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Review

**$\beta$ -Cyclocitral and derivatives: Emerging molecular signals serving multiple biological functions**

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## **ABSTRACT**

$\beta$ -cyclocitral is a volatile short-chain apocarotenoid generated by enzymatic or non-enzymatic oxidation of the carotenoid  $\beta$ -carotene.  $\beta$ -cyclocitral has recently emerged as a new bioactive compound in various organisms ranging from plants and cyanobacteria to fungi and animals. In vascular plants,  $\beta$ -cyclocitral and its direct oxidation product,  $\beta$ -cyclocitric acid, are stress signals that accumulate under unfavorable environmental conditions such as drought or high light. Both compounds regulate nuclear gene expression through several signaling pathways, leading to stress acclimation. In cyanobacteria,  $\beta$ -cyclocitral functions as an inhibitor of competing microalgae and as a repellent against grazers. As a volatile compound, this apocarotenoid plays also an important role in intra-species and inter-species communication. This review summarizes recent findings on the multiple roles of  $\beta$ -cyclocitral and of some of its derivatives.

## **1. Introduction**

$\beta$ -cyclocitral (2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde) is a volatile compound derived from the oxidation of the carotenoid  $\beta$ -carotene (Figure 1). It is an odorant molecule with a characteristic hey-like, mild floral flavor (Mahattanatawee et al., 2005) that has some use in food, beverage and pharmaceutical applications. The volumes of industrial use of  $\beta$ -cyclocitral as a fragrance ingredient appear, however, to be relatively modest compared to the highly demanded odorant apocarotenoids  $\alpha$ - and  $\beta$ -ionone (Lalko et al., 2007).  $\beta$ -Cyclocitral has been found in leaves, fruits or flowers of a variety of terrestrial vascular plant species (*e.g.* Ramel et al., 2012a; Tieman et al., 2012; Rubio-Moraga et al., 2009; Chen et al. 2017).  $\beta$ -cyclocitral participates in the organoleptic properties of  $\beta$ -carotene-containing fruits, such as tomato or watermelon (Lewinsohn et al., 2005). It has also been measured in cyanobacteria and microalgae (Arii et al.,

2015), freshwater plants (Yu et al., 2019), lichens (Garcia-Plazaola et al., 2017) and mosses (Saritas et al., 2001). However, non-photosynthetic organisms such as fungi (Zorn et al., 2003a) and animals (McLean et al., 2019) have also been reported to contain detectable levels of this compound.  $\beta$ -Cyclocitral is not a mere degradation product of  $\beta$ -carotene, but it is a bioactive compound fulfilling several physiological functions best understood in plants. Recent studies have highlighted new important roles for  $\beta$ -cyclocitral in cellular signaling, in communication between individuals and in defense mechanisms against abiotic and biotic stresses, which are summarized in this review article, focusing on discoveries in plants.

## 2. $\beta$ -Cyclocitral formation in photosynthetic organisms

$\beta$ -cyclocitral can be formed either by an enzymatic pathway or by direct oxidation of  $\beta$ -carotene by reactive oxygen species, especially singlet oxygen ( $^1\text{O}_2$ ). The enzymatic cleavage of carotenoids is catalyzed in plants by a family of non-heme iron-dependent enzymes, the carotenoid cleavage dioxygenases (CCDs) and the 9-*cis*-epoxycarotenoid cleavage dioxygenases (NCEDs), which lead to oxidation products called apocarotenoids (Giuliano et al., 2003; Hou et al., 2016). NCED enzymes cleave the 11,12 (11',12') double bond of 9-*cis*-violaxanthin or 9-*cis*-neoxanthin, catalyzing the first step in abscisic acid (ABA) biosynthesis (Schwartz et al., 1997; Nambara and Marion-Poll, 2005). In contrast, CCD enzymes do not share cleavage specificities with NCED enzymes. In *Arabidopsis*, there are 4 CCDs (CCD1, CCD4, CCD7, CCD8). So far, it is not clear whether one of these CCDs can produce  $\beta$ -cyclocitral from  $\beta$ -carotene in plant leaves.  $\beta$ -Cyclocitral accumulation was not affected in *Arabidopsis* mutants deficient in each of the 4 CCDs, suggesting that the CCD-mediated oxidation of  $\beta$ -carotene is not a major source of this apocarotenoid in this species (Ramel et al., 2013b), although functional redundancy between

CCDs cannot be excluded. Similarly, no CCD serving  $\beta$ -cyclocitral formation was found in cyanobacteria (Cui et al., 2012).

CCD1 is cytosolic, contrary to the other CCDs which are plastidial, and preferentially cleaves the 9,10 (9',10') double bonds of a wide range of monocyclic or acyclic carotenoids and apocarotenoids to generate divergent apocarotenoids and volatiles. CCD7 and CCD8 are devoted to the biosynthesis of strigolactones (Al-Babili and Bouwmeester, 2015). CCD4 has a selective 9,10 (9',10') and 5,6 (5',6') double bond cleavage activity and therefore is not supposed to produce  $\beta$ -cyclocitral (Giuliano et al., 2003; Hou et al., 2016). Moreover, CCD4 is strongly down-regulated in high light conditions that trigger  $\beta$ -cyclocitral accumulation (Ramel et al., 2013b). However, in *Citrus*, a CCD4 enzyme, CCD4b, was reported to cleave  $\beta$ -carotene at the position 7,8 (7',8'), hence producing  $\beta$ -cyclocitral (Rodrigo et al., 2013). Similarly, in the stigma of *Crocus*, another CCD4, CCD4c, is able to cleave carotenoids at the position 9-10 (9',10') leading to  $\beta$ -ionone and, with a much lower efficiency, at position 7,8 (7',8') producing  $\beta$ -cyclocitral (Rubio-Moraga et al., 2014). Expressing *Vitis vinifera* CCD4b gene in carotenoid-accumulating yeast strains also resulted in the production of  $\beta$ -cyclocitral (Meng et al., 2019). Interestingly, lipoxygenase could provide an alternative route for carotenoid oxidation (Hayward et al., 2017). Accordingly, knockout mutants of Arabidopsis and tomato for the 13-lipoxygenase LOX2 were found to contain reduced levels of  $\beta$ -cyclocitral in leaves (Gao et al., 2019). However, it is not known if this enzyme is involved in the accumulation of  $\beta$ -cyclocitral under high light and  $^1\text{O}_2$  stress although LOX2 is induced under such conditions (Ramel et al., 2013a). Finally, it can be mentioned that a peroxidase has been purified from the extracellular liquid of the fungus *Lepista irina* which can cleave  $\beta$ -carotene into  $\beta$ -cyclocitral and other volatile apocarotenoids (Zorn et al., 2003b)

$\beta$ -Cyclocitral can also be generated by  $\beta$ -carotene autooxidation, especially upon attack by the reactive species  $^1\text{O}_2$ . In plants,  $^1\text{O}_2$  is produced mainly from excited chlorophylls in the triplet state, acting as photosensitizers (Krieger-Liszkay, 2004; Triantaphylides and Havaux, 2009). The main source of  $^1\text{O}_2$  is the reaction center of PSII (Krieger-Liszkay, 2004; Pospisil, 2016; D'Alessandro and Havaux, 2019) which contains  $\beta$ -carotene molecules, the function of which is to quench  $^1\text{O}_2$  produced therein.  $\beta$ -carotene is also present in the chlorophyll antennae of PSI (Thayer and Bjorkman, 1992). However, it is generally considered that this photosystem does not produce  $^1\text{O}_2$  in significant amounts compared to PSII (D'Alessandro and Havaux, 2019).

The main mechanism of  $^1\text{O}_2$  quenching by carotenoids is the physical one, involving energy transfer from  $^1\text{O}_2$  to the carotenoid followed by thermal decay of the excited quencher (Edge et al., 1997). However,  $\beta$ -carotene can also be oxidized by  $^1\text{O}_2$ , allowing chemical quenching of  $^1\text{O}_2$ . In fact,  $^1\text{O}_2$  is an electrophilic molecule having a high affinity for double bonds (Triantaphylides and Havaux, 2009), and each double bond in the carotenoid molecules can be oxidized by  $^1\text{O}_2$ , producing a variety of apocarotenoids, including  $\beta$ -cyclocitral in the case of  $\beta$ -carotene (Stratton et al., 1993; Ramel et al., 2012a; Ramel et al., 2012b).  $\beta$ -Cyclocitral was found to accumulate in *Arabidopsis* leaves exposed to high light stress (Ramel et al., 2012a; Lundquist et al., 2013), a condition that is known to generate  $^1\text{O}_2$ . Together with  $\beta$ -ionone,  $\beta$ -cyclocitral is the major oxidation product of  $\beta$ -carotene in microalgae (Watson et al., 2016; Kim et al., 2019), which can release amounts of this compound during summer blooms (Ma et al., 2013). Incidentally, this phenomenon can be problematic because it can generate unpleasant odor and can deteriorate water quality.  $\beta$ -Cyclocitral content of the cyanobacterium *Microcystis* was shown to be increased by high light or high temperature (Zheng et al., 2020) and by nutrient deficiency (Zuo et al., 2018; Ye et al., 2018).

### 3. $\beta$ -Cyclocitral derivatives

In *Arabidopsis* leaves,  $\beta$ -cyclocitral rapidly oxidizes into  $\beta$ -cyclocitric acid (2,2,6-trimethyl cyclohexene-1-carboxylic acid, Figure 1), which appears to be the main form accumulating under stress conditions (D'Alessandro et al., 2019). For instance, in plants exposed to drought stress, the  $\beta$ -cyclocitric acid levels rose by a factor of 15 while the  $\beta$ -cyclocitral content was doubled only, and exposing plants to volatile  $\beta$ -cyclocitral led to a marked accumulation of  $\beta$ -cyclocitric acid (D'Alessandro et al., 2019). In tomato leaves and fruits, the  $\beta$ -cyclocitric acid levels are noticeably higher than the  $\beta$ -cyclocitral levels (D'Alessandro et al., 2019). Although  $\beta$ -cyclocitral can spontaneously convert to  $\beta$ -cyclocitric acid in water (Tomita et al., 2016), the reaction *in planta* appears to be much faster (D'Alessandro et al., 2019), suggesting the possibility of enzymatic catalysis. Although this putative enzymatic activity remains to be identified, one can imagine the involvement of a Baeyer-Villiger monooxygenase that produces esters from ketones and carboxylic acids from aldehydes (van Berkel et al., 2006). This type of enzyme has been reported for the oxidation of castasterone to brassinolide in brassinosteroid biosynthesis (Kim et al., 2005). Interestingly, the transcriptome of  $\beta$ -cyclocitral-treated *Arabidopsis* plants contains a number of induced monooxygenases (Ramel et al., 2012), constituting potential candidates for this function which should be investigated in the future.  $\beta$ -cyclocitral treatment also induces several glycosyl transferases (Ramel et al., 2012a), and  $\beta$ -cyclocitral can occur in plants in glycosylated form (Mi et al., 2018). Glycosylation of  $\beta$ -cyclocitral could represent a regulatory mechanism modulating the  $\beta$ -cyclocitral-mediated signaling. However, glycosylated  $\beta$ -cyclocitral was found to represent a small fraction (< 2%) of the total  $\beta$ -cyclocitral pool (Mi et al., 2018).

Exposure to UV light converts  $\beta$ -cyclocitral into various compounds, such as 2,6-dimethyl-cyclohexanol and 2,2,6-trimethyl-cyclohexanone (Kim et al., 2019). The latter

compound was found in the stress-tolerant Mediterranean shrub *Cistus ladanifer* (Frazao et al., 2018). It is also one of the main compounds released during cyanobacterial blooms in natural lakes (Arii et al., 2015). The function of those  $\beta$ -cyclocitral derivatives is unknown.  $\beta$ -cyclocitral can also be derived into various lactones with potential bioactivities, which can be produced by chemical synthesis ( $\delta$ -iodo- $\gamma$ -lactone,  $\delta$ -chloro- $\gamma$ -lactone,  $\delta$ -bromo- $\gamma$ -lactone, 1,2,2,6-Tetramethyl-9-oxabicyclo[4.3.0]nonan-8-one) (Mazur et al., 2014). Although no systematic study has been conducted to establish their occurrence *in planta*, one of those bioactive lactones, dihydroactinidiolide, is present in plant leaves and accumulates under photooxidative stress conditions (Ramel et al., 2012a; Shumbe et al., 2014).

#### **4. Functions in vascular plants**

$\beta$ -Cyclocitral has recently emerged as a new plant signal triggering plant stress tolerance. Exposing *Arabidopsis* plants to volatile  $\beta$ -cyclocitral changed the expression of hundreds of nuclear-encoded genes (Ramel et al., 2012a) and enhanced plant tolerance to high light-induced oxidative stress (Ramel et al. 2012a) and to drought stress (D'Alessandro et al., 2019). Accordingly, overexpression of the *Crocus* CCD4b, an enzyme that can produce  $\beta$ -cyclocitral from  $\beta$ -carotene (Rubio-Moraga et al., 2014), in *Arabidopsis* imparts tolerance to environmental stresses, such as dehydration, salt and oxidative stresses (Baba et al., 2015). Interestingly, the transcriptomic changes induced by  $\beta$ -cyclocitral strongly overlap with the network of genes regulated by  $^1\text{O}_2$  (Ramel et al., 2012a). The majority of the induced genes encodes proteins involved in the interactions with the environment, in stress responses and in cellular transport, whereas many repressed genes are related to development, growth, and biogenesis of cellular components (Ramel et al., 2012a). Thus,  $\beta$ -cyclocitral appears to function as an intermediate in the



$^1\text{O}_2$  signaling pathway which can reprogram gene expression shifting plant cells from active growth to cellular defense toward stress. These effects were not observed with  $\beta$ -ionone (Ramel et al., 2012a), another short-chain apocarotenoid produced by  $^1\text{O}_2$  oxidation of  $\beta$ -carotene with a chemical structure close to that of  $\beta$ -CC (cleavage at the 9,10 position instead of the 7,8 double bond).

Key players in  $\beta$ -cyclocitral-dependent signaling have been recently identified, revealing the existence of several independent branches in the pathway (Figure 2). In particular,  $\beta$ -cyclocitral was shown to induce MBS1 (Shumbe et al., 2017), a small zinc finger protein (METHYLENE BLUE SENSITIVITY 1) required for the correct expression of  $^1\text{O}_2$ -responsive genes under normal or light stress conditions (Shao et al., 2013).  $\beta$ -cyclocitral not only increased MBS1 protein levels, but it induced a relocalization of the protein to the nucleus (Shumbe et al. 2017), as does high light stress (Shao et al., 2013). The Arabidopsis *mbs1* mutant, deficient in MBS1, was insensitive to  $\beta$ -cyclocitral, lacking the enhancement of phototolerance after  $\beta$ -cyclocitral treatment (Shumbe et al., 2017). Moreover, the absence of MBS1 led to de-regulation of specific  $^1\text{O}_2$  marker genes and ROS-responsive transcription factors in response to  $\beta$ -cyclocitral. Together, those results indicate that MBS1 is situated downstream of  $\beta$ -cyclocitral in the  $^1\text{O}_2$  signaling pathway, but the exact function of MBS1 is still unknown. In contrast,  $\beta$ -cyclocitral-dependent signaling was found to be independent of EXECUTER1 (Ramel et al., 2012a), a protein involved in the  $^1\text{O}_2$  signaling pathway leading to programmed cell death (Laloi and Havaux, 2015).

The transcriptome of  $\beta$ -cyclocitral-treated Arabidopsis plants showed the induction of various detoxification mechanisms, among which the induction of several glutathione-S-transferases (GST) and UDP-glycosyltransferases (Ramel et al., 2012a), which participate in the

xenobiotic detoxifying process (Sandermann, 1992). In fact, in plants, exogenous reactive chemicals (herbicides, pollutants, ...) are inactivated by a set of detoxifying enzymes that eliminate these compounds in three phases: modification (introduction or modification of side groups in the toxic molecule), conjugation (conjugation of the modified compound to sugar moieties or glutathione) and compartmentalization (Riechers et al., 2010; Sandermann, 1992). While the conjugation phase has been reported to be induced under many different stresses, the modification phase has been characterized more recently (Riechers et al., 2010; Mueller et al., 2008; Ramel et al., 2012c). In particular, in the modification phase, the GRAS protein SCARECROW LIKE 14 (SCL14) and the glutaredoxin GRX480/ROXY19 compete for binding with TGAI transcription factors and mediate the activation or the inhibition, respectively, of a large set of detoxifying enzymes (Huang et al., 2016; Fode et al., 2008; Köster et al., 2012; Ndamukong et al., 2007).

SCL14-controlled xenobiotic detoxification is strongly induced by  $\beta$ -cyclocitral and by photooxidative stress conditions (D'Alessandro et al., 2018). A *sc14* knockout mutant did not respond to  $\beta$ -cyclocitral and remained sensitive to high light stress after  $\beta$ -cyclocitral treatment (D'Alessandro et al., 2018). Mimicking  $\beta$ -cyclocitral treatment, overexpressor lines of SCL14 were more tolerant both to excessive light and to drought stress (D'Alessandro et al., 2018, 2019). Owing to previous works on xenobiotic detoxification (Fode et al., 2008; Köster et al., 2012), a few components in the signaling cascade downstream of  $\beta$ -cyclocitral have been identified. In particular,  $\beta$ -cyclocitral increased expression of SCL14, favoring its interaction with TGAI transcription factors. The SCL14/TGA II complex then modulates the transcriptional levels of the ANAC102 transcription factor which controls the downstream ANAC002, ANAC031 and ANAC081 transcription regulators and finally the redox enzymes of the first

phase of the detoxification response (D'Alessandro et al., 2018), hence boosting the cellular detoxification capacities.

The xenobiotic detoxification response is also activated by endogenous toxicants, such as reactive carbonyl species (RCS) deriving from the spontaneous decomposition of lipid peroxides (Mueller et al., 2008; Mano, 2012; Mano et al., 2019). Lipid peroxides are not only the signature of photooxidation but also directly contribute to its toxicity. They decay and form reactive aldehydes, such as acrolein or 4-hydroxynonenal (HNE), which can further hinder the physiological functioning of macromolecules and induce cell death (Mano, 2012; Biswas and Mano, 2016; Mueller et al., 2008; Mano et al., 2019). By triggering the SCL14-dependent xenobiotic response,  $\beta$ -cyclocitral could prepare the cell against the accumulation of toxic, pro-death lipid peroxides occurring under excessive light or drought stress through increasing detoxification capacities (D'Alessandro et al., 2018). Surprisingly,  $\beta$ -cyclocitral-induced cellular detoxification was not affected by the loss of MBS1 (D'Alessandro et al., 2018). This indicates the existence of at least two pathways in  $\beta$ -cyclocitral signaling, one pathway regulated by SCL14 and another one dependent of MBS1 (Figure 2).

Another branch in the  $\beta$ -cyclocitral-induced signaling pathway was recently shown to rely on the conversion of  $\beta$ -cyclocitral into its oxidized form,  $\beta$ -cyclocitric acid (D'Alessandro et al., 2019). While  $\beta$ -cyclocitral is a volatile lipid-soluble compound,  $\beta$ -cyclocitric acid is water soluble and can be more easily applied to plants through the irrigation water or by spraying the plants. Similarly to  $\beta$ -cyclocitral,  $\beta$ -cyclocitric acid was shown to act as a signal molecule triggering changes in gene expressions, and  $\beta$ -cyclocitric acid-treated *Arabidopsis* plants exhibited a marked increase in drought tolerance (D'Alessandro et al., 2019). This effect was confirmed in plants of agronomic importance such as tomato and pepper. The molecular bases of the  $\beta$ -cyclocitric acid-induced enhancement of plant drought tolerance are unknown, but they are

different from known responses such as stomatal closure, osmotic adjustment and jasmonate signaling. Also the protective effect of  $\beta$ -cyclocitric acid was not cancelled in the *mbs1* mutants, indicating that  $\beta$ -cyclocitric acid signaling is independent of the MBS1-mediated signaling. However, whereas  $\beta$ -cyclocitral and  $\beta$ -cyclocitric acid were able to enhance drought stress tolerance in plants, only  $\beta$ -cyclocitral was found to enhance tolerance to high light in the absence of water stress. This indicates that the  $\beta$ -cyclocitral and  $\beta$ -cyclocitric acid pathways do not fully overlap, with  $\beta$ -cyclocitric acid possibly mediating only a branch of the  $\beta$ -cyclocitral signaling pathway. Taken together, the available data suggest that  $\beta$ -cyclocitral could trigger an array of signaling pathways that remain to be characterized in more detail.

$\beta$ -Cyclocitral can also impact on root development. Application of  $\beta$ -cyclocitral on *Arabidopsis* seedlings grown on solid medium in Petri dishes was reported to stimulate the growth of primary roots (Dickinson et al., 2019). This effect, which was also observed in tomato and rice, was independent of auxin and brassinosteroid signaling pathways. Enhancement of root development may be advantageous under water stress conditions by favoring soil exploration and water uptake. The positive effect of  $\beta$ -cyclocitral was also observed under salt stress conditions: application of  $\beta$ -cyclocitral to salt-stressed rice roots counteracted the inhibitory effect of salt on root depth (Dickinson et al., 2019). However, it is not yet clear whether this root growth stimulation is a direct effect of  $\beta$ -cyclocitral on cell division/elongation or is indirect, *e.g.* through the induction of cellular detoxification and tolerance against oxidative stress, as described in shoots. Indeed, homeostasis of reactive oxygen species is known to be perturbed in roots when grown in the light (Yokawa et al., 2011), as is usually the case in Petri dishes. Furthermore, treatments with volatile  $\beta$ -cyclocitral require to seal the Petri dishes, reducing gas exchange and favoring photorespiration and  $H_2O_2$  production, which can repress cell cycle genes and meristem growth (Tsukagoshi, 2016). It is therefore possible that, under those unfavorable, pro-oxidative

conditions, root growth can be rescued by  $\beta$ -cyclocitral-induced protection against peroxidative damage. Concerning  $\beta$ -cyclocitric acid, the effect on root growth was very limited compared to  $\beta$ -cyclocitral (D'Alessandro et al., 2019), and this could be related to its water solubility that did not impose sealing of the Petri dishes. The link between drought tolerance and root growth in  $\beta$ -cyclocitric acid-treated plants has not yet been established. Further studies will have to clarify the molecular mechanisms behind the modulation of root development by  $\beta$ -cyclocitral.

## 5. Functions in non-vascular plants

Microalgae release volatile organic compounds (VOC), including  $\beta$ -cyclocitral. The functions of VOC in algae are similar to those in vascular plants: they transfer stress information to homogeneous algae to induce defense, and they play allelopathic roles on heterogeneous algae and aquatic macrophytes for competing nutrients, or they protect against predators (Achyuthan et al., 2017; Andrade et al., 2018; Zuo, 2019).  $\beta$ -cyclocitral is one of the major VOC emitted by the cyanobacterium *Microcystis*, which showed inhibitory effects on the growth of the green microalga *Chlorella pyrenoidosa* (Ikawa et al., 2001). Meanwhile,  $\beta$ -cyclocitral can cause cell rupture of the diatom *Nitzschia palea* (Chang et al., 2011). The toxicity of  $\beta$ -cyclocitral to *Chlamydomonas* cells was associated with caspase 9- and caspase 3-like activities, nuclear variation and DNA laddering, suggesting the induction of a programmed cell death mechanism (Sun et al., 2020). The presence of a  $\beta$ -cyclocitral microzone around *Microcystis* colonies also acts a powerful repellent and a signal of poor quality food to grazers, such as the planktonic crustacean *Daphnia* (Jüttner et al., 2010). A *Nannochloropsis salina* culture infested by a marine rotifer emitted volatile  $\beta$ -cyclocitral which could provide an early biomarker of algal pond crashes (Reese et al., 2019).

However, high concentrations of  $\beta$ -cyclocitral can impact on the growth of *Microcystis* itself, by causing cell lysis (Ozaki et al., 2008). During summer bloom, the oxidation of  $\beta$ -cyclocitral produced by *Microcystis* to  $\beta$ -cyclocitric acid leads to water acidification, causing chlorophyll loss, cell lysis and a typical blue coloration due to release of phycocyanin pigments (Arii et al., 2015). So, during cyanobacterial bloom,  $\beta$ -cyclocitral toxicity may occur indirectly through an acid stress. These results suggest that  $\beta$ -cyclocitral and other volatile apocarotenoids may be the main allelopathic agents in cyanobacterial VOC, but at high concentrations these agents may be detrimental to the emitters. However,  $\beta$ -cyclocitral derived from *Microcystis* has stronger inhibitory activity against other cyanobacteria and algae, indicating that  $\beta$ -cyclocitral gives *Microcystis* competitive advantage and plays an important role in the ecology of aquatic environments. So far, there is no investigation on low  $\beta$ -cyclocitral concentrations as inducers of defense mechanisms in photosynthetic microorganisms like those reported in vascular plants.

Mosses also emit VOC (Saritas et al., 2001) which could be involved in communication between species (Vicherova et al., 2020). The moss *Hamatocaulis vernicosus* reacted to its common competitor *Sphagnum flexuosum* VOC by enhancing its growth rate and altering its own VOC emission, specifically increasing production of a compound (methyl 2,6,6-trimethyl-1-cyclohexene-1-carboxylate, Fig. 1) structurally related to  $\beta$ -cyclocitral, which is also released by *H. vernicosus*. This could constitute an alarm mechanism signaling the competitive strength of their neighbors.

## **6. Some considerations on $\beta$ -cyclocitral in animals**

Animals use chemical signals for social communication and feeding behavior. Apocarotenoids derived from  $\beta$ -carotene, including  $\beta$ -cyclocitral, are involved in these functions, being present in

the composition of chemical signals such as pheromones (Tagua et al., 2012). For instance, the supracaudal or tail gland of the red fox emits a mixture of apocarotenoids which contains  $\beta$ -cyclocitral (McLean et al., 2019). Some insect species, such as *Drosophila* species, are attracted by  $\beta$ -cyclocitral (Versace et al., 2016; Keeseey et al., 2015). The invasive spotted-wing drosophila, *Drosophila suzukii*, is more attracted to leaf odors than *D. melanogaster* in behavioral trials, and this differential behavioral preference towards leaves was specifically related to  $\beta$ -cyclocitral (Keeseey et al., 2015).  $\beta$ -cyclocitral synergizes the response of *D. suzukii* to fruit-based and leaf-based compounds in a sex-dependent manner (Bolton et al., 2019; Pinero et al., 2019). This olfactory sensitivity to leaf chemistry in *D. suzukii* appears to be regulated by the “ab3A” neuron, an olfactory sensitive neuron that has been shown repeatedly to play a role in species differences for feeding and oviposition preference (Keeseey et al., 2015).

Rather than an attractive compound,  $\beta$ -cyclocitral can also act as a repellent for pathogens and herbivores. Exogenous applications of  $\beta$ -cyclocitral inhibited infestation by the two-spotted spider mite (*T. urticae* Koch) in African spider plants (Niyalala et al., 2013).  $\beta$ -cyclocitral applications also reduced disease symptoms caused by the plant pathogenic oomycete *Plasmopara viticola* in grapevines (Lazazzara et al., 2018). A number of  $\beta$ -cyclocitral-derived compounds, such as lactones with hydroxy functions, have been shown to have biological functions, in particular as antifeedants towards storage product pests (Mazur et al., 2014). These examples support the idea that  $\beta$ -cyclocitral could have some beneficial effects in crop protection against biotic stresses, which would clearly deserve to be explored in future studies.

It is becoming apparent that nonretinoid apocarotenoids, including those originating from  $\beta$ -carotene oxidation, have the potential to regulate a broad spectrum of critical cellular functions in mammals, thus influencing mammalian health (Harrison and Quadro, 2018). In particular, short-chain  $\beta$ -carotene-derived apocarotenoids, such as  $\beta$ -ionone, have been shown to exhibit

anticancer properties by inhibiting cell proliferation and inducing apoptosis (Liu et al., 2004; Sharoni et al., 2012). However, very little information is available on  $\beta$ -cyclocitral in human cells, although the compound is present in the human metabolome database (<https://hmdb.ca>), and a similar anti-cancer activity is not documented for this molecule. The functions of  $\beta$ -cyclocitral in mammalian cells is unexplored and remain to be investigated in the future.

## 7. CONCLUSIONS

This review has gone through the multiple biological activities of  $\beta$ -cyclocitral which occur throughout the life kingdoms. Our current knowledge on these functions is the most advanced for vascular plants in which  $\beta$ -cyclocitral and its oxidation product  $\beta$ -cyclocitric acid act as stress signals triggering acclimatory responses and enhancing plant stress tolerance through several pathways (Figure 2). The primary target of  $\beta$ -cyclocitral in plant cells is still unknown. It has been reported that apocarotenoids can stimulate the electrophile/antioxidant response element transcription system in human cells (Linnewiel et al., 2019). However, the protective effect of  $\beta$ -cyclocitral against photooxidative stress appears to be specific since it is not shared with  $\beta$ -ionone, another short-chain apocarotenoid resulting from  $\beta$ -carotene oxidation (Ramel et al., 2012a). Consequently, the signaling activity of  $\beta$ -cyclocitral cannot be explained by its aldehydic and electrophilic nature since the electrophilicity of  $\beta$ -ionone is higher than that of  $\beta$ -cyclocitral. Moreover, the transcriptomic response of *Arabidopsis* to  $\beta$ -cyclocitral overlapped very poorly with the gene expression changes induced by other volatile reactive electrophile species such as malondialdehyde and methyl vinyl ketone (Ramel et al., 2012a). This could suggest the existence of a receptor that could transduce the  $\beta$ -cyclocitral signal into specific cellular responses. The identification of this receptor will constitute an important step in the elucidation of the functions of  $\beta$ -cyclocitral. It is also unknown if  $\beta$ -cyclocitral, as a volatile



molecule, can fulfill its functions in long-distance signaling between different plants. This is at variance with other organisms, such as non-vascular photosynthetic organisms, in which  $\beta$ -cyclocitral serves in intra-species or inter-species communication. It is clear that new and exciting avenues are now opening for elucidating the mode of action and the molecular bases of the  $\beta$ -cyclocitral bioactivities and possibly for exploiting its properties in biotechnological applications.

### **Declaration on competing interests**

The author declares that he has no conflict of interest.

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### **References**

- Achyuthan, K.E., Harper, J.C., Manginell, R.P., Moorman, M.W. 2017. Volatile metabolites emission by in vivo microalgae - An overlooked opportunity? *Metabolites* 7, 39
- Al-Babili, S., Bouwmeester, H.J. 2015. Strigolactones, a novel carotenoid-derived plant hormone. *Annu. Rev. Plant Biol.* 66, 161-186
- Andrade, L.M., Andrade, C.J., Dias, M., Nascimento, C.A.O., Mendes, M.A. 2018. Chlorella and spirulina microalgae as sources of functional foods, nutraceuticals, and food supplements; an overview. *MOJ Food Process. Technol.* 6, 45–58.
- Arii, S., Tsuji, K., Tomita, K., Hasegawa, M., Bober, B., Harada, K.I., 2015. Cyanobacterial blue color formation during lysis under natural conditons. *Appl. Environ. Microbiol.* 81, 2667-2675

- Baba, S.A., Jain, D., Abbas, N., Ashraf, N. 2015. Overexpression of *Crocus* carotenoid cleavage dioxygenase, CsCCD4b, in *Arabidopsis* imparts tolerance to dehydration, salt and oxidative stresses by modulating ROS machinery. *J. Plant Physiol.* 189, 114-125
- Biswas, M.S., Mano, J. 2016. Reactive carbonyl species activate caspase-3-like protease to initiate programmed cell death in plants. *Plant Cell Physiol.* 57,1432-1442
- Bolton, L.G., Piñero, J.C., Barrett, B.A. 2019. Electrophysiological and behavioral responses of *Drosophila suzukii* (Diptera: *Drosophilidae*) towards the leaf volatile  $\beta$ -cyclocitral and selected fruit-ripening volatiles. *Environ Entomol.* 48,1049-1055.
- Chang, D.W., Hsieh, M.L., Chen, Y.M., Lin, T.F., Chang, J.S. 2001. Kinetics of cell lysis for *Microcystis aeruginosa* and *Nitzschia palea* in the exposure to  $\beta$ -cyclocitral. *J. Hazard Mater.* 185, 1214-1220.
- Chen, W.-K., Yu, K.-J., Liu, B., Lan, Y.-B., Suna, R.-Z., Li, Q., He, F., Pan, Q.-H., Duan, C.-Q., Wang, J. 2017. Comparison of transcriptional expression patterns of carotenoid metabolism in 'Cabernet Sauvignon' grapes from two regions with distinct climate. *J. Plant Physiol.* 213, 75-86
- Cui, H., Wang, Y., Qin, S. 2012. Genomewide analysis of carotenoid cleavage dioxygenases in unicellular and filamentous cyanobacteria. *Comp. Funct. Genomics* 2012, 164690
- D'Alessandro, S., Ksas, B., Havaux, M. 2018. Decoding  $\beta$ -cyclocitral-mediated retrograde signaling reveals the role of a detoxification response in plant tolerance to photooxidative stress. *Plant Cell* 30, 2495-2511
- D'Alessandro, S., Havaux, M. 2019. Sensing  $\beta$ -carotene oxidation in photosystem II to master plant stress tolerance. *New Phytol.* 223, 1776-1783
- D'Alessandro, S., Mizokami, Y., Légeret, B., Havaux, M. 2019. The Apocarotenoid  $\beta$ -Cyclocitric Acid Elicits Drought Tolerance in Plants. *iScience.* 19, 461-473
- Dickinson, A. J., Lehner, K., Mi, J., Jia, K.-P., Mijar, M., Dinneny, J., et al. 2019.  $\beta$ -Cyclocitral is a conserved root growth regulator. *Proc. Natl. Acad. Sci. USA* 116, 10563–10567.
- Edge R., McGarvey, D.J., Truscott, T.G. 1997. The carotenoids as anti-oxidants - a review. *J. Photochem. Photobiol. B.* 41,189-200.
- Fode, B., Siemsen, T., Thurow, C., Weigel, R., Gatz, C. 2008. The *Arabidopsis* GRAS protein SCL14 interacts with Class II TGA transcription factors and is essential for the activation of stress-inducible promoters. *Plant Cell* 20, 3122–3135.

- Frazão, D.F., Raimundo, J.R., Domingues, J.L., Quintela-Sabarís, C., Gonçalves, J.C., Delgado, F. 2018. *Cistus ladanifer* (Cistaceae): a natural resource in Mediterranean-type ecosystems. *Planta* 247, 289-300.
- Gao, L. , Gonda, I., Sun, H. , Ma, Q. , Bao, K. , Tieman, D. M. , et al. 2019. The tomato pan-genome uncovers new genes and a rare allele regulating fruit flavor. *Nat. Genet.* 51, 1044
- García-Plazaola, J.I., Portillo-Estrada, M., Fernández-Marin, B., Kännaste, A., Niinemets, U. 2017. Emissions of carotenoid cleavage products upon heat shock and mechanical wounding from a foliose lichen. *Environ. Exp. Bot.* 133, 87-97.
- Giuliano, G., Al-Babili, S., von Lintig, J. 2003. Carotenoid oxygenases: cleave it or leave it. *Trends Plant Sci.* 8 , 145–149.
- Harrison, E.H., Quadro, L. 2018. Apocarotenoids: Emerging roles in mammals. *Annu. Rev. Nutrit.* 38, 153-172
- Hayward, S., Cilliers, T., Swart, P. 2017. Lipoxygenases: from isolation to application. *Compr. Rev. Food Sci.* 16, 199–211.
- Hou, X., Rivers, J., León, P., McQuinn, R. P., Pogson, B. J. 2016. Synthesis and function of apocarotenoid signals in plants. *Trends Plant Sci.* 21, 792–803
- Huang, L.-J., Li, N., Thurow, C., Wirtz, M., Hell, R., Gatz, C. 2016. Ectopically expressed glutaredoxin ROXY19 negatively regulates the detoxification pathway in *Arabidopsis thaliana*. *BMC Plant Biol.* 16, 200–211.
- Ikawa, M., Sasner, J.J., Haney, J.F. 2001. Activity of cyanobacterial and algal odor compounds found in lake waters on green alga *Chlorella pyrenoidosa* growth. *Hydrobiologia* 443, 19-22
- Jüttner, F., Watson, S.B., von Elert, E., Köster, O.J. 2010.  $\beta$ -cyclocitral, a grazer defence signal unique to the cyanobacterium *Microcystis*. *Chem Ecol.* 36,1387-1397.
- Keeseey, I.W., Knaden, M., Hansson, B.S.J. 2015. Olfactory specialization in *Drosophila suzukii* supports an ecological shift in host preference from rotten to fresh fruit. *Chem Ecol.* 41, 121-128.
- Kim, T.-W., Hwang, J.Y., Kim, Y.S., Joo, S.H., Chang, S.C., Lee, J.S., Takatsuto, S., Kim, S.K. 2005. *Arabidopsis* CYP85A2, a cytochrome P450, mediates the Baeyer-Villiger oxidation of castasterone to brassinolide in brassinosteroid biosynthesis. *Plant Cell* 17, 2397–2412.
- Kim, T., Kim, T.-K., Zoh, K.-D. 2019. Degradation kinetics and pathways of  $\beta$ -cyclocitral and  $\beta$ -ionone during UV photolysis and UV/chlorination reactions. *J. Environ. Manag.* 239, 8-16

- Köster, J., Thurow, C., Kruse, K., Meier, A., Iven, T., Feussner, I., Gatz, C. 2012. Xenobiotic- and jasmonic acid-inducible signal transduction pathways have become interdependent at the Arabidopsis CYP81D11 promoter. *Plant Physiol.* 159, 391–402.
- Krieger-Liszkay, A. 2004. Singlet oxygen production in photosynthesis. *J. Exp. Bot.* 56, 337–346.
- Lalko, J., Lapczynski, A., McGinty, D., Bhatia, S., Letizia, C.S., Api, A.M. 2007. Fragrance material review on ionone. *Food Chem. Toxicol.* 45, S251-S257
- Laloi, C., Havaux, M. 2015. Key players of singlet oxygen-induced cell death in plants. *Front Plant Sci* 6: 39
- Lazazzara, V., Bueschl, C., Parich, A., Ilaria, P., Scuhmacher, R., Perzzolli, M. 2018. Downy mildew symptoms on grapevines can be reduced by volatile organic compounds of resistant genotypes. *Sci. Rep.* 8, 1618
- Lewinsohn, E., Sitrit, Y., Bar, E., Azulay, Y., Meir, A., Zamir, D., Tadmor, Y. 2005. Carotenoid pigmentation affects the volatile composition of tomato and watermelon fruits, as revealed by comparative genetic analyses. *J. Agric. Food Chem.* 8, 3142-3148
- Linnewiel, K., Ernst, H., Caris-Veyrat, C., Ben-Dor, A., Kampf, A., Salman, H., Danilenko, M., Levy, J., Sharoni, Y. 2009. Structure activity relationship of carotenoid derivatives in activation of the electrophile/antioxidant response element transcription system. *Free Radic. Biol. Med.* 47, 659–667
- Liu, J.-R., Chen, B.-Q., Yang, B.-F., Dong, H.-W., Sun, C.-H., Wang, Q., Song, G., Song, Y.-Q. 2004. Apoptosis of human gastric adenocarcinoma cells induced by b-ionone. *World J. Gastroentero.* 10, 348-351
- Lundquist, P.K., Poliakov, A., Giacomelli, L., Friso, G., Appel, M., McQuinn, R.P., Krasnoff, S.B., Rowland, E., Ponnala, L., Sun, Q., van Wijk, K.J. 2013. Loss of plastoglobule kinases ABC1K1 and ABC1K3 causes conditional degreening, modified prenyl-lipids, and recruitment of the jasmonic acid pathway. *Plant Cell* 25, 1818-1839.
- Ma, Z., Niu, Y., Xie, P., Chen, J., Tao, M., Deng, X. 2013. Off-flavor compounds from decaying cyanobacterial blooms of Lake Tai. *J. Environ. Sci.* 25, 495-501
- Mahattanatawee, K., Rouseff, R., Valim, M.F., Naim, M. 2005. Identification and aroma impact of norisoprenoids in orange juice. *J. Agric. Food Chem.* 53, 393-397
- Mano, J. 2012. Reactive carbonyl species: Their production from lipid peroxides, action in

- environmental stress, and the detoxification mechanism. *Plant Physiol. Biochem.* 59, 90–97.
- Mano, J., Biswas, M.S, Sugimoto, K. 2019. Reactive carbonyl species: A missing link in ROS signaling. *Plants (Basel)* 8, 391
- Mazur, M., Gładkowski, W., Podkowik, M., Bania, J., Nawrot, J., Białońska, A., Wawrzęczyk, C. 2014. Lactones 43. New biologically active lactones:  $\beta$ -cyclocitral derivatives. *Pest Manag. Sci.* 70, 286-294.
- McLean, S., Davies, N.W., Nichols, D.S. 2019. Scent chemicals of the tail gland of the red fox, *Vulpes Vulpes*. *Chem Senses* 44, 215-224
- Meng N., Yan, G.-L. , Zhang, D. , Li, X.-Y. , Duan, C.-Q. , Pan, Q.-H. 2019. Characterization of two *Vitis Vinifera* carotenoid cleavage dioxygenases by heterologous expression in *Saccharomyces Cerevisiae*. *Mol. Biol. Rep.* 46, 6311-6323
- Mi, J., Jia, K.-P., Balakrishna, A., Wang, J.Y., Al-Babili, S. 2019. An LC-MS profiling method reveals a route for apocarotene glycosylation and shows its induction by high light stress in *Arabidopsis*. *Analyst.* 144, 1197-1204
- Mueller, S., Hilbert, B., Dueckershoff, K., Roitsch, T., Krischke, M., Mueller, M.J., Berger, S. 2008. General detoxification and stress responses are mediated by oxidized lipids through TGA transcription factors in *Arabidopsis*. *Plant Cell* 20, 768–785.
- Nambara, E., Marion-Poll, A. 2005. Abscisic acid biosynthesis and catabolism. *Annu. Rev. Plant Biol.* 56, 165-185
- Ndamukong, I., Abdallat, A. Al, Thurow, C., Fode, B., Zander, M., Weigel, R., Gatz, C. 2007. SA-inducible *Arabidopsis* glutaredoxin interacts with TGA factors and suppresses JA-responsive PDF1.2 transcription. *Plant J.* 50, 128–139.
- Nyalala, S., Petersen, M. Grout, B. 2013. Volatile compounds from leaves of the African spider plant (*Gynandropsis gynandra*) with bioactivity against spider mite (*Tetranychus urticae*). *Annal. Appl. Biol.* 162, <https://doi.org/10.1111/aab.12021>
- Ozaki, K., Ohta, A., Iwata, C., Horikawa, A., Tsuji, K., Ito, E., Ikai, Y., Harada, K. 2008. Lysis of cyanobacteria with volatile organic compounds. *Chemosphere* 71,1531-1538.
- Piñero, J.C., Barrett, B.A., Bolton, L.G., Follett, P.A. 2019.  $\beta$ -cyclocitral synergizes the response of adult *Drosophila suzukii* (Diptera: *Drosophilidae*) to fruit juices and isoamyl acetate in a sex-dependent manner. *Sci. Rep.* 9,10574.

- Pospisil, P. 2016. Production of reactive oxygen species by Photosystem II as a response to light and temperature stress. *Front. Plant Sci.* 7, 1950
- Ramel, F., Birtic, S., Ginies, C., Soubigou-Taconnat, L., Triantaphylidès, C., Havaux, M. 2012a. Carotenoid oxidation products are stress signals that mediate gene responses to singlet oxygen in plants. *Proc Natl Acad Sci USA* 109, 5535–5540
- Ramel, F., Birtic, S., Cuiné, S., Triantaphylides, C., Ravanat, J.L., Havaux, M. 2012b. Chemical quenching of singlet oxygen by carotenoids in plants. *Plant Physiol.* 158, 1267-1278.
- Ramel, F., Sulmon, C., Serra, A.A., Gouesbet, G., Couée, I. 2012c. Xenobiotic sensing and signalling in higher plants. *J. Exp. Bot.* 63, 3999–4014.
- Ramel, F., Ksas, B., Akkari, E., Mialoundama, A.S., Monnet F., Krieger-Liszkay, A., Ravanat, J.L., Mueller, M.J., Bouvier, F., Havaux, M. 2013a. Light-induced acclimation of the *Chlorinal* mutant to singlet oxygen. *Plant Cell* 25, 1445-1462.
- Ramel, F., Mialoundama, A.S., Havaux, M. 2013b. Nonenzymic carotenoid oxidation and photooxidative stress signalling in plants. *J. Exp. Bot.* 64, 799-805
- Reese, K.L., Fisher, C.L., Lane, P.D., Jaryenneh, J.D., Moorman, M.W., Jones, A.D., Frank, M., Lane, T.W. 2019. Chemical profiling of volatile organic compounds in the headspace of algal cultures as early biomarkers of algal pond crashes. *Sci. Rep.* 9, 13866
- Riechers, D.E., Kreuz, K., Zhang, Q. 2010. Detoxification without intoxication: Herbicide safeners activate plant defense gene expression. *Plant Physiol.* 153, 3–13.
- Rodrigo, M.J., Alquézar, B., Alós, E., Medina, V., Carmona, L., Bruno, M., Al-Babili, S., Zacarías, L. 2013. A novel carotenoid cleavage activity involved in the biosynthesis of *Citrus* fruit-specific apocarotenoid pigments. *J. Exp. Bot.* 64, 4461-4478.
- Rubio-Moraga, A., Rambla, J.L., Fernández-de-Carmen, A., Trapero-Mozos, A., Ahrazem, O., Orzáez, D., Granell, A., Gómez-Gómez, L. 2014. New target carotenoids for CCD4 enzymes are revealed with the characterization of a novel stress-induced carotenoid cleavage dioxygenase gene from *Crocus sativus*. *Plant. Mol. Biol.* 86, 555-569
- Rubio Moraga, A., Rambla, J.L., Ahrazem, O., Granell, A., Gomez-Gomez, L. 2019. Metabolite and target transcript analyses during *Crocus sativus* stigma development. *Phytochemistry* 70, 1009-1016
- Sandermann, H. 1992. Plant metabolism of xenobiotics. *Trends Biochem. Sci.* 17, 82–84.

- Saritas, Y., Sonwa, M.M., Iznaguen, H., König, W.A., Muhle, H., Mues, R. 2001. Volatile constituents in mosses (*Musci*). *Phytochemistry* 7, 443-457
- Schwartz, S. H., Qin, X., Zeevaart, J. A. 2001. Characterization of a novel carotenoid cleavage dioxygenase from plants. *J. Biol. Chem.* 276, 25208– 25211
- Shao, N., Duan, G. Y., Bock, R. A. 2013. Mediator of singlet oxygen responses in *Chlamydomonas reinhardtii* and *Arabidopsis* identified by a luciferase-based genetic screen in algal cells. *Plant Cell* 25, 4209–4226.
- Sharoni, Y., Khanin, M., Salman, H., Veprik, A., Danilenko, M., Levy, J. 2012. Carotenoids and apocarotenoids in cellular signaling related to cancer: A review. *Mol. Nutr. Food Res.* 56, 259-269
- Shumbe, L., Bott, R., Havaux, M. 2014. Dihydroactinidiolide, a high light-induced  $\beta$ -carotene derivative that can regulate gene expression and photoacclimation in *Arabidopsis*. *Mol. Plant* 7, 1248-1251
- Shumbe, L., D'Alessandro, S., Shao, N., Chevalier, A., Ksas, B., Bock, R., Havaux, M. 2017. METHYLENE BLUE SENSITIVITY 1 (MBS1) is required for acclimation of *Arabidopsis* to singlet oxygen and acts downstream of  $\beta$ -cyclocitral. *Plant Cell Environ.* 40, 216–226.
- Stratton, S.P., Schaefer, W.H., Liebler, D.C. 1993. Isolation and identification of singlet oxygen oxidation products of beta-carotene. *Chem. Res. Toxicol.* 6, 542–547.
- Sun, Q., Zhou, M., Zuo, Z. 2020. Toxic mechanism of eucalyptol and  $\beta$ -cyclocitral on *Chlamydomonas reinhardtii* by inducing programmed cell death. *J. Hazard Mater.* 389, 121910
- Tagua, V.G., Medina, H.R., Martín-Domínguez, R., Eslava, A.P., Corrochano, L.M., Cerdá-Olmedo, E., Idnurm, A. 2012. A Gene for carotene cleavage required for pheromone biosynthesis and carotene regulation in the fungus *Phycomyces blakesleeanus*. *Fungal Genet. Biol.* 49, 398-404
- Thayer, S.S., Bjorkman, O. 1992. Carotenoid distribution and deepoxidation in thylakoid pigment–protein complexes from cotton leaves and bundle-sheath cells of maize. *Photosynth. Res.* 33, 213–225.
- Tieman, D. et al. 2012. The chemical interactions underlying tomato flavor preferences. *Curr. Biol.* 22, 1035-1039

- Tomita, K., Hasegawa, M., Arie, S., Tsuji, K., Bober, B., Harada, K. 2016. Characteristic oxidation behavior of  $\beta$ -cyclocitral from the cyanobacterium *Microcystis*. *Environ. Sci. Pollut. Res.* 23, 11998–12006.
- Triantaphylides, C., Havaux, M. 2009. Singlet oxygen in plants: production, detoxification and signaling. *Trends Plant Sci.* 14, 219–228.
- Tsukagoshi, H. 2016. Control of root growth and development by reactive oxygen species. *Curr. Opin. Plant Biol.* 29, 57–63.
- van Berkel, W. J. H., Kamerbeek, N. M., Fraaije, M. W. 2006. Flavoprotein monooxygenases, a diverse class of oxidative biocatalysts. *J. Biotechnol.* 124, 670–689.
- Versace, E., Eriksson, A., Rocchi, F., Castellan, I., Sgadò, P., Haase, A. 2016. Physiological and behavioral responses in *Drosophila melanogaster* to odorants present at different plant maturation stages. *Physiol. Behav.* 163, 322–331.
- Vicherova, E., Glinwood, R., Hajek, T., Smilauer, P., Ninkovic, V. 2020. Bryophytes can recognize their neighbours through volatile organic compounds. *Sci. Rep.* 10, 7405
- Watson, S.B., Monis, P., Baker, P., Giglio, S. 2016. Biochemistry and genetics of taste-and-odor-producing cyanobacteria. *Harmful Algae* 54, 112–127
- Ye, C., Yang, Y., Xu, Q., Ying, B., Zhang, M., Gao, B., Ni, B., Yakefu, Z., Bai, Y., Zuo, Z. 2018. Volatile organic compound emissions from *Microcystis aeruginosa* under different phosphorus sources and concentrations. *Phycol. Res.* 66, 15–22
- Yokawa, K., Kagenishi, T., Kawano, T., Mancuso, S., Baluska, F. 2011. Illumination of *Arabidopsis* roots induces immediate burst of ROS production. *Plant Signal. Behav.* 6, 1460–1464
- Yu, C., Shi, C., Ji, M., Xu, X., Zhang, Z., Ma, J., Wang, G. 2019. Taste and odor compounds associated with aquatic plants in Taihu Lake: distribution and producing potential. *Env. Sci. Pollut. Res.* 26, 34510–34520.
- Zheng, T., Zhou, M., Yang, L., Wang, Y., Wang, Y., Meng, Y., Liu, J. 2020. Effects of high light and temperature on *Microcystis aeruginosa* cell growth and  $\beta$ -cyclocitral emission. *Ecotoxicol. Environ. Safety* 192, 110313
- Zorn, H., Langhoff, S., Scheibner, M., Berger, R.G. 2003a. Cleavage of  $\beta,\beta$ -carotene to flavor compounds by fungi. *Appl. Microbiol. Biotechnol.* 62, 331–336



Zorn, H., Langhoff, S., Scheibner, M., Nimtz, M., Berger, R.G. 2003b. A peroxidase from *Lepista irina* cleaves beta,beta-carotene to flavor compounds. J. Biol. Chem. 384,1049-1056.

Zuo, Z., Yang, L., Chen, S., Ye, C., Han, Y., Wang, S., Ma, Y. 2018. Effects of nitrogen nutrients on the volatile organic compound emissions from *Microcystis aeruginosa*. Ecotoxicol. Environ. 161, 214-220

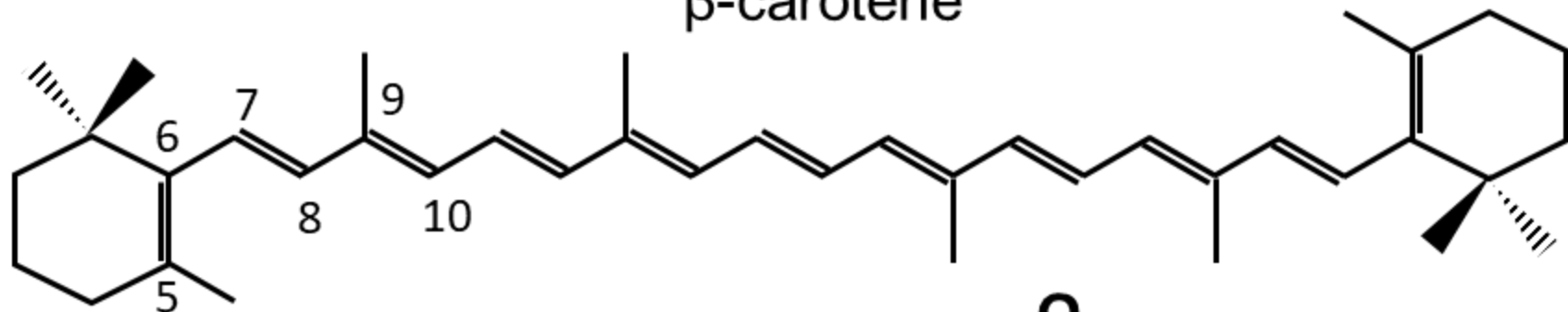
Zuo, Z. 2019. Why algae release volatile organic compounds – the emission and roles. Front. Microbiol. 10, 491

### Figure legends

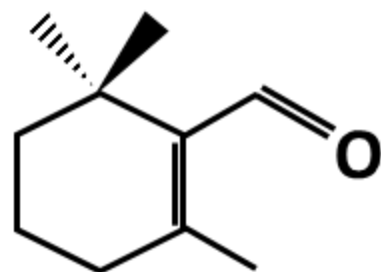
Fig. 1. Chemical structure of  $\beta$ -carotene and some of its oxidation products:  $\beta$ -cyclocitral ( $\beta$ -CC),  $\beta$ -cyclocitric acid ( $\beta$ -CCA),  $\beta$ -ionone and methyl-2,6,6-trimethyl-1-cyclohexene-1-carboxylate.  $\beta$ -CC and  $\beta$ -ionone are oxidation products of  $\beta$ -carotene.  $\beta$ -CCA derived from the oxidation of  $\beta$ -CC. Methyl-2,6,6-trimethyl-1-cyclohexene-1-carboxylate is a methylated form of  $\beta$ -CCA.

Fig. 2. Signaling pathways induced by  $\beta$ -cyclocitral ( $\beta$ -CC) in vascular plants. In yellow boxes, metabolites; in blue boxes, proteins; in red boxes, functions.  $\beta$ -CCA,  $\beta$ -cyclocitric acid; MBS1, METHYLENE BLUE SENSITIVITY 1; SCL14, SCARECROW LIKE 14; ANAC, member of the NAC (NAM/ATAF/CUC) transcription factor family.

$\beta$ -carotene

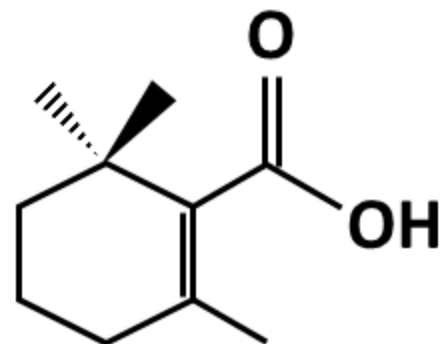


$\beta$ -CC

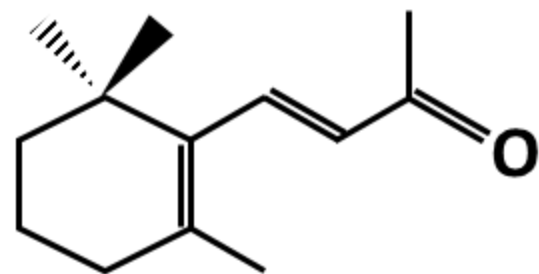


OH

$\beta$ -CCA



$\beta$ -ionone



O-CH<sub>3</sub>

Methyl 2,6,6-trimethyl-1-cyclohexene-1-carboxylate

