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Review Article

Recent progress in the chemistry and biochemistry of strigolactones

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Strigolactones (SLs) are plant secondary metabolites derived from carotenoids. SLs play important roles in the regulation of plant growth and development *in planta* and coordinate interactions between plants and other organisms including root parasitic plants, and symbiotic and pathogenic microbes in the rhizosphere. In the 50 years since the discovery of the first SL, strigol, our knowledge about the chemistry and biochemistry of SLs has advanced explosively, especially over the last two decades. In this review, recent advances in the chemistry and biology of SLs are summarized and possible future outcomes are discussed.

Keywords: arbuscular mycorrhizal fungi, germination stimulant, plant hormone, root parasitic weed, strigolactone.

Introduction

Strigolactones (SLs) are plant secondary metabolites derived from carotenoids. SLs play important roles in the regulation of plant growth and development through crosstalk with other plant hormones *in planta* and in the chemical communications with beneficial and detrimental soil microorganisms and root parasitic plants (weeds) in the rhizosphere.[1,2] In the 50 years since the discovery of the first SL, strigol (**1**),[3] our knowledge of the chemistry and biochemistry of SLs has advanced explosively, especially during the last two decades. For example, only five natural SLs, strigol, strigyl acetate (**2**),[3] sorgolactone (**5**),[4] alectrol[5] (orobanchyl acetate, **4**),[6] and orobanchol (**3**)[7] were known by the end of the last century, and now more than 30 natural SLs have been identified.[8,9] In addition, the biosynthetic pathway of SLs from carotenoids, the SL receptors in plants and root parasitic weeds, but not in microorganisms including arbuscular mycorrhizal (AM) fungi, and the SL signal transduction system in plants have mostly been clarified.[2] In this review, recent advances in the chemistry and biochemistry of SLs are summarized and possible future outcomes are discussed. To minimize overlaps in explanations and discussions with those in recently published reviews, I will omit some aspects of SL chemistry and biochemistry that have been discussed extensively. Please refer to reviews[2,10–13] and books.[14,15]

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1. Chemistry of SLs

Root parasitic weeds of the Orobanchaceae family, witchweeds (*Striga* spp.) and broomrapes (*Orobanche* and *Phelipanche* spp.), cause devastating damage to agricultural production all over the world.[16] The seeds of these root parasites germinate only when they perceive chemicals called germination stimulants produced by and released from host roots.[1,2,17] Among the germination stimulants, SLs are the most potent and widely distributed chemicals in the plant kingdom.[1,2] Strigol (**1**), the first identified SL, was isolated from cotton (*Gossypium hirsutum*) root exudates as a germination stimulant of *S. lutea* (*S. asiatica*).[3,18] The term "strigolactone" was then coined by Butler for the *Striga* germination stimulants structurally related to strigol[19] which contains the ABC-ring, the core, connected to the methyl-butenolide D ring moiety *via* an enol-ether bridge. These typical SLs are called "canonical SLs" (Fig. 1).[2] SLs include another group of compounds called "non-canonical SLs" with a more structurally diverse core and the common enol-ether–D ring moiety (Fig. 2).[2,9,20] Although non-canonical SLs have been characterized only in the last 10 years, most SLs characterized recently have been non-canonical. It is expected that the number of non-canonical SLs will soon exceed that of canonical SLs as the former allow more structural diversity; any compounds showing SL-like activity shall be called non-canonical SLs if they contain the enol-ether–D ring moiety which has been repeatedly proposed to be essential for SL activity.[21] Synthetic SL agonists that lack an enol-ether but contain the D ring have been developed,[22,23] indicating that only the D ring is essential for SL activity. The stereochemistry at the asymmetric carbon, C2′ in the canonical SLs, is an *R*-configuration in all natural SLs so far characterized (Fig. 1).

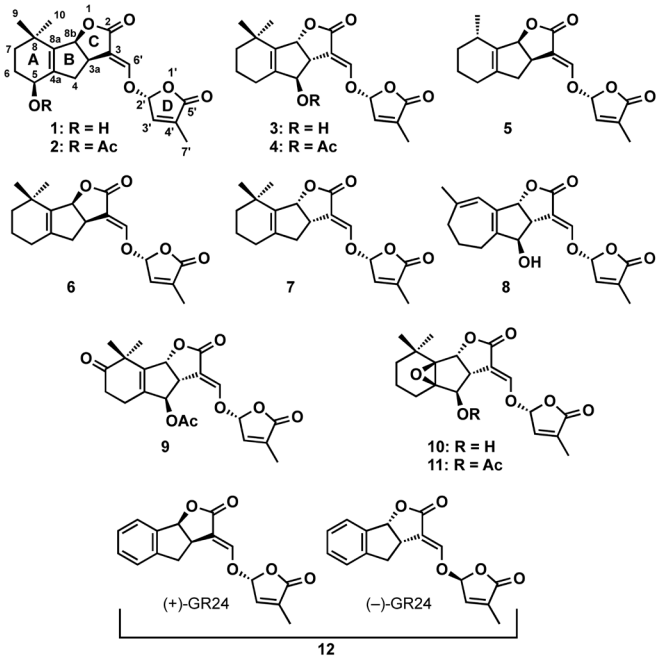

Fig. 1. Structures of canonical strigolactones and *rac*-GR24.

1.1. Canonical SLs

Until the discovery of carlactone (CL, **13**) as a precursor for SLs,[24] all known natural SLs contained the ABC-ring connected to the D ring. All of them, except for medicaol (**8**), which has a seven-membered A ring,[25] contain a fused 6-5-5 membered ring as the ABC-ring system. They have no, one, two, or three hydroxyl or acetyloxyl groups on the A and/or B ring. These canonical SLs can be divided into two groups depending on the C ring orientations.[26] Strigol- and orobanchol-type SLs contain a β-oriented and an α-oriented C ring, respectively. Some plant species like cotton and rice (*Oryza sativa*) apparently produce only strigol- and orobanchol-type SLs, respectively, whereas others like tobacco (*Nicotiana tabacum*) produce both types.[8,9] It might be that all plant species have the ability to produce both types at very different levels and thus only one type of SL is present at detectable levels in the root exudates.

1.2. Non-canonicals SLs

As mentioned above, non-canonical SLs lack A, B, or C rings but have the enol-ether–D ring moiety. The first reported non-canonical SL was CL (**13**), which was proposed as a precursor for SLs from *in vitro* experiments[24] and later confirmed as an endogenous compound which is converted to SLs *via* carlactonoic acid (CLA, **14**) by the cytochrome P450 MORE AXILLARY GROWTH 1 (MAX1) oxidation at C19 *in planta*.[27,28] Most of the non-canonical SLs identified as germination stimulants for root parasitic weeds are C_{20} compounds whereas the core structure of canonical SLs is a C_{19} compound, indicating that these non-canonical SLs derive from methyl carlactonoate (MeCLA, **15**), the first C_{20} compound in the SL biosynthetic pathway.[28] Non-canonical SLs isolated as germination stimulants of root parasitic weeds are avenaol (**16**),[29] heliolactone (**17**),[30] zealactone (**18**)[31] (methyl zealactonoate),[32] pyranozealactone (**19**),[33] and lotuslactone (**20**).[34] In addition to these, several SL-like compounds, very likely non-canonical SLs, have been detected from root exudates of rice, tall goldenrod (*Solidago altissima*),[9] hemp (*Cannabis sativa*) (J.-B. Pouvreau, unpublished results), and other plant species. Some of these compounds appear to be difficult to purify due to their instability (see below) and the presence of hardly separable impurities and/or their isomers.

1.3. Stability of SLs

In general, non-canonical SLs are less stable than canonical SLs, and this is one reason why non-canonical SLs were only recently characterized. For example, CL decomposes rapidly when concentrated to dryness and thus should be kept in a solution of inert organic solvents.[9] A similar instability was observed with other non-canonical SLs; however, canonical SLs can be stored safely without organic solvents.

Even canonical SLs are chemically unstable and decompose rapidly in the soil.[35–38] For example, the half-life of 5-deoxystrigol (5DS, **6**), a relatively stable canonical SL, in water was 1.5 days.[39] The half-life would be much shorter under alkaline conditions. Such instability of SLs allows obligate biotrophs, including AM fungi and root parasitic weeds, to use SLs as a reliable signal of living host roots nearby. However, SLs would remain longer in the slightly acidic rhizosphere than would be expected in bulk soil,[40] because they are more stable under weakly acidic conditions. In addition, various organic compounds that are mostly exuded from plant roots protect SLs from rapid degradation.[40] It should be noted that, in most cases, SL degradation experiments have been conducted at relatively high concentrations (>mM) far above the water solubility of SLs, and SL degradation is often monitored by a high performance liquid chromatograph equipped with UV-visible or photodiode array detector by which detection of SLs at concentrations lower than μM is rather difficult.[41] In contrast, biologically active concentrations of SLs in the rhizosphere would be lower than μM; therefore, it is im-

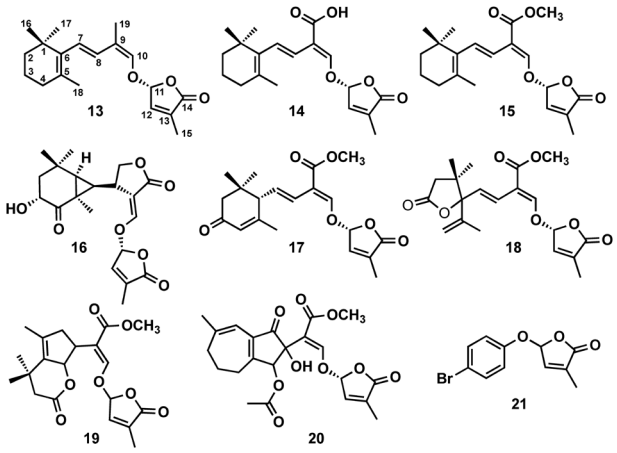

Fig. 2. Structures of non-canonical strigolactones and debranone.

portant to conduct SL degradation experiments at these biological concentrations. For example, typical concentrations of SLs in the culture media of plants grown hydroponically under phosphate deficiency are at nM levels and no significant degradation of SLs in the media was detected when the growth media was left at room temperature overnight after removal of the plants (K. Yoneyama, unpublished).

2. SL biosynthesis

2.1. Biosynthetic pathway of SLs

SLs are plant secondary metabolites derived from carotenoids and produced by all higher plant species including root parasitic weeds.[42] Although the biosynthetic pathway of SLs has not been fully clarified, most of the enzymes involved have been identified.[2] The first enzyme, carotenoid isomerase DWARF27 (D27), converts all-*trans*-*β*-carotene to 9-*cis*-*β*-carotene, which is then cleaved by CAROTENOID CLEAVAGE DIOXYGENASE 7 (CCD7). CCD7 cleaves not only 9-*cis*-*β*-carotene but also other 9-*cis*-carotenoids, and CCD8 subsequently catalyzes further cleavage and cyclization of the CCD7 reaction products leading to CL and hydroxy-CLs (HO-CLs).[43] The oxidation of CL by the cytochrome P450 MAX1 (CYP711A) affords CLA in thale cress (*Arabidopsis thaliana*)[28] and 4-deoxyorobanchol (4DO, **7**) and orobanchol in rice.[44] These reactions were confirmed with recombinant enzymes *in vitro*. One of the rice MAX1 homologs, Os900 (carlactone oxygenase), converts CL to 4DO, which is then hydroxylated to orobanchol by another homolog Os1400 (orobanchol synthase).[44] The HO-CLs are also converted to HO-CLAs by MAX1 homologs.[43] Characterization of enzymatic functions of MAX1 homologs from rice, maize (*Zea mays*), tomato (*Solanum lycopersicum*), poplar (*Populus trichocarpa)*, and the lycophyte spike moss (*Selaginella moellendorffii*), classified these homologs into three types: A-1 type (CYP711A1) converting CL to CLA, A-2 type (CYP711A2) converting CL to 4DO *via* CLA, and A-3 type (CYP711A3) catalyzing the oxidation of CL to CLA and also 4DO to orobanchol.[45] However, in some plant species like cowpea (*Vigna unguiculata*) and moonseed (*Menispermum dauricum*), hydroxy-SLs, orobanchol and strigol, respectively, are not formed from the corresponding deoxy-SLs, 4DO and 5DS.[46] Recently, another clade of cytochrome P450 CYP722C functioning downstream of MAX1 was shown to directly convert CLA to orobanchol, presumably *via* 18-HO-CLA, although formation of the B/C ring structure with the recombinant enzyme was not stereoselective.[47] In birdsfoot trefoil (*Lotus japonicus*), 18-HO-CLA has been detected as an endogenous compound[48] and also in other plant species (K. Yoneyama, unpublished data). These results clearly indicate that the biosynthesis from *β*-carotene to CLA is a common pathway for SLs but further steps after formation of CLA may differ with each plant species and thus with each SL. However, as discussed earlier, HO-CLs seem to be derived from the corresponding HO-carotenoids as in the case of 3-HO-CL, and modifications of the A ring may occur before formation of the CL structure. This allows further diversifications of biosynthesis and chemistry of SLs. In *Arabidopsis* and probably also in other plant species, LATERAL BRANCHING OXIDOREDUCTASE (LBO) seems to function downstream of MAX1.[49] LBO has been shown to produce an unknown oxygenated compound (MeCLA+16 Da) from MeCLA. This LBO product was determined to be hydroxymethyl carlactonoate (1′-HO-MeCLA) and detected as an endogenous compound in *Arabidopsis*.[50] MAX1 homologs from rice and spike moss convert CL to 4DO *in vitro*, and the *in vitro* conversion of CLA to 5DS by a CYP722C from cotton (*Gossypium arboretum*) has recently been achieved.[51]

To date, enzymes involved in the biosynthesis of the non-canonical SLs—avenanol, heliolactone, zealactone, pyranozealactone, and lotuslactone—have not been identified although they appear to be derived from MeCLA or its derivatives as they are C_{20} compounds. Among plant species producing these SLs, wild oat (*Avena strigosa*), sunflower (*Helianthus annuus*), maize, and *L. japonicus*, only *L. japonicus* produces canonical SL, 5DS,[52] and the others do not produce detectable levels of known canonical SLs. Therefore, *L. japonicus* is a good model plant to identify enzymes involved in the biosynthesis of the canonical SL, 5DS, and the non-canonical SL, lotuslactone.[34] Since MAX1 homologs of these non-canonical SL-producing plant species expressed in yeast catalyze only the conversion of CL into CLA,[45] additional enzymes should function in SL biosynthesis at least in these plant species. Recently, a cytochrome P450 (CYP722C) and a 2-oxoglutarate-dependent dioxygenase were shown to be involved in the biosynthesis of 5DS and lotuslactone, respectively, in *L. japonicus*.[53]

2.2. Regulation of SL biosynthesis

2.2.1. Mineral nutrients

Nutrient availability has been shown to profoundly affect biosynthesis and exudation of SLs. Such an effect is related to the nutrient acquisition strategy of plants. Under phosphate deficiency, in general, plants increase SL production and exudation, allocate more resources to roots, and promote AM symbiosis to enhance phosphate uptake.[52,54,55] Although nitrogen deficiency little affects SL production in leguminous plants such as red clover (*Trifolium pratense*) that form symbiotic relationships with root nodule bacteria, non-leguminous mycotrophic plants like sorghum (*Sorghum bicolor*) increase SL production under nitrogen deficiency as well as phosphate deficiency,[56] indicating that these species depend on AM fungi for the supply of both phosphate and nitrogen. Upregulation of expression of SL biosynthesis genes under phosphate starvation has been observed in rice,[57] petunia (*Petunia hybrida*),[58] and barrel medic (*Medicago truncatula*).[59] A similar upregulation of SL biosynthesis genes was also observed under nitrogen deficiency in rice plants.[57] In addition, in rice plants, sulfur deficiency increased SL levels *via* upregulation of *D27*, while the expression of other SL biosynthesis genes *D10*, *D17*, and *OsMAX1* was unaffected.[60]

2.2.2. Effects of other hormones

Auxin is a potent, positive regulator of SL biosynthesis.[61–64] Auxin upregulates SL biosynthesis genes and promotes SL production. By contrast, decapitation (*i.e.*, removal of the auxin-

producing apical bud) promotes axillary bud outgrowth due to a strong downregulation of SL biosynthesis genes. Auxin also promotes SL biosynthesis indirectly *via* reduction of cytokinin (CK) biosynthesis,[64,65] because CKs antagonize SLs.[66,67] The SLs appear to reduce CK levels by promoting metabolism.[68] Although both CKs and SLs are mainly synthesized in roots and move upward to shoots, CKs promote shoot branching[66] and delay leaf senescence,[69] but SLs inhibit shoot branching[70,71] and promote leaf senescence.[72–74] Since biosynthesis of CKs and SLs in roots is responsive to soil nitrate[75–77] and phosphate availability, respectively, plants may use CK and SL levels together as information on soil nutrient status.

Gibberellins (GAs) and SLs have similarities, especially in their signal transduction systems. The receptors of GAs and SLs, GIBBERELLIN INSENSITIVE DWARF 1 (GID1)[78] and DWARF14 (D14),[79] respectively, belong to the superfamily of α/β-hydrolases, and degradation of suppressors (DELLA for GAs and DWARF53 (D53)/SUPPRESSOR OF MAX2 1-LIKE (SMXLs) for SLs) occurs in response to ligand binding mediated by the SKIP1-CULLIN-F-BOX (SCF) ubiquitin ligase complex. Details of SL perception and signal transduction will be discussed in the next section. Although GAs have been proposed to negatively regulate SL biosynthesis,[80] GAs and SLs seem to regulate separate processes[81] and function independently in the stem elongation.[82,83] It has been reported that GA treatment during seed conditioning of root parasitic weeds greatly enhances germination responsiveness to SLs and SL agonists,[84,85] probably due to reduced SL biosynthesis in the conditioned parasite seeds[42] thus enhancing their sensitivity to exogenous SLs.

Abscisic acid (ABA) is another plant hormone derived from carotenoids.[86] Since mutants impaired in ABA biosynthesis produce lower levels of SLs, ABA has been suggested to regulate SL biosynthesis.[87,88] Additionally, treatment of *P. ramosa* seeds with the synthetic SL GR24 (**12**) induces the expression of *PrCYP707A1*, encoding an ABA-catabolizing enzyme, and reduces the ABA level.[89] Therefore, some direct and indirect interactions between ABA and SLs seem to occur, but no clear evidence has been reported so far. Other plant hormones, brassinosteroids,[90] jasmonic acid,[87] salicylic acid, ethylene,[74,91] and peptide hormones,[92] may also interact with SLs, but further studies are needed to draw a clear conclusion.

3. Perception and signal transduction of SLs

3.1. Receptors

Structure–activity relationship (SAR) studies of SLs in germination stimulation of root parasitic weed seeds,[93] hyphal branching of AM fungi,[39] and inhibition of shoot branching,[94] have demonstrated that structural requirements of SLs in these biological activities are generally similar but not identical, suggesting that receptors and signal transduction systems are also somewhat similar. In other words, an active SL would bind to receptors in plants, root parasitic weeds, and AM fungi and other microbes with different affinities. In fact, all the natural SLs, except for 5DS, the first identified hyphal branching factor of AM fungi, have been isolated as germination stimulants for root parasitic weeds and are active as AM fungi hyphal branching factors and as inhibitors of shoot branching.

Among the SL receptors, D14 was the first one identified as involved in inhibition of shoot branching.[79] As expected, *d14* mutants accumulate SLs and display a hyper branching (tillering) phenotype which is not rescued by application of SLs. Since all plant species have a single D14 homolog, each SL binds to the D14 homolog in different plant species with a similar affinity. However, as mentioned earlier, different plant species or even different cultivars of the same species produce mixtures of different SLs. This implies that shoot branching inhibiting hormones (BIHs) are not the SLs so far identified. In addition, non-canonical SLs with unique structures like avenaol may not be BIHs as they seem to be species-specific. Structure- and stereo-specific transport of SLs from roots to shoots also supports this possibility,[95] because root-applied strigol, which was not transported from roots to shoots in rice plants, strongly inhibited tillering,[71] suggesting that metabolites of SLs or other signaling compounds downstream of SLs are the true BIHs. Recently, PLEIOTROPIC DRUG RESISTANCE 1 (PDR1), an ABC-type transporter in petunia, which participates in the cell to cell transport of SLs,[96] was shown to be responsible for short-distance transport but only partially for long-distance transport of SLs.[97] These results together suggest that BIHs are substrates of PDR1 and that PDR1 is involved in short-distance transport of BIHs and regulating shoot branching. The possibility that BIHs are not canonical SLs is further supported by the observation that targeted mutation in *SlCYP722C*, a gene responsible for the conversion of CLA to orobanchol in tomato, resulted in a significant reduction in germination stimulation activity of root exudates due to blockage of orobanchol biosynthesis but did not show the apparent phenotypes of the SL-deficient mutant *Slccd8*.[47]

When the root parasitic plants evolved around 40–60 million years ago,[98,99] SLs already existed in the rhizosphere as the host signals for AM fungi[100] with which land plants established symbiotic relationships 460 million years ago.[100–103] It is likely that SLs evolved 750–1200 million years ago.[13,104] The root parasitic plants have developed very sensitive detection systems of SLs with paralogs of D14 called D14-like (D14L), KARRIKIN INSENSITIVE2 (KAI2), or HYPOSENSITIVE TO LIGHT (HTL).[105,106] Although the corresponding protein KAI2 in non-parasitic plants such as *Arabidopsis* has a binding domain that can accommodate karrikins (KARs) but not SLs, those in root parasites are large enough for SLs to bind. KARs are butenolide compounds produced by combustion of plant materials and elicit seed germination of fire-prone plants.[107] For example, *S. hermonthica* has 11 paralogs, with ShHTL7 being most sensitive to 5DS.[106] It is likely that during evolution of parasitism, diversifications of KAI2/HTL genes provided these parasites with highly specific and sensitive detection systems of SLs. These KAI2 or HTL homologs seem to have different affinities to SLs and therefore, at least several of them are involved in sensing different SLs during seed germination stimulation.[106]

Table 1. Germination stimulation of *Orobanche minor* and *O. cumana* seeds by strigolactones and their mixtures

Broomrape	Germination (%)				
	Strigol	Orobanchol	7-Oxoorobanchyl acetate	Strigol+orobanchol	Orobanchol+7-oxoorobanchyl acetate
O. minor	92	88	83	91	80
O. cumana	19	64	94	24	0

Conditioned seeds were treated with strigolactone and strigolactone mixture (100 nM) (M. Fernández-Aparicio, unpublished data).

There may be additive, synergistic, and/or antagonistic effects among SLs in the root exudate on parasite seed germination stimulation. In a preliminary experiment, as shown in Table 1, the seeds of clover broomrape (*O. minor*), a generalist, responded almost equally to strigol, orobanchol, and 7-oxoorobanchyl acetate (**9**), and the mixtures of two of them. Therefore, in the seed germination stimulation of *O. minor*, only additive effects were observed between the two SLs. By contrast, the seeds of sunflower broomrape (*O. cumana*), which attacks exclusively sunflower, were less sensitive to strigol than orobanchol and most sensitive to 7-oxoorobanchyl acetate among the SLs tested. The equimolar mixture of strigol and orobanchol induced germination at a level similar to that induced by strigol alone, indicating that strigol may antagonize orobanchol in *O. cumana* germination stimulation. A clear antagonistic effect was observed between orobanchol and 7-oxoorobanchyl acetate; although they were active when applied individually, the mixture was totally inactive in *O. cumana* seed germination (M. Fernández-Aparicio, unpublished data). Similar results were obtained when seeds of various root parasitic weeds were treated with root exudates of their host and non-host plants. The seeds of weedy broomrape species with a wide host range including *O. minor*, *P. aegyptiaca*, and *P. ramosa*, germinated well when treated with root exudates of garden pea (*Pisum sativum*), *L. japonicus*, fava bean (*Vicia faba*), cowpea, maize, and sunflower. By contrast, *O. cumana* seeds hardly responded to the root exudates of these fabaceous plants which produce orobanchol (5DS by *L. japonicus*) as a major SL. Maize root exudates, containing zealactone and pyranozealactone, did not elicit *O. cumana* seed germination, either.[108] These results suggest that effects of SL mixtures on seed germination need to be examined to understand the host recognition system of root parasitic weeds, because not only quantitative but also qualitative differences of SLs in root exudates contribute to the seed germination. The composition of SL mixtures may also affect host recognition of AM fungi and other soil microbes, although SL receptors in microbes remain elusive.

3.2. Perception and signal transduction

In the absence of SL, the transcriptional repressors D53/SMXLs together with the corepressors TOPLESS (TPL)/TPL-RELATED (TPRs) proteins repress the expression of SL-responsive genes through the interaction with SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) family transcription factors.[109–111] Upon binding of SL, D14 interacts with an F-box protein (MAX2 in *Arabidopsis* and D3 in rice) from an SCF ubiquitin ligase complex that targets the transcriptional repressors D53/SMXLs for polyubiquitination and proteasomal degradation, resulting in the activation of SL-responsive genes.

The SL receptor D14 is an α/β-hydrolase capable of binding and hydrolyzing SLs.[110,112] In the previously proposed model, D14 binds SL and hydrolyzes it into the ABC-CHO and the HO-D ring. Although the former is released, the latter covalently binds to D14, termed a covalently linked intermediate molecule (CLIM), and triggers conformational change to form a D14–$SCF^{D3/MAX2}$ complex.[110,113] In this model, perception of SL depends on the degradation of SL. However, recent findings demonstrated that specific binding of SL to D14 is enough to induce conformational change of D14 to interact with other components from the SL signaling pathway.[114–116] This model can explain why compounds like debranones (**21**), which lack the site of hydrolysis, are active SL agonists. Hydrolytic degradation of SLs by D14 may help maintain homeostatic level of SLs.[116] Further studies are needed to clarify when and how SL degradation occurs after signal perception.[117]

4. Biological functions of SLs

SLs were originally identified as germination stimulants for root parasitic weeds and then as hyphal branching factors for AM fungi. In addition to their roles in the rhizosphere, SLs are involved in the regulation of shoot and root architecture, secondary growth, seed germination, leaf senescence, and drought and salinity tolerance. Here, I will omit well-documented subjects and focus on those less discussed.

4.1. Seed germination stimulation of root parasitic weeds

SLs are potent germination stimulants for seeds of root parasitic weeds, and most natural SLs have been characterized as germination stimulants because the parasite seed germination test is highly sensitive and selective to SLs. There are several protocols for seed germination tests and a standardized method has been proposed.[118] The seeds of root parasites require "conditioning" prior to exposure to germination stimulants. For conditioning, the seeds are kept in a warm moist environment in the dark for 1–2 weeks during which the seeds become responsive to germination stimulants. We noticed some differences in germination stimulation activities of SLs among different assay protocols. For example, in our germination test, sample solutions containing 0.1% acetone (v/v) are added directly to the conditioned seeds. In this assay, hydroxy-SLs appear to exhibit stronger activities than those obtained in the proposed standardized assay. Therefore, orobanchol (it was listed as 2′-*epi*-orobanchol) was most active against *O. minor* seeds.[93] Furthermore, root exudates from *Striga-*

susceptible and -resistant sorghum cultivars induced similar levels of *S. hermonthica* seed germination.[119] By contrast, root exudates of *Striga*-resistant sorghum cultivars that produced orobanchol, a poor germination stimulant for *S. hermonthica*, induced only low germination using the standardized assay method.[120]

As previously mentioned, plants produce not a single SL but a mixture of SLs. For example, garden pea produces at least five canonical SLs: 4DO, orobanchol, orobanchyl acetate, fabacol (**10**), and fabacyl acetate (**11**). Time-course characteristics of SL production by hydroponically-grown pea seedlings may clarify which SL or SLs among these five canonical SLs are important for host recognition of root parasitic weeds which generally establish parasitism 2–3 weeks after germination of host plants. Of course, as discussed earlier, the profile of the SL mixture may also be involved in host recognition by the root parasites and the time-course characteristics of SL production may totally differ under field conditions.

4.2. Promotion of AM colonization

The most ancient and important function of SLs in the rhizosphere appears to be promotion of AM colonization. Although SL biosynthesis mutants show reduced levels of AM colonization,[70,121] *d14* mutants impaired in SL perception form normal AM colonization,[122] indicating an original role of SLs as a host-rhizosphere recognition signal for AM fungi.[13] Detailed phylogenetic analysis of SMXL proteins has also suggested that SLs first evolved as rhizosphere signals.[104] Rice *d3* and *d14l* mutants were not colonized due to impaired hyphopodium formation,[123] indicating possible involvement of KAR signals in AM colonization. However, exogenous KARs showed no effects on AM colonization. An uncharacterized natural substrate for D14L (KAI2), KAR ligand (KL), may participate in the communications between AM fungi and their host plants.[123]

SLs and their mixtures along with other metabolites in plant root exudates may affect the microbiome in the rhizosphere.[124] For example, higher diversities were detected in fungal but not bacterial communities in the rhizosphere of wild-type compared to *max4* mutants of *Arabidopsis*, a non-mycotrophic species.[125] In the case of mycotrophic plant maize, there were no distinct differences in AM fungal communities between the *Striga*-susceptible and -resistant cultivars examined.[126] Although a SAR study of SLs on the hyphal branching in the AM fungus *Gigaspora margarita* was reported,[39] other AM fungal species may respond differently to individual SLs and mixtures of SLs. To understand effects of SL mixtures on AM colonization of a crop, it is necessary to identify all SLs in the root exudates of the crop and AM fungal species in the rhizosphere. In addition to the positive effect of SLs on AM symbiosis, SLs have been shown to promote symbiosis with root nodule bacteria.[127,128] Furthermore, SLs have been suggested to promote root-knot nematode infection by reducing jasmonic acid levels in rice plants.[129]

4.3. Regulation of drought and salinity response by SLs

SLs promote AM symbiosis, which improves water and nutrient uptake by host plants, and therefore SLs indirectly alleviate drought stress and nutrient deficiencies. Recently, SLs were shown to participate directly in adaptive responses to abiotic stresses such as drought and salinity.[130–132] In fact, SL-deficient and signaling mutants are hypersensitive to drought and salinity, and exogenous SL rescues the phenotype of the SL-deficient mutants but not that of the signaling mutants.[130] These mutants show higher stomatal conductance, presumably due to decreased sensitivity to ABA or impaired transport of ABA.[133] These results suggest that SLs and ABA may together regulate stomatal development and function to mitigate drought stress. Exogenous *rac*-GR24 induces stomatal closure in *Arabidopsis*, tomato, and fava bean,[130,134,135] but not in *d14* and *kai2* mutants of *Arabidopsis*, indicating that both SLs and KL are involved in the response to drought at least in *Arabidopsis*.[136]

In addition to abiotic stresses, SLs have been suggested to enhance resistance to biotic stresses. For example, an SL-deficient *ccd8* mutant line of tomato was more susceptible to airborne pathogenic fungi *Botrytis cinerea* and *Alternaria alternata*.[87] However, *ccd8* mutants of pea and wild-type plants were equally susceptible to the soilborne pathogen *Fusarium oxysporum*.[137] Therefore, effects of SLs may vary with the pathosystem examined.[138] Additionally, SLs indirectly enhance plant resistance to pathogens by promoting AM symbiosis, which induces pathogen resistance.[139]

Conclusions and perspectives

SLs are derived from carotenoids, which are rich sources of biologically active compounds. For example, two biologically active apocarotenoids, zaxinone[140] and anchorene,[141] were recently identified, but biologically active apocarotenoids remain to be characterized.[142,143] It is an intriguing challenge to examine molecular evolution of SLs; ancient SLs may have evolved non-enzymatically from carotenoids.

So far, most biological activities of SLs described in the literature have been obtained using a mixture of four stereoisomers or a racemic mixture of the synthetic SL, GR24, although we now know that these stereoisomers may exhibit different activities.[144] There may be no, additive, synergistic, or antagonistic interactions among stereoisomers. Therefore, some biological data may be artifacts and should be interpreted carefully. For example, most of the effects on *Arabidopsis* root development attributed to SL signaling have been shown to be mediated by the KAI2 signaling pathway.[145] To decipher biological functions of SLs, it is preferable to use natural SLs or optically pure isomers. For this, SL-producing cell cultures or a cell-free system need to be established. As previously mentioned, it is also important to examine biological activities of SL mixtures.

Extensive studies have been conducted to obtain more active and more stable SL agonists. In addition, SL antagonists and SL biosynthetic inhibitors targeting different enzymes have been developed.[146] These compounds can be used to regulate biosynthesis, perception, and functions of SLs for regulating plant growth and development, promoting AM symbiosis, enhancing resistance

to abiotic and biotic stresses, and managing root parasitic weeds.

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