New Phytologist

Review

Research review

Sensing β-carotene oxidation in photosystem II to master plant stress tolerance

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Received: *28 March 2019*
Accepted: *8 May 2019*

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New Phytologist (2019)
doi: 10.1111/nph.15924

Key words: apocarotenoid, photooxidative stress, photosystem, signaling, singlet oxygen, β-carotene, β-cyclocitral.

Summary

Stressful environmental conditions lead to the production of reactive oxygen species in the chloroplasts, due to limited photosynthesis and enhanced excitation pressure on the photosystems. Among these reactive species, singlet oxygen ($^1O_2$), which is generated at the level of the PSII reaction center, is very reactive, readily oxidizing macromolecules in its immediate surroundings, and it has been identified as the principal cause of photooxidative damage in plant leaves. The two β-carotene molecules present in the PSII reaction center are prime targets of $^1O_2$ oxidation, leading to the formation of various oxidized derivatives. Plants have evolved sensing mechanisms for those PSII-generated metabolites, which regulate gene expression, putting in place defense mechanisms and alleviating the effects of PSII-damaging conditions. A new picture is thus emerging which places PSII as a sensor and transducer in plant stress resilience through its capacity to generate signaling metabolites under excess light energy. This review summarizes new advances in the characterization of the apocarotenoids involved in the PSII-mediated stress response and of the pathways elicited by these molecules, among which is the xenobiotic detoxification.

Introduction: Photosynthesis and photooxidation, two sides of the same coin

Photosynthesis of green organisms inevitably produces harmful reactive oxygen species (ROS) (Apel & Hirt, 2004; Li *et al.*, 2009) due to an increased O_2 concentration in the chloroplasts (Steiger *et al.*, 1977) and its interaction with the photosynthetic electron transport chain. In addition, chlorophyll molecules can act as photosensitizers when in the triplet excited state, generating singlet oxygen ($^1O_2$). $^1O_2$ is highly reactive and readily oxidizes its local environment leading to decreased photosynthetic efficiency (Triantaphylidès & Havaux, 2009; Fischer *et al.*, 2013). The lifetime of $^1O_2$ in plant tissues is therefore short, < 1 μs (Redmond & Kochevar, 2007).

Photoproduction of $^1O_2$ and other ROS is enhanced under many environmental stress conditions, when photosynthesis is restricted and light energy is absorbed in excess of its utilization by the photosynthetic process, promoting transfer of electrons and excitation energy to O_2 (Apel & Hirt, 2004; Li *et al.*, 2009). $^1O_2$ is the predominant ROS produced in Arabidopsis cell suspensions exposed to high light (Gonzalez-Perez *et al.*, 2011) and, since photooxidation is associated with a lipid peroxidation signature typical of $^1O_2$ attack on lipids, it has been concluded that $^1O_2$ is the major ROS involved in photooxidative damage to plant leaves (Triantaphylides *et al.*, 2008). However, besides its direct toxicity, $^1O_2$ also acts as a signaling molecule that triggers a transcriptional response, leading to programmed cell death or to acclimation to photooxidative stress, depending on its level (Laloi & Havaux, 2015; Dogra *et al.*, 2018). Through ROS production, chloroplasts can thus indicate absorbed light energy to the nucleus, which regulates gene expression and hence adjusts plant metabolism to the incident light energy and its use in photosynthesis.

Photosystem II as a $^1O_2$ photo-generator

The main source of $^1O_2$ in plant leaves is photosystem II (PSII). Triplet–triplet energy transfer from chlorophyll to O_2 can occur in both the PSII antenna complexes (LHCII) and the PSII reaction center (RC) (Krieger-Liszkay, 2004; Vass, 2012; Pospisil, 2016). In the LHCII, triplet chlorophylls can be formed by a

photosensitization mechanism, whereas in the PSII RC, triplet chlorophyll ^{3}P680 is formed by charge recombination of the triplet radical pair ^{3}[P680$^+$ Pheophytin$^-$], where P680 is the RC chlorophyll molecule. $^1O_2$ is believed to be generated predominantly from the PSII RC by the latter mechanism, particularly when reduction of the PSII electron acceptors is enhanced (Vass, 2012). An additional channel for ^{3}[P680$^+$ Pheophytin$^-$] state formation is via a backflow of electrons from the first stabilized charge separation state, P680$^+$ Q_A^- (Vass, 2011, 2012). The presence of O_2 during illumination of isolated PSII RC drastically shortens the ^{3}P680 lifetime from 1 ms to *c.* 30 μs (Durrant *et al.*, 1990), indirectly providing $^1O_2$ formation. More direct evidence of $^1O_2$ in PSII RC preparations was obtained from measurements of its luminescence at 1270 nm (Telfer *et al.*, 1994). In contrast, the LHCII contain many xanthophyll carotenoids located in close proximity to the chlorophyll molecules, especially lutein and zeaxanthin, which can directly quench triplet chlorophylls (Dall'Osto *et al.*, 2006, 2012), thus limiting the release of $^1O_2$. Moreover, the PSII antennae are equipped with non-photochemical quenching mechanisms that can dissipate excess light energy, thus limiting overexcitation and avoiding triplet chlorophyll and $^1O_2$ formation (Demmig-Adams *et al.*, 2014). Accordingly, $^1O_2$ release by the PSII centers is drastically enhanced in the Arabidopsis *ch1* mutant that is completely devoid of LHCII (Dall'Osto *et al.*, 2010). In contrast, the PSII core complex contains only β-carotene molecules, with two molecules in the RC itself (Ferreira *et al.*, 2004). These two β-carotene molecules are bound to two homologous PSII-center polypeptides, D1 and D2, and are thus distanced form the chlorophyll molecules (Trebst, 2003). As a consequence, they are not able to quench the ^{3}P680 triplet state, and the function of β-carotene in the PSII centers is principally to scavenge the $^1O_2$ molecules produced therein. The probability of $^1O_2$ generation in the PSII center is therefore much higher than in the LHCII.

The principal mechanism for quenching $^1O_2$ by carotenoids is physical quenching, involving energy transfer and producing the carotenoid triplet state that deactivates through thermal decay (Triantaphylidès & Havaux, 2009; Edge & Truscott, 2018). However, β-carotene in PSII can occasionally be oxidized by $^1O_2$, generating a variety of derivatives including the $^1O_2$-specific β-carotene endoperoxide (Ramel *et al.*, 2012a). This latter compound accumulates in leaves upon exposure to high light. In contrast, lutein/zeaxanthin endoperoxides remain at a low level in high light-exposed leaves. Accordingly, ^{14}C pulse-chase labeling experiments revealed a continuous flux of newly fixed carbon into β-carotene in photosynthesizing leaves transferred to high light (Beisel *et al.*, 2010), suggesting rapid turnover of this pigment. No evidence was found for ^{14}C incorporation into xanthophylls, and this could be related to the maintenance of a low level of lutein/zeaxanthin endoperoxide observed in high light-exposed leaves. Taken together, these findings suggest a low turnover of the xanthophylls compared to β-carotene, probably reflecting selective chemical quenching of $^1O_2$ by the latter carotenoid in PSII. Interestingly, β-carotene endoperoxide as well as $^1O_2$-specific lipid peroxidation products were found in leaves even in low light (Triantaphylides *et al.*, 2008; Ramel *et al.*, 2012a), indicating that $^1O_2$ is chronically produced in PSII.

Because the two β-carotene molecules of the PSII center are distant from the source of $^1O_2$, their ability to scavenge $^1O_2$ is partial, and $^1O_2$ can oxidize other targets such as the PSII protein D1, leading to a rapid turnover of this protein even at low light intensities (Mattoo *et al.*, 1984; Keren *et al.*, 1995). Damaged D1 protein is degraded, and PSII is repaired by the assembly of newly synthesized D1 in the so-called PSII repair cycle (Theis & Schroda, 2016). The controlled degradation of the D1 protein acts as a safety valve, as selective destruction of a specific protein, rather than the whole PSII complex, is likely to be advantageous both in terms of energy cost and release of potentially harmful pigments. In fact, further degradation of chlorophyll-binding subunits may lead to the production of free chlorophylls, which are dangerous photosensitizers and can provoke disastrous production of $^1O_2$. To minimize this effect, free chlorophyll in the thylakoids can be transiently managed by stress-inducible specialized proteins of the LHC family, such as ELIP, SEP and OHP (Hutin *et al.*, 2003; Engelken *et al.*, 2012). These stress proteins have been proposed to transiently bind chlorophylls during biogenesis/turnover of chlorophyll-binding proteins and/or to regulate chlorophyll biosynthesis. Accordingly, small LHC-like proteins were found to prevent $^1O_2$ formation during PSII damage (Sinha *et al.*, 2012).

Chlorophyll precursors are also $^1O_2$ photosensitizers, but they normally do not accumulate in plant leaves. Photodynamic damage by chlorophyll precursors was observed under specific conditions, such as in the Arabidopsis *flu* mutant after dark adaptation (Laloi & Havaux, 2015; Dogra *et al.*, 2018) or in leaves treated with aminolevulinic acid (Chakraborty & Tripathy, 1992).

The production of $^1O_2$ by PSI is not considered significant (Suh *et al.*, 2000). Using EPR spectroscopy and a $^1O_2$ spin probe, it was showed that neither PSI nor LHCII produce $^1O_2$ in high light (Hideg & Vass, 1995). In line with these observations, the β-carotene endoperoxide, a specific marker of $^1O_2$ oxidation, was not induced when PSI was selectively illuminated with high intensity far-red light (Ramel *et al.*, 2012a). Under special conditions, triplet state P700 can be formed by charge recombination, as is the case for P680 in PSII. However, the lifetime of the state ^{3}P700 is not shortened by O_2 (Sétif *et al.*, 1981), indicating that P700 is screened from O_2, hence precluding $^1O_2$ formation. Consistently, the lifetime of PSI is considerably longer than that of PSII (Yao *et al.*, 2012). Taken together, these results lead to the conclusion that PSI is not a major source of $^1O_2$, although Cazzaniga *et al.* (2016) reported $^1O_2$ production from isolated PSI–LHCI complex exposed to very high light intensity, as measured by an increase in fluorescence intensity of the SOSG probe (Singlet Oxygen Sensor Green). However, the interpretation of SOSG fluorescence changes in terms of $^1O_2$ concentrations must be considered with caution because this technique has some drawbacks, including the fact that SOSG has photosensitizing properties that could lead to artefactual $^1O_2$ detection, particularly in very high light (Ragas *et al.*, 2009). Moreover, destruction of photosystems at very high light can uncouple or release chlorophyll molecules which can secondarily generate $^1O_2$.

Interestingly, another source of $^1O_2$ may exist: the cytochrome b6/f complex, which contains a chlorophyll *a* molecule at an approximate 1 : 1 stoichiometry (Stroebel *et al.*, 2003). This

complex is able to generate $^1O_2$ in the light (Jung & Kim, 1990; Suh *et al.*, 2000) and requires the presence of a neighboring β-carotene to avoid its autoxidation (Zhang *et al.*, 1999). Cytochrome b6/f is therefore a potential source of $^1O_2$ and oxidized β-carotene derivatives, but its contribution to the *in vivo* $^1O_2$ production is assumed to be minor compared to PSII-generated $^1O_2$.

Carotenoid oxidation and stress signaling surveillance of PSII

The $^1O_2$ is a strong electrophile agent that has high reactivity towards double bonds in biological molecules, producing a variety of oxidized derivatives, such as aldehydes, ketones, endoperoxides, epoxides or lactones (Triantaphylidès & Havaux, 2009). Actually, each double bond in the β-carotene molecule can be oxidized by $^1O_2$ (Stratton *et al.*, 1993; Ramel *et al.*, 2012a, 2012b) (Fig. 1a). Oxidative cleavage of the double bond of the β-carotene polyene chain at position 7,8 generates a volatile, short-chain compound called β-cyclocitral (β-CC) (Fig. 1b) and the long-chain β-apo-8′-carotenal, which can be further oxidized to generate another molecule of β-CC and crocetindialdehyde (Frusciante *et al.*, 2014). Cleavage at position 9,10 leads to β-ionone (Fig. 1e), which can be further oxidized by $^1O_2$ resulting in lactone dihydroactinidiolide (dhA) (Havaux, 2014) (Fig. 1f).

Under high light stress, a multitude of β-carotene-derived metabolites, such as β-CC, β-ionone and dhA, are generated in leaves at the PSII level (Ramel *et al.*, 2012b). Emission of those compounds was also reported in lichens and in cyanobacteria exposed to stress (García-Plazaola *et al.*, 2017). Although a large fraction of β-carotene is located in PSI (Thayer & Bjorkman, 1992), the low capacity of this photosystem to generate $^1O_2$ makes it unlikely that it contributes to β-CC production in planta under physiologically relevant conditions.

Unlike β-carotene endoperoxide, β-ionone and other oxidized carotenoid products are not specific markers of $^1O_2$ oxidation since they can be also produced enzymatically by carotenoid cleavage dioxygenases (CCD), which cleave carotenoids at specific positions (Auldridge *et al.*, 2006; Harrison & Bugg, 2014). In Arabidopsis there are nine CCD, categorized according to their enzymatic specificities, among which five members are in the nine-cis-epoxy carotenoid dioxygenase subfamily (NCED2,3,5,6,9) that mediates cleavage at the 11–12 position and participates in ABA biosynthesis. CCD1, CCD4 and CCD7 preferentially mediate cleavage at the 9,10 position and have broad substrate specificity, while CCD8 may be specific for strigolactone biosynthesis. In addition, CCD2 has been identified in *Crocus sativus*, where it mediates the cleavage at the 7,8 position of zeaxanthin (but not β-carotene) to generate 3-OH-β-cyclocitral, a precursor of safranal (Auldridge *et al.*, 2006; Harrison & Bugg, 2014).

In Arabidopsis, β-ionone can be enzymatically produced by CCD1 and CCD7, while it is not clear whether a CCD could generate β-CC from β-carotene *in vivo*. In *C. sativus*, β-CC seems to be a minor cleavage product of CCD4 (Rubio-Moraga *et al.*, 2014). Therefore, β-CC and β-ionone may correspond to two different pathways in apocarotenoid signaling, with β-ionone being generated also via an enzymatic reaction and β-CC having mainly an autooxidative origin. However, *CDD* genes were not found to be induced under high light stress, with *CCD4* even being strongly repressed (Ramel *et al.*, 2013). Moreover, high light-induced accumulation of β-CC and β-ionone was not inhibited in Arabidopsis mutants deficient in each individual CCD (Ramel *et al.*, 2013), and the concentrations of the two molecules are comparable both under physiological conditions and in high light (Ramel *et al.*, 2012b). Those findings undermine the role of enzymatic oxidation in the production of oxidized carotenoid metabolites during photooxidative stress and support a role for these molecules in PSII oxidation surveillance.

Both β-CC and dhA were shown to act as signaling molecules, inducing changes in the expression of a wide set of nuclear-encoded $^1O_2$-responsive genes (Ramel *et al.*, 2012b; Shumbe *et al.*, 2014). A general feature of this transcriptomic response was the induction of genes related to cellular defense against stress and the down-regulation of genes related to cell growth and development. This phenomenon was not observed with β-ionone, indicating

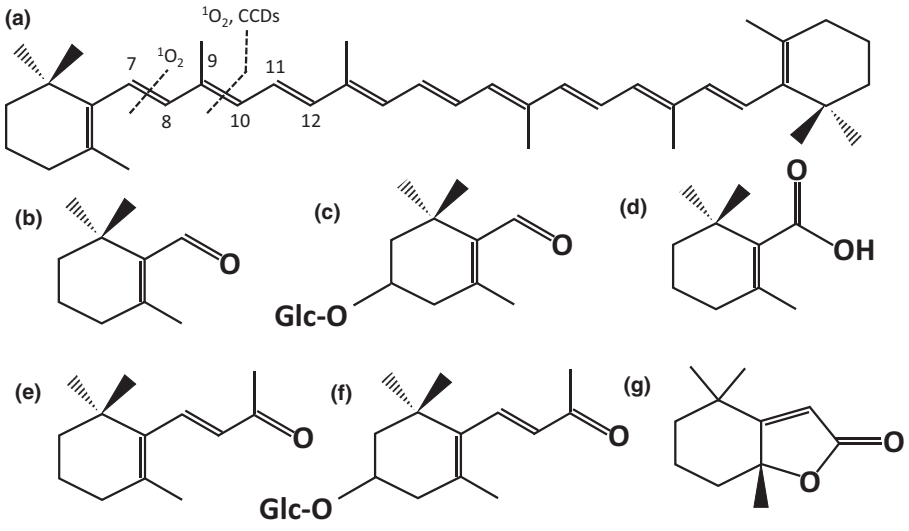

Fig. 1 Chemical structure of β-carotene (a) and of some of its oxidized derivatives: β-cyclocitral (b), its glycosylated form GAPO7 (c), β-cyclocitric acid (d), β-ionone (e), its glycosylated form GAPO9 (f) and dihydroactinidiolide (g). Some cleavage sites of $^1O_2$ and/or of carotenoid cleavage dioxygenase enzymes (CCD) in β-carotene are shown in (a). Oxidative cleavage of the double bond at the position 7,8 generates compound (b) while cleavage at the position 9,10 leads to compound (e). Glc, glucose.

specificity of the transcriptomic responses to some carotenoid metabolites. It also indicates that the effects of β-CC or dhA cannot be assumed to be a mere response to reactive electrophiles since the electrophilicity of β-ionone is higher than that of β-CC and dhA. Moreover, the transcriptome of β-CC-treated Arabidopsis plants overlaps only moderately with the transcriptome of plants treated with typical reactive electrophilic species, such as malondialdehyde or methyl vinyl ketone (Weber *et al.*, 2004; Ramel *et al.*, 2012b).

As discussed by Vass (2011, 2012), primary charge separation in PSII and formation of ^{3}[P680$^+$ Pheophytin$^-$], the source of ^{3}P680, can occur after light saturation of photosynthetic electron transport. Since the rate of primary charge separation in closed PSII centers linearly depends on light intensity, the yield of ^{3}P680, and therefore the production of $^1O_2$, are expected to increase linearly with light intensity. It would be interesting to test whether β-CC production exhibits a similar relationship with light intensity.

The relation of β-CC to other retrograde signaling pathways

Tremendous progress has been made in identifying signaling components mediating chloroplastic control of nuclear gene expression. In particular, a prominent role of metabolites has been described in retrograde signaling, including tetrapyrroles (*e.g.* Mochizuki *et al.*, 2001; Page *et al.*, 2017), 3-phosphoadenosine 5-phosphate (PAP) (Estavillo *et al.*, 2011), dihydroxyacetone phosphate (DHAP) (Lorenc-Kukula *et al.*, 2012) and carotenoid derivatives, among which are β-CC and dhA. In order to transmit a signal to the nucleus, these molecules need to move out of the chloroplasts or impose a signaling mechanism from the chloroplast to the nucleus. H_2O_2 produced at the level of PSI, for example, can directly move to the nucleus (Exposito-Rodriguez *et al.*, 2017). Other polar molecules, such as PAP and DHAP, requires specific transporters. Conversely, β-CC and dhA are small, lipid soluble and volatile. Therefore, they may diffuse through membranes, escape the chloroplast and assist in the communication between different organelles. Hence, they are potential carriers of the *in vivo* $^1O_2$ signal from the chloroplast to the nucleus. However, migration of β-CC to the nucleus remains to be demonstrated experimentally. Also, no receptors for β-CC or dhA have been discovered so far, and identification of the primary target of these metabolic signals will be a major challenge in the future.

In the cyanobacterium *Microcystis*, the only organism in which true β-carotene CCD-dependent 7,8 cleavage activity has been described (Jüttner *et al.*, 2010), the rapid burst of β-CC production is concomitant with its oxidation to a carboxylic acid, β-cyclocitric acid (β-CCA) (Fig. 1d). This oxidation occurs spontaneously in water (Tomita *et al.*, 2016). We recently demonstrated that this molecule exists in Arabidopsis at higher concentrations than β-CC (D'Alessandro *et al.*, 2018a). Therefore, we can hypothesize several fates for β-CC, depending on which side of the thylakoid membrane it is released from PSII. In the case of a release on the luminal side of the thylakoid membranes, we can imagine a fast oxidation due the oxidizing environment (Steiger *et al.*, 1977), especially under illumination. In this compartment, the majority of β-CC could be transformed in the carboxylic acid β-CCA, which has a predicted pKa between 4.5 and 4.85. At the acidic pH of the thylakoid lumen under illumination (Takizawa *et al.*, 2007), i.e. when β-CC is mainly generated, only a fraction of the molecule would be present in the neutral form, and β-CCA would be trapped in the lumen unless hypothesizing the presence of transporters. In any case, once in the stroma, β-CCA would be almost completely present in the ionic form, and again only the presence of transporters could allow the molecule to exit the chloroplast. In contrast, if β-CC were generated in the chloroplast stroma, the reducing environment (−360 mV) (Takizawa *et al.*, 2007) could impede the immediate oxidation to β-CCA, therefore β-CC could pass through chloroplast membranes and directly act as a retrograde signal, being transformed into β-CCA only in a second phase, for example in the cytosol, which is slightly less reducing (−320 mV).

A possible chloroplastic action of β-CC/β-CCA cannot be excluded, and the connection between β-CC and other known elements of chloroplastic retrograde signaling has been tested. β-CC signaling appears to take place independently of the tetrapyrrole pathway as the mutant lines *gun 1, 4, 5,* representatives of the Mg-Protoporphyrin IX pathway, and *gun3* and *fc1,* for the heme pathway, were not affected in the genetic response to β-CC (unpublished). The EXECUTER 1 and 2 (EX1, EX2) proteins, responsible for mediating $^1O_2$-induced cell death, also resulted a distinct pathway (Ramel *et al.*, 2012b; Dogra *et al.*, 2018). From recent analyses of the transcriptome of β-CC-treated plants, a possible interconnection between β-CC and PAP signaling arose from the observation that the 3′(2′),5′-bisphosphate nucleotidase SAL1, responsible for PAP degradation, is down-regulated by β-CC (Ramel *et al.*, 2012b). Furthermore, β-CC induces the ST2A sulfotransferase that uses PAPS as sulfate donor, hence generating PAP. Therefore, β-CC may induce PAP accumulation and mediate PAP regulation in response to altered photosynthesis under drought and excessive light stress.

In line with the interconnection with PAP signaling, gene reprogramming by β-CC and dhA was associated with a substantial increase in plant tolerance to photooxidative stress. Arabidopsis plants pre-treated with volatile β-CC or dhA exhibited lower lipid peroxidation and lower PSII photoinhibition after high light stress compared to untreated plants (Ramel *et al.*, 2012b; Shumbe *et al.*, 2014, 2017). β-CC and β-CCA were also found to enhance drought tolerance of Arabidopsis plants (D'Alessandro *et al.*, 2018a). Both stresses are known to modulate PAP accumulation (Estavillo *et al.*, 2011).

The METHYLENE BLUE SENSITIVITY (MBS) proteins 1 and 2 are small zinc-finger proteins that mediate the transcriptomic response to $^1O_2$ in *Chlamydomonas* and Arabidopsis (Shao *et al.*, 2013), suggesting an upstream position in the $^1O_2$ signaling pathway. The *mbs1* mutant was found to be insensitive to β-CC and dhA (Shumbe *et al.*, 2017). Treatments with β-CC that enhanced photooxidative stress tolerance in WT plants did not decrease the photosensitivity of *mbs1* mutant plants, indicating that the MBS1 protein is required for β-CC-dependent $^1O_2$ signaling. Moreover, β-CC brought about an accumulation of MBS1 in WT plants, as does high light stress, with partial re-localization to the nuclei (Shumbe *et al.*, 2017). Despite its involvement in β-CC signaling, the relation of MBS1 with other components of the $^1O_2$

response, such as the EXECUTER proteins, and its exact function remain, however, to be established.

β-CC as an endogenous safener

Finally, a mechanism for β-CC-induced acclimation/tolerance to excess light was recently proposed (D'Alessandro *et al.*, 2018b). Plants, like other eukaryotes, have developed a xenobiotic detoxification system that involves thousands of proteins, including transcription factors, redox enzymes and transporters (Sandermann, 1992). This mechanism processes ectopic molecules entering the cell through redox modification, conjugation and compartmentalization to avoid toxic effects due to their chemical reactivity (Sandermann, 1992). Part of the system is transcriptionally controlled by TGAII transcription factors and their interaction with the GRAS protein SCL14 (Fode *et al.*, 2008). We demonstrated that SCL14-controlled xenobiotic detoxification is strongly induced by β-CC and by photooxidative conditions (D'Alessandro *et al.*, 2018b). Furthermore, the so-called 'xenobiotic response' is also activated by endogenous toxicants, such as reactive carbonyl species (RCS) deriving from the spontaneous decomposition of lipid peroxides (Mano, 2012). Therefore, lipid peroxides not only are the signature of photooxidation but also directly contribute to its toxicity by decaying into reactive aldehydes, such as acrolein or 4-hydroxynonenal, which can further hinder the physiological functioning of macromolecules (Mano, 2012) and can induce cell death (Biswas & Mano, 2016). By inducing SCL14-dependent xenobiotic response, β-CC prepares the cell for the accumulation of lipid peroxides occurring under excess light or drought stress by increasing detoxification of toxic RCS (D'Alessandro *et al.*, 2018b). Similar to β-CC treatment, the overexpressor lines of SCL14 were more tolerant both to excess light and to drought stress (D'Alessandro *et al.*, 2018a, 2018b). Drought and oxidative tolerance was also confirmed in rice by the overexpressor lines of the SCL14 homologue OsGRAS23 (Xu *et al.*, 2015).

Thanks to previous works on xenobiotic detoxification (Fode *et al.*, 2008; Köster *et al.*, 2012), it was possible to place an entire signaling cascade downstream of β-CC. In particular, the interaction between SCL14 and TGAII transcription factors modulates the transcriptional level of the ANAC102 chloroplastic transcription factor (Inzé *et al.*, 2012), which controls the downstream ANAC002, ANAC031 and ANAC081 transcription regulators (D'Alessandro *et al.*, 2018b), and finally the redox enzymes of the first phase of the detoxification response. In other words, β-CC acts as an endogenous safener. The latter type of compounds, usually artificial, are used in agronomy to induce the detoxification pathway without harming the plants, hence leading to tolerance to toxicants such as herbicides (Riechers *et al.*, 2010; Brazier-Hicks *et al.*, 2018). The SCL14-dependent detoxification response is independent of MBS1 regulation (D'Alessandro *et al.*, 2018b), indicating the existence of at least two pathways downstream of β-CC.

Homeostasis of β-CC

The presence of β-CC can induce the xenobiotic detoxification pathway (D'Alessandro *et al.*, 2018b), but the question arises as to whether it is also a substrate for these detoxifying chemical modifications. A possible answer to this question can be inferred from the recent demonstration that apocarotenoid hydroxylation and glycosylation are enhanced under high light conditions (Mi *et al.*, 2018). In this context, it is interesting to note that $^1O_2$ as well as β-CC have a strong up-regulating effect on the expression of several glycosyl transferases in Arabidopsis leaves (Ramel *et al.*, 2012b, 2013). When plants are subjected to strong illumination,

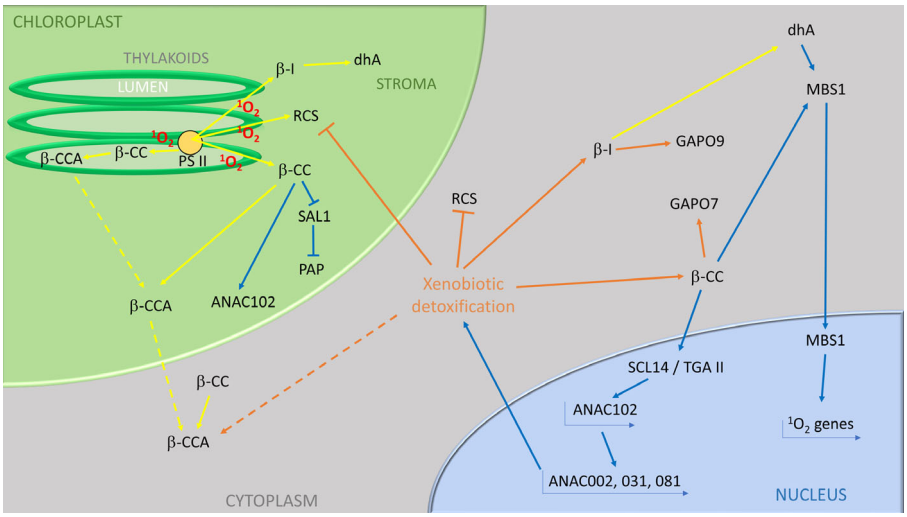

Fig. 2 Apocarotenoid production from PSII and its signaling effect in plant leaves. $^1O_2$ oxidation of β-carotene in PSII generates apocarotenoids such as β-cyclocitral (β-CC), β-ionone (β-I) and dihydroactinidiolide (dhA). Further oxidation (yellow arrows) leads to β-cyclocitric acid (β-CCA) and RCS oxidation. Active transfer of the hydrophilic molecules or passive diffusion of the volatile apocarotenoids to the cytoplasm brings about the activation of at least two signaling pathways (blue arrows) mediated by MBS1 or by TGAII transcription factors interacting with SCL14. These signaling pathways induce changes in the expression of $^1O_2$-responsive genes and of genes involved in cellular detoxification. The latter mechanism (orange arrows) targets toxic RCS (reactive carbonyl species). Dotted lines indicate hypothetical processes. PAP, 3-phosphoadenosine 5-phosphate.

the concentrations of many hydroxylated and glycosylated apocarotenoids (GAPO) increased. Although dhA and β-CCA were not present in the study, the concentrations of β-CC and β-ionone under control conditions were comparable to previous measurements (Ramel *et al.*, 2012b). Conversely, the levels of the derived glycosylated forms, GAPO7 and GAPO9, respectively (Fig. 1c), were very different, with GAPO9 concentrations being 200 times higher than GAPO7 in Arabidopsis leaves in control conditions. Massive glycosylation of β-ionone to GAPO9 could explain why a molecule with a known biosynthetic pathway via CCD shows unconjugated levels in the same range as β-CC that does not possess such a pathway. Although the measured levels of GAPO7 are just a fraction of the β-CC concentration (1.67%), while most of the β-ionone is present as GAPO9 (575%), this process suggests that β-carotene derivatives are targeted by detoxifying mechanisms. Furthermore, unlike hydroxylated β-ionone, hydroxy-β-cyclocitral was not detectable, suggesting a fast conversion to the glycosylated form and therefore the importance of this process in β-CC homeostasis and in the regulation of β-CC signaling (Mi *et al.*, 2018). The β-CC metabolization described above limits its signaling role and may constitute a negative feedback mechanism to return $^1O_2$ signaling back to unstressed levels.

Is β-CC involved in more than stress tolerance?

In addition to its role in stress response, β-CC has recently been shown to be involved in root development (Dickinson *et al.*, 2019; Wurtzel, 2019). In these new results, β-CC is able to induce primary root growth and lateral root capacity without passing through the major pathways affecting meristem growth, such as auxin or brassinosteroids (Dickinson *et al.*, 2019). However, cell growth and development pathways are down-regulated in the shoot by β-CC treatment (Ramel *et al.*, 2012b). Furthermore, involvement of β-CC in growth, as recently reviewed (Wurtzel, 2019), would strongly argue towards the existence of a biosynthetic pathway leading to β-CC production, which has yet to be described in plants.

ROS, such as H_2O_2 and superoxide, directly impact root growth (Tsukagoshi, 2016), and recently a role for $^1O_2$ in root response to osmotic stress was elucidated (Chen & Fluhr, 2018). ROS homeostasis is altered when roots are exposed to light, like in the majority of *in vitro* studies on root development (Yokawa *et al.*, 2011). Furthermore, the use of Parafilm to seal the Petri dishes reduces gas exchange and enhances photorespiration (Kerchev *et al.*, 2013), increasing H_2O_2 concentration, which is known to repress cell cycle genes and consequently meristem growth (Tsukagoshi, 2016). Interestingly, natural auxin and auxin-like molecules are able to alleviate the negative effects of photorespiration (Kerchev *et al.*, 2013) and to induce the SCL14-dependent xenobiotic response (Fode *et al.*, 2008). We suggest, therefore, that in parallel to a yet undiscovered root development mechanism, β-CC may mediate enhanced root growth by increasing root tolerance to peroxidative damage, both *in vitro* and in soil, in a similar way as it acts in the shoot (D'Alessandro *et al.*, 2018b).

Conclusions

Despite the presence of many protective mechanisms (energy dissipation by the NPQ mechanism, state transition, cyclic electron flow), PSII is likely the most vulnerable component of the photosynthetic chain to several environmental constraints, such as excess light energy (Tyystjärvi, 2013), UV-B radiation (Szilard *et al.*, 2007) and heat stress (Allakhverdiev *et al.*, 2008). Not only $^1O_2$ but also β-carotene oxidation products are released at the level of PSII due to stress-induced photooxidation, and plants have evolved mechanisms to decode these retrograde signals and consequently to regulate gene expression so as to adjust metabolism and alleviate the effects of PSII-damaging conditions, as summarized in Fig. 2.

β-Carotene has been present in PSII since early cyanobacteria, and this long-lasting relationship likely allowed the evolution of very rich and redundant response mechanisms to β-carotene derivatives. In this review, we focused on the apocarotenoids derived from β-carotene and, in particular, on β-ionone, dhA and β-CC. Most of the presented studies fit with the recently proposed role of these apocarotenoids in stress response via the xenobiotic detoxification mechanism, but an impact of β-CC on PAP retrograde signaling and the existence of more than one pathway downstream of these molecules is also possible. A new and exciting avenue towards new discoveries in the multiple effects of ROS-induced carotenoid metabolites is now opening. It is clear that a larger effort towards a better understanding of the signaling and of the mechanisms elicited by these apocarotenoids is required, especially regarding the possible existence of a receptor protein.

Acknowledgements

We would like to acknowledge funding from the French National Research Agency (ANR project SLOSAM, 14-CE02-0010-02).

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