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Strigolactones, how are they synthesized to regulate plant growth and development?

Kaori Yoneyama[1,2] and Philip B Brewer[3]

Abstract

Strigolactones (SLs) are multifunctional plant metabolites working not only as allelochemicals in the rhizosphere, but also as a novel class of hormones regulating growth and development *in planta*. To date, more than 30 SLs have been characterized, but the reason why plants produce structurally diverse SLs and the details of their biosynthetic pathway remain elusive. Recent studies using transcriptomics and reverse genetic techniques have paved the way to clarify the entire biosynthetic pathway of structurally diverse SLs. In this review, we discuss how various SLs are synthesized and what SL structural diversity means for plant growth and development.

Addresses

[1] Graduate School of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama, 790-8566, Japan

[2] PRESTO, JST, Japan

[3] ARC Centre of Excellence in Plant Energy Biology, Waite Research Institute, School of Agriculture, Food and Wine, The University of Adelaide, Glen Osmond SA 5064, Australia

Corresponding authors: Yoneyama, Kaori (yoneyama.kaori.wx@ehime-u.ac.jp); Brewer, Philip B (philip.brewer@adelaide.edu.au)

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Introduction

It is now widely recognized that strigolactones (SLs) are a novel class of phytohormones that regulate broad aspects of plant growth and development, with shoot branching inhibition being the first observed hormonal effect [1,2]. SLs also act as signaling molecules exuded from roots into the rhizosphere to promote symbiosis with arbuscular mycorrhizal fungi (AMF); SLs induce hyphal branching, and this morphological change is observed only in the vicinity of host roots [3]. On the other hand, root parasitic weeds are devastating agricultural pests that make use of SLs as cues to find host roots; their seeds germinate only when they perceive SLs [4].

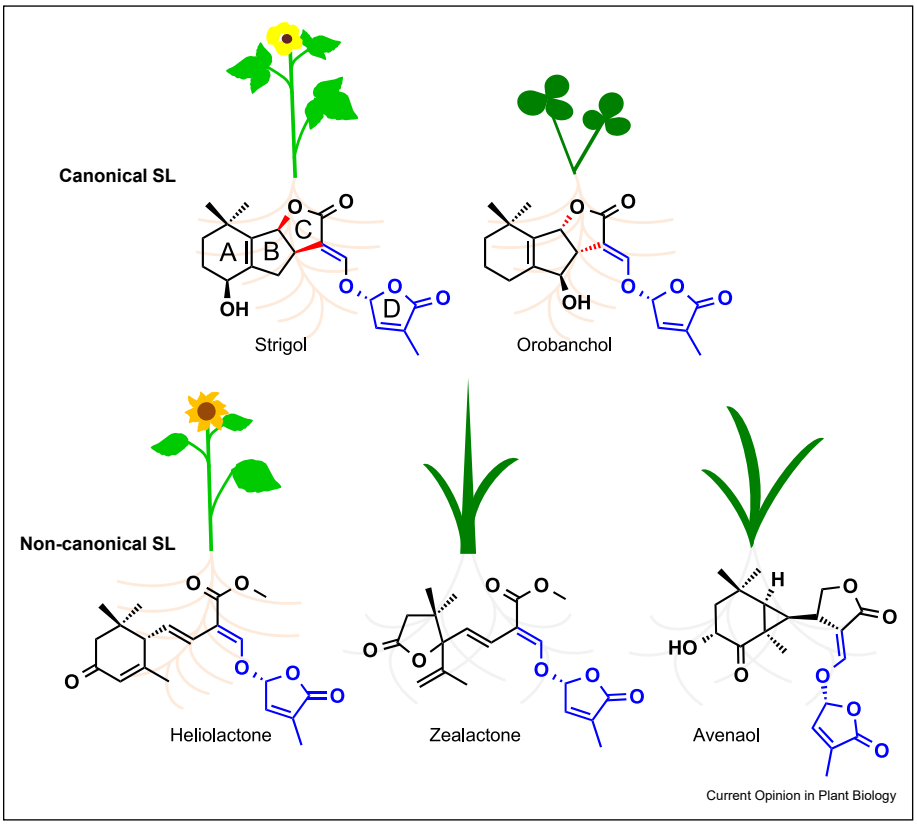
To date, more than 30 SLs have been characterized, mainly as germination stimulants for root parasitic weeds. Plants produce only small amounts of SLs (pg-ng/g root FW) that are chemically unstable. Root exudates are a preferable source for SL detection, as the exudates contain only compounds released from the roots. So far, the details of the SL biosynthetic pathways in plants, and the reason why plants produce structurally diverse SLs, remain elusive. Recent studies using transcriptomics and reverse genetics have led the way to clarify the overall biosynthetic pathway of structurally diverse SLs. In this review, we discuss how various SLs are synthesized and how important their structural diversities are in plant growth and development.

What is a strigolactone?

Strigol was the first SL isolated as a germination stimulant for the root parasitic weed, *Striga*, from root exudates of cotton [4]. Strigol contains an ABC-ring connected via an enol-ether bridge to a methylbutenolide D-ring (Figure 1). Orobanchol was the first germination stimulant identified for another group of root parasites, *Orobanche*, from root exudates of red clover [5]. Natural SLs can be classified into two types based on the orientation of the C-ring; strigol-type SLs with a β-oriented C-ring and orobanchol-type SLs with an α-oriented C-ring. Most plant species examined produce and exude one type, while tobacco plants (*Nicotiana tabacum* cv Michinoku No.1) produce and exude both types including orobanchol and 5-deoxystrigol [6]. It will be informative to discover how tobacco enzymes have evolved to produce both types of SLs and if that is a recent diversification.

SLs having the ABC-ring system are called canonical SL, and those lacking canonical A, B, or C-rings are non-canonical SLs. Avenaol, heliolactone, zealactone, and lotuslactone have been isolated as germination stimulants for root parasitic weeds from root exudates of wild

Figure 1



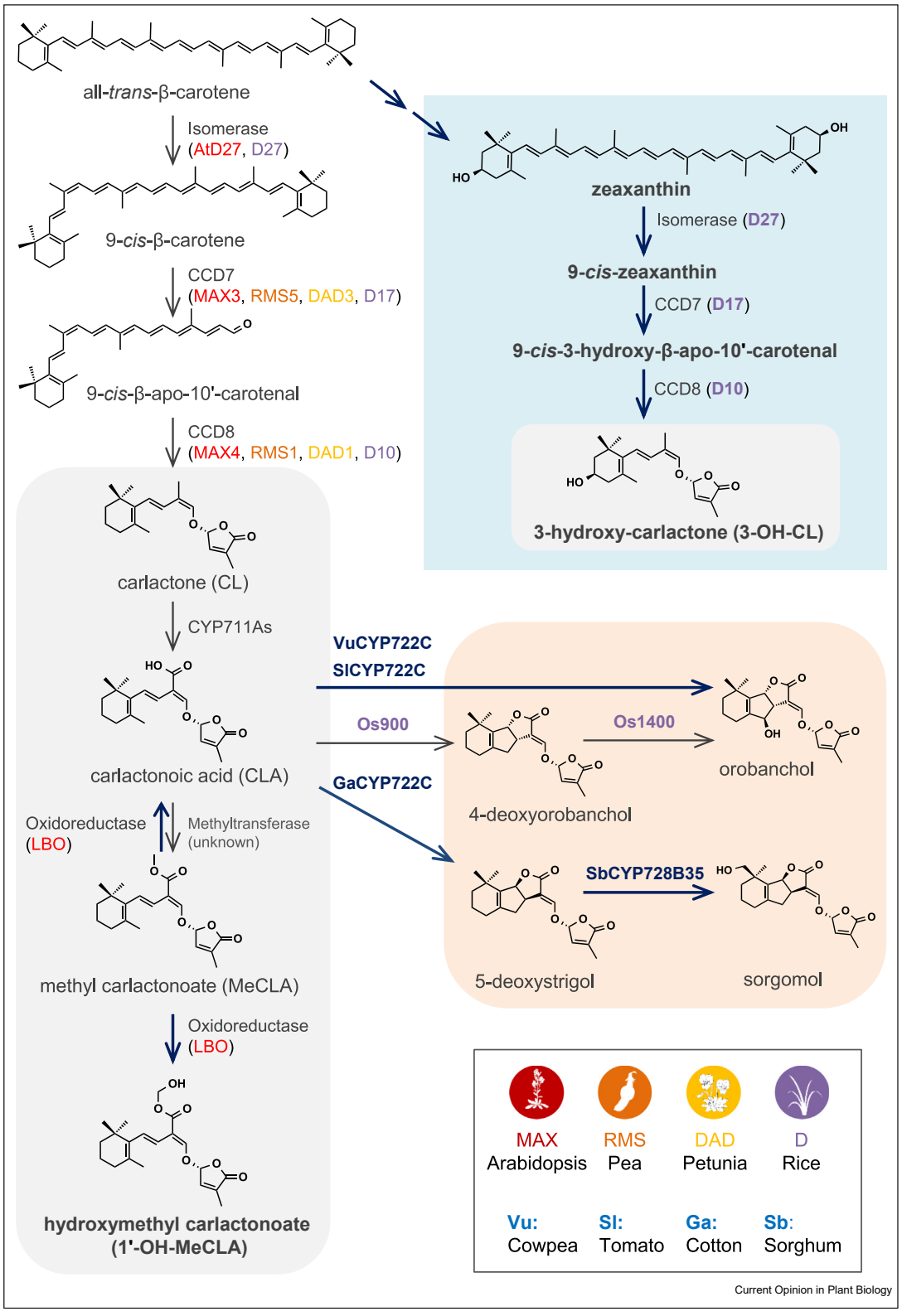
Structures of canonical and non-canonical strigolactones. Strigolactones (SLs) having the ABC-ring system are called canonical SLs (e.g., strigol), which are further classified into strigol and orobanchol types based on the orientation of the C-ring; strigol-type SLs with a β-oriented C-ring and orobanchol-type SLs with an α-oriented C-ring. Non-canonical SLs lack the A, B, or C-ring, but have an enol-ether connected to a methylbutenolide D-ring (blue line), which is essential for bioactivity. Strigol, and especially orobanchol, appear to be widely distributed in the plant kingdom, whereas heliolactone, zealactone, and avenaol may be unique to the plant species from which they were originally isolated.

oat, sunflower, maize, and *Lotus japonicum*, respectively, and are non-canonical SLs with unique structures [7] (Figure 1).

How are the various structures of strigolactones synthesized?

Carotenoid isomerase D27, carotenoid cleavage dioxygenases CCD7 and CCD8, and cytochrome P450 monooxygenases have been identified as SL biosynthesis enzymes by genetic screening for shoot branching mutants. As shown in Figure 2, the core of SL biosynthesis starts from all-*trans*-β-carotene which is converted to 9-*cis*-β-carotene by D27 isomerase, and then, sequential reactions catalyzed by CCD7 and CCD8 convert 9-*cis*-β-carotene to carlactone (CL) having the A and D-ring structure. CL was first discovered in an *Escherichia coli in vitro* system and found to inhibit the tillers of rice plants and induce the germination of root parasitic plants [8] and was later shown to be an endogenous precursor for canonical and non-canonical SLs in *Arabidopsis thaliana* and rice [9]. In *Arabidopsis*, oxidation of CL by the cytochrome P450 MORE AXILLARY GROWTH (MAX)1 (CYP711A1) produces carlactonoic acid (CLA), which is then converted by an unknown methyltransferase to methyl carlactonoate (MeCLA) [10]. We identified *LATERAL BRANCHING OXIDOREDUCTASE (LBO)*, encoding a 2-oxoglutarate and Fe (II)-dependent dioxygenase, using transcriptomics and reverse genetics. *LBO* was highly co-expressed with SL biosynthesis genes under specific conditions including removal of the shoot tip and treatment with an auxin transport inhibitor that influences shoot branching [11]. Then, it was found that the LBO enzyme coverts MeCLA into hydroxymethyl carlactonoate (1′−OH−MeCLA) (recombinant LBO *in vitro*) [12]. However, CLA is also produced in the reaction. LBO might produce CLA by enzymatic demethylation of MeCLA or indirectly from spontaneous reversion of 1′−OH−MeCLA to CLA. Demethylation can occur with 2-oxoglutarate and Fe (II)-

Figure 2



The biosynthetic pathway of strigolactones. The genes of SL biosynthesis were identified and named *MAX* (*MORE AXILLARY GROWTH*) in *Arabidopsis* [red], *RMS* (*RAMOSUS*) in pea [orange], *DAD* (*DECREASED APICAL DOMINANCE*) in petunia [yellow] and *D* (*DWARF*) in rice [purple] from mutants with excessive shoot branching. Recent studies using transcriptomics and reverse genetic approaches revealed new pathways and identified novel genes, which are shown in dark blue color. In rice, a new pathway upstream of CL was shown to produce 3-OH-CL via zeaxanthin [light blue box]. Several hydroxy-CLs are predominant in *Arabidopsis* and these SLs may be produced upstream of CL. The names of new SLs recently characterized are shown in bold type. Canonical SLs are in a beige box and non-canonical SLs are in gray boxes. The genes involved in the biosynthesis of various non-canonical SLs exuded into the rhizosphere including heliolactone, zealactone, avenaol (Figure 1) have not yet been identified and are required to fully understand the various structures of SLs.

dependent dioxygenases [13]. Perhaps CLA production occurs if 1′−OH−MeCLA does not undergo further processing. Thus, it will be important to resolve whether LBO-dependent CLA production occurs *in planta*.

In contrast to *Arabidopsis*, rice has five MAX1 homologs and among them, Os900 (CYP711A2) and Os1400 (CYP711A3) convert CL to CLA, the same as *Arabidopsis* MAX1 [14]. However, Os900 has the additional function to catalyze the B−C ring closure and stereoselectively convert CLA into 4-deoxyorobanchol (4DO), a major rice SL [15]. Then, Os1400 catalyzes the hydroxylation of 4DO into orobanchol.

In contrast to rice, tomato plants produce orobanchol, but not 4DO. Moreover, tomato has only one MAX1 homolog (CYP711A21), which lacks the ability to convert 4DO into orobanchol [14]. The production step for orobanchol was unknown until a recent breakthrough. *CYP722C* is from a completely different cytochrome P450 clade to *MAX1* and was identified in cowpea using transcriptomics [16]. CYP722C converts CLA directly into orobanchol, downstream of MAX1, with no 4DO intermediate [16]. Orobanchol is widely distributed in legumes and Asteraceae plants, but only some of them produce 4DO [17]. The relationship between CYP711A and CYP722C could be elucidated by examining genomic and transcriptomic sequences in plants that produce 4DO compared to those that do not.

It should be noted that cotton CYP722C converts CLA to 5-deoxystrigol (5DS) [18]. Then there is an additional P450 belonging to yet another clade, CYP728B35 from sorghum, that converts 5DS into sorgomol [19]. *CYP728B35* was discovered by screening P450s genes upregulated under low phosphate stress. Thus, three P450 clades are now involved in canonical SL production from CLA. In contrast, MeCLA seems to be the precursor for non-canonical SLs; for example, in sunflower, MeCLA can been converted into heliolactone [20]. Enzyme(s) involved in the production of heliolactone need to be identified to confirm this hypothesis and it is also important to confirm if MeCLA is essential for the synthesis of other non-canonical SLs including avenaol and zealactone.

Not only the enzymes downstream of CL in SL biosynthesis but also those upstream may be important for the formation of structurally diverse SLs. Carotenoid isomerase, CCD7, and CCD8 convert all-*trans*-β-carotene into CL and also 3-hydroxy-carlactone (3-OH-CL) via zeaxanthin (Figure 2) [21]. Although hydroxy-carlactone derivatives are the predominant SLs in *Arabidopsis* [12], their roles in the regulation of plant growth and development have not yet been clarified. These non-canonical SLs are less stable than canonical SLs [7], which hinders studies on biological functions of non-canonical SLs.

What are the multifunctional roles of strigolactone?

Why do SLs function both as internal signals to control plant responses and as exogenous signals to induce mutualisms? A key to connect between the two different phenomena is inorganic nutrient. Plants respond to nutrient availability, especially phosphate, and regulate SL production and exudation; phosphate starvation significantly promotes SL production, while phosphate sufficiency suppresses it in mycotrophic plants [22]. Since AMF symbiosis plays pivotal roles in the acquisition of inorganic nutrients by host plants, especially phosphate, plants increase SL exudation to promote AMF symbiosis. At the same time, plants need to minimize resource use for shoot growth by repressing branching, while increasing the root area [23−26] to increase nutrient absorption when they begin to encounter nutrient starvation. Furthermore, SLs may act as early modulators of plant responses to P starvation in *Arabidopsis* and tomato plants; an applied SL analog mimicked P starvation responses, including induced expression of phosphate starvation marker genes, activation of acid phosphatase and anthocyanin accumulation [27,28].

Nitrogen starvation also enhances SL production and exudation in some plant species including sorghum, maize, and lettuce, suggesting that these plants depend on AMF for nitrogen supply [22]. In rice, sulfate deficiency also promotes SL production [29]. Phosphate deficiency increases the expression of all SL biosynthesis genes [30,31], whereas only *D27* is strongly expressed in sulfate deficiency, suggesting that *D27* may play an important role in effective S acquisition via AMF symbiosis [29]. Other plant hormones, including gibberellins and cytokinins, are negative [31,32] while auxin is a positive regulator [33,34] of SL biosynthesis and their biosynthesis pathways are also influenced by mineral nutrients [35−37]. Furthermore, a recent study suggested that photosynthetic sugar controls a key integrator of circadian rhythms, which impacts the SL pathway and shoot branching in rice plants [38]. The molecular mechanism that regulates SL biosynthesis and exudation in response to other plant hormones and the availability of nutrients remains elusive so far and requires much deeper analysis.

How do structural variations of SL influence its functions?

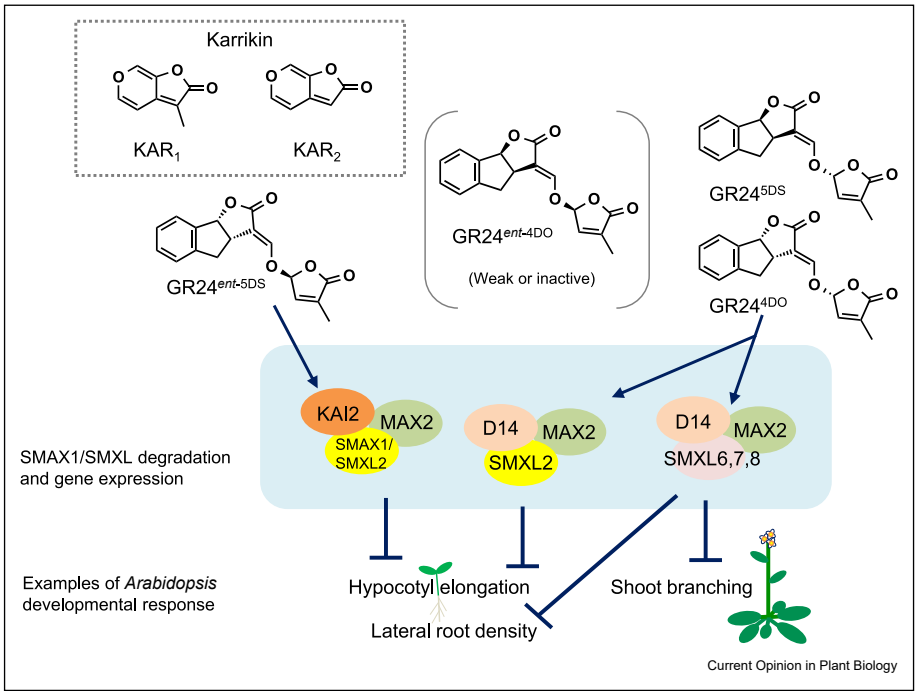
Different SLs can display variation in bioactivity, for example, when added to plants to repress branching [39]. However, it is unclear if this is due to uptake, transport, stability, metabolism, or receptor-binding specificity. SLs are perceived by an SCF ubiquitin-ligase complex that includes the MAX2 F-box protein and an α/β-fold hydrolase, DWARF14 (D14), which has the additional ability to enzymatically hydrolyze and

inactivate SLs by splitting the D-ring from the ABC-ring structure [40]. Scaffidi et al. [41] first demonstrated that *Arabidopsis* could recognize structural variations of the synthetic SL, GR24, which has 4 stereoisomers and display different physiological responses. This may relate to D14 hydrolysis, which can correlate with the bioactivity of GR24 varieties [42], but not always [43]. Specific GR24 stereoisomers can also activate specific downstream transcriptional and growth responses [44–46] (Figure 3).

Some versions of GR24 are perceived by the karrikin receptor (KARRIKIN INSENSITIVE2, KAI2), which is an α/β-fold hydrolase (related to D14) that also associates with MAX2 (Figure 3). Karrikins are smoke-derived seed germination stimulators thought to mimic an undiscovered plant hormone called KAI2-ligand (KL) [47]. KAI2 in *Arabidopsis* has a preference for compounds with a *2′S*-configured D-ring or a desmethyl D-ring [41,43]. Perception of SLs and KL is transmitted through various SUPPRESSOR OF MAX2 1(SMAX1) and SMAX1-LIKE (SMXL) transcriptional repressor proteins that are degraded to induce tissue-specific responses [44,48,49]. However, SLs can act independently of gene expression in the repression of auxin transport [50,51], which may require SMXLs [52] and may include KL [51]. GR24 can even induce responses independently of MAX2 [24,49,50,53–57], and D14 and KAI2 [54]. There has been a heavy reliance on GR24 in experiments, so use of natural SLs of various structures may lead to the discovery of unexpected information about SL functions. For example, bioassays using non-canonical SLs (racemic structure) revealed distinctive effects on primary root growth [58]. Interestingly, KAI2 has evolved SL perception in parasitic weed seed germination and shows expansion in gene copy number [47]. Weed species are sensitive to SL structural variation [59], which, combined with diversification of weed receptors and diversification of SL biosynthesis in hosts (see above), may represent counter-adaptations between weeds and host plants.

Deciphering the origins of SL and KL can help understand function in seed plants. Charophyte fresh water algae have SL biosynthesis-like genes and may produce SLs, and exogenously applied GR24 can stimulate rhizoid elongation of *Chara coralina* [60]. However, there are no obvious *D14* genes in non-seed plants, including the charophytes [61]. AMF symbiosis appeared approximately 450 million years ago [62] as an important

Figure 3



Scheme of GR24 stereoisomer influence on *Arabidopsis* development. GR24 has four stereoisomers and each one differentially influences the signal transduction and *Arabidopsis* development. Karrikins (e.g., KAR_1 and KAR_2) are related chemical compounds derived from smoke that stimulate seed germination after fire. The receptor of KARs is a homolog of SL receptor D14 and named KARRIKIN INSENSITIVE2 (KAI2, also HTL or D14L). In SL perception/signaling, D14 forms a complex with F-box protein MAX2 and SMXLs (SUPPRESSOR OF MAX2 1(SMAX1)-LIKEs) transcriptional repressors. Interestingly, KARs also use the same F-box protein, MAX2, for signal transduction, but different SMXLs. Recent studies demonstrated that exogenously applied KAR surrogates $GR24^{ent\text{–}5DS}$ and suppresses hypocotyl elongation and lateral root density via induction of KAI2 association with SMAX1 and SMXL2, whereas $GR24^{5DS}$ and $GR24^{4DO}$ do via enhancement of interaction between D14 and SMXL2. $GR24^{5DS}$ and $GR24^{4DO}$ also inhibit shoot branching *via* SMXL6,7,8 degradation. $GR24^{ent\text{–}4DO}$ is reported to show only weak or zero activity.

SL-related innovation for plants as they colonized mineral limited land, but charophytes diverged from land plants before AMF symbiosis. So, was the ancestral function of SLs as a hormone for rhizoid growth? Was that connected to nutrient limitation, and if so, was that SL response later exploited for AMF symbiosis? Did the ancestral SL function through a KAI2-like receptor, or through an unknown receptor? If unknown, then is it independent of MAX2, was it inherited in seed plants, and what SL structural variation does it recognize? Further studies into SL pathway mutants and responses to various SLs, particularly in non-seed plants, are needed to resolve these questions.

In contrast to rhizobia, which have strict host-specificity, AMF host plants can be colonized by different fungal taxa, although there is often a preference [63]. Some plants, including *Arabidopsis*, have lost the ability to be colonized by AMF [62]. In the structure of SLs, the ABC-ring system and the enol-ether-D-ring moiety are important for exhibiting a high hyphal branching activity in an AM fungus *Gigaspora margarita* [64]. Studies on the structure–activity relationships of SLs in other AMF have not been conducted. So, it remains unknown if structural differences of SLs influence the quality and quantity of the AMF community. Other soil microbes may be sensitive to SL structure. *Arabidopsis* SL production seems to influence rhizosphere community composition [65] and varieties of sorghum that exude different types of SL displayed different soil bacterial community composition in non-fertile soil [66].

When deciding SL structure–activity relationships, it is important to keep in mind that exogenous SLs may undergo further processing in plants. Mutants that lack SL biosynthesis have been useful for this. For example, exogenously applied CL inhibits shoot branching [8]. However, CL accumulates in *Arabidopsis max1* mutants, indicating that CL is an inactive intermediate that needs to be converted by MAX1, and not a direct shoot branching inhibitor [10]. On the other hand, orobanchol and solanacol are absent in the *cyp722c* knock-out mutant in tomato (in root exudates) (see above), but mutant plants do not show increased branching [16], as seen with other tomato SL mutants, *ccd7*, *ccd8* and *max1* [67–69]. So, it could be that endogenous SLs produced by CYP722C are not involved in branching inhibition and/or are not mobile into the shoot. Interestingly, the *lbo* mutant has increased branching, but wild-type rootstock is poor at repressing *lbo* shoot branching when grafted [11]. The 1′–OH–MeCLA product of LBO is very unstable, so it would make sense that 1′–OH–MeCLA is ineffective over a graft. In contrast, a downstream product of MAX1 is very active over a graft [70]. This product is likely to be CLA, which is not bioactive. Thus, the movement and conversion of CLA into a bioactive, non-canonical SL that is unstable and short ranged may be the main driver of branching inhibition.

There are hints that individual rice *MAX1* genes could be involved in different developmental processes and stress responses, based on *in silico* analyses of transcriptional regulation [71]. More extensive mutant analyses and/or use of specific inhibitors at different biosynthetic steps may unveil the nature and biosynthesis of the shoot branching inhibiting hormone.

Perspective

Manipulation of SL biosynthesis would be an important target of agrochemicals or genetic modifications to improve crop yield and quality in sustainable low-input agriculture and to mitigate damage by root parasitic weeds [72,73]. Advanced sequencing, gene editing, biochemistry, and synthetic biology technologies have the potential to accelerate use of SLs in agriculture, but progress will be slow unless we also decipher the many unknown SL reaction steps and the functions of the SL products *in planta*.

Gene expression studies and reverse genetics have replaced forward genetics in SL mutant discovery. However, other screening methods will be needed if missing enzyme genes are not altered in expression. Also, the instability of some bioactive SLs makes further research very difficult. A greater range of novel synthetic SL mimics and higher resolution detection methods in smaller tissue samples may be required. Moreover, KL and its biosynthesis pathway urgently requires discovery, along with any ancestral or alternative SL receptors. Ultimately, the tissue-specific gene targets and cellular mechanisms of SLs and KL will be required to fully understand how SL structure relates to function.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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- •• of outstanding interest

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