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DOI
10.1201/9780429455612-13

Publication date
2020

Document Version
Submitted manuscript

Published in
Biology of Plant Volatiles

Chapter 11

Biosynthesis and regulation of below-ground signaling molecules

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Abstract:

Plants emit signaling molecules to interact with their below-ground environment and these interactions are mainly localized in a small area adjacent to the plant root, called the rhizosphere. Signaling molecules so far identified in the rhizosphere mostly belong to the specialized metabolites, such as the terpenoids (including the strigolactones) and phenolics (including the flavonoids) and indole-derived and fatty acid derived compounds. The biological roles of these four classes of molecules in the rhizosphere and their biosynthesis are discussed, as well as the regulation of their formation by biotic stresses, both above- and below-ground, such as herbivores, pathogens and beneficial microorganisms. Abiotic stress, especially nutrient deficiency, also affects the production of some of these signaling molecules. Integration of the recently emerging metabolomics, metagenomics, metabolic engineering and systems biology approaches will help to unravel the mechanisms underlying the role of signaling molecules in the interaction between plants and soil biota and will provide the basis for a better exploitation of the hidden, below-ground, part of plants.

Contents

11.1 Introduction

A crucial process for the interaction of plants with other organisms in their environment, is the conversion of photosynthetically fixed carbon to signaling molecules, through a series of enzymatic reactions, and emitting them to the air surrounding them or the soil in which they grow. Volatile signals that are emitted above-ground can travel long distances and thus also mediate the interaction with organisms at long distances (as detailed elsewhere in this book). Below-ground, however, most of the signals and interactions happen in a small volume of soil bordering the plant root, called the rhizosphere, in which plant roots, soil and the soil biota tightly interact with each other (Lynch and de Leij 2001). Studies indicate that roughly 40% of photosynthetically fixed carbon is invested belowground (Jones, Nguyen, and Finlay 2009). Nineteen percent of fixed carbon is invested in root biomass, and the remaining 21% is deposited into the soil. The carbon deposited into the soil is composed of compounds actively secreted by the plant root or exuded passively in a gradient-dependent manner (together they are called the root exudate), as well as dead root cells and/or sloughed-off of root border cells (Hawes et al. 2000). The composition and quantity of root exudates is affected by plant development, soil properties and abiotic and biotic environmental factors and they are exuded into the rhizosphere as the result of the interaction network between a plant and its environment (Jones, Nguyen, and Finlay 2009, Badri and Vivanco 2009). Vice versa, these root exudates modify properties of the soil they are exuded into, for example change the pH

and detoxify heavy metals to improve nutrient uptake and alleviate other abiotic stresses (Hinsinger 2001, Walker et al. 2003).

Although we are just beginning to understand the full complexity and biological relevance of these root exudates, in recent years quite a number of reviews and research papers were dedicated to this topic, especially in the emerging area of the plant and soil microbiome interaction (Venturi and Keel 2016, Walker et al. 2003, van Dam and Bouwmeester 2016, Chaparro, Badri, and Vivanco 2014, Lundberg et al. 2012). Plant root exudates serve as chemo-attractants and chemo-repellents to attract beneficial organisms and repel harmful ones like pathogens and herbivores, respectively (Walker et al. 2003). This seems to have started of an arm's race, in which other organisms hijack the root exudates as signaling molecules for host detection or use them as food source (Walker et al. 2003, el Zahar Haichar et al. 2014). Examples of this are the strigolactones and flavonoids that have both positive and negative roles, which will be further discussed below (Ruyter-Spira et al. 2013, Falcone Ferreyra, Rius, and Casati 2012). Signaling molecules so far identified in the rhizosphere mostly belong to the specialized metabolites, such as the terpenoids (including the strigolactones) and phenolics (including the flavonoids) and indole-derived and fatty acid derived compounds. Below, we will briefly describe the biological roles of these four classes of molecules in the rhizosphere (see more details in Chapter 18) and discuss their biosynthesis and its regulation.

11.2 Terpenoid rhizosphere signals

11.2.1 Biological role of rhizosphere terpenoids

Terpenoids play a crucial role in the below-ground communication between plants and other organisms. The volatile, low molecular weight, monoterpenoids, sesquiterpenoids and some diterpenoids contribute to long-range communication. The non-volatile terpenoids, such as diterpenoids, sesterterpenoids, triterpenoids, tetraterpenoids and some of their breakdown

products, are secreted/deposited from the epidermal cell layer of the root and are involved in communication with organisms in the rhizosphere (Stipanovic et al. 1975, Bell et al. 1975, Mylona et al. 2008). Avenacins, for example, accumulate in root epidermal cells of oat roots (Mylona et al. 2008) and these compounds confer broad-spectrum resistance to soil pathogens. In addition to an effect against soil pathogens, terpenoids exuded by plants roots have been reported to inhibit growth of neighboring plant roots (Xu et al. 2012), repel herbivores, have antimicrobial activity (Stipanovic et al. 1975, Bell et al. 1975), attract entomopathogenic nematodes (Köllner et al. 2008) and other predators of plant enemies (Rioja et al. 2016). But intriguingly, they have also been hijacked as host detection signals, for example by plant parasitic nematodes (Schenk et al. 1999). Some of the non-volatile terpenoids were found to be biologically active in very low concentrations, which suggests their significance as signaling molecules. For instance, the triterpenoids in the root exudate of several plant species that are responsible for the induction of hatching of cyst nematodes, glycinoeclepin A in soybean (Fukuzawa, Furusaki, et al. 1985), glycinoeclepin B and C in kidney bean (Fukuzawa, Matsue, et al. 1985) and solanoeclepin A in potato (Schenk et al. 1999) that are active in nanomolar concentrations (Tanino et al. 2011). Another example are the diterpene momilactones that have been isolated from the seed husk of rice and were reported to inhibit the growth of rice roots at less than 100 ppm (Kato et al. 1973). In contrast to these non-volatile terpenoids, volatile compounds (such as for example camphor, cineole, and camphene) - detected in the soil around Salvia leucophylla roots – displayed allelopathy in rather high concentrations (>400µM) (Nishida et al. 2005). These monoterpenes inhibited seed germination and seedling growth of Brassica campestris by inhibiting both cell-nuclear and organelle DNA synthesis in the root apical meristem.

11.2.2 Biosynthesis of rhizosphere terpenoids

Terpenoids (isoprenoids) are derived from the isomeric 5-carbon building blocks (IDP) and dimethylallyl diphosphate (DMADP). IDP and DMADP are produced through two independent pathways in plants, the methylerythritol phosphate (MEP) pathway that is located in the plastids and the mevalonic acid (MVA) pathway in the cytosol (McGarvey and Croteau 1995). IDP and DMADP are also available in the mitochondria where isoprenoid derived compounds such as ubiquinone and heme A are produced (Kappers et al. 2005). The IDP in the mitochondria is imported from the cytosol and the DMADP generated by a mitochondrially located IDP isomerase (Disch, Hemmerlin, and Rohmer 1998, Phillips et al. 2008, Guirimand et al. 2012).

IDP and DMADP are condensed to form bigger (C10, C15, C20, etc) molecules by the activity of prenyl transferases or isoprenyl diphosphate synthases (Wang and Ohnuma 2000). The condensation of one DMADP molecule with one IDP molecule in a head-to-tail manner yields the C10 monoterpene precursor geranyl diphosphate (GDP) or its cisoid isomer neryl diphosphate (NDP). Interestingly, (heterologously expressed and artificially targeted) GDP synthase can use mitochondrial, cytosolic, and plastid localized IDP and DMADP and produce GDP in the corresponding compartment (Dong et al. 2016). This GDP can also be exchanged between these compartments but not equally effective. GDP produced in the cytosol is not transferred to the plastids, while the GDP produced in the mitochondria is entirely transferred to the plastids (Dong et al. 2016). However, only 7% of GDP produced in the plastids is available in the mitochondria. The condensation of two IDP and one DMADP units results in the C15 sesquiterpene precursor farnesyl diphosphate (2*E*,6*E*-FDP) or its isomers (2*Z*,6*Z*-FDP and 2*Z*,6*E*-FDP). The enzymes catalyzing this reaction, FDP synthases, were shown to be present in the mitochondria (Cunillera, Boronat, and Ferrer 1997), cytosol (Laule et al. 2003), plastids (Sanmiya et al. 1999) and peroxisomes (Krisans et al. 1994). The

condensation of three IDP and one DMADP units results in the C20 precursor geranylgeranyl diphosphate (GGDP), a reaction catalyzed by GGDP synthase (Okada et al. 2000). Just five years ago it was discovered that GGDP can combine with another IDP molecule to produce the C25 prenyl diphosphate precursor (geranylfarnesyl diphosphate), a precursor for the production of sesterterpenoids, and this step is catalyzed by a geranylfarnesyl diphosphate synthase (Liu et al. 2016). Interestingly, FDP and GGDP molecules can also be self-doubled (with the loss of both diphosphate groups) by enzymes beyond the family of prenyl transferaseses, squalene synthase (SS) and phytoene synthase (PSY) leading to the C30 triterpenoid and phytosterol precursor, squalene, and the C40 carotenoid precursor, phytoene, respectively (Thimmappa et al. 2014, Hirschberg 2001).

Isoprenyl diphosphate synthases are duplicated in many plant species. For example, there are 12 GGDP synthases (GGDPS) in the Arabidopsis genome (Beck et al. 2013). They differ in their expression, subcellular localization and metabolic process in which they are involved (Ruiz-Sola et al. 2016). *GGDPS11* has the highest expression level compared to all other paralogs in all organs (Ruiz-Sola et al. 2016). *GGDPS1* and *GGDPS2* are also ubiquitously expressed in all organs but with much lower expression level, whereas expression of *GGDPS3, 4, 6, 7, 8, 9, 10* is confined to specific organs (Ruiz-Sola et al. 2016). At the subcellular level, GGDPS1 was shown to localize to the mitochondria, GGDPS3 and GGDPS4 to the ER, and GGDPS2, 6, 7, 8, 9, 10 and 11 to the plastids (Ruiz-Sola et al. 2016). Except for GGDPS5 and GGDPS12, all other GGDPSs were shown to display GGDPS activity in *Escherichia coli* (Beck et al. 2013). The different GGDPSs were suggested to be specific for certain metabolic processes. Mutant lines for *GGDPS2*, *GGDPS6*, *GGDPS7*, *GGDPS8*, *GGDPS9* and *GGDPS10* did not show any developmental defects compared to wild-type plants, while *GGDPS11* was shown to be required for the production of most photosynthesis related isoprenoids and to be essential for plant development (Ruiz-Sola et al.

2016). Like the GGDPSs, GDS (Bouvier et al. 2000), FDS (Cunillera et al. 1996) and PSY (Welsch et al. 2008) were also shown to be duplicated, which indicates that duplication and possibly neofunctionalization are important processes for the evolution of isoprenoids and/or the organ- and/or conditon-specific regulation of their production.

After formation of the prenyl diphosphate precursors GDP, FDP and GGDP, monoterpenes, sesquiterpenes and diterpenes, respectively, are generated through the action of a large family of enzymes known as terpene synthases (Tholl 2006). Some of these terpene synthases produce a (virtually) single product, such as the monoterpene synthases geraniol synthase (Dong et al. 2013) and linalool synthase (Pichersky, Lewinsohn, and Croteau 1995), the sesquiterpene synthases amorpha-4,11-diene synthase (Wallaart et al. 2001) and germacrene A synthase (Bouwmeester et al. 2002), and the diterpene synthases geranyllinalool synthase (Herde et al. 2008) and taxadiene synthase (Köksal et al. 2011). The majority of the terpene synthases, however, produce multiple products. For example, TPS12 in tomato was shown to catalyze the formation of the sesquiterpenes β-caryophyllene and α-humulene from 2*E*,6*E*-FDP while TPS14 catalyzes the formation of several bisabolene isomers from either all-*trans*-*E*,*E*-FPP, or from all-*cis*-*Z*,*Z*-FPP as substrate (Falara et al. 2011). Another multi-substrate enzyme described in one of the earliest studies on this topic, is the *Mentha x piperita* β-farnesene synthase (Crock, Wildung, and Croteau 1997). It converts FDP to mainly (*E*)-β-farnesene (85%) and lower amounts of (*Z*)-β-farnesene (8%) and δ-cadinene (5%). But it can also use GDP as substrate and produce several different monoterpene products such as limonene, terpinolene, and myrcene (Crock, Wildung, and Croteau 1997).

Many of the terpenoids are direct products of terpene synthase, while others are formed through modification of the primary terpene skeleton by hydroxylation, acetylation, dehydrogenation, glycosylation and other types of reactions. Below-ground produced terpenoids that (potentially) play a role in rhizosphere signaling have been investigated much

less than the above-ground terpenoids, due to their more difficult accessibility. However, below we will discuss some examples, hoping to demonstrate and raise the awareness of how diverse the biosynthesis of below-ground terpenoids is. Their biosynthesis largely shares the same principles as described above, yet, with some interesting particularities.

11.2.3 Biosynthesis of volatile rhizosphere terpenoids

Just as above-ground, volatiles produced below-ground play an important role in direct and indirect plant defense against biotic stresses. An example of a direct defense below-ground volatile is the monoterpene 1,8-cineole (Steeghs et al. 2004). Cineole is not present in normal or mechanically injured *A. thaliana* roots, but upon pathogen infection is rapidly produced and released. In addition to induced direct defense compounds such as 1,8-cineole, plants also constitutively produce volatiles for direct defense. The semi-volatile diterpene, rhizathalene A, for example is constitutively released from *A. thaliana* roots, and was shown to play a role in the belowground resistance towards root-feeding insects (Vaughan et al. 2013). An example of indirect defense is represented by the sesquiterpene, (*E*)-β-caryophyllene, which is released from maize (*Zea mays*) roots upon feeding by corn root worm larvae *Diabrotica virgifera virgifera* and emitted from the leaves in response to attack by lepidopteran larvae like *Spodoptera littoralis.* Below-ground the induced (*E*)-β-caryophyllene attracts an entomopathogenic nematode, a natural enemy of the corn rootworm larvae, while above-ground a parasitic wasp is attracted (Köllner et al. 2008). The *A. thaliana* genome contains over 32 genes potentially encoding terpene synthases (TPSs), 15 of which are expressed primarily or exclusively in the roots (Vaughan et al. 2013). Interestingly, the formation from GDP of 1,8-cineole, which is only detected in the rhizosphere and not in the roots, was catalyzed by two TPSs (*At3g25820*/*At3g25830*), which were exclusively expressed in *A.*

thaliana roots (Chen et al. 2004). In contrast, (*E*)-β-caryophyllene synthase (TPS23) in *Z. mays* is expressed in both roots and above-ground organs (Köllner et al. 2008).

11.2.4 Volatile derived non-volatile rhizosphere terpenoids

One of the earliest studies showing that there are terpenoids in the root exudate of plants is the short communication by Hunter *et al.* in 1978 (Hunter et al. 1978a). The authors reported that the terpenoid aldehydes, desoxyhemigossypol, desoxy-6-methoxyhemigossypol, hemigossypol, 6-methoxyhemigossypol, gossypol, 6-methoxygossypol, and 6,6′-dimethoxygossypol, previously reported to be present in the epidermis of cotton roots (*Gossypium hivsutum* L.) (Stipanovic et al. 1975, Bell et al. 1975), were also exuded to an absorbing surface adjacent to roots (Hunter et al. 1978a). These terpenoid aldehydes have antimicrobial activity and offer natural resistance to cotton against insects and are thus considered a phytoalexin (Tian et al. 2016, Hunter et al. 1978a). From the above mentioned terpenoid aldehydes, gossypol is a C30 compound ($C_{30}H_{30}O_8$) that has multiple phenol-like rings, making it hard to establish whether this is a triterpenoid (Benbouza et al. 2002) or a phenolic compound (Dodou 2005). However, gossypol and its derivatives are derived from a volatile sesquiterpene. Early experiments with C14 labelled precursors showed that it is produced by cyclization of farnesyl pyrophosphate (Heinstein et al. 1970). This was supported by the discovery of a (+)-δ-cadinene synthase in cotton that catalyses the first committed step in the gossypol biosynthetic pathway (Chen et al. 1995). Interestingly, a year after, Chen et al identified a second (+)-δ-cadinene synthase from *Gossypium arboreum* which belongs to a different subfamily (80% identity with the other one) but has the same activity as the first identified one (Chen et al. 1996). In 2018, Tian et al characterized a number of the other biosynthetic steps in this biosynthetic pathway. Three cytochrome P450s (CYP706B1, *CYP82D113 and* CYP71BE79) were demonstrated to catalyze the C8, C7 and C11 oxidation of (+)-δ-cadinene, 7-hydroxy-(+)-δ-cadinene, and 8-hydroxy-7-keto-δ-cadinene, respectively.

In addition, Tian et al identified an alcohol dehydrogenase and one 2-oxoglutarate/Fe(II)-dependent dioxygenase which are also involved in gossypol biosynthesis up to the intermediate 3-hydroxy-furocalamen-2-one (Tian et al. 2018). A few steps downstream from this intermediate, a specific (S-adenosyl-L-methionine) methyltransferase methylates the 6-position of desoxyhemigossypol to form desoxyhemigossypol-6-methyl ester also identified in cotton (Liu et al. 1999).

11.2.5 Non-volatile derived volatile terpenoids

The unusual acyclic C11 homoterpene (*E*)-4, 8-dimethyl-1,3,7-nonatriene (DMNT) was initially isolated and identified from the essential oil of *Elettaria cardamomurn* (Maurer, Hauser, and Froidevaux 1986). But soon DMNT was found in the headspace of many plant species in response to herbivore attack (De Moraes et al. 1998, McCall et al. 1994). DMNT was shown to be an attractant for several insect species, both herbivorous as well as predators and parasites of other insects, among which *Cydia pomonella* (codling moth), *Neoseiulus womersleyi* (predatory mite), *Myllocerinus aurolineatus* (Tea weevil), *Phytoseiulus persimilis* (predatory mite), *Orseolia oryzivora* (African rice gall midge), *Cotesia marginiventris* (parasitoid wasp) and *Microplitis croceipes* (braconid wasp) (Rioja et al. 2016). In cucumber and maize, it was shown that (*E*)-nerolidol synthase catalyzes the conversion of FDP to the sesquiterpene nerolidol, the likely first committed step of DMNT biosynthesis, based on the direct incorporation of deuterium-labeled (E)-nerolidol into DMNT and the close correlation between (*E*)-nerolidol synthase activity and DMNT emission after herbivore damage (Degenhardt and Gershenzon 2000, Bouwmeester et al. 1999). Only 16 years later, exploiting the variation in herbivore-induced volatile formation among 26 maize inbred lines using nested association mapping and genome-wide association analysis, a P450 monooxygenase (CYP92C5) was identified that can convert nerolidol into DMNT (Richter et al. 2016). The cytochrome P450 enzyme encoded by the *A. thaliana CYP82G1*, which is induced in the

Arabidopsis Inflorescences and leaves upon insect feeding, produces DMNT and its C16-analog (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) *in vitro* by the oxidative breakdown of (E)-nerolidol and (E,E)-geranyl linalool, respectively. However, in *A. thaliana* leaves CYP82G1 only functions as a TMTT synthase because of the presence of (E,E)-geranyllinalool but not (E)-nerolidol in *A. thaliana* leaves (Lee et al. 2010). Intriguingly, the roots of *A. thaliana* also emit DMNT, and emission of DMNT was increased ~7-fold over constitutive background levels upon inoculation of detached axenically cultivated roots with soil-borne pathogen *Pythium irregulare*, but did not proceed via (*E*)-nerolidol (Lee et al. 2010). Instead, in *A. thaliana* roots DMNT is produced via degradation of a non-volatile triterpenoid arabidiol (Sohrabi et al. 2015), representing an intriguing example of convergent evolution. The reaction is catalyzed by the *Brassicaceae*-specific cytochrome P450 monooxygenase CYP705A1, which clusters with the arabidiol synthase in the genome of *A. thaliana* (Sohrabi et al. 2015).

The formation of volatile compounds by degradation of non-volatile terpenoid precursors also occurs in the formation of apocarotenoids such as safranal, ionones, citral, and β-damascenone by oxidative cleavage of C40 carotenoid precursors which is catalyzed by carotenoid cleavage dioxygenases (CCDs) (Walter, Floss, and Strack 2010). Interestingly, analogs of the ionones called irones are assumed to be produced by oxidative degradation of iridal triterpenes in rhizomes of *Iris* species (Jaenicke and Marner 1990). However, until now, no genes responsible for this conversion have been identified.

11.2.6 Non-volatile terpenoids

Like the volatiles discussed above, non-volatile compounds also play important roles in the rhizosphere interaction between plants and their environment. Saponins, for example, are triterpenoid glycosides and have been implicated as phyto-anticipins to fungal attack (Osbourn 1996). The avenacins, produced by oats (*Avena* spp.), have become an important

model to study saponin biosynthesis and their interaction with soil-borne pathogens (Faizal and Geelen 2013). The first committed step in avenacin biosynthesis is the cyclization of 2,3-oxidosqualene to the simple triterpene β-amyrin by an oxidosqualene cyclase (Kemen et al. 2014). β-Amyrin is then oxidized by the CYP450 enzyme CYP51H10 (Thimmappa et al. 2014), and further modified by a series of downstream enzymes such as glycosyltransferase, methyltransferase, and a serine carboxypeptidase-like acyltransferase to give the pathway end-product, avenacin (Mugford et al. 2009, Mugford et al. 2013).

Strigolactones are another interesting case of non-volatile below-ground signals that received a lot of attention in the past decade or so due to their multiple roles in plant biology. Strigolactones were initially discovered as root exuded signals that induce the germination of parasitic plants such as *Striga* and *Orobanche* species (Cook et al. 1966, Bouwmeester et al. 2003). Under phosphate deficiency, strigolactone secretion is upregulated reportedly as symbiotic signal for arbuscular mycorrhizal fungi, which facilitate the uptake of phosphate by plants (Bouwmeester et al. 2003, Yoneyama et al. 2007). In 2008 and 2011, strigolatones were reported to also be endogenous signals, plant hormones, that regulate shoot branching and root development, respectively (Gomez-Roldan et al. 2008, Koltai 2011, Ruyter-Spira et al. 2011). Even though strigolactones have a hormonal function also in the shoot, they are primarily biosynthesized - though there are indications not exclusively - in the roots. Strigolactones occur widespread in the plant kingdom, and their biosynthetic pathway is a perfect example for divergent evolution. All of the so far identified strigolactones derive from the same precursor, all-*trans*-β-carotene and it seems that the initial three enzymatic steps are highly conserved. From all-*trans*-β-carotene three sequential reactions are catalyzed by β-carotene isomerase (D27) and two carotenoid cleavage dioxygenases (CCDs) 7 and 8, resulting in the formation of the already bioactive strigolactone precursor carlactone (Seto and Yamaguchi 2014). However, from carlactone over 30 different strigolactones are produced

with individual plant species producing specific blends of up to about 8 different strigolactones, such as 4-deoxyorobanchol, orobanchol, 5-deoxystrigol, strigol, zealactone, and heliolactone (Wang and Bouwmeester 2018). The enzymes involved in the conversion of carlactone to all these different strigolactones are still largely unknown. A class-III cytochrome P450 monooxygenase (MAX1) was shown to catalyze the conversion of carlactone to carlactonoic acid in *A. thaliana* (Abe et al. 2014), which is further converted to methyl carlactonate by an unknown methyl transferase, and to an as yet unidentified oxidised product by LATERAL BRANCHING OXIDOREDUCTASE (LBO) (Brewer et al. 2016). In rice, two MAX1 homologs are responsible for the formation of 4-deoxyorobanchol from carlactone and orobanchol from 4-deoxyorobanchol (Zhang et al. 2014). Identification of the other enzymes involved in strigolactone structural diversification should help us to better understand the biological and evolutionary relevance of the strigolactone structural diversity in plants.

11.3 Phenolic rhizosphere signals

11.3.1 Function of phenolic compounds as signaling molecules

Phenolics are metabolites that have an aromatic ring (or rings) decorated with one or more hydroxyl groups. As antioxidants, pigments, auxin transport regulators, defense and signaling compounds, phenolic compounds in general are being recognized for their profound impact on plant growth, development, reproduction, UV protection, and defense (Croteau, Kutchan, and Lewis 2000). An increasing number of phenolic compounds are recognized for their signaling role in the rhizosphere. In *A. thaliana*, phenolic-related compounds were shown to be positively correlated with a higher number of unique rhizosphere microbiome species compared with other groups of compounds (*i.e.* sugars, sugar alcohols, and amino acids). For instance, salicylic acid levels in the rhizosphere positively correlated with the presence of

microbial species of the *Corynebacterineae*, *Pseudonocardineae* and *Streptomycineae.* This suggests that salicylic acid acts as specific substrate or signaling molecule for certain microbial species in the soil (Badri et al. 2013). Also larger organisms, such as the root knot nematode *Meloidogyne incognita* was shown to be attracted to volatile compounds released by roots of *Capsicum annum*, such as methyl salicylate, α-pinene, limonene, and tridecane (Kihika et al. 2017), and the phenolic compound methyl salicylate was the most potent one. The phenolic compound rosmarinic acid (RA), a caffeic acid ester widely present in the plant kingdom, presumably works as a defense compound (Petersen et al. 2009). Upon pathogen attack RA secreted by *Ocimum basilicum* roots as part of the root exudates, was shown to be highly inhibitory against an array of rhizosphere microorganisms (Bais et al. 2002). Recently, however, a new role was discovered for RA (Corral-Lugo et al. 2016). Bacteria monitor their own cell density using quorum sensing (QS) molecules, such as the homoserine lactones (Parsek et al. 1999). RA was demonstrated to be a homoserine-lactone mimic and can evoke several QS regulator controlled phenotypes like virulence factor biosynthesis or biofilm formation (Corral-Lugo et al. 2016).

Phenolics also play a crucial role in the interaction of legumes with symbiotic nitrogen-fixing bacteria or rhizobia. Nodules are root organelles that are developed through signal exchange between the plant roots and the rhizobia to facilitate nitrogen fixation and nodulation gene (*nod*) expression in rhizobium was shown to be essential for this process. Depending on their structure, the phenolic compounds can induce or suppress *nod* gene expression. Two flavonoids that acted as *nod* gene expression inducers were isolated from *Medicago sativa* (luteolin) (Peters, Frost, and Long 1986) and *Trifolium repens* (7,4' dihydroxyflavone in 1986 (Redmond et al. 1986). In contrast, the isoflavonoids medicarpin and coumestrol, isolated from *M. sativa* have been shown to negatively regulate *nod* gene expression in *Sinorhizobium meliloti* (Zuanazzi et al. 1998).

Just as for their role in plant-microbe interaction, phenolic compounds also play dual roles in plant-plant interaction. Parasitic weeds of the *Striga* genus, such as *Striga hermonthica* and *Striga asiatica*, constitute one of the major problems in African agriculture with yield losses up to 100% in large parts of sub-Saharan Africa (Gressel et al. 2004). The use of the legume *Desmodium uncinatum* as a 'push–pull' intercrop was proposed for smallholder farmers to control *Striga* infection in maize (Khan et al. 2006, Hooper et al. 2009). The strong suppressing effect of Desmodium on *Striga* infection seems to be caused by the presence of isoflavonoids in the Desmodium root exudate, which were demonstrated to prevent attachment of *Striga* to its host (Hooper et al. 2010, Khan et al. 2010). However, on the other hand, phenolic compounds, such as 2,6-dimethoxy-p-benzoquinone (DMBQ), isolated from sorghum roots (Chang and Lynn 1986) were shown to induce haustorium formation in *Striga*, a process required for attachment to and penetration of its host (Estabrook and Yoder 1998).

11.3.2 Biosynthesis and diversification of phenolic compounds

There are about 8000 naturally occurring plant phenolics, and phenolic compounds represent a large proportion of the metabolites present in root exudates (Baxter, Harborne, and Moss 1998). Several classes of phenolics have been categorized according to their basic skeletons: C6 (simple phenols, benzoquinones), C6–C1 (phenolic acids and aldehydes), C6–C2 (acetophenones, phenylacetic acids), C6–C3 (hydroxycinnamic acids, coumarins, phenylpropanes, chromones, monolignols), C6–C4 (naphthoquinones), C6–C1–C6 (xanthones), C6–C2–C6 (stilbenes, anthraquinones), C6–C3–C6 (flavonoids, isoflavonoids, anthocyanins), (C6–C3–C6)2,3 (bi-, triflavonoids, proanthocyanidin dimers, trimers), (C6–C3)2 (lignans, neolignans), (C6–C3)n (lignins), (C6)n (catechol melanins, phlorotannins) and (C6–C3–C6)n (condensed tannins) (Cheynier et al. 2013). Phenolics are derived from the shikimate pathway, beginning with an aldol-type condensation of phosphoenolpyruvic acid (PEP) from the glycolysis pathway, and D-erythrose-4-phosphate, from the pentose phosphate

cycle, to produce 3-deoxy-D-arabino-heptulosonic acid 7-phosphate (DAHP) (Santos-Sánchez et al. 2019) (Figure 11.1). Then six more enzymatic steps result in the formation of the key branch-point compound, chorismic acid, the final product of the shikimate pathway and also the common precursor for three aromatic amino acids, tryptophan, phenylalanine, and tyrosine. Of these three amino acids, phenylalanine and tyrosine can both serve as precursor for the biosynthesis of phenolic acids, tannins, flavonoids, and isoflavonoids. Interestingly, in most vascular plants, phenylalanine is the preferred substrate for this, but the monocot enzymes can utilize both phenylalanine and tyrosine (Croteau, Kutchan, and Lewis 2000). The core pathway of phenolic biosynthesis from phenylalanine and tyrosine starts with the removal of the amino group by phenylalanine ammonia lyase (PAL) to form cinnamic acid (Wanner et al. 1995), while the amino group of tyrosine can be removed by two different enzymes, tyrosine aminotransferase (or tyrosine transaminase TAT) that produces 4-hydroxyphenylpyruvic acid or tyrosine ammonia lyase that produces *p*-coumaric acid (Schenck and Maeda 2018) (Figure 11.1). The further transformation of phenylalanine derived cinnamic acid is catalyzed by the enzyme of the general phenylpropanoid pathway, cinnamic acid 4-hydroxylase (C4H) to form *p*-coumaric acid. In the tyrosine derived pathway, 4-hydroxyphenylpyruvic acid is further reduced to 4-hydroxyphenyllactic acid by hydroxyphenylpyruvate reductase. 4-Coumaric acid CoA-ligase (4CL) will use both phenylalanine and tyrosine derived *p*-coumaric acid and form *p*-coumaroyl-CoA (Figure 11.1).

While most of these aromatic compounds are usually non-volatile, the volatile subset is represented by benzenoid (C6–C1), phenylpropanoid (C6–C3) and phenylpropanoid-related compounds (C6–C2) (Peled-Zehavi et al. 2015). Generally, C6-C1 compounds such as benzyl alcohol, methyl benzoate, and benzyl benzoate are formed from cinnamic acid as a precursor, C6-C2 compounds such as 2-pheylethanol and phenyl acetaldehyde from phenylalanine, and

C6-C3 compounds such as eugenol, methyl eugenol and chavicol from 4-coumaroyl-CoA (Dudareva et al. 2013).

All other phenolic compounds are produced either from phenylalanine or from tyrosine from the intermediary precursors from the core pathway (Figure 11.1). For example, coumarins derive from cinnamic acid and ubiquinons, lignins, flavonoids, and anthocyanins derive from *p*-coumaric acid (Falcone Ferreyra, Rius, and Casati 2012). *p*-Hydroxyphenylpyruvic acid is needed for the biosynthesis of tocopherols and plastoquinones (Lushchak and Semchuk 2012). There are also some phenolic compounds derived from both pathways, for example, the above mentioned rosmarinic acid. *p*-Coumaroyl-CoA and *p*-hydroxyphenyllactic acid are coupled by ester formation and with the release of coenzyme A, 4-coumaroyl-4'-hydroxyphenyllactic acid is formed. The reaction is catalysed by ‘‘rosmarinic acid synthase” (RAS; 4-coumaroylCoA:40-hydroxyphenyllactic acid 4-coumaroyltransferase). The 3-and 30-hydroxyl groups are finally introduced by cytochrome P450-dependent monooxygenase reactions (CYP98A) (Petersen et al. 2009) (Figure 11.1). Some phenolic compounds have several biosynthetic routes, for example, salicylic acid. Stressed *A. thaliana* synthesizes salicylic acid primarily via an isochorismate-utilizing pathway derived from chorismic acid. A distinct pathway utilizing phenylalanine derived cinnamic acid as the substrate also may contribute to salicylic acid production, although to a much lesser extent (D'Maris Amick Dempsey, Vlot, and Daniel 2011) (Figure 11.1).

11.4 Benzoxazinoid- and fatty acid derived rhizosphere signaling molecules

Besides the ubiquitous terpenoids and phenolics, miscellaneous compounds such as benzoxazinoids, fatty acids derived compounds, alkaloids, gamma-aminobutyric acid, and sulfur containing compounds have also been shown to have important roles in shaping below-ground biota. In this chapter, however, we will only focus on the benzoxazinoids and fatty

acids derived compounds because they have important below-ground signaling roles, contain also volatiles and have well-studied biosynthetic routes.

11.4.1 Benzoxazinoids

The most well-studied examples of benzoaxizinoid signaling is represented by the volatile 6-methoxy-2-benzoxazolinone (MBOA) and DIMBOA (2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one). They are found mainly in monocot species of the Poales (Gramineae), including major agricultural crops such as maize, wheat and rye (Frey et al. 2009). Outside the Poales, benzoxazinoids are only detected in single species in the Ranunculales and the Lamiales (Frey et al. 2009). Their biosynthesis and biological function have been most thoroughly studied in maize. It was shown that larvae of the specialist western corn rootworm can hijack the signal released from maize roots (MBOA) for host detection (Bjostad and Hibbard 1992). In contrast to MBOA, DIMBOA secreted by maize seedling was demonstrated to confer a strong beneficial effect to the vulnerable seedlings in this early developmental stage, by not only repelling harmful bacteria and insects, such as soft rot bacteria (*Erwinia* spp.) (Lacy et al. 1979) and the European corn borer (*Ostrinia nubilalis*) (Klun, Tipton, and Brindley 1967) but also attracting the plant-beneficial bacterium (*Pseudomonas putida*) (Neal et al. 2012).

The biosynthesis of the benzoaxizinoids starts from indole, which is produced from indole-3-glycerolphosphate by indole-3-glycerolphosphate lyase (BX1) (Melanson et al. 1997). The following four oxygenation reactions catalyzed by four cytochrome P450 enzymes (CYP71 family) convert indole into DIBOA (Frey et al. 1995). Glucosylation, hydroxylation and methylation of DIBOA are catalyzed by two UDP-glucosyltransferases to form DIBOA glucoside (BX8 and BX9)(Von Rad et al. 2001, Jonczyk et al. 2008), followed by oxidation by a 2-oxoglutarate dependent dioxygenase (BX6) (Frey et al. 2003) and methylation by an *O*-methyltransferase (BX7) (Jonczyk et al. 2008) to form DIMBOA glucoside (Von Rad et al.

2001, Jonczyk et al. 2008). DIMBOA and MBOA seem to be produced from DIMBOA glucoside through the action of glucosidases and other reaction mechanisms which have not been fully elucidated yet (Hu et al. 2018, Wouters, Gershenzon, and Vassão 2016).

11.4.2 Fatty acid derived compounds

Fatty acids (FA) are carboxylic acids with an aliphatic chain and they are important structural and metabolic constituents of plant cells. In addition, fatty acids are being recognized for their signaling roles in plant and between plant and other organisms. Jasmonic acid and its volatile methyl ester (MeJA) are by far the best-studied fatty acid-derived signals in plants. Jasmonic acid signalling is crucial for plant stress responses to wounding, ultraviolet light, pathogens infection and insect attack (Weber 2002). Both compounds are often applied to plants to induce stress responses and study specialized metabolism (De Geyter et al. 2012). Jasmonic acid is also important for anther dehiscence and pollen development (An et al. 2018) and root stem cell activation and promoting root regeneration (Zhou et al. 2019). Both exogenous treatment with jasmonic acid and disrupted jasmonic acid signaling have been shown to alter root exudate profiles and the composition of root-associated microbial communities (Carvalhais et al. 2015, Doornbos et al. 2011). Also a number of other fatty acids such as 13(*S*)-hydroxy octadecatrienoic acid and 9-keto-octadecadienoic acid (9-KODE) and 13-KODE were shown to increase after wounding and infection by a pathogen (Weber 2002). Newly hatched larvae of the cabbage root fly react to fatty acid derived volatile stimuli (hexanol, hexanal and cis-3-hexenol) and can orient themselves by concentration gradients of these stimuli (Košťál 1992).

Fatty acids differ by length of their aliphalic chains, and they are arbitrarily categorized as short chain (5 or less C), medium chain (6 to 12 C), long chain (13 to 21 C) and very long chain (22 or more C) fatty acids. Fatty acid volatiles biosynthetically derive from the C18 unsaturated fatty acids, linoleic or linolenic acid. Linolenic acid is further oxidised to 13-

hydroperoxy linolenic acid – by 13-hydroperoxide lyase - the precursor for hexanal formation, and to 9-hydroperoxy linolenic acid – by 9-hydroperoxide lyase - the precursor for nonenal formation. Alcohol dehydrogenases can reduce the C6 and C9 aldehydes to alcohols and form hexanol and nonenol, respectively. Methyl jasmonate biosynthetically originates from 13-hydroperoxy linolenic acid too, however, unlike the other volatiles that are produced in a single step, multiple enzymatic steps are involved in its formation through the octadecanoid pathway. First, 12-oxo-phytodienoic acid (OPDA) is formed through the action of plastid localised allene oxide synthase and allene oxide cyclase. Subsequently, in the peroxisomes, OPDA is reduced by OPDA reductase (OPR3) to give 3-oxo-2(2′[*Z*]-pentenyl)-cyclopentane-1-octanoic acid (OPC8), after which OPC-8:0 CoA ligase converts OPC8 to OPC8-CoA. The final steps needed to produce jasmonic acid consist of 3 cycles of β-oxidation catalyzed by acyl-CoA oxidase - a multifunctional protein having both 2-trans-enoyl-CoA hydratase and L-3-hydroxyacyl-CoA dehydrogenase activities - and 3-ketoacyl-CoA thiolase (Li et al. 2005). Methyl jasmonate is formed through methylation of jasmonic acid by jasmonic acid carboxyl methyltransferase (Cheong and Do Choi 2003).

11.5 Regulation of signal molecule production

11.5.1 Biotic factors

11.5.1.1 Herbivores

Plants under attack by arthropod herbivores emit volatiles from their leaves that attract natural enemies of the herbivores. The sesquiterpene (*E*)-β-caryophyllene was the first identified insect-induced belowground plant signal, induced upon feeding by larvae of the western corn rootworm and is highly attractive to an entomopathogenic nematode (Rasmann et al. 2005). Likewise, monoterpenes including α-pinene, camphene, β-pinene, *p*-cymene and 1,8-cineole were shown to be released from roots of *Populus trichocarpa* and *Populus nigra* after

cockchafer larvae-damage and camphor was released from the roots of apple after *Melolontha melolontha* larvae attack (Abraham, Giacomuzzi, and Angeli 2015). The expression profiles of terpene synthases *PtTPS1, PtTPS*4, *PtTPS14, PtTPS*16 and *PnTPS21* responsible for the production of these monoterpenes in poplar all showed significant upregulation upon root herbivory, suggesting that monoterpene emission from roots is mainly determined transcriptionally (Lackus et al. 2018).

11.5.1.2 Pathogens

Root released signaling molecules are also induced by pathogen infection. Infection of cotton hypocotyls by *Rhizoctonia solani* increased the concentration of gossypol like terpenoids in the root exudate (Hunter et al. 1978a, b). Formation of the volatile homoterpene DMNT was transiently induced in a jasmonate-dependent manner upon infection by the root-rot pathogen *P. irregulare*, and two biosynthetic genes (arabidiol synthase and CYP705A1) were up-regulated in the roots (Sohrabi et al. 2015). Although DMNT is especially known for its role in the attraction of natural enemies in tritrophic interactions, in this case DMNT played a role in Arabidopsis resistance against *P. irregulare*.

11.5.1.3 Arbuscular mycorrhizal fungi

Both the quantity and composition of root exudates were altered by the presence of *arbuscular mycorrhizal* (AM) fungi, and these changes were plant species specific. Lendzemo *et al.* demonstrate that in sorghum (*Sorghum bicolor*) AM colonization results in a reduction of Striga infection, possibly through the down regulation of strigolactone production (Lendzemo et al. 2007). This is possibly supported by the fact that plants colonized by AM fungi seem to negatively regulate further mycorrhization via their root exudates (Pinior et al. 1999). The authors showed that root exudates from non-mycorrhizal cucumber plants stimulated hyphal growth, whereas root exudates from AM fungi (*Glomus intraradices* or *Glomus mosseae*) colonized cucumber plants showed no stimulation of the hyphal growth

of *Gigaspora rosea* (Pinior et al. 1999). In addition to down regulation of the secretion of rhizosphere signals, mycorrhizal sorghum plant roots exuded more alcohols, alkenes, ethers, and acids but fewer linear-alkanes (Sun and Tang 2013). Root volatile isoprenoid emission in tomato (*Solanum lycopersicum*) was decreased when roots were colonized by AM fungi (Asensio, Rapparini, and Peñuelas 2012).

11.5.1.4 Plant growth promoting rhizobacteria

Plant root exudates can be influenced by volatile compounds produced by plant growth promoting rhizobacteria (PGPR). It was shown that root exudates of sorghum exposed to volatile compounds from different PGPR strains differ in terms of types, numbers, and concentrations of compounds (Hernández-Calderón et al. 2018). For example, exudates produced by plants exposed to volatile compounds from *Arthrobacter agilis* were more diverse and accumulated in higher concentrations than those of plants exposed to other bacterial strains such as *Bacillus methylotrophicus* and *Sinorhizobium meliloti* (Hernández-Calderón et al. 2018). In non-inoculated and *A. agilis* inoculated plants, the root exudates were also shown to be different with one compound only found in the non-inoculated exudates whereas 6 compounds were only found in exudates of plants treated with the *A. agilis* (Hernández-Calderón et al. 2018). The authors speculate that of these 6 compounds, citric acid is likely used as carbon source by the bacteria, ferulic acid acts as a chemo-attractant signal compound for beneficial rhizospheric bacteria, while three fatty acids (nonadecanoic, eicosanoic, and tetracosanoic acids) may possibly affect plant growth.

11.5.1.5 Elicitors

Elicitors are chemicals or biological factors that can trigger physiological and morphological responses and/or phytoalexin accumulation (Zhao, Davis, and Verpoorte 2005). Biotic elicitors are for example compounds produced by fungi, bacteria, viruses or herbivores, plant cell wall components, as well as chemicals that are released at the attack site by plants upon

pathogen or herbivore attack, such as salicylic acid (SA), jasmonates (MeJA and JA) and nitric oxide (NO) (Zhao, Davis, and Verpoorte 2005). Exogenous application of these elicitors induces the accumulation of a wide range of secondary metabolites (Zhao, Davis, and Verpoorte 2005). Hydroponically cultivated *Lupinus luteus*, for example, secreted 10-fold higher genistein (phenolic) levels upon SA treatment (Kneer et al. 1999). In *A. thaliana*, treatment with SA, MeJA and NO affected different metabolic pathways in the roots, with limited overlap between the 3 elicitors (Badri et al. 2008). Eight phytochemicals including several kaempferol glycosides and methyl indolyl-3-carboxylate increased over 2-fold 3 h after treatment with MeJA, while only two unidentified compounds increased over 2-fold with SA and NO treatments. In addition, exogenous treatment with JA has recently been shown to alter root exudate profiles and the composition of root-associated bacterial communities (Carvalhais et al. 2015). The authors investigated the effect on root exudate profiles and the relative abundance of bacteria and archaea in the rhizosphere by disruption of JA secretion into the rhizosphere. In their study, two *A. thaliana* mutants that are disrupted in different branches of the jasmonate signaling pathway, namely *myc2* and *med25*, were used. Compared with the wild type, both mutants showed distinct exudation patterns, including lower amounts of asparagine, ornithine, and tryptophan, as well as a distinct bacterial and archaeal community composition, as illustrated by an increased abundance of *Streptomyces*, *Bacillus*, and *Lysinibacillus* taxa in the *med25* rhizosphere and of an Enterobacteriaceae population in *myc2* (Carvalhais et al. 2015).

11.5.1.6 Above-ground stresses

Above-ground stresses were also shown to influence belowground chemical signaling. One example was revealed by gas chromatography–mass spectrometry and proton transfer reaction-mass spectrometry analysis on the grass root volatile compounds with above-ground infection by the endophyte fungus *Neotyphodium uncinatum* (Rostás, Cripps, and Silcock

2015). The roots emitted less volatile compounds compared with controls, which reduced the attraction of the root herbivore *Costelytra zealandica*.

11.5.2 Abiotic factors

11.5.2.1 Nutrient deficiency

The composition of root exudates is also altered in response to abiotic signals in the rhizosphere. Phenolics, for example, are especially responsive to phosphate and nitrogen deficiency (Malusà et al. 2006, Sugiyama et al. 2016). Phenolic compounds are known to stimulate AM fungi hyphal growth that facilitate phosphate uptake (Abdel-Lateif, Bogusz, and Hocher 2012). Under phosphate starvation, total soluble phenolic content of bean (*Phaseolus vulgaris* L.) root exuduates increased whereas the content of phenolic compounds in the root decreased (Malusà et al. 2006). The increase of phenolic exudates might be explained by an increased gene expression of *L-phenylalanine ammonia-lyase* (Malusà et al. 2006). Induction of phenolic compounds (isoflavonoid, anthocyanin and cinnamoyl putrescines) by phosphate deficiency in the root exudates was also found in other plant species such as maize (Weisskopf et al. 2006), tomato (Khavari-Nejad, Najafi, and Tofighi 2009), and tobacco (Knobloch and Berlin 1981). As described above, phenolic compounds secreted by legume roots are chemoattractants and nod gene inducers for the symbiotic Rhizobia. Under nitrogen deficiency, both expression of the flavonoid biosynthesis genes, *chalcone synthase* and *isoflavone reductase*, and exudation of flavonoids and isoflavonoids increased in alfalfa (*Medicago sativa* L.) roots (Coronado et al. 1995). Soybean roots secrete isoflavonoids, daidzein and genistein, to attract rhizobia and nitrogen deficiency resulted in a strong increase in their secretion (Sugiyama et al. 2016).

Terpenoid derived strigolactones are another type of signaling molecules in the rhizosphere that are induced by phosphate and nitrogen deficiency. Phosphate deficiency induces strigolactone biosynthesis in tomato, and strongly increased *Phelipanche ramosa* seed

germination and hyphal branching of AM fungi compared with control plants grown with normal phosphate supply (López-Ráez et al. 2008). Mutants of striolactone signaling (*max2*) or biosynthesis mutants (*max4*) in *A. thaliana* showed reduced response to low Pi conditions, such as a lower root hair density,lower level of expression for starvation-induced genes, and higher shoot branching than the wild type suggesting that strigolactones play a role in the plant response to low phosphate conditions (Ruyter-Spira et al. 2011, Mayzlish-Gati et al. 2012). Similarly, low levels of either phosphate or nitrogen stimulated strigolactone biosynthesis in rice roots (Sun et al. 2014). Strigolactone deficient mutants (d10 and d27) and strigolactone signalling mutant (d3) displayed a lost sensitivity of the root response to phosphate and nitrogen deficiency. For example, seminal root length was increased by 50% and 29% in WT plants but by only approximately 18% and 14% in the three mutants when grown under low phosphate and low nitrogen solution, respectively.

11.5.2.2 Other abiotic signals

The composition of root exudates is also affected by other abiotic signals in the rhizosphere. There is evidence that flavonoids play a role in resistance to aluminum toxicity and silicon induced amelioration of aluminum toxicity in maize (Kidd et al. 2001). Roots of maize plants that were exposed to aluminum and silicon exuded high levels of phenolics compounds such as catechol, catechin, and quercetin, and an aluminum-resistant variety exuded a 15-fold higher level of phenolics when pretreated with silicon than when no such pre-treatment was applied (Kidd et al. 2001). These results might be due to the metal-binding activity of many flavonoids rather than that they have a signaling role. The exudation of phenolic compounds was also shown to display seasonal changes. During summer, the secretion of flavonoids by *Cistus ladanifer*, increased approximately three- to four-fold compared with the secretion measured in spring. Mimicking summer conditions, drought and high temperatures, in a glasshouse and culture room experiment showed that methylated flavonoids (kaempferols and

7-methylated apigenins) in the exudate increased perhaps because the methylated flavonoids are less hydrophylic than the less methylated ones, therefore the secretion of more methylated flavonoids is considered as part of the defense mechanism of the plant against the drought stress of summer (Chaves, Escudero, and Gutierrez-Merino 1997). Other abiotic factors like light (Hughes et al. 1999), CO2 (Watt and Evans 1999), and soil moisture (Xia and Roberts 1994) were all shown to affect root exudate composition in black alder, white lupin and maize, respectively, but there is so far no evidence linking this to a signaling role of the exuded compounds.

11.6 Conclusions and future perspective

In this book chapter, we described the current knowledge on the biosynthesis of a number of important classes of signaling molecules exuded by plants from their roots into the rhizosphere. Compared with above-ground signaling molecules, common and root specific biosynthetic routes were discussed and the plasticity and regulation of the biosynthesis of these signaling molecules reviewed. The biosynthesis and exudation of below-ground signaling molecules are mostly regulated by abiotic and biotic factors in the soil but there is evidence that also above-ground factors can influence below-ground signaling.

We are just beginning to explore the importance of the vast repertoire of below-ground signaling molecules. Structure elucidation of the unknown signaling molecules of low abundance in the rhizosphere is a first challenge. Establishment of the link between signaling molecules and single or multiple organisms from the enormous diversity of soil-dwelling organisms is the next. Using the recently emerging metabolomics and metagenomics tools and systems biology approaches to unravel the relationship between signaling molecules and soil biota from our biological ‘big data’ will likely advance our knowledge rapidly. Verifying postulated signaling relationships will rely on metabolic engineering approaches.

CRISPR/Cas9 as a new approach for metabolic engineering is evolving rapidly from only gene knock-out applications to knock-in and precise gene editing with targeted nucleotide modification (Swinnen, Goossens, and Colinas 2019). In addition, metabolic engineering approaches such as virus-induced-gene-silencing and transgenic hairy roots are greatly shortening the process to discover new signaling relationships (Liscombe and O'Connor 2011, Groten et al. 2015, Häkkinen and Oksman-Caldentey 2018). In addition to metabolic engineering, modern breeding technique such as Targeting Induced Local Lesions IN Genomes (TILLING) and eco-TILLING to find EMS-induced or natural mutations, respectively, in genes of interest can be used for gene discovery and to study the effect of certain signaling molecules on their environment (Reddy and Saiprasad 2015, Lin, Wagner, and Alper 2017).

A better understanding of the molecular mechanisms underlying biosynthesis and regulation of below-ground signals and their biological function together with advanced metabolic engineering/breeding approaches will provide the basis for a better exploitation of the hidden, below-ground, part of plants and the creation of a more sustainable agriculture with less inputs of fertilizers and pesticides.

Acknowledgments

The preparation of this book chapter was supported by the ERC (Advanced grant CHEMCOMRHIZO, 670211 to HJB) and the EU (Marie Curie grant NemHatch, 793795 to LD).

Figure Legend

Figure 11.1 Biosynthesis of phenolic compounds. The core phenylpropanoid pathway is highlighted with color.

DAHP synthase, 3-deoxy-d-arabino-heptulosonate 7-phosphate synthase; ICS, isochorismate synthase; IPL, isochorismate pyruvate lyase; BA2H, benzoic acid 2-hydroxylase; PAL, phenylalanine ammonialyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumaroyl CoA ligase; TAT, tyrosine aminotransferase; HPPR, hydroxyphenylpyruvate reductase; RAS, 4-coumaroylCoA:40-hydroxyphenyllactic acid 4-coumaroyltransferase; CYP98A, cytochrome P450-dependent monooxygenase 98A family; CHI, chalcone isomerase; CHS, chalcone synthase

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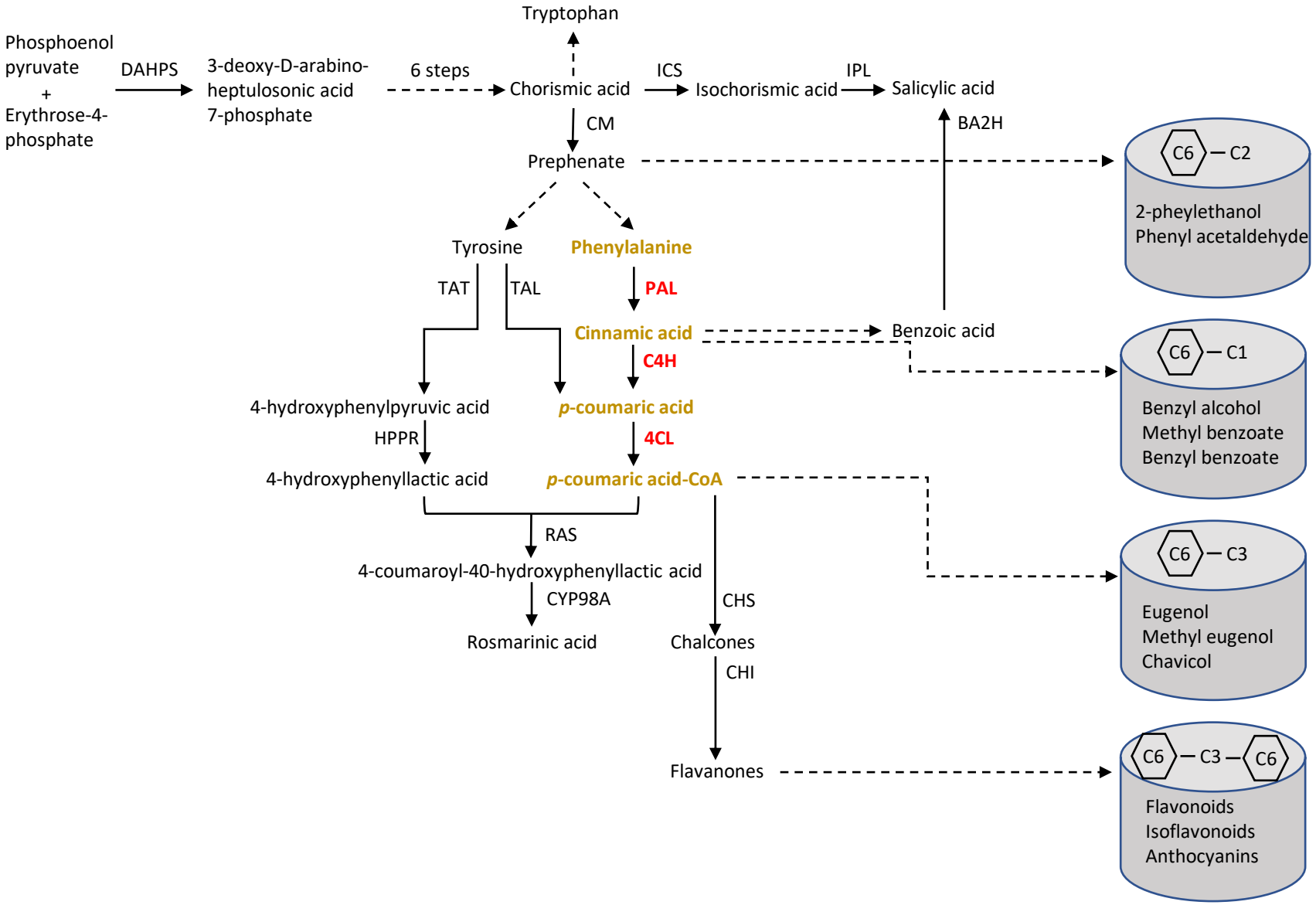
Tryptophan
Phosphoenol pyruvate + Erythrose-4-phosphate
DAHPS
3-deoxy-D-arabino-heptulosonic acid 7-phosphate
6 steps
Chorismic acid
ICS
Isochorismic acid
IPL
Salicylic acid
CM
BA2H
Prephenate
C6 — C2
2-pheylethanol
Phenyl acetaldehyde
Tyrosine
Phenylalanine
TAT
TAL
PAL
Cinnamic acid
Benzoic acid
C4H
C6 — C1
Benzyl alcohol
Methyl benzoate
Benzyl benzoate
4-hydroxyphenylpyruvic acid
p-coumaric acid
HPPR
4CL
4-hydroxyphenyllactic acid
p-coumaric acid-CoA
RAS
C6 — C3
Eugenol
Methyl eugenol
Chavicol
4-coumaroyl-40-hydroxyphenyllactic acid
CYP98A
CHS
Rosmarinic acid
Chalcones
CHI
Flavanones
C6 — C3 — C6
Flavonoids
Isoflavonoids
Anthocyanins