes –as complex as seed germination, root formation and quiescence, leaf senescence, bud dormancy, and growth inhibition, among others–, to environmental stresses (for review see: Simkin, 2021, and references therein). For instance, the study of Liu et al. (2019) revealed that high temperature and drought stress resulted in the accumulation of more ABA in imbibing seeds, mainly via enhanced ABA biosynthesis rather than suppressed ABA catabolism. As a result, seed germination and seedling growth are reduced, possibly due to the arrest of embryo development, inhibition of radicle growth, and inhibition of water uptake. This is in agreement with results obtained by Nambara et al. (2010), which reported inhibition of seed germination due to ABA accumulation under abiotic stress. The expression of ABA biosynthesis genes is reported to show a direct impact on seed germination along with abiotic stresses (Vishal and Kumar, 2018). The identification and characterization of NCED genes revealed the tissue-specific expression (e.g., *NCED6* gene in the endosperm and *NCED9* in both embryo and endosperm during *Arabidopsis* seed development) and that the resultant modulation of endogenous ABA level at different developmental stages is responsible for the regulation (inhibition) of seed germination and seed maturation, besides response to abiotic stresses (Vishal and Kumar, 2018). Further, and as stated in the review by Hong, and references within et al. (2013), ABA also reduces root meristem and primary root length by promoting the inactive status of quiescent centre cells along with the suppression of cell differentiation in the columella stem cell layer. Abscisic acid is also involved in adaptive “deeper” root growth, and other aspects of architectural modification, including lateral root emergence, root growth and morphology, and root-to-shoot ratios (De Smet et al., 2006), under nitrogen deficiency (Zhang et al., 2006), heat stress (Hong et al., 2013) or under water-limited conditions (Sah et al., 2016).

Strigolactones, such as strigol, orobanchol and sorgolactone, are involved in variable aspects of plant growth and development such as lateral root initiation, main root elongation, nodulation and the establishment of the plant-mycorrhizal symbiosis (McAdam et al., 2017)

(Section 2.4; Fig. 3). The SLs also inhibit shoot lateral branching, an essential determinant of plant architecture, and contribute to leaf senescence (for review see Felemban et al., 2019; Jia et al., 2018; Simkin, 2021). Strigolactones are particularly relevant for dealing with restricted nutrient availability in the rhizosphere, especially inorganic phosphate (Pi) (Al-Babili and Bouwmeester, 2015). When Pi is limited, SLs inhibit axillary bud outgrowth to avoid the nutrient-demanding growth of shoots, thereby reducing sink tissues (Kapulnik and Koltai, 2016). Moreover, the SL-mediated promotion of leaf senescence and reduction of leaf surface area may allow a reallocation of resources to other parts of the plant during Pi starvation (Bennett et al., 2016). Below ground, under Pi-limiting conditions, SLs induce lateral initiation/formation and promote root-hair-elongation (Kapulnik and Koltai, 2016).

A recent study conducted by Wang et al. (2019) unraveled zaxinone (C<sub>18</sub>) as a member of the ubiquitous family of carotenoid-derived signalling molecules and hormones that triggers the growth of crown. Zaxinone is classified as an apocarotenoid formed by the action of the zaxinone synthase, a member of a less-characterized plant CCD sub-family that cleaves apo-100-zeaxanthinal (C<sub>27</sub>) at the 13,14 double bond (Liang et al., 2021). In soil, zaxinone application also increased the shoot length and biomass of zaxinone-deficient mutant (*zas*) and rescued root phenotype. Expression analysis of treated *zas* mutant and wild-type plants suggested that zaxinone suppressed SL biosynthesis at the transcript level under Pi-limiting conditions. Interestingly, zaxinone is required for optimal colonization of roots by mycorrhizal fungi (see Section 2.4.1), which in turn, are known to improve plant growth under adverse environmental conditions.

### 2.3.2. Volatile carotenoid derivatives as growth regulators

Plant growth and development as well as the response of plants to environmental changes and stress factors are regulated not only by the above-mentioned phytohormones. Volatile compounds such as  $\beta$ -Cyc, DHA, of 3-hydroxy- $\beta$ -ionone (Section 2.2.), loliolide and anchorene also regulate plant growth, development and metabolism. Recent studies underpin the biological importance of  $\beta$ -Cyc as an enhancer of root growth and branching under salt and drought stress conditions (D'Alessandro et al., 2019; Ramel et al., 2012; Simkin, 2021). However, it is not yet clear whether this root growth stimulation is a direct effect of  $\beta$ -Cyc on cell division/elongation in meristems of lateral and primary roots or is indirect, e.g. through the induction of cellular detoxification and tolerance against oxidative stress (Havaux, 2020). Dickinson et al. (2019) showed that when  $\beta$ -Cyc was applied to salt-stressed rice roots, it compensated for the negative effect of salt on root depth, generated a more compact crown root system and increased the vigour of rice plants. Similarly, Deshpande et al. (2021) observed that  $\beta$ -Cyc-treated drought-exposed tomato plants showed no wilting, higher relative water content and stomatal conductance, and unchanged ABA levels. Moreover,  $\beta$ -Cyc significantly enhanced root growth, even after long-term drought exposure. This finding consequently suggests that larger root growth favours soil exploration and enhances the water uptake capacity of the plant and maintains relative water content.

Dihydroactinidiolide was reported to accumulate in *Arabidopsis* leaves under high light stress (Ramel et al., 2012; Shumbe et al., 2014). Although little is known about the actions of DHA in vascular plants, some studies have classified DHA as a potent inhibitor of seed germination and seedling growth in watercress, radish (Stevens and Merrill, 1980) and wheat (Kato et al., 2003).

The symmetrical cleavage of Zea at the 9,10 (9',10') positions leads to the formation of 3-hydroxy- $\beta$ -ionone. This compound may have a function in the light-induced inhibition of hypocotyl elongation beans (Kato-Noguchi et al., 1993). Kato-Noguchi et al. (2010) showed that 3-hydroxy- $\beta$ -ionone produced by the moss *Rhynchosostegium pallidifolium* (Mitt.), which typically forms large colonies on rocks and soils, inhibited the growth of hypocotyls and roots of cress (*Lepidium sativum* L.).

Defined as a photo-oxidative or thermally degraded product of

carotenoid precursors (such as Lut, Vio, Zea, and fucoxanthin), loliolide is a C<sub>11</sub>-terpene lactone ubiquitously found in many algae and plants (Liang et al., 2021) (Fig. 1). Loliolide exhibits various physiological roles in stress responses in plants: it inhibits germination, hypocotyl and root growth (Bich and Kato-Noguchi, 2014), induces resistance against herbivory (e.g., such as two-spotted spider mite and the common cutworm) and may exhibit allelopathic and phytotoxic activities (Bich and Kato-Noguchi, 2014; Li et al., 2020) (see also Section 2.4).

Carotenoid-derived dialdehyde products (diapocarotenoids) such as anchorene remained understudied mainly because of their instability, which makes it difficult to detect in biological systems and identify their possible biological activity (Felemban et al., 2019). Recently, Jia et al. (2019) performed and screened for known and predicted diapocarotenoids involved in plant development. Anchorene treatment of *Arabidopsis* plants led to a wider root system due to the increased anchor root number, and enhanced root (about 50%) and shoot fresh biomass (about 30%). Anchorene treatment can also increase root length in both *Arabidopsis* and rice revealing that it is an enhancer of plant growth (Jia et al., 2019). Interestingly, nitrogen deficiency led also to an increase in anchorene content, suggesting a possible function of this metabolite in regulating the response of the plant to this deficiency (Jia et al., 2019).

#### 2.4. Functions at the individuals/ecosystem level: carotenoids as messengers in plant-mediated belowground/aboveground interactions

##### 2.4.1. Secondary carotenoids as mediators in belowground communication

Belowground in the rhizosphere, plants mainly interact with bacterial and fungal communities via chemical signals, which are largely based on monoterpenes (C<sub>10</sub>) and sesquiterpenes (C<sub>15</sub>) and less frequently (but not less important) on apocarotenoids such as SLs (Huang et al., 2019). The SLs released and exuded to the rhizosphere by plant roots are the best-characterized mediators (Al-Babili and Bouwmeester, 2015). Known for their important roles in signalling (Section 2.2) and development (Section 2.3.1), SLs also act as inter-specific communication cues to induce hyphal branching in arbuscular mycorrhizal fungi (Jia et al., 2018; Moreno et al., 2021) to form a symbiotic association under P limitation (Al-Babili and Bouwmeester, 2015) by the development of fungal adhesion structures on root epidermis (Fig. 3). This relationship provides benefits to the host plants, by improving the mineral nutrient uptake and tolerance to stresses (Fiorilli et al., 2019). Strigolactones were also shown to stimulate nodulation in the legume-rhizobium interaction process (Foo and Davies, 2011), although the underlying mechanism is unknown. Moreover, SLs participate in the belowground plant-plant communication (van Dam and Bouwmeester, 2016). They can induce the germination of the obligate heterotrophic parasitic plants, such as witchweeds (*Striga* spp.) and broomrapes (*Phelipanche* and *Orobancha* spp.) (Sato et al., 2005), which recognize specifically the SLs produced by plants as a germination signal ensuring host availability. These parasitic plants are a worldwide agricultural concern and a major threat to global food security (Nelson, 2021). Recently, it has been proposed that other apocarotenoids with growth-promoting activity, as zaxinone (Section 2.3.1.), can alleviate the infestation by the root parasitic plants by decreasing the SLs production (Wang et al., 2019). Other carotenoids derivatives, such as those derived from cyclohexanone, blumenol and mycorradicin (non-volatiles), act as signalling molecules (Section 2.2) and they are involved in controlling beneficial mycorrhizal fungi symbiosis by regulating arbuscule turnover and hyphal branching in the rhizosphere (Simkin, 2021; Walter et al., 2010) (Fig. 3).

##### 2.4.2. Primary and secondary carotenoids as communication messenger in aboveground interactions

Plants are in constant dialogue through volatile compounds with other organisms in their environment. For instance, plant reproduction and plant-animal aboveground communication are aided by carotenoids and apocarotenoids that attract pollen and seed dispersal animals (birds

and insects) both by bright colours and scents (Schaefer et al., 2004, see also Section 3). Apocarotenoids  $\alpha$ - and  $\beta$ -ionone,  $\beta$ -Cyc and  $\beta$ -damascenone, and citral serve as chromophores and/or at the same time as flavouring and odouring agents (Lewinsohn et al., 2005) (Section 3). Besides, carotenoids found in fruits may be a cue of ripeness and nutritional value associated with better health due to an increase in the antioxidant system (Pérez-Rodríguez, 2009).

Moreover, apocarotenoids are important mediators of insect-plant interactions (Heath et al., 2013). They regulate attraction or repulsion on mutualistic or parasite plant-insect relationships (Simkin, 2021, and references within). For example,  $\beta$ -ionone regulates plant and herbivorous insect interaction acting as a feeding deterrent (Gruber et al., 2009). The  $\beta$ -hydroxy-ionone and  $\beta$ -Cyc can play an important ecological role by acting as allelopathic agents (Kato-Noguchi et al., 2010) and repellents for insect pests, including spider mites and thrips, and as antifungal compounds, preventing plant diseases (Moreno et al., 2021).

We also find shreds of evidence of plant-plant interaction via  $\beta$ -Cyc (Fig. 3). The  $\beta$ -Cyc can function in long-distance and serves in intra- and inter-species communication (Havaux, 2020), even in the most ancestral land plants, the bryophytes. Vicherová et al. (2020) observed that the growth pattern of one bryophyte species was altered by the  $\beta$ -Cyc emitted by other bryophyte species.

### 3. Parallel carotenoids functions in plant and animals of environmental concern

While all photosynthetic organisms and some non-photosynthetic fungi and bacteria can synthesize carotenoids, *de novo* biosynthesis is rare in the Animal Kingdom. Only a few arthropods such as pea aphids, spider mites and gall midges have recently been described to perform it (Dhami and Cazzonelli, 2020). This ability is because they have acquired carotenoids by horizontal gene transfer from fungi (Toews et al., 2017). All non-carotenogenic organisms, including humans, need a dietary intake of carotenoid-rich foods (i.e., fruits and vegetables) to fulfill the carotenoid requirement. Then, these carotenoids are distributed across all organs and tissues, with a marked preference for the retina, skin or liver. Besides, most animals are equipped with enzymes encoded by highly conserved genes that allow the metabolization of carotenoids to their derivatives (i.e., apocarotenoids) (Toews et al., 2017). As they do in plants (Fig. 4), carotenoids play vital roles in the metabolism and physiology of animals (e.g., in photoprotection and serving as photoreceptors, antioxidants, and signalling molecules), enhancing the fitness of individuals in response to environmental conditions (Svensson and Wong, 2011; Swapnil et al., 2021). Indeed, dietary carotenoids and derived apocarotenoids are bioactive compounds that promote health and prevent or act against diseases in animals and especially in humans (Meléndez-Martínez and Mapelli-Brahm, 2021; Swapnil et al., 2021). In the subsequent subsections, we will break down the most relevant functions of carotenoids in animals (including humans) following the order set out previously in the Section 2.

#### 3.1. Carotenoid functions: photoreception, photoprotection and membrane stabilizers

Animals can convert dietary carotenoids, such as  $\alpha$ -Car,  $\beta$ -Car and  $\beta$ -cryptoxanthin, into vitamin A (all-*trans* retinol/ROL). Vitamin A is the precursor of 11-*cis* retinal, the chromophore that covalently binds to the opsin protein to form rhodopsin in rod cells of the retina to detect light/dark contrast and in the cone cells to detect colours. It is now theorized that the trichromatic colour vision (blue, green, red) of primates and humans probably coevolved with the colours of fruits and leaves, leading to more effective identification and selection by colours (Osorio and Vorobyev, 1996). A cycle of *cis*-to-*trans* isomerization of the visual chromophore is the basis of animal vision (see von Lintig et al., 2021 and references within). Interestingly, opsins are present in eyespots of metazoans and motile green algae, but they have not been found in



higher plants. In both animal and unicellular flagellated algae, rhodopsin responds to a light signal and mediate a phototransduction process: (i) in animals' eye, light energy is converted into an electrical signal in the visual process (Ridge and Palczewski, 2007), meanwhile (ii) in the "algae eye" rhodopsin is the receptor of phototaxis activating flagellar movements to guide the algae to places with optimal light conditions for photosynthesis (Hegemann, 1997). This is an example of parallelism of the light-harvesting function of plants in animals (see Section 2.1.2).

Lately, rhodopsin has also been found in human skin as part of a photo-sensitive system for visible radiation that modulates physiological processes of the skin as wound healing, photoaging, melanogenesis and even hair growth (Suh et al., 2020, and references within). In this regard, topical carotenoid-based sunscreens applied directly to the skin, together with the intake of endogenous carotenoids, can achieve effective photoprotection (Zerres and Stahl, 2020).

Regarding the function of energy dissipation (see Section 2.1.2), the xanthophylls Lut, meso-Zea, Zea and their oxidized metabolites (mainly present in the macula lutea region of the human retina) protect the eye from light damage, macular diseases and other vision pathologies (see Sauer et al., 2019, and references within). This is performed by acting as effective high-energy visible blue light (450–500 nm) filters, absorbing 40–90% light (Arunkumar et al., 2018). Additionally, xanthophylls decrease membrane susceptibility to lipid oxidation by enhancing the rigidity of the lipid bilayer (Arunkumar et al., 2020). This membrane rigidity is due to the orientation of Lut (which occupies parallel or perpendicular orientations) and Zea (only perpendicular) to the plane of the membrane (Arunkumar et al., 2020; as noted in Section 2.1.1).

### 3.2. Antioxidant protection

As in plants, carotenoids are not only involved in eye photoprotection in animals but also in antioxidant protection (see Section 2.1.3). This is vital because in animals oxidative stress and inflammation response are interconnected and both contribute to the development of chronic diseases (Chatterjee, 2016; Demmig-Adams et al., 2020). Carotenoids and their derivatives (i.e., apocarotenoids) can exert their effects at multiple biochemical levels in humans: (i) they may act as signalling molecules triggering reactions cascades that ultimately activate cytoprotective genes to induce antioxidant response (Pall and Levine, 2015); (ii) they can react directly with ROS (Gammone et al., 2015), as macular carotenoids; (iii) they can interact with other non-enzymatic antioxidants (e.g., ascorbic acid, tocopherol, docosahexaenoic acid and eicosapentaenoic acid), increasing their effectiveness in a synergistic manner (Gammone et al., 2015); and (iv) their orientation in biological membranes is crucial to elucidate antioxidant or pro-oxidant properties of them (McNulty et al., 2007).

The effectiveness of carotenoids as antioxidants is influenced by multifactorial elements, in fact under some metabolic conditions they may have a dual role as antioxidants or pro-oxidants (Ribeiro et al., 2018). Under high oxygen partial pressure, high concentrations of  $\alpha$ - and  $\beta$ -Car in cells are autoxidized and display a pro-oxidant activity (Ribeiro et al., 2018). At present, it is difficult to reach a definitive conclusion on the anti- or pro-oxidant properties of carotenoids as these are strongly affected by metabolic and structural factors of cells (McNulty et al., 2007; Ribeiro et al., 2018). In plants, the accumulation of  $\beta$ -Car seems to be controlled by strict regulation mechanisms, avoiding the excessive accumulation of carotenoids and maintaining homeostasis of these endogenous multifunction compounds. In any case, recent studies recommend avoiding the intake of excessive doses of  $\beta$ -Car as a diet supplement, especially for smokers.  $\beta$ -Car can easily be converted into pro-oxidant in the oxidative environment of the lung and lead to the so-called "antioxidative stress", blocking the natural adaptive intracellular signalling pathways mediated by low levels of ROS (Astori et al., 2021). To date, no pro-oxidant activity has been observed in plants similar to that described in humans.

In mammals, especially in humans, carotenoids regulate specific processes at the molecular level absent in plants, related to disease prevention and health promotion (Swapnil et al., 2021). However, this knowledge is still insufficient and more progress is needed to understand the interactions of apocarotenoids in the metabolism of plants and animals to elucidate their biological roles. Increasing evidence at the biochemical and epidemiological levels demonstrated the benefits of carotenoids as health-promoting compounds. Thus, this area is of great interest to agriculture, the food industry and medicine due to the positive effect on human health.

### 3.3. Carotenoids-based signals in bio-communication

Plant carotenoids are one of the most common ornamental compounds used by plants and animals to communicate with each other by complex intra- or interspecific ecological interactions (Svensson and Wong, 2011; see also Section 2.4). They have been proposed as visual signal molecules that reflect good health status (Bertrand et al., 2006; Pérez et al., 2008; Svensson and Wong, 2011) or social status in animals (Lozano, 2001). Indeed, a parallel role for carotenoids in reproductive success can be found in some animals that accumulate carotenoids as sexually selected ornaments. The function of this accumulation is to attract the opposite sex (to promote mating), as carotenoid colouring could indicate mate quality and social status (Svensson and Wong, 2011). There is experimental evidence on the relationship between the accumulation of carotenoids in birds and fishes with higher metabolic cost (negative effect) or on the contrary with animal health (positive effect) (Lozano, 2001; Svensson and Wong, 2011). However, it remains contradictory whether the beneficial effects are directly due to the action of the carotenoids themselves or just reflect a general health status due to other non-visible protective molecules. In this case, ornamental carotenoids can reflect a good provision of other more effective colourless antioxidants (i.e., vitamins C and E) (Bertrand et al., 2006).

Plant carotenoids and apocarotenoids can play other ecologically relevant roles in bio-communication with plants (Section 2.4), but also between animals, such as in mimicry, aposematic colouration, camouflage, and social dominance of animals (Svensson and Wong, 2011). An example of the latter is the role of  $\beta$ -Cyc,  $\beta$ -cyclocitric acid, DHA,  $\beta$ - and  $\alpha$ -ionone used by red fox as odorant molecules in chemical signalling for social communication (McLean et al., 2019).

## 4. Connections of carotenoids with the functioning of terrestrial ecosystems

Carotenoids, together with chlorophylls, are a part of the photosynthetic apparatus and they are fundamental to the energy and carbon exchange into plant biomass. However, exists a trade-off between photosynthesis and photoprotection (modulated by carotenoids; Section 4.1) that may have important consequences in the context of whole-leaf and whole-plant function in the environment. Plants regulate the amounts of carotenoids to respond to environmental conditions (Esteban et al., 2015a). From the applied point of view, the carotenoid content in plants is related to the plant's physiological status and the functioning of the ecosystems. Therefore, carotenoids are central to evaluating plant health both at the organism and ecosystem levels (Section 4.2).

### 4.1. Photosynthesis-photoprotection trade-off by carotenoids

There is evidence that losses in plant productivity caused by unfavourable environmental stresses that potentially lead to photoinhibition are counteracted by increased photoprotection (e.g., Demmig-Adams et al., 2014). Since most strategies of photoprotection involve lowering the efficiency of light energy conversion, it has been postulated the existence of a trade-off between photosynthesis and photoprotection. Carotenoids are one the basis of most of these mechanisms (achieved by

dissipation of excess energy by xanthophyll cycles –see Section 2.2.1–, by light shielding–see Section 2.1.4– and scavenging of ROS –see Section 2.2.2–), which inevitably has implications for global production. This is exemplified in desiccation-tolerant plants. These species, also known as resurrection plants, synthesize large amounts of Zea in response to desiccation, even in darkness (Fernández-Marín et al., 2021). Zeaxanthin contributes to thylakoid protection in the desiccated state, by favouring energy dissipation and antioxidant activity. In the same way, tomato plants can also compensate for the photoinhibition caused under chilling stress by increasing photoprotection through the accumulation of Zea (Wang et al., 2010). However, complex crosstalk between redox and hormonal signalling has been described in chloroplast function to regulate photosynthesis and photoprotection at the whole plant level (Müller and Munné-Bosch, 2021) (see Section 2.1). Indeed, it has been illustrated that, while losses in productivity by abiotic stresses can be improved by augmented thermal dissipation in some cases (Demmig-Adams et al., 2014 and references herein), plant resistance to abiotic stress (e.g., pests and pathogens) can be either lowered or increased by augmented thermal dissipation (Demmig-Adams et al., 2014 and references herein). Still, much work is needed regarding this point, especially under the combination of different stresses.

Another example of the previous trade-off is the light shielding by secondary carotenoids as retrocarotenoids (see Section 2.1.4) is an economically cheap mechanism to reduce energy absorption. Unlike

other secondary metabolites, carotenoids lack nitrogen or other valuable nutrients and once produced, they are remarkably stable and do not require continuous recycling or metabolic inputs (Solovchenko and Neverov, 2017). The main disadvantage of such a mechanism is that, unlike flexible mechanisms such as dynamic thermal dissipation, it is not reversible in a short time. It implies that the accumulation of secondary carotenoids decreases the efficiency of light absorption by chlorophyll, thereby reducing photosynthetic rates. This is probably the reason why the accumulation of secondary carotenoids is only observed under conditions of sustained or severe stress (Hong et al., 2013; Solovchenko and Neverov, 2017). An escape from such a trade-off between photosynthesis and photoprotection has been described in *Euglena sanguinea*, a microalga that contains red-coloured astaxanthin-containing lipid bodies (Laza-Martínez et al., 2019). These structures move freely through the cytoskeleton and can be located facing the surface under conditions of high illumination or in the center of the cells when light is limited. These movements lead to rapid and dramatic changes in the colour (from red to green and vice-versa) of their colonies.

#### 4.2. Using carotenoids as tools to evaluate plant health

Even beginners in gardening know that yellowish foliage indicates that plants are suffering any kind of stress. This is simply because observable phenotypic signs of loss of plant performance and health

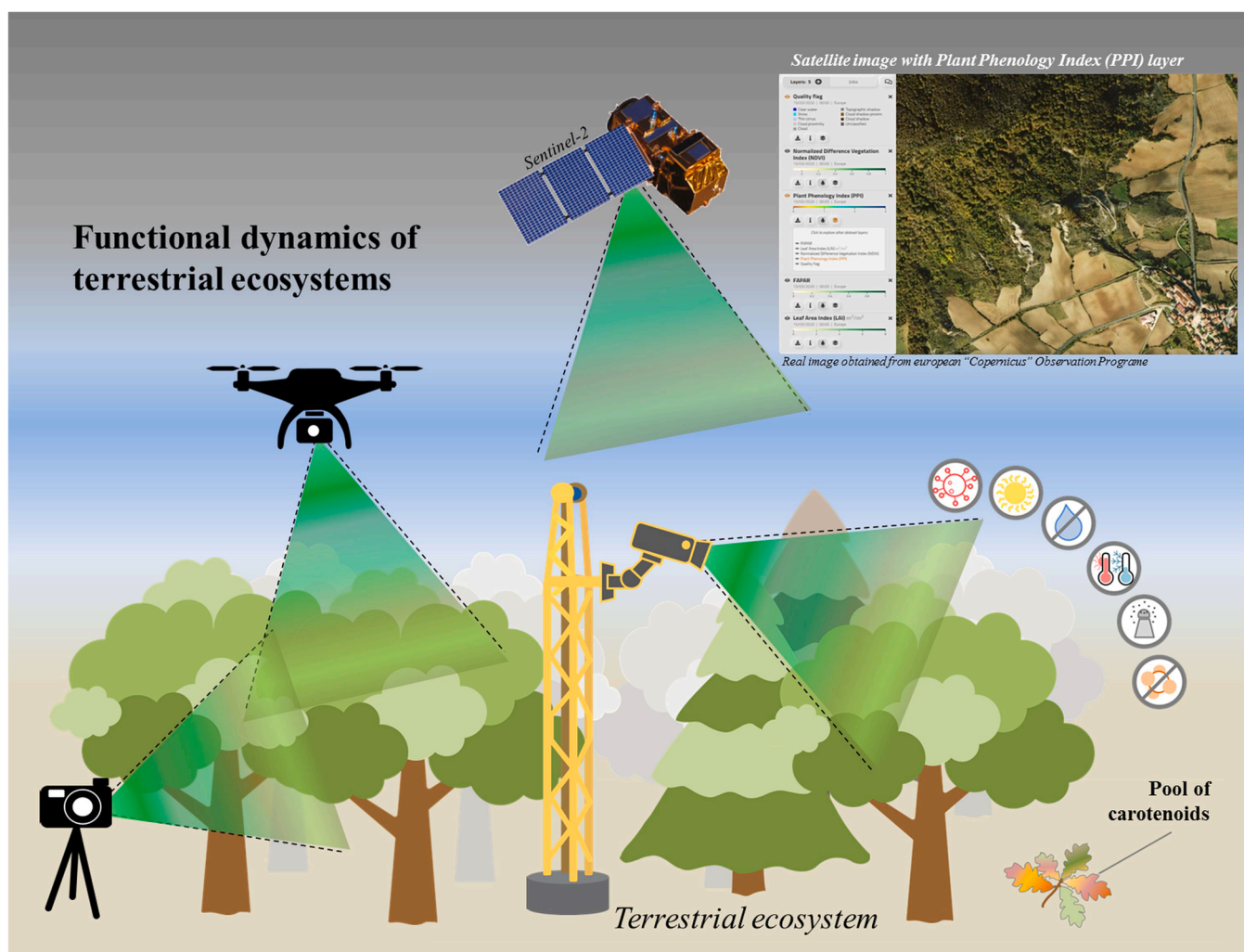


Fig. 5. Overview of monitoring devices for open vegetation sensitive to variations in carotenoid content. From left to right: tripod-mounted digital camera set up in the field, drone with sensors (i.e., hyperspectral) onboard, tower-based digital camera for forest canopy, and coarse-scale satellite remote sensing. Quantitative data on vegetation colour can be directly extracted from the images obtained by all these devices and transformed into vegetation indices.

include anatomical, morphological, and physiological characteristics (traits) resulting from the biochemical adjustments triggered at the leaf level (Fernández-Marín et al., 2017). This in turn reflects profound modifications of the photosynthetic apparatus (Esteban et al., 2015a) and plant metabolism (Rivas-Ubach et al., 2016), although the latter do not always imply a visual phenotypic change. Interestingly, the spatial and temporal dynamics of plant carotenoids are connected to the functional dynamics of ecosystems, due to their multiple biological roles in optimal and stressed conditions. The enhancement of carotenoids triggered under stress is widely employed as early stress markers to evaluate and predict plant physiological status and as early warning signals (Fenollosa and Munné-Bosch, 2018). Hence, carotenoids may be considered a tool of high capacity for the diagnosis of early vigor losses at the plant level, and even the ecosystem level (Encinas-Valero et al., 2022). Still challenging, field-based carotenoid approaches provide the opportunity to scale up leaf responses and assess these traits from the leaf/tree level into pixels by a variety of techniques, such as digital images from near-surface and remote sensing technology (Fig. 5). The use of digital cameras to track temporal changes in the vegetation structure and leaf phenology is becoming of global use due to their low cost and high resolution (Alberton et al., 2017; Junker and Ensminger, 2016). Through the quantification of the red, green, and blue colour channels, vegetation indices may be related to senescence processes and the carotenoid contents (Junker and Ensminger, 2016). Regarding remote sensing technology, vegetation optical indices are widely employed to monitor ecosystems, as they target reflectance and absorption changes related to pigment composition. The photochemical reflectance index compares reflectance at 531 and 570 nm, correlates with the xanthophyll cycle activity and stress responses in plants (Wong and Gamon, 2015) and the chlorophyll carotenoid index (Lyapustin et al., 2012) is closely linked to changes in colour (due to stoichiometric changes of the photosynthetic apparatus). These indices can be obtained by satellite images (i.e., MODIS, Sentinel) or with drone platforms with sensors onboard (D'Odorico et al., 2021). Remote-sensing and high throughput plant carotenoids phenotyping are fast, low cost and reliable and could be of interest in most ecophysiological studies. However, further evaluations of these techniques during the coming years are required.

## 5. Concluding remarks and prospects

The global and comprehensive approach of this review sheds light on the multifunctional character of carotenoids in plant-environment interactions. Much progress is being made in understanding the mechanisms that control the biosynthesis, modification, and degradation of carotenoids, as well as the properties related to light relation (i.e., photoprotection, light harvesting or antioxidants). Even so, the implications of several secondary carotenoids in plant physiology are not yet fully understood. Further research on plant-microbiome and plant-plant interactions is needed to better understand the role of carotenoids in these linkages. Another important line of research could involve integrating carotenoid functions with phytohormones and redox signalling. Finally, fine-tuning of high-resolution monitoring of carotenoid dynamics (including primary and secondary carotenoids) is still needed. Understanding plant-environment interactions at the biochemical, physiological and ecological scales, can lead to advances in fields such as ecology, agriculture, food quality/safety and human health; all of them particularly pertinent given the current climatic crisis.

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## CRediT authorship contribution statement

**Raquel Esteban:** Conceptualization, Supervision. **María Teresa Gómez-Sagasti, Marina López-Pozo, Unai Artetxe, José María Becerril, Antonio Hernández, José Ignacio García-Plazaola, Raquel Esteban:** Design, Writing – original draft. **María Teresa Gómez-Sagasti, Marina López-Pozo:** Figures design. **María Teresa Gómez-Sagasti, Raquel Esteban:** Writing – review & editing. **José María Becerril, José Ignacio García-Plazaola, Raquel Esteban:** Funding acquisition.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

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