

1 Flavonoids and saponins in plant rhizospheres

2
3 **Flavonoids and saponins in plant rhizospheres: roles, dynamics, and the potential**
4 **for agriculture**

5
6 Akifumi Sugiyama

7
8 Research Institute for Sustainable Humanosphere, Kyoto University, Gokasho, Uji, 611-
9 0011, Japan

10
11 ***Corresponding author: Akifumi Sugiyama**

12 Address: Laboratory of Plant Gene Expression, Research Institute for Sustainable
13 Humanosphere, Kyoto University, Uji 611-0011, Japan.

14 Tel: +81-774-38-3617, Fax: +81-774-38-3623, E-mail: sugiyama.akifumi.4m@kyoto-
15 u.ac.jp

Abstract

Plants are in constant interaction with a myriad of soil microorganisms in the rhizosphere, an area of soil in close contact with plant roots. Recent research has highlighted the importance of plant specialized metabolites (PSMs) in shaping and modulating the rhizosphere microbiota; however, the molecular mechanisms underlying the establishment and function of the microbiota mostly remain unaddressed. Flavonoids and saponins are a group of PSMs whose biosynthetic pathways have largely been revealed. Although these PSMs are abundantly secreted into the rhizosphere and exert various functions, the secretion mechanisms have not been clarified. This review summarizes the roles of flavonoids and saponins in the rhizosphere with a special focus on interactions between plants and the rhizosphere microbiota. Furthermore, this review introduces recent advancements in the dynamics of these metabolites in the rhizosphere and indicates potential applications of PSMs for crop production and discusses perspectives in this emerging research field.

Keywords

flavonoid, holobiont, rhizosphere, saponin, soybean

Introduction

Plants produce a diverse array of low molecular weight compounds, the number of which exceeds 200,000 (Ferne 2007; Afendi et al. 2012). A large portion of these metabolites are not necessarily essential for growth and development but often have biological activities such as protection against biotic and abiotic stress and modulation of interactions with other organisms. These metabolites are called plant specialized metabolites (PSMs), and it is thought that plants acquired the ability to biosynthesize these metabolites during evolution for adaptation to the ecosystem (Kim and Buell 2015; Lichman et al. 2020).

In nature, plants are in constant interaction with a myriad of microorganisms ranging from pathogens, commensals, and beneficial microbes. It is believed that plants have evolved to benefit from beneficial microorganisms while protecting themselves from pathogens; however, how to distinguish these microbes at the molecular level remains unclear. How do plants engage with beneficial microorganisms while at the same time restricting pathogens is among the top unanswered questions about molecular plant-microbe interactions (Harris et al. 2020), and answering this question is necessary for the utilization of these microbes for sustainable crop production (Finkel et al. 2017; Zhang et al. 2017). The rhizosphere, the region of soil in proximity to plant roots, is a hotspot for these interactions, and it harbors unique repositories of microbes and metabolites (Hartmann et al. 2008). Plant roots exude a substantial amount (up to 40%) of photosynthesis-derived carbons into the rhizosphere (Lynch and Whipps 1990; Badri and Vivanco 2009; Haichar et al. 2016). The majority of them are primary metabolites including sugars, amino acids, and organic acids, which nourish rhizosphere microbes (Canarini et al. 2019). In addition to the primary metabolites, plant roots secrete PSMs

that have important roles in the interactions between plants and soil microbes.

Root-derived metabolites, along with the physicochemical properties of soil and environment factors, influence soil microbial communities and affect the formation of rhizosphere microbiota characterized by abundant and active microbial communities having reduced diversity, as compared with bulk soil (Wang et al. 2020; Vieira et al. 2020). Recent evidence supports the importance of the rhizosphere microbiota in plant growth, immunity, and fitness, emphasizing the potential for utilization in crop production (Canto et al. 2020; Compant et al. 2019). The involvement of PSMs in shaping the root/rhizosphere microbiota was demonstrated using plant mutants disrupted in a particular biosynthetic pathway and has been summarized in recent reviews (Pascale et al. 2020; Jacoby et al. 2021; Pang et al. 2021). In addition to the loss-of-function approach, we have employed an artificial rhizosphere treatment method to reveal the functions of flavonoids and saponins in shaping the rhizosphere microbiota (Okutani et al. 2020; Fujimatsu et al. 2020) (Nakayasu et al. 2021) (Figure 1). In this review, we focus on two types of PSMs—flavonoids and saponins—that are abundantly secreted from roots to summarize their functions and dynamics in the rhizosphere and highlight future challenges in harnessing rhizosphere microbiota for plant robustness and sustainable crop production.

Flavonoids

Synthesis and accumulation

Flavonoids are phenolic compounds comprising more than 8,000 distinct molecules (Pietta 2000). Flavonoids confer protection from UV-B radiation, pathogen, and herbivores, attract pollinators, regulate auxin transport, and modulate reactive oxygen

species and fertility in plants (Ferreyra et al. 2012). Additionally, flavonoids are secreted from roots and exert diverse roles in the rhizosphere (see below). Flavonoids in plants are biosynthesized through the phenylpropanoid and the acetate-malonate pathways to form molecules composed of 15 carbon atoms arranged as C₆-C₃-C₆. Chalcone synthase (CHS) catalyzes the first step in flavonoid biosynthesis using *p*-coumaroyl-CoA and malonyl-CoA as substrates (Yonekura-Sakakibara et al. 2019). Based on the oxidation and substitution of the C (C₃) ring, flavonoids are further categorized into the subgroups, including anthocyanins, flavonols, flavanones, flavones, flavanols, isoflavones, chalcones, catechins, and aurones (Panche et al. 2016). Isoflavones are predominantly found in legume plants (Figure 2). Daidzein and genistein in soybean are biosynthesized from liquiritigenin and naringenin, respectively, by isoflavone synthase (IFS), a P450 protein, to form 2-hydroxyisoflavanone (Akashi et al. 1999; Jung et al. 2000), followed by dehydration to produce daidzein and genistein either spontaneously or via 2-hydroxyisoflavanone dehydratase (HID) (Akashi et al. 2005). Glycitein is biosynthesized from liquiritigenin by flavonoid 6-hydroxylase to form 6-hydroxyliquiritigenin (Latunde-Dada et al. 2001). 6-hydroxyliquiritigenin is then converted to 6-hydroxydaidzein by IFS and HID and subsequently converted to glycitein by isoflavone *O*-methyltransferase (Uchida et al. 2020).

Although direct biochemical evidence is lacking, most flavonoids in plant cells are assumed to be accumulated in vacuoles in the form of glucosides. Vacuolar sequestration of flavonoids involves transporters, glutathione S-transferase, and vesicle trafficking (Zhao 2015). Both ATP-binding cassette (ABC) transporters and multidrug and toxin extrusion (MATE) transporters have been identified as being involved in the vacuolar sequestration of flavonoids (Zhao 2015). Most of the research on vacuolar

sequestration has analyzed the sequestration of anthocyanins and proanthocyanidins (Zhao 2015; Shitan and Yazaki 2020); however, biochemical transport analysis using yeast membrane vesicles revealed that AtABCC2 of *Arabidopsis thaliana* transports flavonoid glucosides such as luteolin 7-*O*-glucoside and apigenin 7-*O*-glucoside, in addition to anthocyanins (Behrens et al. 2019), and MtMATE2 of *Medicago truncatula* transports apigenin 7-*O*-glucoside, apigenin 7-*O*-glucoside malonate, kaempferol 7-*O*-glucoside, and kaempferol 7-*O*-glucoside malonate, in addition to anthocyanins (Zhao et al. 2011).

Secretion into the rhizosphere

Root exudates and root border cells are the primary sources of rhizosphere flavonoids (Sasse et al. 2018; Hassan and Mathesius 2012). ABC transporters have been suggested to mediate the secretion of flavonoids into the rhizosphere. A biochemical transport assay using plasma membrane vesicles of soybean revealed the involvement of ABC transporters in the secretion of genistein, an isoflavone acting as a signal for nod gene expression in rhizobia (Sugiyama et al. 2007). Tobacco BY-2 cells expressing MtABCG10 showed the efflux of isoliquiritigenin from cells (Biala et al. 2017).

Soybean is a suitable model plant to study flavonoid secretion because of its relatively large leaves and roots and the importance of secreted flavonoids in its interactions with rhizosphere microbes (Kosslak et al. 1987; Sugiyama 2019; Okutani et al. 2020). Three types of isoflavone aglycones—daidzein, genistein, and glycitein—are biosynthesized in the cytosol of soybean, and their glucosides and malonylglucosides are presumably accumulated in the vacuoles (Figure 2A). These aglycones are also secreted from roots and found in root exudates (Pueppke et al. 1998). In addition to the ATP-

dependent active transport of isoflavone aglycones, secretion of isoflavone glucosides stored in vacuoles into the apoplast has also been proposed (Suzuki et al. 2006; Sugiyama 2019). Secreted isoflavone glucosides are hydrolyzed to aglycones by isoflavone conjugate hydrolyzing β -glucosidase (ICHG) (Suzuki et al. 2006).

The secretion of isoflavones from soybean roots has been analyzed both in hydroponic conditions and in the field. Both daidzein and genistein induce *nod* genes of the rhizobia, leading to a symbiosis for biological nitrogen fixation (Kosslak et al. 1987; Pueppke et al. 1998). Under nitrogen deficiency, the secretion of these isoflavones is increased approximately 10-fold in hydroponic conditions (Sugiyama et al. 2016) (Figure 3). Daidzein is the predominant isoflavone in root exudates throughout the growth stages; a higher amount of daidzein is secreted during the vegetative stages than the reproductive stages, and the secretion of malonyldaidzin and daidzin is increased during the reproductive stages (Sugiyama et al. 2016). The amount of daidzein and genistein in root exudates is stable during the day. However, genes for transcription factors and biosynthesis of isoflavone metabolism show diurnal regulation with increased expression during the daytime (Matsuda et al. 2020). GmMYB176, a transcription factor of isoflavone biosynthesis highly expressed in roots, induces isoflavone biosynthetic genes from dawn to noon, followed by the induction of isoflavone biosynthetic genes at noon, and a slight increase of daidzein aglycone in roots in the afternoon (Matsuda et al. 2020). Conceivably, the secretion pathway from vacuolar glucosides to apoplast would be induced during the nighttime when the expression of *ICHG* is increased to maintain the daidzein level in root exudates.

In field-grown soybean, the secretion of daidzein is also higher in the early vegetative stages than in the reproductive stages, but the amount is increased up to

10,000-fold, compared with hydroponic conditions (Toyofuku et al. 2021) (Figure 3). The increase of secretion is possibly due to the presence of microbes in the field; however, soil particles in the field may influence the root morphology and the rate of isoflavone secretion because the particle size and chemistry of growth substrates such as sand, clay, and glass beads, affect root morphology and exudation in *Brachypodium distachyon* (Sasse et al. 2020). It remains unclear whether both pathways of isoflavone secretion, i.e. active transport of isoflavone aglycones and secretion of vacuole-stored isoflavone glucosides, operate simultaneously or if one operates conditionally. Although higher daidzein secretion is suggested during the early vegetative stages, the rhizosphere isoflavone contents are slightly higher in the reproductive stage due to relative stability of the compounds in the soil (Sugiyama et al. 2017). It is of particular importance to analyze the microbial communities and physicochemical properties of soils together with degradation kinetics since the stability of flavonoids depends on the soil (Sugiyama and Yazaki 2014).

Roles in the rhizosphere

Flavonoids display a broad range of biological activities not only in plants but in animals and microbes. The biological activities in humans include antioxidative activity, free radical scavenging capacity, anti-inflammatory capacity, and anticancer activities (Gorniak et al. 2019), and flavonoids are widely used as phytomedicines. Flavonoids inhibit a range of root pathogens in the rhizosphere (Hassan and Mathesius 2012; Mierziak et al. 2014) because of their antipathogenic properties, such as the ability to disrupt membrane integrity (Weinstein and Albersheim 1983; Wu et al. 2019) and inhibit DNA gyrase (Wu et al. 2013). These defensive flavonoids can be exuded into the soil

either constitutively or inductively; for example, an increased level of glyceollin I, a phytoalexin of soybean, is found in the root exudate upon exposure to pathogens and non-symbiotic rhizobia (Schmidt et al. 1992; Lozovaya et al. 2004), isoflavones in the root exudates of white lupin are increased upon treatments with various elicitors (Gagnon and Ibrahim 1997). Other roles of flavonoids in the rhizosphere include mediation of allelopathy, chelation, and reduction of metals in soil (Hassan and Mathesius 2012; Cesco et al. 2012; Weston and Mathesius 2013). The following sections highlight the functions of flavonoids as regulators of the rhizosphere microbiome.

Roles as chemoattractants

In addition to functioning as a nutrient source for rhizosphere microorganisms, plant metabolites (particularly PSMs) exert both attractive and repellent effects on soil microbes and shape the rhizosphere microbiota (Pascale et al. 2020; Jacoby et al. 2021). Flavonoids have been thought of as an attractant for the rhizobia, as the chemical signals from plant roots to symbiotic rhizobia have been determined to be flavonoids. Several reports have shown a chemotactic activity of *nod* gene-inducing flavonoids, including luteolin, 4',7-dihydroxyflavone, 4',7-dihydroxyflavanone, and 4,4'-dihydroxy-2-methoxychalcone for *Ensifer meliloti* (Caetanoanollés et al. 1988; Dharmatilake and Bauer 1992) and also apigenin and luteolin for *Rhizobium leguminosarum* (Aguilar et al. 1988); however, a recent comprehensive analysis revealed that *E. meliloti* did not show a chemotactic response to flavonoids such as hyperoside, luteolin, luteolin-7-*O*-glucoside, quercetin, and chrysoeriol in alfalfa seed exudates (Compton et al. 2020). Amino acids and quaternary ammonium compounds, which are 10-fold more abundant than flavonoids in alfalfa root exudates, are the primary chemoattractants of *E. meliloti* (Compton et al.

207 2020). Bacterial chemoreceptors for these metabolites have also been identified in *E.*
208 *meliloti* (Webb et al. 2014; Webb et al. 2017). In the case of soybean-rhizobia interactions,
209 *Bradyrhizobium japonicum* is most attracted to succinate, glutamate, and malonate and is
210 not attracted to luteolin, daidzein, or genistein (Barbour et al. 1991). *Bradyrhizobium*
211 *japonicum* is also attracted to cinnamic acid and hydroxycinnamic acids, such as *p*-
212 coumaric acid, caffeic acid, ferulic acid, and sinapinic acid (Kape et al. 1991). Together,
213 these studies do not favor the contribution of flavonoids in the recruitment of rhizobia to
214 the proximity of plant roots but indicate the contribution of amino acids, dicarboxylic
215 acids, and quaternary ammonium compounds. Future research is needed to highlight the
216 functions of these chemoattractants in the plant rhizosphere with consideration for the
217 secretion, degradation, and distribution within the context of the soil community where
218 multiple interactions occur.

219 *Roles as nod gene inducers*

220 Once attracted to the vicinity of the root surface, chemical signal exchange between
221 legume plants and the rhizobia occurs (Janczarek et al. 2015; Liu and Murray 2016).
222 Luteolin and 7,4'-dihydroxyflavone were the first signaling compounds discovered in
223 alfalfa and white clover, respectively, using a *nod-lacZ* expression system, which was also
224 used to identify other signaling flavonoids in other legume species, such as daidzein and
225 genistein in soybean (Peters et al. 1986; Redmond et al. 1986; Kosslak et al. 1987; Kape
226 et al. 1991). Different classes of flavonoids, including flavones, flavonols, flavanones,
227 isoflavones, and chalcones, have since been identified as plant signals for induction of
228 *nod* genes in the rhizobia in addition to non-flavonoid metabolites (Janczarek et al. 2015;
229 Liu and Murray 2016). Most of these flavonoids induce *nod* genes at low micromolar
230

concentrations within the range of the rhizospheric concentrations, at least for daidzein (Sugiyama et al. 2017; Toyofuku et al. 2021). The specificity of flavonoid profiles in each legume plant and the specific perception of flavonoids by NodD, a LysR-type transcription regulator, in the rhizobia are responsible for the first level of host specificity for legume-rhizobia symbiosis. Upon perception of the signal, the rhizobia synthesize and secrete lipo-chitoooligosaccharide Nod factors (NFs) to be recognized by receptors on the root surface (Gourion et al. 2015; Buhian and Bensmihen 2018). Genetic signaling pathways governing NF detection and nodule organogenesis are now quite well understood in the model legume plants, *Medicago truncatula* and *Lotus japonicus* (Roy et al. 2020). In addition to NFs, type III secretion systems, together with exported proteins, are induced by genistein in *Sinorhizobium fredii* USDA257, a symbiont of soybean and other legume plants (Krishnan et al. 2003), and *B. elkanii* SEMIA587 (de Campos et al. 2011). Genistein also induces the expression of resistance-nodulation-division (RND) efflux pumps in *B. japonicum* (Takeshima et al. 2013). A *B. japonicum* mutant deficient in this efflux pump is sensitive to genistein (but not to daidzein) and shows reduced nodulation and nitrogen fixation when this mutant is inoculated in soybean roots. The expression of this efflux pump is negatively regulated by a TerR-like regulator (BdtR) (Han et al. 2020). Mutation of this regulator results in higher extracellular genistein levels and decreased susceptibility to genistein because of induction of the efflux pump. In contrast, the induction of *nod* genes is reduced in the mutant. These results suggest that the rhizobia maintain intracellular genistein homeostasis to induce *nod* genes for nodulation while the toxic effect of this isoflavone is alleviated.

Roles in modulating the microbiota

Flavonoids in the rhizosphere impact the microbiota as well as the rhizobia. The application of pure flavonoid compounds modifies both bacterial and fungal communities in soil. Daidzein and genistein applied to the soil resulted in different microbial community structures, as revealed by phospholipid fatty acid profiling (Guo et al. 2011). The treatment of soils with 7,4'-dihydroxyflavone, a *nod* gene-inducing flavonoid of alfalfa roots, modified bacterial communities at a concentration found in root exudates, with an increase in Acidobacteria, suggesting multiple functions of this flavonoid in the rhizosphere beyond the establishment of symbiosis (Szoboszlay et al. 2016). Both bacterial and fungal communities of the peanut rhizosphere were modified when luteolin was continuously applied to the soil (Wang et al. 2018). The growth and nodule formation of peanuts treated with luteolin were reduced, suggesting the inhibitory effects of luteolin in continuous mono-cropped peanut systems, leading to reduced productivity. Daidzein also modifies bacterial communities in soil (Okutani et al. 2020). The relative abundance of Comamonadaceae was increased in a concentration-dependent manner, and the bacterial communities became more similar to the rhizosphere of soybean grown in fields rather than bulk soil. The rhizosphere bacterial communities of soybean hairy roots silenced with the *IFS* gene showed a slight change, depending on the gene silencing and hairy root transformation (White et al. 2017). Comamonadaceae were not reduced in the IFS-silenced hairy root rhizosphere, suggesting the involvement of multiple metabolites in modulating the rhizosphere microbiota. In maize, flavones such as apigenin and luteolin are secreted from the roots and promote the enrichment of Oxalobacteraceae in the rhizosphere (Yu et al. 2021). Oxalobacteraceae isolates belonging to the genus *Massilia* improve the growth of maize under nitrogen-deficient conditions via alteration of root development, suggesting a network of root architecture and the microbial taxa in

the rhizosphere, resulting in improved plant growth under nutrient deficiency.

Saponins

Synthesis and accumulation

Saponins are a group of PSMs widely distributed in higher plants (Vincken et al. 2007). They contain an aglycone hydrophobic backbone bound to hydrophilic saccharides such as glycosides, resulting in amphiphilicity and the formation of a soap-like foam when agitated in water. The name saponin is derived from the Latin word 'sapo', meaning soap, and they are typically subdivided into triterpenoid and steroid glycosides based on the carbon skeletons (Vincken et al. 2007). Both types of compounds are biosynthesized from a common precursor, 2,3-oxidosqualene, via multiple reactions such as cyclization, oxidation, and glycosylation. In the plant kingdom, dicotyledonous plants mainly accumulate triterpenoid saponins, while monocotyledonous plants mainly synthesize steroidal saponins (with some exceptions) (Sparg et al. 2004; Moses et al. 2014).

The triterpenoid and steroidal aglycone backbones are synthesized from isopentenyl diphosphate units derived from the mevalonate pathway. Condensation of two farnesyl diphosphate by squalene synthase (SQS) generates squalene, which is then epoxidized to 2,3-oxidosqualene catalyzed by squalene epoxidase (SQE). Oxidosqualene cyclases (OSCs) catalyze the cyclization of 2,3-oxidosqualene, and they are positioned at a key metabolic branch point between primary metabolism for plant sterols (phytosterols) and brassinosteroid hormones and specialized metabolism for triterpenoids. 2,3-Oxidosqualene can be cyclized into a diverse range of compounds with triterpene backbones, including dammaranes, tirucallanes, lupanes, hopanes, oleananes, taraxasteranes, ursanes, lanostanes, and cucurbitanes (Vincken et al. 2007). OSCs

catalyzing the cyclization of 2,3-oxidosqualene are either specific or multifunctional, leading to either a single product or multiple products from a single reaction (Moses et al. 2014). Following cyclization, triterpene aglycones are oxidized by cytochrome P450 and further modified by an array of transferases such as UDP-dependent glycosyltransferases (UGTs), acyltransferases, malonyltransferases, and methyltransferases (Thimmappa et al. 2014; Seki et al. 2015).

Soyasaponins are triterpenoid saponins commonly found in legume plants. They are composed of aglycone and oligosaccharide moieties. Soyasaponins are subdivided into four groups based on the aglycone structures: soyasaponin group A, group B, and group E (derived from soyasapogenol A, soyasapogenol B, and soyasapogenol E, respectively), and DDMP saponin consisting of soyasapogenol B with a DDMP (2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one) residue at the C-22 position of the aglycone (Figure 2B). Soyasapogenol B is biosynthesized by the hydroxylation of β -amyrin at C-22 and C-24, soyasapogenol A has an additional hydroxylation at C-21, and soyasapogenol E has a carbonyl group at C-22 (Zhang and Popovich 2009; Seki et al. 2015; Krishnamurthy et al. 2019). These soyasapogenols are further diversified by glycosylation catalyzed by UGTs to form the soyasaponin groups A, B, and E. DDMP saponin is biosynthesized from group B saponin, possibly via a UGT encoded by the Sg-9 locus (Sundaramoorthy et al. 2019) (Figure 4).

Cycloartenol synthase (CAS) catalyzes the cyclization of 2,3-oxidosqualene to cycloartenol, which is a precursor for phytosterols including cholesterol, campesterol, and β -sitosterol. Cholesterol is then oxidized and glycosylated to form steroidal saponins (Figure 4). Steroidal glycoalkaloids, typically found in species of *Solanum* as toxic substances, are also biosynthesized from cholesterol as a precursor but incorporate an

amine group at C-26 to generate aglycones such as tomatidine and solanidine (Harrison 1990; Cardenas et al. 2015), which are subsequently glycosylated at the C3 hydroxy group and accumulated as α -tomatine in tomato (*Solanum lycopersicum*) and α -solanine in potato (*Solanum tuberosum*) (Friedman 2006). Genes involved in saponin biosynthesis have been identified from various plant species (Thimmappa et al. 2014; Lee et al. 2019; Jozwiak et al. 2020; Chung et al. 2020) and are often organized as a cluster in the genome (Itkin et al. 2013; Nutzmam et al. 2018) (Akiyama et al. 2021).

Secretion into the rhizosphere

Transporters of saponins for vacuolar accumulation or secretion to the rhizosphere have not been identified so far (Francisco and Martinoia 2018). The transporter responsible for the relocation of α -tomatine from the vacuole to the cytosol has recently been identified in tomato (Kazachkova et al. 2021) (Figure 3). Secretion of soyasaponins from soybean roots and tomatine from tomato roots have been investigated both in hydroponic culture and in field conditions. The secretion of soyasaponins from soybean roots was discovered during a metabolomic analysis of soybean root exudates (Tsuno et al. 2018) as a strong peak on the total ion chromatogram that did not show clear UV absorption typical to aromatic rings. A detailed assessment of m/z values and MS/MS spectra, together with authentic standard specimens, identified this peak as soyasaponin Bb.

Soyasaponins are secreted during growth in hydroponic culture but peak at the early growth stage, as is the case for isoflavones of soybean (Tsuno et al. 2018) (Figure 4).. Soybean roots secrete both group A and group B soyasaponins, but the secretion of DDMP saponins is limited despite the predominant accumulation in soybean roots. The differential composition of soyasaponins between roots and root exudates suggests the

involvement of regulatory mechanisms such as transporters and apoplastic enzymes to be elucidated in future studies. Other legume plants including, but not limited to, *Lotus japonicus*, alfalfa (*Medicago sativa*), and pea (*Pisum sativum*), secrete soyasaponins at varying concentrations and compositions, but DDMP saponins are not detectable from root exudates in these legume species (Tsuno et al. 2018). The contents of the major soyasaponins, soyasaponin Ab and soyasaponin Bb, in root exudates have no apparent diurnal pattern, although the gene expression levels of β -amyrin synthase, cytochrome P450, and UDP-glucuronosyltransferase involved in soyasaponin biosynthesis (Krishnamurthy et al. 2019; Seki et al. 2015) are higher at night (Matsuda et al. 2020). The amount of soyasaponins in the rhizosphere of soybean grown in field is slightly increased during the growth stages, but the composition is stable, with group B soyasaponins representing about 60% of the total soyasaponins, followed by group A, E, and DDMP soyasaponins. Soyasaponin Bb represents up to 70% of group B soyasaponins, and soyasapogenols are limited throughout the growth stages (Fujimatsu et al. 2020).

Tomatine and its aglycone tomatidine were also found in root exudates of tomato (Kirwa et al. 2018). In hydroponically grown tomato, the concentrations of tomatine and tomatidine in root exudates are higher during the early growth stages than the later growth stages, consistent with the secretion of isoflavones and soyasaponins during growth (Nakayasu et al. 2021) (Figure 4). The regulations of tomatine secretion remain elusive, but it is known that it is regulated systemically by the addition of glycosylated azelaic and possibly by soil microbiota (Korenblum et al. 2020). In field-grown tomatoes, rhizosphere tomatine contents are comparable between the flowering and green-fruit stages. Neither tomatine nor tomatidine is detectable in bulk soil, suggesting that tomatine is secreted from field-grown tomato plants and accumulates in the rhizosphere throughout the growth

stages (Nakayasu et al. 2021).

Roles in the rhizosphere

Saponins exert a diverse range of biological properties relevant to rhizosphere interactions including antibacterial, antifungal, and insecticidal activities, in addition to various pharmaceutical effects in humans (Cheok et al. 2014; Vincken et al. 2007; Augustin et al. 2011). The ecological significance of saponins has long been of particular interest for their relevance in crop production. Biological activities against pathogens have been reported; for example, minotosides extracted from *Allium minutiflorum* have antimicrobial activities against various fungal and oomycete pathogens such as *Alternaria alternata*, *Botrytis cinerea*, *Fusarium oxysporum*, *F. solani*, *Pythium ultimum*, and *Rhizoctonia solani* (Barile et al. 2007), alliospirosides extracted from *Allium cepa* have antifungal activities against a range of fungi including *Botrytis cinerea* and *Colletotrichum gloeosporioides* (Teshima et al. 2013); and aescin from *Aesculus hippocastanum* shows antifungal activities against *Microdochium nivale*, *Pyrenophora teres*, and *Leptosphaeria maculans* (Trda et al. 2019). Additionally, steroidal glycoalkaloids such as α -solanine, α -chaconine, and α -tomatine show hatching stimulation activity in potato cyst nematode eggs, albeit weaker than solanoecelepin A, a triterpene secreted from potato roots (Shimizu et al. 2020).

Roles as allelochemicals

Saponins have also long been recognized as allelochemicals (Oleszek W. 1992). Saponins in the roots of alfalfa (*Medicago sativa*) and red clover (*Trifolium pratense*) shows inhibitory effects on seed germination and seedling growth of wheat (*Triticum aestivum*)

(Oleszek and Jurzysta 1987; Oleszek 1993), and medicagenic acid saponins exhibit autotoxic effects and significantly lower the germination rate of alfalfa seeds as well (Ghimire et al. 2019). Ginsenosides from *Panax notoginseng* exhibit autotoxicity and these saponins accumulated in soil are suggested to cause the replant failure of this plant (Yang et al. 2015).

Roles in modulating microbiota

The function of saponins in modulating the rhizosphere microbiota has recently been identified for saponins. An oat *sad1* mutant deficient in avenacin, a triterpenoid saponin, harbors altered rhizosphere microbial communities, compared with the wildtype (Turner et al. 2013). We used tomato as a model system to investigate the role of saponins in shaping the rhizosphere microbiota. Tomatine and tomatidine treatments of field soil *in vitro* resulted in the enrichment of ten and two families, respectively, and the depletion of 35 and 78 families, respectively. The bacterial communities of both tomatine- and tomatidine-treated soils are more similar to those of the tomato rhizosphere than of bulk soil, as shown by a smaller weighted UniFrac distance between PSM-treated soil and the rhizosphere than between PSM-treated soils and bulk soil. Sphingomonadaceae was the only family enriched in both PSM-treated soil and tomato rhizosphere soil. Among Sphingomonadaceae, the genus *Sphingobium* was particularly enriched, and this increase was attributable to one amplicon sequence variant (ASV) among the 15 ASVs observed in these soil samples (Figure 4). Sphingomonadaceae was also enriched in soil treated with soyasaponin Bb (Fujimatsu et al. 2020); operational taxonomic units annotated as *Novosphingobium* were particularly enriched in soyasaponin Bb-treated soil as well as the soybean rhizosphere (Figure 4). It is of particular interest to investigate whether

differences in the carbon skeletons of saponins affect the influence of saponins in rhizosphere bacterial communities. While the effect on bacterial communities is not known, ginsenosides (triterpenoid saponins in Sanqi ginseng) alter fungal communities with the enrichment of potentially pathogenic taxa including *Alternaria* and *Fusarium*, and with the depletion of potentially beneficial taxa such as *Acremonium*, *Mucor*, and *Ochroconis* (Li et al. 2020), which is a potential cause of replanting failure in Sanqi ginseng.

Dynamics of flavonoids and saponins in the rhizosphere

The dynamics and interactions of PSMs and microbes in the rhizosphere are of particular importance for gaining insight into the functions of PSMs. Traditionally, the amount of PSMs secreted from roots has been analyzed using hydroponic cultures because of the ease of applicability compared with field sampling (Oburger and Jones 2018). Although research in our laboratory has revealed that the secretion of daidzein from roots follows a similar pattern during the growth stages (i.e., higher secretion in early growth stages than later growth stages), the amount of daidzein secreted from roots was much higher in the field (Toyofuku et al. 2021), pointing toward the importance of measurement of metabolites in field-grown plants. Along with the differences in the amount and rate of secretion, it is technically challenging to analyze the positional secretion rate using a hydroponic system. In hydroponic culture, the total amount of metabolites secreted from whole roots is usually measured; in reality, the secretion rate varies based on root area (Pearson and Parkinson 1960; McDougall and Rovira 1970; Weisskopf et al. 2005). Recently, analysis using matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) showed a distribution of various metabolites including PSMs in the

rhizosphere (Velickovic et al. 2020). In this study, a polyvinylidene fluoride (PVDF) membrane was placed against the soil-root interface of plants grown in a rhizobox. The PVDF membrane was then mounted on a MALDI target plate for mass imaging. Biosensors are also useful for revealing the distribution of metabolites in the rhizosphere. Fux fusion bioreceptors have been developed in *Rhizobium leguminosarum* to detect specifically for sugars, polyols, amino acids, organic acids, and flavonoids (Pini et al. 2017). Although a bacterial association with the plant root system needs to be validated, these biosensors are potentially applicable for a broader range of bacteria and metabolites to investigate the spatiotemporal distribution of PSMs in the rhizosphere.

Degradation

The amount of secretion and the stability of PSMs need to be analyzed to understand the dynamics of PSMs in the rhizosphere. Once secreted, PSMs are degraded by soil microbes. Despite the rather toxic properties of flavonoids and saponins, these PSMs are also a carbon source for microbes possessing catabolic enzymes. The stability of PSMs in the rhizosphere varies depending on the metabolites and soil microbial communities. In contrast to primary metabolites that are degraded within a few minutes (Gunina and Kuzyakov 2015), flavonoids are rather stable in the soil. The majority of naringenin and formononetin is degraded within 96 hours (Shaw and Hooker 2008), while the half-life of apigenin, kaempferol, and their derivatives ranges from 4.4 to 14.7 days with a seasonal variation (Sosa et al. 2010). The half-life of daidzein is about seven days, measured in soil collected from a soybean farm (Okutani et al. 2020) (Figure 3). The half-life of saponins in the soil is comparable with flavonoids. The allelopathic effects of saponins from alfalfa and red clover last for one to two weeks depending on the soil (Oleszek and

Jurzysta 1987), and most soyasaponin Bb and tomatine in soil are degraded within a week (Fujimatsu et al. 2020) (Nakayasu et al. 2021).

Aerobic flavonoid biodegradation has been reported for a wide range of bacterial species (Shaw et al. 2006). *Nod* gene-inducing daidzein and genistein are also to be degraded by rhizobial species via multiple C-ring fission, although genes involved in this pathway have not been identified (Rao and Cooper 1994, 1995). The metabolic pathways for flavonoid degradation have been characterized in intestinal microbes; daidzein, when consumed as a part of soy products, is converted to a reduction product, equol, and a C-ring cleavage product, *O*-demethylangolensin, by anaerobic intestinal bacteria (Feng et al. 2018). These products have not been identified in rhizosphere soil to our knowledge. Screening of mutants defective in naringenin catabolism in *Herbaspirillum seropedicae* revealed the involvement of a monooxygenase, FdeE, in the cleavage of naringenin, possibly together with FdeD, a putative Rieske protein in fed operon (Marin et al. 2013; Marin et al. 2016). Other proteins encoded by the fde operon include FdeH, a cupin family protein that contains quercetin 2,3-dioxygenase for cleaving quercetin into 2-protocatechuoyl-phloroglucinol carboxylic acid and carbon monoxide, identified in *Bacillus subtilis* (Bowater et al. 2004; Barney et al. 2004) and *Streptomyces* sp. (Merkens et al. 2007). Most of the genes involved in flavonoid catabolism, especially those for isoflavones, have not been identified.

Intestinal and fecal microorganisms capable of metabolizing saponins have also been reported (Hu et al. 2004; Dong et al. 2017; del Hierro et al. 2018). Sapogenins such as oleanolic acid, hederagenin, serjanic acid, diosgenin, and soyasapogenol B accumulate when plant extracts rich in saponins (e.g., quinoa, lentil, and fenugreek) are fermented by gut microbiota (del Hierro et al. 2020). Sapogenins are absorbed by the gastrointestinal

tract, and no gut microorganisms capable of degrading sapogenin have been identified. In contrast, it is not well understood what kind of microorganisms decompose compounds in the soil. Microorganisms capable of degrading tomatine into tomatidine have been reported (Ford et al. 1977; Okmen et al. 2013), and *Sphingobium* spp. isolated from tomatine-treated soil degrade tomatine and tomatidine and use them as a carbon source (Nakayasu et al. 2021). Saponins such as ginsenosides and soyasaponins, are also metabolized by intestinal and fecal microbes (Hu et al. 2004; Dong et al. 2017). Yet, the pathways and genes involved in the degradation of aglycones have not been characterized, particularly for rhizosphere microbes.

Adsorption

In addition to the degradation by soil microbes, adsorption by organic matter and clay minerals reduces the distribution of metabolites in the rhizosphere. We used daidzein as a model to investigate the adsorption to grey lowland soil collected from a soybean farm. Possible adsorption sites are humic substances and clay minerals. Humic substances contain hydroxyl and phenolic hydroxyl groups involved in the formation of complexes with organic substances (Pei Gan and Yau Li 2013). Decomposition of organic matter in grey lowland soil reduced daidzein adsorption, suggesting the involvement of humic substances in limiting the distribution of daidzein in the rhizosphere. In contrast, adsorption of daidzein to clay minerals such as kaolinite, a 1:1-type silicate mineral, is much lower than that to grey lowland soil, and adsorption to illite, a 2:1-type silicate material, is undetectable (Okutani et al. 2020) (Figure 3).

Simulation

The distribution of mineral ions and water in the soil surrounding plant roots has been simulated (Duncan et al. 2018; Zarebanadkouki et al. 2014; Vereecken et al. 2016). For PSMs in rhizosphere soil, the advection-diffusion (dispersion) equation has recently been used to simulate the dynamics of daidzein in the rhizosphere (Okutani et al. 2020). A single root of diameter 2 mm and length 10 cm was set in the center of soil with a diameter of 20 cm and a depth of 20 cm. The root length and diameter were assumed to be constant, and daidzein secretion was assumed to be equal from all parts of the roots. Daidzein distribution was predicted to within a few millimeters of the root surface during the early growth stages (14 days). Although daidzein distribution was shown to be within 2 mm in a rhizobox experiment (Okutani et al. 2020), this simulation is based on constant soil environmental conditions, the absence of root growth, and equal secretion from all parts of the roots. It is necessary to incorporate, at a minimum, the soil water contents (i.e., wet conditions on rainy days and dry conditions on sunny days) and root growth with differing secretion rates of metabolites to precisely simulate the distribution of metabolites in rhizosphere soil in field-grown conditions. Also, simulation based on the secreted amount of daidzein in hydroponic culture underestimates the amount of rhizosphere daidzein. When the daidzein secretion rate measured in field-grown soybean was used for the simulation, the estimated daidzein concentration in the soybean rhizosphere was within the range of that from the rhizosphere in the soybean field (Toyofuku et al. 2021). So far, the adsorption coefficient has been analyzed for flavonoids and saponins in addition to organic xenobiotics and estrogens in soil (Shaw and Hooker 2008; Caron et al. 2010) (Fujimatsu et al. 2020); thus, simulations based on a fluid model can be applied to predict the rhizosphere distribution of PSMs.

Conclusion and future perspectives

Research in the past few decades has identified a vast array of roles of flavonoids and saponins in the rhizosphere, especially for mediating interactions between plants and microbes. Recent advancement of multi-omics analysis revealed a tight network between host plants and microbiota, which opens up a holistic approach toward a comprehensive understanding of plants and microbes. This concept considers the host plant and its microbiota as a unique biological entity called “holobiont,” in which the host and microbiota interact to affect morphology, development, and physiology among others (Rosenberg and Zilber-Rosenberg 2016; Hassani et al. 2018). It remains largely unclear how the metabolic network in the holobiont is established and how it affects plant growth, fitness, and robustness to changing environments. For crop species, the domestication process has affected root microbiota, mediated at least partially by the alteration of root exudates (Iannucci et al. 2017; Escudero-Martinez and Bulgarelli 2019). The metabolic network in the holobiont provides a valuable basis for designing an optimized microbiota to confer robustness against both biotic and abiotic stresses and, eventually, to improve crop yields. Flavonoids and saponins are key metabolites for such an approach since these bioactive compounds mediate the interaction between the plant and the microbiota, and recent evidence has revealed the possibility of fine-tuning the interactions (Fujimatsu et al. 2020; Nakayasu et al. 2021).

The direct application of metabolites is the first step toward this goal. Flavonoids applied to seeds improve the nodulation in several legume crops (Mabood et al. 2014), and saponins applied to seeds improve salinity stress tolerance in quinoa and soybean (Yang et al. 2018; Soliman et al. 2020). There remain possibilities to utilize these PSMs in agriculture directly, but an obstacle is the stability of these metabolites in the soil.

Multiple applications are necessary to exert bioactive properties during a crop season, and the application of PSMs to the rhizosphere is technically unfeasible. Designing a holobiont-metabolic network utilizing both plant breeding and microbial inoculation would be promising to circumvent these problems. Designing rhizosphere microbiomes for crop productions has been proposed in reviews (Mueller and Sachs 2015; de Souza et al. 2020; Pascale et al. 2020), and the holobiont-metabolic network could be a key component.

Metagenomic analysis revealed key bacterial taxa in the microbiome for beneficial traits. By comparing the rhizosphere metagenomes of resistant and susceptible tomato varieties to pathogenic *Ralstonia solanacearum*, it was found that *Flavobacterium* was abundant in the rhizosphere of resistant varieties, and isolated *Flavobacterium* suppressed *R. solanacearum* when inoculated in pots (Kwak et al. 2018). Metagenomics on disease suppressive soil identified a consortium of *Chitinophaga* and *Flavobacterium* for the suppression of the fungal root pathogen *Rhizoctonia solani*, and gene clusters encoding the production of nonribosomal peptide synthetases and polyketide synthases in *Flavobacterium* were found to be essential for disease suppression (Carrion et al. 2019). In contrast to metagenomics, the identities and functions of metabolites in the rhizosphere largely remained unknown because of the instability and limited amounts of metabolites in soil and difficulties with extraction from the rhizosphere. In addition to PSMs, microbial metabolites exert diverse ranges of influence on host plants and co-occurring microbes (Backer et al. 2018; Weisskopf et al.). The rhizosphere metabolome is a prominent approach for uncovering the novel metabolites in the rhizosphere. We recently found okaramine A, B, and C in the rhizosphere of hairy vetch (*Vicia villosa*), a cover crop or a green manure crop (Sakurai et al. 2020). Okaramines were first identified as

insecticides from *okara* inoculated with *P. simplicissimum* AK-40 (Hayashi et al. 1989), but they had not been identified in nature. Okaramine B was also detected in the rhizosphere of soybean grown after hairy vetch but not in soybean without previous hairy vetch cultivation, suggesting an interspecies soilborne legacy or an indirect defense of plants against pests (Sakurai et al. 2020; Matsuda 2018).

Multi-omics analyses integrating metagenomics and rhizosphere metabolomics are prerequisites for designing microbiomes based on holobiont-metabolic networks. The rhizosphere is vital for plant growth and crop production. Unlike the accumulation of microbial genomics, metabolomics has only revealed the tip of the iceberg. It will be of particular importance to design a holobiont metabolic network by combining the identification of new key metabolites, metagenomic analysis, isolation, and functional characterization, and plant breeding to enable plants to grow more robustly (Figure 5).

Acknowledgments

All research in our laboratory was performed with graduate students, post-docs, technicians, and collaborators. I would like to thank all members who participated in this research. I would like to express my sincere gratitude to Dr. Kazufumi Yazaki (professor at Kyoto University), Dr. Jiro Sekiya (emeritus professor at Kyoto University), and Dr. Nobukazu Shitan (professor at Kobe Pharmaceutical University) for continuous guidance and encouragement. I would like to thank Dr. Masaru Nakayasu for the critical reading of the manuscript, and Ms. Keiko Kanai for preparing the illustration.

Funding

This work was supported in part by JST CREST grant JPMJCR17O2, JSPS KAKENHI

grants 18H02313 and 21H02329, and funds from the Research Institute for Sustainable
Humanosphere, Kyoto University.

Disclosure statement

No potential conflict of interest was reported by the author.

Data Availability Statement

The data generated during this study are available from the corresponding author upon
reasonable request.

References

- Afendi FM, Okada T, Yamazaki M et al. KNApSAcK Family Databases: Integrated
Metabolite-Plant Species Databases for Multifaceted Plant Research. *Plant and
Cell Physiology* 2012;**53**:1-12.
- Aguilar JMM, Ashby AM, Richards AJM et al. CHEMOTAXIS OF RHIZOBIUM-
LEGUMINOSARUM BIOVAR PHASEOLI TOWARDS FLAVONOID
INDUCERS OF THE SYMBIOTIC NODULATION GENES. *Journal of General
Microbiology* 1988;**134**:2741-2746.
- Akashi T, Aoki T, Ayabe S Cloning and functional expression of a cytochrome P450
cDNA encoding 2-hydroxyisoflavanone synthase involved in biosynthesis of the
isoflavonoid skeleton in licorice. *Plant Physiology* 1999;**121**:821-828.
- Akashi T, Aoki T, Ayabe S Molecular and biochemical characterization of 2-
hydroxyisoflavanone dehydratase. Involvement of carboxylesterase-like proteins
in leguminous isoflavone biosynthesis. *Plant Physiology* 2005;**137**:882-891.
- Akiyama R, Watanabe B, Nakayasu M et al. The biosynthetic pathway of potato
solanidanes diverged from that of spirosolanones due to evolution of a dioxygenase.
Nat Commun 2021;**12**:1300.
- Augustin JM, Kuzina V, Andersen SB et al. Molecular activities, biosynthesis and
evolution of triterpenoid saponins. *Phytochemistry* 2011;**72**:435-457.
- Backer R, Rokem JS, Ilangumaran G et al. Plant Growth-Promoting Rhizobacteria:
Context, Mechanisms of Action, and Roadmap to Commercialization of

646 Biostimulants for Sustainable Agriculture. *Frontiers in Plant Science* 2018;**9**:17.
 647 Badri DV, Vivanco JM Regulation and function of root exudates. *Plant Cell and*
 648 *Environment* 2009;**32**:666-681.
 649 Barbour WM, Hattermann DR, Stacey G CHEMOTAXIS OF BRADYRHIZOBIUM-
 650 JAPONICUM TO SOYBEAN EXUDATES. *Applied and Environmental*
 651 *Microbiology* 1991;**57**:2635-2639.
 652 Barile E, Bonanomi G, Antignani V et al. Saponins from *Allium minutiflorum* with
 653 antifungal activity. *Phytochemistry* 2007;**68**:596-603.
 654 Barney BM, Schaab MR, LoBrutto R et al. Evidence for a new metal in a known active
 655 site: purification and characterization of an iron-containing quercetin 2,3-
 656 dioxygenase from *Bacillus subtilis*. *Protein Expression and Purification*
 657 2004;**35**:131-141.
 658 Behrens CE, Smith KE, Iancu CV et al. Transport of Anthocyanins and other Flavonoids
 659 by the Arabidopsis ATP-Binding Cassette Transporter AtABCC2. *Scientific*
 660 *Reports* 2019;**9**:
 661 Biala W, Banasiak J, Jarzyniak K et al. *Medicago truncatula* ABCG10 is a transporter of
 662 4-coumarate and liquiritigenin in the medicarpin biosynthetic pathway. *Journal of*
 663 *Experimental Botany* 2017;**68**:3231-3241.
 664 Bowater L, Fairhurst SA, Just VJ et al. *Bacillus subtilis* YxaG is a novel Fe-containing
 665 quercetin 2,3-dioxygenase. *Febs Letters* 2004;**557**:45-48.
 666 Buhian WP, Bensmihen S Mini-Review: Nod Factor Regulation of Phytohormone
 667 Signaling and Homeostasis During Rhizobia-Legume Symbiosis. *Frontiers in*
 668 *Plant Science* 2018;**9**:
 669 Caetanoanollés G, Cristóbal DK, Bauer WD CHEMOTAXIS OF RHIZOBIUM-
 670 MELILOTI TO THE PLANT FLAVONE LUTEOLIN REQUIRES
 671 FUNCTIONAL NODULATION GENES. *Journal of Bacteriology*
 672 1988;**170**:3164-3169.
 673 Canarini A, Kaiser C, Merchant A et al. Root Exudation of Primary Metabolites:
 674 Mechanisms and Their Roles in Plant Responses to Environmental Stimuli.
 675 *Frontiers in Plant Science* 2019;**10**:
 676 Canto CD, Simonin M, King E et al. An extended root phenotype: the rhizosphere, its
 677 formation and impacts on plant fitness. *Plant Journal* 2020;**103**:951-964.
 678 Cardenas PD, Sonawane PD, Heinig U et al. The bitter side of the nightshades: Genomics
 679 drives discovery in Solanaceae steroidal alkaloid metabolism. *Phytochemistry*
 680 2015;**113**:24-32.
 681 Caron E, Fahrenhorst A, Zvomuya F et al. Sorption of four estrogens by surface soils from

- 41 cultivated fields in Alberta, Canada. *Geoderma* 2010;**155**:19-30.
- Carrion VJ, Perez-Jaramillo J, Cordovez V et al. Pathogen-induced activation of disease-suppressive functions in the endophytic root microbiome. *Science* 2019;**366**:606-685 +.
- Cesco S, Mimmo T, Tonon G et al. Plant-borne flavonoids released into the rhizosphere: impact on soil bio-activities related to plant nutrition. A review. *Biology and Fertility of Soils* 2012;**48**:123-149.
- Cheok CY, Salman HAK, Sulaiman R Extraction and quantification of saponins: A review. *Food Research International* 2014;**59**:16-40.
- Chung SY, Seki H, Fujisawa Y et al. A cellulose synthase-derived enzyme catalyses 3-O-glucuronosylation in saponin biosynthesis. *Nature Communications* 2020;**11**:
- Compant S, Samad A, Faist H et al. A review on the plant microbiome: Ecology, functions, and emerging trends in microbial application. *Journal of Advanced Research* 2019;**19**:29-37.
- Compton KK, Hildreth SB, Helm RF et al. An Updated Perspective on Sinorhizobium meliloti Chemotaxis to Alfalfa Flavonoids. *Frontiers in Microbiology* 2020;**11**:
- de Campos SB, Deakin WJ, Broughton WJ et al. Roles of flavonoids and the transcriptional regulator TtsI in the activation of the type III secretion system of Bradyrhizobium elkanii SEMIA587. *Microbiology-Sgm* 2011;**157**:627-635.
- de Souza RSC, Armanhi JSL, Arruda P From Microbiome to Traits: Designing Synthetic Microbial Communities for Improved Crop Resiliency. *Frontiers in Plant Science* 2020;**11**:
- del Hierro JN, Cueva C, Tamargo A et al. In Vitro Colonic Fermentation of Saponin-Rich Extracts from Quinoa, Lentil, and Fenugreek. Effect on Saponins Yield and Human Gut Microbiota. *Journal of Agricultural and Food Chemistry* 2020;**68**:106-116.
- del Hierro JN, Herrera T, Fornari T et al. The gastrointestinal behavior of saponins and its significance for their bioavailability and bioactivities. *Journal of Functional Foods* 2018;**40**:484-497.
- Dharmatilake AJ, Bauer WD CHEMOTAXIS OF RHIZOBIUM-MELILOTI TOWARDS NODULATION GENE-INDUCING COMPOUNDS FROM ALFALFA ROOTS. *Applied and Environmental Microbiology* 1992;**58**:1153-1158.
- Dong WW, Xuan FL, Zhong FL et al. Comparative Analysis of the Rats' Gut Microbiota Composition in Animals with Different Ginsenosides Metabolizing Activity. *Journal of Agricultural and Food Chemistry* 2017;**65**:327-337.
- Duncan SJ, Daly KR, Sweeney P et al. Mathematical modelling of water and solute

718 movement in ridged versus flat planting systems. *Eur J Soil Sci* 2018;**69**:967-979.

719 Escudero-Martinez C, Bulgarelli D Tracing the evolutionary routes of plant-microbiota
720 interactions. *Curr Opin Microbiol* 2019;**49**:34-40.

721 Feng X, Li Y, Brobbey Oppong M et al. Insights into the intestinal bacterial metabolism
722 of flavonoids and the bioactivities of their microbe-derived ring cleavage
723 metabolites. *Drug Metab Rev* 2018;**50**:343-356.

724 Fernie AR The future of metabolic phytochemistry: Larger numbers of metabolites,
725 higher resolution, greater understanding. *Phytochemistry* 2007;**68**:2861-2880.

726 Ferreyra MLF, Rius SP, Casati P Flavonoids: biosynthesis, biological functions, and
727 biotechnological applications. *Frontiers in Plant Science* 2012;**3**:

728 Finkel OM, Castrillo G, Paredes SH et al. Understanding and exploiting plant beneficial
729 microbes. *Curr Opin Plant Biol* 2017;**38**:155-163.

730 Ford JE, McCance DJ, Drysdale RB DETOXIFICATION OF ALPHA-TOMATINE BY
731 FUSARIUM-OXYSPORUM F SP LYCOPERSICI. *Phytochemistry*
732 1977;**16**:545-546.

733 Francisco RD, Martinoia E The Vacuolar Transportome of Plant Specialized Metabolites.
734 *Plant and Cell Physiology* 2018;**59**:1326-1336.

735 Friedman M Potato glycoalkaloids and metabolites: Roles in the plant and in the diet.
736 *Journal of Agricultural and Food Chemistry* 2006;**54**:8655-8681.

737 Fujimatsu T, Endo K, Yazaki K et al. Secretion dynamics of soyasaponins in soybean
738 roots and effects to modify the bacterial composition. *Plant Direct* 2020;**4**:

739 Gagnon H, Ibrahim RK Effects of various elicitors on the accumulation and secretion of
740 isoflavonoids in white lupin. *Phytochemistry* 1997;**44**:1463-1467.

741 Ghimire BK, Ghimire B, Yu CY et al. Allelopathic and Autotoxic Effects of *Medicago*
742 *sativa*-Derived Allelochemicals. *Plants-Basel* 2019;**8**:

743 Gorniak I, Bartoszewski R, Kroliczewski J Comprehensive review of antimicrobial
744 activities of plant flavonoids. *Phytochemistry Reviews* 2019;**18**:241-272.

745 Gourion B, Berrabah F, Ratet P et al. Rhizobium-legume symbioses: the crucial role of
746 plant immunity. *Trends in Plant Science* 2015;**20**:186-194.

747 Gunina A, Kuzyakov Y Sugars in soil and sweets for microorganisms: Review of origin,
748 content, composition and fate. *Soil Biology & Biochemistry* 2015;**90**:87-100.

749 Guo ZY, Kong CH, Wang JG et al. Rhizosphere isoflavones (daidzein and genistein)
750 levels and their relation to the microbial community structure of mono-cropped
751 soybean soil in field and controlled conditions. *Soil Biology & Biochemistry*
752 2011;**43**:2257-2264.

753 Haichar FE, Heulin T, Guyonnet JP et al. Stable isotope probing of carbon flow in the

754 plant holobiont. *Current Opinion in Biotechnology* 2016;**41**:9-13.

755 Han F, He XQ, Chen WW et al. Involvement of a Novel TetR-Like Regulator (BdtR) of
756 Bradyrhizobium diazoefficiens in the Efflux of Isoflavonoid Genistein. *Molecular*
757 *Plant-Microbe Interactions* 2020;**33**:1411-1423.

758 Harris JM, Balint-Kurti P, Bede JC et al. What are the Top 10 Unanswered Questions in
759 Molecular Plant-Microbe Interactions? *Molecular Plant-Microbe Interactions*
760 2020;**33**:1354-1365.

761 Harrison DM STEROIDAL ALKALOIDS. *Natural Product Reports* 1990;**7**:139-147.

762 Hartmann A, Rothballer M, Schmid M Lorenz Hiltner, a pioneer in rhizosphere microbial
763 ecology and soil bacteriology research. *Plant and Soil* 2008;**312**:7-14.

764 Hassan S, Mathesius U The role of flavonoids in root-rhizosphere signalling:
765 opportunities and challenges for improving plant-microbe interactions. *J Exp Bot*
766 2012;**63**:3429-3444.

767 Hassani MA, Duran P, Hacquard S Microbial interactions within the plant holobiont.
768 *Microbiome* 2018;**6**:17.

769 Hayashi H, Takiuchi K, Murao S et al. STRUCTURE AND INSECTICIDAL ACTIVITY
770 OF NEW INDOLE ALKALOIDS, OKARAMINE-A AND OKARAMIN-B
771 FROM PENICILLIUM-SIMPLICISSIMUM AK-40. *Agricultural and Biological*
772 *Chemistry* 1989;**53**:461-469.

773 Hu J, Zheng YL, Hyde W et al. Human fecal metabolism of soyasaponin I. *Journal of*
774 *Agricultural and Food Chemistry* 2004;**52**:2689-2696.

775 Iannucci A, Fragasso M, Beleggia R et al. Evolution of the Crop Rhizosphere: Impact of
776 Domestication on Root Exudates in Tetraploid Wheat (Triticum turgidum L.).
777 *Frontiers in Plant Science* 2017;**8**:

778 Itkin M, Heinig U, Tzfadia O et al. Biosynthesis of Antinutritional Alkaloids in
779 Solanaceous Crops Is Mediated by Clustered Genes. *Science* 2013;**341**:175-179.

780 Jacoby RP, Koprivova A, Kopriva S Pinpointing secondary metabolites that shape the
781 composition and function of the plant microbiome. *J Exp Bot* 2021;**72**:57-69.

782 Janczarek M, Rachwal K, Marzec A et al. Signal molecules and cell-surface components
783 involved in early stages of the legume-rhizobium interactions. *Applied Soil*
784 *Ecology* 2015;**85**:94-113.

785 Jozwiak A, Sonawane PD, Panda S et al. Plant terpenoid metabolism co-opts a component
786 of the cell wall biosynthesis machinery. *Nature Chemical Biology* 2020;**16**:740-+.

787 Jung W, Yu O, Lau SMC et al. Identification and expression of isoflavone synthase, the
788 key enzyme for biosynthesis of isoflavones in legumes. *Nature Biotechnology*
789 2000;**18**:208-212.

790 Kape R, Parniske M, Werner D CHEMOTAXIS AND NOD GENE ACTIVITY OF
 791 BRADYRHIZOBIUM-JAPONICUM IN RESPONSE TO
 792 HYDROXYCINNAMIC ACIDS AND ISOFLAVONOIDS. *Applied and*
 793 *Environmental Microbiology* 1991;**57**:316-319.

794 Kazachkova Y, Zemach I, Panda S et al. The GORKY glycoalkaloid transporter is
 795 indispensable for preventing tomato bitterness. *Nature Plants* 2021;
 796 Kim J, Buell CR A Revolution in Plant Metabolism: Genome-Enabled Pathway Discovery.
 797 *Plant Physiology* 2015;**169**:1532-1539.

798 Kirwa HK, Murungi LK, Beck JJ et al. Elicitation of Differential Responses in the Root-
 799 Knot Nematode *Meloidogyne incognita* to Tomato Root Exudate Cytokinin,
 800 Flavonoids, and Alkaloids. *Journal of Agricultural and Food Chemistry*
 801 2018;**66**:11291-11300.

802 Korenblum E, Dong YH, Szymanski J et al. Rhizosphere microbiome mediates systemic
 803 root metabolite exudation by root-to-root signaling. *Proceedings of the National*
 804 *Academy of Sciences of the United States of America* 2020;**117**:3874-3883.

805 Kossalak RM, Bookland R, Barkei J et al. INDUCTION OF BRADYRHIZOBIUM-
 806 JAPONICUM COMMON NOD GENES BY ISOFLAVONES ISOLATED
 807 FROM GLYCINE-MAX. *Proceedings of the National Academy of Sciences of the*
 808 *United States of America* 1987;**84**:7428-7432.

809 Krishnamurthy P, Fujisawa Y, Takahashi Y et al. High-Throughput Screening and
 810 Characterization of a High-Density Soybean Mutant Library Elucidate the
 811 Biosynthesis Pathway of Triterpenoid Saponins. *Plant and Cell Physiology*
 812 2019;**60**:1082-1097.

813 Krishnan HB, Lorio J, Kim WS et al. Extracellular proteins involved in soybean cultivar-
 814 specific nodulation are associated with pilus-like surface appendages and
 815 exported by a type III protein secretion system in *Sinorhizobium fredii* USDA257.
 816 *Molecular Plant-Microbe Interactions* 2003;**16**:617-625.

817 Kwak MJ, Kong HG, Choi K et al. Rhizosphere microbiome structure alters to enable wilt
 818 resistance in tomato. *Nature Biotechnology* 2018;**36**:1100-+.

819 Latunde-Dada AO, Cabello-Hurtado F, Czittrich N et al. Flavonoid 6-hydroxylase from
 820 soybean (*Glycine max* L.), a novel plant P-450 monooxygenase. *Journal of*
 821 *Biological Chemistry* 2001;**276**:1688-1695.

822 Lee HJ, Nakayasu M, Akiyama R et al. Identification of a 3 beta-Hydroxysteroid
 823 Dehydrogenase/3-Ketosteroid Reductase Involved in alpha-Tomatine
 824 Biosynthesis in Tomato. *Plant and Cell Physiology* 2019;**60**:1304-1315.

825 Li Y, Dai S, Wang B et al. Autotoxic Ginsenoside Disrupts Soil Fungal Microbiomes by

826 Stimulating Potentially Pathogenic Microbes. *Appl Environ Microbiol* 2020;**86**:
827 Lichman BR, Godden GT, Buell CR Gene and genome duplications in the evolution of
828 chemodiversity: perspectives from studies of Lamiaceae. *Curr Opin Plant Biol*
829 2020;**55**:74-83.

830 Liu CW, Murray JD The Role of Flavonoids in Nodulation Host-Range Specificity: An
831 Update. *Plants-Basel* 2016;**5**:
832 Lozovaya VV, Lygin AV, Zernova OV et al. Isoflavonoid accumulation in soybean hairy
833 roots upon treatment with *Fusarium solani*. *Plant Physiology and Biochemistry*
834 2004;**42**:671-679.

835 Lynch JM, Whipps JM SUBSTRATE FLOW IN THE RHIZOSPHERE. *Plant and Soil*
836 1990;**129**:1-10.

837 Mabood F, Zhou XM, Smith DL Microbial signaling and plant growth promotion.
838 *Canadian Journal of Plant Science* 2014;**94**:1051-1063.

839 Marin AM, de la Torre J, Oliveira ARM et al. Genetic and functional characterization of
840 a novel meta-pathway for degradation of naringenin in *Herbaspirillum*
841 seropedicae SmR1. *Environmental Microbiology* 2016;**18**:4653-4661.

842 Marin AM, Souza EM, Pedrosa FO et al. Naringenin degradation by the endophytic
843 diazotroph *Herbaspirillum seropedicae* SmR1. *Microbiology-Sgm* 2013;**159**:167-
844 175.

845 Matsuda H, Nakayasu M, Aoki Y et al. Diurnal metabolic regulation of isoflavones and
846 soyasaponins in soybean roots. *Plant Direct* 2020;**4**:
847 Matsuda K Okaramines and other plant fungal products as new insecticide leads. *Current*
848 *Opinion in Insect Science* 2018;**30**:67-72.

849 McDougall BM, Rovira AD SITES OF EXUDATION OF C-14-LABELLED
850 COMPOUNDS FROM WHEAT ROOTS. *New Phytologist* 1970;**69**:999-+.

851 Merkens H, Sielker S, Rose K et al. A new monocupin quercetinase of *Streptomyces* sp
852 FLA: identification and heterologous expression of the *queD* gene and activity of
853 the recombinant enzyme towards different flavonols. *Arch Microbiol*
854 2007;**187**:475-487.

855 Mierziak J, Kostyn K, Kulma A Flavonoids as Important Molecules of Plant Interactions
856 with the Environment. *Molecules* 2014;**19**:16240-16265.

857 Moses T, Papadopoulou KK, Osbourn A Metabolic and functional diversity of saponins,
858 biosynthetic intermediates and semi-synthetic derivatives. *Critical Reviews in*
859 *Biochemistry and Molecular Biology* 2014;**49**:439-462.

860 Mueller UG, Sachs JL Engineering Microbiomes to Improve Plant and Animal Health.
861 *Trends in Microbiology* 2015;**23**:606-617.

862 Nakayasu M, Ohno K, Takamatsu K et al. Tomato roots secrete tomatine to modulate the
 863 bacterial assemblage of the rhizosphere. *Plant Physiol* 2021;
 864 Nutzmam HW, Scazzocchio C, Osbourn A (2018) Metabolic Gene Clusters in Eukaryotes.
 865 In: Bonini NM (ed) Annual Review of Genetics, Vol 52, vol 52. Annual Review
 866 of Genetics. Annual Reviews, Palo Alto, pp 159-183. doi:10.1146/annurev-genet-
 867 120417-031237
 868 Oburger E, Jones DL Sampling root exudates - Mission impossible? *Rhizosphere*
 869 2018;**6**:116-133.
 870 Okmen B, Etalo DW, Joosten M et al. Detoxification of -tomatine by *Cladosporium*
 871 *fulvum* is required for full virulence on tomato. *New Phytologist* 2013;**198**:1203-
 872 1214.
 873 Okutani F, Hamamoto S, Aoki Y et al. Rhizosphere modelling reveals spatiotemporal
 874 distribution of daidzein shaping soybean rhizosphere bacterial community. *Plant*
 875 *Cell and Environment* 2020;**43**:1036-1046.
 876 Oleszek W ALLELOPATHIC POTENTIALS OF ALFALFA (MEDICAGO-SATIVA)
 877 SAPONINS - THEIR RELATION TO ANTIFUNGAL AND HEMOLYTIC
 878 ACTIVITIES. *Journal of Chemical Ecology* 1993;**19**:1063-1074.
 879 Oleszek W, Jurzysta M THE ALLELOPATHIC POTENTIAL OF ALFALFA ROOT
 880 MEDICAGENIC ACID GLYCOSIDES AND THEIR FATE IN SOIL
 881 ENVIRONMENTS. *Plant and Soil* 1987;**98**:67-80.
 882 Oleszek W. JM, Górski P.M. (1992) Alfalfa saponins — the allelopathic agents.
 883 Allelopathy. Springer. doi:https://doi.org/10.1007/978-94-011-2376-1_11
 884 Panche AN, Diwan AD, Chandra SR Flavonoids: an overview. *Journal of Nutritional*
 885 *Science* 2016;**5**:
 886 Pang Z, Chen J, Wang T et al. Linking Plant Secondary Metabolites and Plant
 887 Microbiomes: A Review. *Frontiers in Plant Science* 2021;**12**:
 888 Pascale A, Proietti S, Pantelides IS et al. Modulation of the Root Microbiome by Plant
 889 Molecules: The Basis for Targeted Disease Suppression and Plant Growth
 890 Promotion. *Frontiers in Plant Science* 2020;**10**:
 891 Pearson R, Parkinson D The sites of excretion of ninhydrin-positive substances by broad
 892 bean seedlings. *Plant and Soil* 1960;**13**:391-396.
 893 Pei Gan P, Yau Li SF (2013) CHAPTER 4 Biosorption of Elements. In: Element
 894 Recovery and Sustainability. The Royal Society of Chemistry, pp 80-113.
 895 doi:10.1039/9781849737340-00080
 896 Peters NK, Frost JW, Long SR A PLANT FLAVONE, LUTEOLIN, INDUCES
 897 EXPRESSION OF RHIZOBIUM-MELILOTI NODULATION GENES. *Science*

1986;**233**:977-980.

Pietta PG Flavonoids as antioxidants. *Journal of Natural Products* 2000;**63**:1035-1042.

Pini F, East AK, Appia-Ayme C et al. Bacterial Biosensors for in Vivo Spatiotemporal Mapping of Root Secretion. *Plant Physiology* 2017;**174**:1289-1306.

Pueppke SG, Bolanos-Vasquez MC, Werner D et al. Release of flavonoids by the soybean cultivars McCall and Peking and their perception as signals by the nitrogen-fixing symbiont *Sinorhizobium fredii*. *Plant Physiology* 1998;**117**:599-608.

Rao JR, Cooper JE RHIZOBIA CATABOLIZE NOD GENE-INDUCING FLAVONOIDS VIA C-RING FISSION MECHANISMS. *Journal of Bacteriology* 1994;**176**:5409-5413.

Rao JR, Cooper JE Soybean nodulating rhizobia modify nod gene inducers daidzein and genistein to yield aromatic products that can influence gene-inducing activity. *Molecular Plant-Microbe Interactions* 1995;**8**:855-862.

Redmond JW, Batley M, Djordjevic MA et al. FLAVONES INDUCE EXPRESSION OF NODULATION GENES IN RHIZOBIUM. *Nature* 1986;**323**:632-635.

Rosenberg E, Zilber-Rosenberg I Microbes Drive Evolution of Animals and Plants: the Hologenome Concept. *Mbio* 2016;**7**:

Roy S, Liu W, Nandety RS et al. Celebrating 20 Years of Genetic Discoveries in Legume Nodulation and Symbiotic Nitrogen Fixation(OPEN). *Plant Cell* 2020;**32**:15-41.

Sakurai N, Mardani-Korrani H, Nakayasu M et al. Metabolome Analysis Identified Okaramines in the Soybean Rhizosphere as a Legacy of Hairy Vetch. *Frontiers in Genetics* 2020;**11**:

Sasse J, Kosina SM, de Raad M et al. Root morphology and exudate availability are shaped by particle size and chemistry in *Brachypodium distachyon*. *Plant Direct* 2020;**4**:

Sasse J, Martinoia E, Northen T Feed Your Friends: Do Plant Exudates Shape the Root Microbiome? *Trends in Plant Science* 2018;**23**:25-41.

Schmidt PE, Parniske M, Werner D PRODUCTION OF THE PHYTOALEXIN GLYCEOLLIN-I BY SOYBEAN ROOTS IN RESPONSE TO SYMBIOTIC AND PATHOGENIC INFECTION. *Botanica Acta* 1992;**105**:18-25.

Seki H, Tamura K, Muranaka T P450s and UGTs: Key Players in the Structural Diversity of Triterpenoid Saponins. *Plant Cell Physiol* 2015;**56**:1463-1471.

Shaw LJ, Hooker JE The fate and toxicity of the flavonoids naringenin and formononetin in soil. *Soil Biology & Biochemistry* 2008;**40**:528-536.

Shaw LJ, Morris P, Hooker JE Perception and modification of plant flavonoid signals by rhizosphere microorganisms. *Environmental Microbiology* 2006;**8**:1867-1880.

934 Shimizu K, Kushida A, Akiyama R et al. Hatching stimulation activity of steroidal
935 glycoalkaloids toward the potato cyst nematode, *Globodera rostochiensis*. *Plant*
936 *Biotechnology* 2020;**37**:319-325.

937 Shitan N, Yazaki K Dynamism of vacuoles toward survival strategy in plants. *Biochim*
938 *Biophys Acta-Biomembr* 2020;**1862**:10.

939 Soliman MH, Abdulmajeed AM, Alhaithloul H et al. Saponin biopriming positively
940 stimulates antioxidants defense, osmolytes metabolism and ionic status to confer
941 salt stress tolerance in soybean. *Acta Physiol Plant* 2020;**42**:13.

942 Sosa T, Valares C, Alias JC et al. Persistence of flavonoids in *Cistus ladanifer* soils. *Plant*
943 *and Soil* 2010;**337**:51-63.

944 Sparg SG, Light ME, van Staden J Biological activities and distribution of plant saponins.
945 *Journal of Ethnopharmacology* 2004;**94**:219-243.

946 Sugiyama A The soybean rhizosphere: Metabolites, microbes, and beyond-A review.
947 *Journal of Advanced Research* 2019;**19**:67-73.

948 Sugiyama A, Shitan N, Yazaki K Involvement of a soybean ATP-binding cassette - Type
949 transporter in the secretion of genistein, a signal flavonoid in legume-Rhizobium
950 Symbiosis(1). *Plant Physiology* 2007;**144**:2000-2008.

951 Sugiyama A, Yamazaki Y, Hamamoto S et al. Synthesis and Secretion of Isoflavones by
952 Field-Grown Soybean. *Plant and Cell Physiology* 2017;**58**:1594-1600.

953 Sugiyama A, Yamazaki Y, Yamashita K et al. Developmental and nutritional regulation of
954 isoflavone secretion from soybean roots. *Bioscience Biotechnology and*
955 *Biochemistry* 2016;**80**:89-94.

956 Sugiyama A, Yazaki K Flavonoids in plant rhizospheres: secretion, fate and their effects
957 on biological communication. *Plant Biotechnology* 2014;**31**:431-443.

958 Sundaramoorthy J, Park GT, Komagamine K et al. Biosynthesis of DDMP saponins in
959 soybean is regulated by a distinct UDP-glycosyltransferase. *New Phytologist*
960 2019;**222**:261-274.

961 Suzuki H, Takahashi S, Watanabe R et al. An isoflavone conjugate-hydrolyzing beta-
962 glucosidase from the roots of soybean (*Glycine max*) seedlings - Purification,
963 gene cloning, phylogenetics, and cellular localization. *Journal of Biological*
964 *Chemistry* 2006;**281**:30251-30259.

965 Szoboszlay M, White-Monsant A, Moe LA The Effect of Root Exudate 7,4 '-
966 Dihydroxyflavone and Naringenin on Soil Bacterial Community Structure. *Plos*
967 *One* 2016;**11**:

968 Takeshima K, Hidaka T, Wei M et al. Involvement of a Novel Genistein-Inducible
969 Multidrug Efflux Pump of *Bradyrhizobium japonicum* Early in the Interaction

970 with Glycine max (L.) Merr. *Microbes and Environments* 2013;**28**:414-421.

971 Teshima Y, Ikeda T, Imada K et al. Identification and Biological Activity of Antifungal
 972 Saponins from Shallot (*Allium cepa* L. Aggregatum Group). *Journal of*
 973 *Agricultural and Food Chemistry* 2013;**61**:7440-7445.

974 Thimmappa R, Geisler K, Louveau T et al. Triterpene biosynthesis in plants. *Annu Rev*
 975 *Plant Biol* 2014;**65**:225-257.

976 Toyofuku M, Okutani F, Nakayasu M et al. Enhancement of developmentally regulated
 977 daidzein secretion from soybean roots in field conditions as compared with
 978 hydroponic culture. *Biosci Biotechnol Biochem* 2021;

979 Trda L, Janda M, Mackova D et al. Dual Mode of the Saponin Aescin in Plant Protection:
 980 Antifungal Agent and Plant Defense Elicitor. *Frontiers in Plant Science* 2019;**10**:

981 Tsuno Y, Fujimatsu T, Endo K et al. Soyasaponins: A New Class of Root Exudates in
 982 Soybean (*Glycine max*). *Plant and Cell Physiology* 2018;**59**:366-375.

983 Turner TR, Ramakrishnan K, Walshaw J et al. Comparative metatranscriptomics reveals
 984 kingdom level changes in the rhizosphere microbiome of plants. *Isme Journal*
 985 2013;**7**:2248-2258.

986 Uchida K, Sawada Y, Ochiai K et al. Identification of a Unique Type of Isoflavone O-
 987 Methyltransferase, GmIOMT1, Based on Multi-Omics Analysis of Soybean under
 988 Biotic Stress. *Plant Cell Physiol* 2020;**61**:1974-1985.

989 Velickovic D, Lin VS, Rivas A et al. An approach for broad molecular imaging of the
 990 root-soil interface via indirect matrix-assisted laser desorption/ionization mass
 991 spectrometry. *Soil Biology & Biochemistry* 2020;**146**:

992 Vereecken H, Schnepf A, Hopmans JW et al. Modeling Soil Processes: Review, Key
 993 Challenges, and New Perspectives. *Vadose Zone J* 2016;**15**:57.

994 Vieira S, Sikorski J, Dietz S et al. Drivers of the composition of active rhizosphere
 995 bacterial communities in temperate grasslands. *Isme Journal* 2020;**14**:463-475.

996 Vincken JP, Heng L, de Groot A et al. Saponins, classification and occurrence in the plant
 997 kingdom. *Phytochemistry* 2007;**68**:275-297.

998 Wang HW, Tang MJ, Su CL et al. The Alleopathic Compound Luteolin from Peanut Litter
 999 Affects Peanut Nodule Formation and the Rhizosphere Microbial Community.
 1000 *Agronomy Journal* 2018;**110**:2587-2595.

1001 Wang XL, Wang MX, Xie XG et al. An amplification-selection model for quantified
 1002 rhizosphere microbiota assembly. *Science Bulletin* 2020;**65**:983-986.

1003 Webb BA, Compton KK, Saldana RC et al. Sinorhizobium meliloti chemotaxis to
 1004 quaternary ammonium compounds is mediated by the chemoreceptor McpX.
 1005 *Molecular Microbiology* 2017;**103**:333-346.

- Webb BA, Hildreth S, Helm RF et al. Sinorhizobium meliloti Chemoreceptor McpU Mediates Chemotaxis toward Host Plant Exudates through Direct Proline Sensing. *Applied and Environmental Microbiology* 2014;**80**:3404-3415.
- Weinstein LI, Albersheim P Host-Pathogen Interactions : XXIII. The Mechanism of the Antibacterial Action of Glycinol, a Pterocarpan Phytoalexin Synthesized by Soybeans. *Plant Physiol* 1983;**72**:557-563.
- Weisskopf L, Fromin N, Tomasi N et al. Secretion activity of white lupin's cluster roots influences bacterial abundance, function and community structure. *Plant and Soil* 2005;**268**:181-194.
- Weisskopf L, Schulz S, Garbeva P Microbial volatile organic compounds in intra-kingdom and inter-kingdom interactions. *Nat Rev Microbiol* 14.
- Weston LA, Mathesius U Flavonoids: Their Structure, Biosynthesis and Role in the Rhizosphere, Including Allelopathy. *Journal of Chemical Ecology* 2013;**39**:283-297.
- White LJ, Ge XJ, Brozel VS et al. Root isoflavonoids and hairy root transformation influence key bacterial taxa in the soybean rhizosphere. *Environmental Microbiology* 2017;**19**:1391-1406.
- Wu SC, Yang ZQ, Liu F et al. Antibacterial Effect and Mode of Action of Flavonoids From Licorice Against Methicillin-Resistant Staphylococcus aureus. *Frontiers in Microbiology* 2019;**10**:
- Wu T, Zang XX, He MY et al. Structure-Activity Relationship of Flavonoids on Their Anti-Escherichia coli Activity and Inhibition of DNA Gyrase. *Journal of Agricultural and Food Chemistry* 2013;**61**:8185-8190.
- Yang A, Akhtar SS, Iqbal S et al. Saponin seed priming improves salt tolerance in quinoa. *Journal of Agronomy and Crop Science* 2018;**204**:31-39.
- Yang M, Zhang XD, Xu YG et al. Autotoxic Ginsenosides in the Rhizosphere Contribute to the Replant Failure of Panax notoginseng. *Plos One* 2015;**10**:
- Yonekura-Sakakibara K, Higashi Y, Nakabayashi R The Origin and Evolution of Plant Flavonoid Metabolism. *Frontiers in Plant Science* 2019;**10**:
- Yu P, He X, Baer M et al. Plant flavones enrich rhizosphere Oxalobacteraceae to improve maize performance under nitrogen deprivation. *Nature Plants* 2021;**7**:481-499.
- Zarebanadkouki M, Kroener E, Kaestner A et al. Visualization of Root Water Uptake: Quantification of Deuterated Water Transport in Roots Using Neutron Radiography and Numerical Modeling. *Plant Physiology* 2014;**166**:487-499.
- Zhang RF, Vivanco JM, Shen QR The unseen rhizosphere root-soil-microbe interactions for crop production. *Curr Opin Microbiol* 2017;**37**:8-14.

Zhang W, Popovich DG Chemical and Biological Characterization of Oleanane
Triterpenoids from Soy. *Molecules* 2009;**14**:2959-2975.
Zhao J Flavonoid transport mechanisms: how to go, and with whom. *Trends in Plant
Science* 2015;**20**:576-585.
Zhao J, Huhman D, Shadle G et al. MATE2 Mediates Vacuolar Sequestration of
Flavonoid Glycosides and Glycoside Malonates in *Medicago truncatula*. *Plant
Cell* 2011;**23**:1536-1555.

Graphical Abstract Caption

Flavonoids and saponins are secreted into rhizospheres. These plant specialized
metabolites exert various functions in mediating interactions with microbiota.

Figure legends

Figure 1. Soybean rhizosphere. Isoflavones and soyasaponins are secreted into the
rhizosphere (①) and affect the bacterial communities (②).

Figure 2. Chemical structures of isoflavones in soybean

Figure 3. Secretion and fate of daidzein in soybean rhizosphere. (1) Sugiyama et al. (2007),
(2) Suzuki et al. (2006), (3) Sugiyama et al. (2006), (4) Toyofuku et al. (2021), (5) Matsuda
et al. (2020), (6) Sugiyama et al. (2017), (7) Okutani et al. (2020).

Figure 4. Secretion of soyasaponin from soybean roots and tomatine from tomato roots:
cycloartenol synthase (CAS), β -amyrin synthase (BAS), UDP-sugar dependent
glycosyltransferase (UGT), arabinose (Ara), galactose (Gal), glucose (Glc), glucuronic
acid (GlcA), rhamnose (Rha), xylose (Xyl). CAS and BAS are members of OSC family.

1068 Bold arrows represent multiple reactions.

1069

1070 Figure 5. Integration of multi-omics to design holobiont metabolic networks that promote
1071 plant growth and suppress pathogens.

1072