Strigolactone Signaling and Evolution

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Abstract

Strigolactones are a structurally diverse class of plant hormones that control many aspects of shoot and root growth. Strigolactones are also exuded by plants into the rhizosphere, where they promote symbiotic interactions with arbuscular mycorrhizal fungi and germination of root parasitic plants in the Orobanchaceae family. Therefore, understanding how strigolactones are made, transported, and perceived may lead to agricultural innovations as well as a deeper knowledge of how plants function. Substantial progress has been made in these areas over the past decade. In this review, we focus on the molecular mechanisms, core developmental roles, and evolutionary history of strigolactone signaling. We also propose potential translational applications of strigolactone research to agriculture.

Contents

STRIGOLACTONE CHEMISTRY AND BIOSYNTHESIS

Arbuscular mycorrhizal (AM) fungi: symbiotic organisms that receive organic carbon and assist mineral uptake through associations with plant roots

Canonical strigolactones: endogenous, carlactone-derived compounds with an ABC tricyclic lactone linked via an enol-ether bond to a methylbutenolide moiety in a 2′*R* configuration (e.g., orobanchol and strigol)

Strigolactones (SLs) are a class of carotenoid-derived hormones with diverse functions in plant growth and development. They were first discovered in root exudates as potent germination stimulants of the parasitic weed *Striga lutea* (42). Decades later, further research revealed that SLs promote hyphal branching of arbuscular mycorrhizal (AM) fungi and enhance the efficiency of AM symbiosis (3, 19), and also have endogenous roles as upwardly mobile signals that repress shoot branching (73, 170). Since the recognition of SLs as plant hormones, the field has progressed rapidly, and we now have extensive insights into how SLs are made, transported, and perceived.

Canonical SLs consist of a tricyclic lactone (ABC ring) joined to a butenolide moiety (D ring) by an enol-ether bond (**Figure 1**). There are natural and synthetic compounds with SL-like activity that lack the core ABC structure, such as methyl carlactonoate (MeCLA), heliolactone, avenaol, debranones, Yoshimulactone Green, and GC242, but an enol-ether-connected D ring remains a consistent feature of bioactive molecules (1, 26, 27, 46, 67, 68, 99, 137, 140, 167, 168). At least 20 SLs have been discovered in plants (185). Stereochemical differences at the junction of the B and C rings divide canonical SLs into two major classes, strigol type and orobanchol type, but in all SLs found in plants the D ring has a 2′*R* configuration (**Figure 1**). This subtle distinction is significant for reliably assigning SL functions in plant growth. The synthetic SL analog GR24 has been useful for investigating SL responses and continues to be used extensively; however, it is typically a racemic mixture that includes enantiomers in the 2′*S* configuration. Because some 2′*S* molecules can activate a non-SL signaling pathway (147), caution must be exercised in interpreting responses to racemic GR24 (*rac*-GR24).

SL biosynthesis begins with the isomerase DWARF27 (D27), which converts all-*trans*-β-carotene to 9-*cis*-β-carotene (6). Sequential actions of CAROTENOID CLEAVAGE

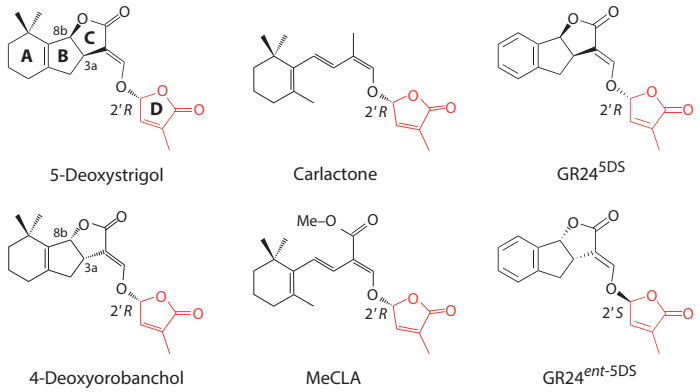


Figure 1

Structures of natural and synthetic SLs. The two major families of canonical SLs, exemplified here by 5-deoxystrigol and 4-deoxyorobanchol, differ by the stereochemistry at the 8b and 3a positions. The biosynthetic precursor for all known SLs is carlactone, a product of carotenoid cleavage. In rice, MAX1 enzymes convert carlactone into orobanchol-type SLs; in *Arabidopsis*, these enzymes convert carlactone into carlactonoic acid, which lacks the canonical ABC tricyclic moiety. This compound is subsequently modified into its methyl ester, MeCLA. Although carlactone-derived compounds with SL-like activity are structurally diverse, the butenolide ring (*red*) is functionally essential. The synthetic SL analog GR24, which has an 8b/3a stereochemistry identical to that of 5-deoxystrigol, is usually synthesized as a racemate of two enantiomeric forms that differ at the 2′ position. Only the 2′*R* enantiomer, known as (+)-GR24 or GR24^{5DS}, accurately recapitulates the bioactivity of natural SLs. Abbreviations: MAX1, MORE AXILLARY GROWTH1; MeCLA, methyl carlactonoate; SL, strigolactone.

DIOXYGENASE7 (CCD7) and CCD8 on this substrate yield carlactone, the last common precursor for all SLs. At this step, the formation and stereochemical orientation of the D ring is complete, but carlactone itself does not have appreciable SL activity. In *Arabidopsis*, the cytochrome P450 enzyme MORE AXILLARY GROWTH1 (MAX1) converts carlactone to carlactonoic acid, which is subsequently methylated by an unknown enzyme to produce MeCLA (1). MeCLA has some activity in shoot branching suppression, a classic SL role, but further action by LATERAL BRANCHING OXIDOREDUCTASE (LBO) converts MeCLA to an unknown compound that may be more potent (33). It is controversial whether *Arabidopsis* makes any canonical SLs, or only MeCLA and derived products (1, 102, 184). Rice, by contrast, has five homologs of MAX1 with diversified functions. One of these, Os01g0700900, functions as a carlactone oxidase that converts carlactone to 4-deoxyorobanchol, the precursor for orobanchol-type SLs (194). A second MAX1 homolog, Os01g0701400, catalyzes the conversion of 4-deoxyorobanchol to orobanchol (194). The enzymes that lead to further diversification of SL structures are unknown. For further reading on the latest discoveries in SL biosynthesis and transport, we refer readers to References 5, 23, and 144.

Butenolide: a lactone with a four-carbon heterocyclic ring structure; it is a common feature of all bioactive strigolactones

Enantiomer: in chiral compounds, one of a pair of stereoisomers that are mirror images of one another (i.e., with opposite stereochemistry)

Racemic GR24 (*rac*-GR24): a commonly used equal mixture of a synthetic strigolactone analog and its enantiomer

THE STRIGOLACTONE SIGNALING MECHANISM

Similarly to the auxin, jasmonate, and gibberellin signaling mechanisms, SL signal transduction is based upon hormone-activated proteolysis (reviewed in 120). In each of these mechanisms, an F-box protein component of a Skp1–Cullin–F-box (SCF) E3 ubiquitin ligase complex targets specific protein substrates for polyubiquitination and degradation by the 26S proteasome. What distinguishes each signaling mechanism are the targets of the F-box protein and how SCF-mediated

polyubiquitination is activated by the hormone; these have been the subject of intensive research by the SL field.

SL responses require the F-box protein MAX2/D3. (For clarity, we use primarily *Arabidopsis* and rice gene nomenclature in this review; for other ortholog names, see **Table 1**.) Loss-of-function *max2/d3* alleles share many phenotypes with SL-biosynthesis mutants, such as increased axillary bud outgrowth, but *max2/d3* cannot be rescued by treatment with exogenous SL analogs (22, 73, 86, 158, 170). Increases in SL production have also been noted in *d3* mutants, consistent with a negative feedback mechanism that controls SL biosynthesis (170). Importantly, *max2* has additional phenotypes not present in SL-biosynthesis mutants, such as increased seed dormancy and reduced seedling photomorphogenesis, indicating that *MAX2* is also likely to be involved in non-SL signaling (129, 150, 151). Consistent with this idea, *max2* alleles were isolated in a screen for karrikin (KAR)–insensitive mutants (129). KARs are a class of molecules found in aqueous smoke extracts and biochar that can promote seed germination of many species (61, 72, 100, 126). Like SLs, they have a conserved butenolide moiety, but the chemical properties of KARs and their effects on plant growth are quite different from those of SLs.

Carlactone: a key intermediate in the biosynthesis of strigolactones, generated by the isomerization and cleavage of all-*trans*-β-carotene

F-box protein: an adapter component of the SCF class of E3 ubiquitin-protein ligase complexes that confers substrate specificity to polyubiquitination

α/β-Hydrolase: a large, diverse class of hydrolytic enzymes that are typified by a core of eight beta strands joined by six alpha helices and contain a catalytic triad

Catalytic triad: three amino acid residues comprising an acid, a base, and a nucleophile (often Asp, His, and Ser, respectively)

Strigolactone Perception

SL perception in angiosperms occurs through the α/β-hydrolase superfamily protein D14 (see **Table 1**). D14 is unusual in comparison with other plant hormone receptors in that it functions as both an enzyme and a receptor. It has a strictly conserved Ser-His-Asp catalytic triad that is necessary for both SL hydrolysis and signaling functions (81, 125, 180). However, the rate of SL hydrolysis by D14 is quite slow in vitro (as low as ~0.3 molecules min^{-1}), suggesting that its function is not to produce a bioactive SL-derived signal that is perceived by another protein (81, 196). Consistent with this, neither the final products of SL hydrolysis—5-hydroxy-3-methylbutenolide and tricyclic lactone—nor the intermediate molecule 2,4,4,-trihydroxy-3-methyl-3-butenal acts as a shoot branching suppression signal (81, 125, 196).

Consequently, it was proposed that D14 undergoes conformational changes during SL binding or hydrolysis that enable SL signal transduction (**Figure 2**). In support of this idea, GR24 induces thermal destabilization of D14, and this response requires an intact catalytic triad (1, 81, 180). GR24 also promotes physical interactions between D14 and MAX2/D3, and MAX2/D3 enhances the magnitude of D14 destabilization (81, 195, 197). Rice D14-D3 association is much more responsive to 2′*R* stereoisomers of GR24 than to 2′*S* stereoisomers (197). Until recently, however, structural evidence for an allosteric signaling model has been lacking, as there are no substantial differences between the crystal structures of apo-D14 and D14 in complex with intact SL, 2,4,4,-trihydroxy-3-methyl-3-butenal, or 5-hydroxy-3-methylbutenolide (81, 95, 125, 196, 197).

An important breakthrough was achieved with the structural characterization of AtD14 in complex with D3 and *Arabidopsis* SKP1-LIKE1 (ASK1) (187). During SL hydrolysis, the D ring is cleaved and forms an intermediate molecule that is covalently linked to the His residue of the catalytic triad (46, 187). This results in D14 adopting a closed-state conformation in which the lid structure collapses from four to three helices and the internal binding pocket volume is reduced. D3-ASK1 stabilizes the closed state and further slows the hydrolysis of SL analogs by D14, suggesting that release of the bound D ring is inhibited (187). Putatively, prior attempts to capture D14 in an active signaling configuration were unsuccessful because it was not stabilized; although SL hydrolysis is enzymatically slow, without D3-ASK1, the closed state simply may not persist on the timescales required for crystallization. A final piece of evidence that D14 is an SL receptor comes from the SL-insensitive d14-5 protein, which has strongly reduced interactions with D3 but actually has a higher rate of SL hydrolysis in vitro than the wild-type protein (187).

Table 1 Orthology of genes related to SL and KAR signaling and SL biosynthesis in model species

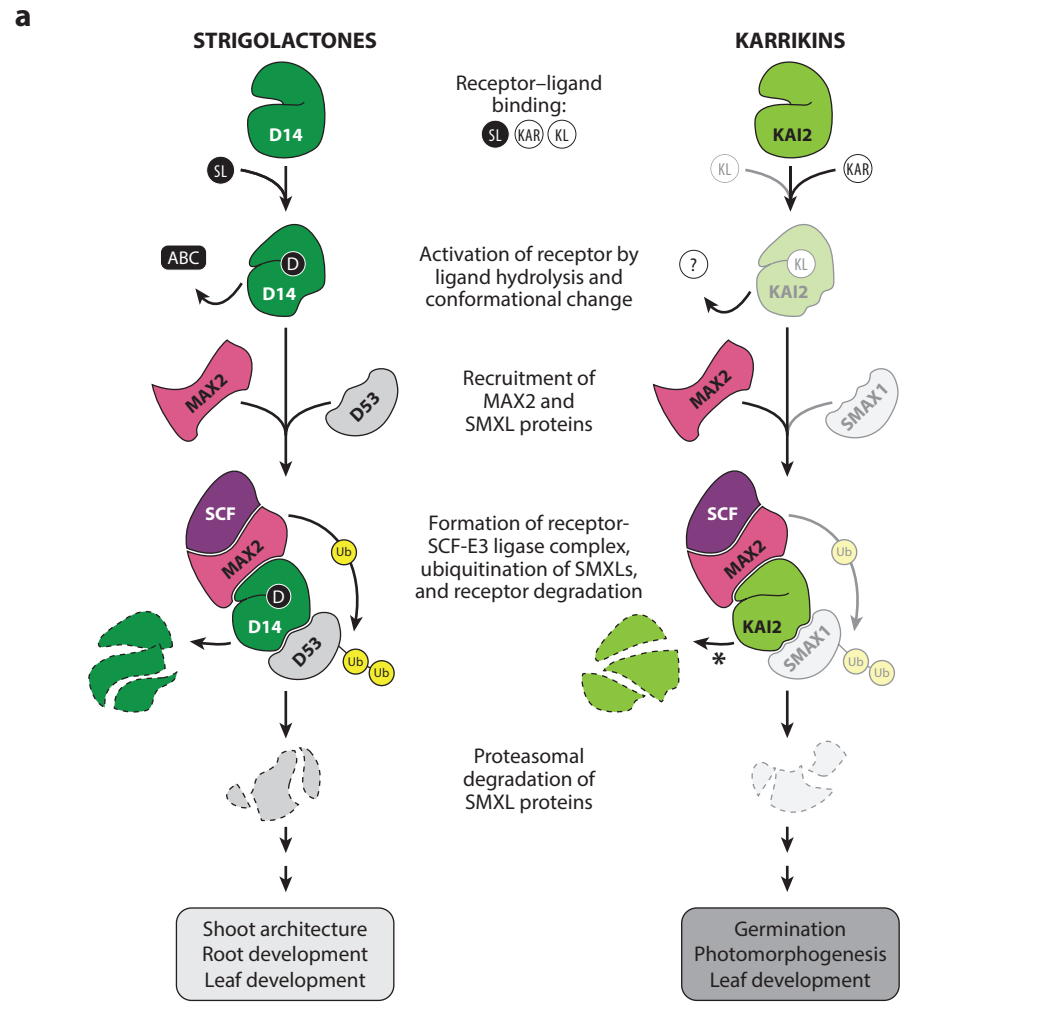
Arabidopsis thaliana	*Oryza sativa*	*Petunia hybrida*	*Pisum sativum*	*Medicago truncatula*	Function of encoded protein	Reference(s)
SL and KAR signaling						
MAX2/ORE9 (At2g42620)	*D3* (Os06g0154200)	*PhMAX2a*, *PhMAX2b*	*RMS4*	Medtr4g109790	F-box component of the SCF complex	38, 53, 86, 94, 158, 182
AtD14 (At3g03990)	*D14/D88/HTD2* (Os03g0203200)	*DAD2*	*RMS3*	Medtr1g023380	α/β-Hydrolase required for SL signaling	9, 46, 70, 81, 106 110, 178
KAI2/HTL (At4g37470)	*D14L* (Os03g0437600)	?	?	MtD14L 1a (Medtr4g095310),[a] MtD14L 1b (Medtr5g016150),[a] MtD14L 1c (Medtr2g089890)[a]	α/β-Hydrolase required for KAR signaling	9, 77, 106, 159, 178
SMAX1 (At5g57710), *SMXL2* (At4g30350)	Os08g15230, Os02g54720	?	?	?	Presumed proteolytic target of KAR signaling; it is weakly similar to class I Clp ATPase but may regulate transcription	156, 157
SMXL6 (At1g07200), *SMXL7* (At2g29970), *SMXL8* (At2g40130)	*D53* (Os11g0104300), *D53-LIKE* (Os12g0104300)	?	?	?	Proteolytic target of SL signaling; it is weakly similar to class I Clp ATPase but may regulate transcription	91, 155, 175, 198
SL biosynthesis						
AtD27 (At1g03350)	*D27* (Os11g0587000)	?	?	*MtD27* (Medtr1g083480),[a] Medtr1g083360[a]	Carotenoid isomerase	6, 38, 108, 109, 177
MAX3 (At2g44990)	*OsCCD7/D17/HTD1* (Os04g0550600)	*DAD3*	*RMS5*	Medtr7g040830	Carotenoid cleavage dioxygenase	21, 38, 52, 94, 106
MAX4 (At4g32810)	*OsCCD8/D10* (Os01g0746400)	*DAD1*	*RMS1*	Medtr3g130620	Carotenoid cleavage dioxygenase	7, 20, 38, 106, 154
MAX1 (At2g26170)	*OsMAX1* (Os01g0700900, Os01g0701400, Os01g0701500,[b] Os02g0221900, Os06g0565100)	?	?	Medtr3g104560, Medtr1g015860[c]	Cytochrome P450 enzyme; specificity varies and includes carlactone oxidase and orobanchol synthase	22, 38, 194
LBO (At3g21420)	Os01g0935400[a]	?	?	Medtr2g437380[a]	2-Oxoglutarate- and Fe(II)-dependent dioxygenase; it acts downstream of MAX1 on MeCLA	33

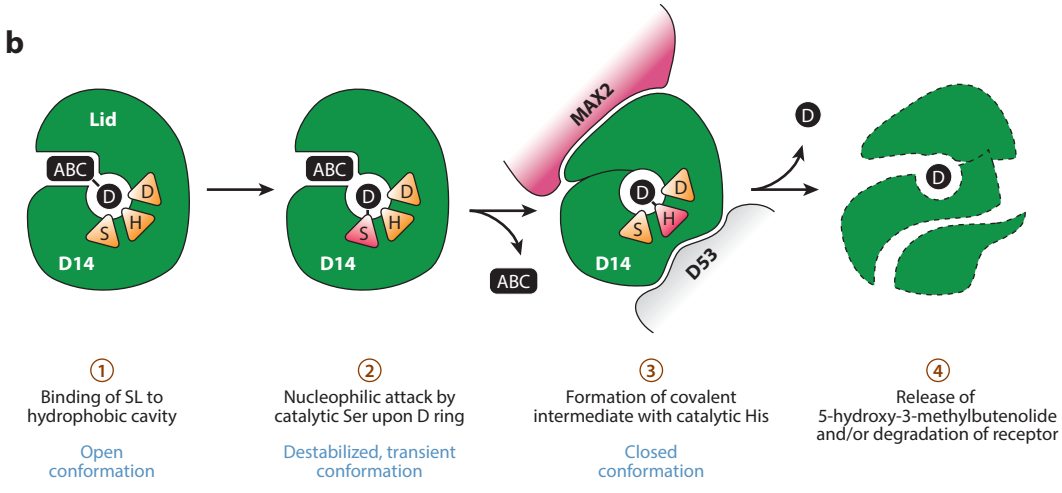
Where applicable, synonyms for the same gene in a species are given in parentheses. Paralogous copies of the same gene in a species are separated by commas. Abbreviations: *CCD*, *CAROTENOID CLEAVAGE DIOXYGENASE*; *D*, *DWARF*; *D14L*, *D14-LIKE*; *DAD*, *DECREASED APICAL DOMINANCE*; *HTD*, *HIGH-TILLERING DWARF*; *HTL*, *HYPOSENSITIVE TO LIGHT*; *KAI2*, *KARRIKIN-INSENSITIVE2*; KAR, karrikin; *LBO*, *LATERAL BRANCHING OXIDOREDUCTASE*; *MAX*, *MORE AXILLARY GROWTH*; MeCLA, methyl carlactonoate; *ORE9*, *ORESARA9*; *RMS*, *RAMOSUS*; SCF, Skp1–Cullin–F-box; SL, strigolactone; *SMAX1*, *SUPPRESSOR OF MAX2 1*; *SMXL*, *SMAX1-LIKE*.

[a]Functional orthology inferred from phylogenetic relationship, with no experimental evidence.

[b]Functional orthology ruled out by experimentation.

[c]Partial or weak functional orthology.


a
STRIGOLACTONES
KARRIKINS
Receptor–ligand binding:
SL
KAR
KL
D14
KAI2
ABC
Activation of receptor by ligand hydrolysis and conformational change
?
MAX2
D53
SMAX1
Recruitment of MAX2 and SMXL proteins
SCF
Ub
Formation of receptor-SCF-E3 ligase complex, ubiquitination of SMXLs, and receptor degradation
*
Proteasomal degradation of SMXL proteins
Shoot architecture
Root development
Leaf development
Germination
Photomorphogenesis
Leaf development


b
Lid
ABC
D
H
S
D14
MAX2
D53
1
Binding of SL to hydrophobic cavity
Open conformation
2
Nucleophilic attack by catalytic Ser upon D ring
Destabilized, transient conformation
3
Formation of covalent intermediate with catalytic His
Closed conformation
4
Release of 5-hydroxy-3-methylbutenolide and/or degradation of receptor

Thus, the enzymatic and signaling functions of D14 have been uncoupled. Similar uncoupling might be found in the d14-2/seto5 protein, which has an altered surface residue (39).

Remarkably, a close homolog of D14, KARRIKIN-INSENSITIVE2 (KAI2)/HYPOSENSITIVE TO LIGHT (HTL)/D14-LIKE (D14L), mediates KAR responses (159, 178). The shared evolutionary origins of these two proteins and several striking similarities suggest that KAI2 functions much like D14, with notable differences in ligand specificity and roles in plant growth. D14 and KAI2 have a high degree of structural similarity overall, but D14 has a larger ligand-binding pocket that can presumably accommodate larger molecules (35, 75, 95). The catalytic triad is conserved among KAI2 proteins and is required for KAI2 function (180). Similarly to D14, KAI2 can undergo thermal destabilization, but it does so only in response to an unnatural 2′*S* SL stereoisomer and not to 2′*R* SLs (180). Binding of KAR_1 to KAI2 has been demonstrated through several techniques (75, 95, 186), but, interestingly, KAI2 does not destabilize in the presence of KARs (180). Two crystal structures of KAI2 with KAR_1 present in the ligand-binding pocket—one from *Arabidopsis* and one from the parasitic plant *Striga hermonthica*—show very different orientations of the KAR_1 molecule (75, 186). Neither structure shows a substantial conformational change in comparison with the apo-KAI2 protein, suggesting that an activated KAI2 state has not yet been captured. KARs might require metabolism in vivo to produce a ligand that is recognizable by KAI2, or perhaps a partner protein such as MAX2 is necessary to produce a detectable active state with KAR ligands in vitro. Protein-protein interactions with KAI2 have been much less explored than those with D14, but yeast two-hybrid assays provide some evidence for interactions between KAI2/HTL and MAX2 (164). Also supporting this, D14 and KAI2 share a well-conserved motif (amino acids 174–186) in the lid that is an integral part of the D14-MAX2 interaction surface (187).

Nevertheless, D14 and KAI2 exhibit distinct ligand specificities. For example, *kai2* mutants are unable to respond to KARs, demonstrating that D14 cannot perceive KARs (147, 178).

Figure 2

(*a*) Models of SL and KAR signaling mechanisms. (*Left*) SL binds to the internal cavity of the receptor D14. Hydrolysis of SL leads to ejection of the ABC moiety and retention of the D ring (46, 187). Hydrolysis also induces a major conformational change that facilitates the association of D14 with MAX2 (187, 197) and probably SMXL proteins such as D53 (91, 107, 169, 175, 198). The order of assembly of these components is unclear, but D14 can associate with both MAX2 and D53-type SMXL proteins independently. After recruitment into the SCF complex, D53 protein is polyubiquitinated and degraded by the proteasome (91, 175, 198). D14 is also degraded in a MAX2-dependent manner (39). Loss of D53/SMXL proteins reduces auxin transport and affects transcriptional activity, such as derepression of *BRC1* transcription (155). (*Right*) The anticipated mechanism for KAI2-dependent signaling parallels that for D14, but less direct evidence is available; hypothetical steps not directly supported by biochemical evidence are shown faded. KARs do not induce a thermal stability change in KAI2 in vitro, but nonnatural, 2′*S* SLs do, and a functional catalytic triad is necessary for KAI2 function (180). A KL is hypothesized to exist (41) and is presumably hydrolyzed like SL. Based on genetic analysis, KAI2 probably regulates growth through physical interaction with SMAX1 and, to a lesser extent, SMXL2 (156, 157). KAI2 is also degraded as a result of its signaling but in a *MAX2*-independent manner (cf. D14) (179). (*b*) Perception of SL by D14. (①) In the open conformation, hydrophobic residues near the entrance to the cavity of D14 stabilize the ABC portion of an SL molecule, orienting the D ring toward the Ser-His-Asp catalytic triad (*triangles*). (②) Hydrolysis of SL initiates with nucleophilic attack of the Ser residue upon the carbonyl group of the D ring. This results in separation of the ABC and D rings, and a transient attachment between the opened D moiety and the Ser residue. (③) The His residue then attacks the Ser-bound moiety, forming a more stable covalent attachment. This catalytic rearrangement induces transition to the closed state, whereby the upper lid portion of D14 flattens and partially collapses the internal cavity. This closed position is stabilized by binding of MAX2 to the newly formed flattened surface of D14, which might also facilitate interaction with SMXL/D53 proteins. (④) After signaling, the D14 protein is degraded. It is unclear whether D14 is activated by SL only once before degradation; in vitro, the hydroxylated D ring (5-hydroxy-3-methylbutenolide) can exit the receptor at a low rate. Abbreviations: *BRC1*, *BRANCHED1*; D, D ring; D14/53, DWARF14/53; KAI2, KARRIKIN-INSENSITIVE2; KAR, karrikin; KL, endogenous KAI2 ligand; MAX2, MORE AXILLARY GROWTH2; SCF, Skp1–Cullin–F-box; SL, strigolactone; SMAX1, SUPPRESSOR OF MAX2 1; SMXL, SMAX1-LIKE; Ub, ubiquitin. Based on data from References 46 and 187.

Promoter-swap experiments confirm that the functional distinction between D14 and KAI2 is not simply a by-product of different spatiotemporal expression patterns (180). Finally, a curious difference between the two receptors is their mode of degradation after the signaling process: D14 is degraded in a *MAX2*-dependent manner, whereas KAI2 is not (39, 179).

Although the mechanism of KAR perception by KAI2 remains uncertain, KAI2 and D14 clearly have distinct roles in plant growth that cumulatively account for *MAX2*-regulated phenotypes. Whereas *d14* mutants have shoot phenotypes that are highly similar to those of SL-deficient mutants, *kai2* mutants do not, instead exhibiting increased seed dormancy, reduced seedling photomorphogenesis, and altered leaf morphology (9, 110, 178). Notably, *kai2* phenotypes are the opposite of growth responses to KAR (127, 128). Because *KAI2* is highly conserved among land plants and most species are not natural fire followers, it has been proposed that the typical function of KAI2 is to recognize an unknown endogenous KAI2 ligand (KL) (41, 63). In some species, such as *Arabidopsis*, KAI2 may have flexibility in its capacity to respond to KARs as well as KL, whereas in others, such as *S. hermonthica*, KAI2 can show subfunctionalized preferences for KARs or KL (40, 41, 163). We discuss the evolutionary history of the SL and KAR/KL pathways further below (see the section titled Evolution of Strigolactone Signaling Pathways).

Proteolytic Targets of Strigolactone Signaling

The direct targets of SCF^{MAX2} action are members of the SUPPRESSOR OF MAX2 1 (SMAX1) or D53 protein family, which is distantly related to the ClpB/HEAT SHOCK PROTEIN100 (HSP100) class of heat shock proteins. SMAX1 was discovered in a screen for genetic suppressors of *max2* phenotypes at the seed and seedling stage (157). Shortly thereafter, the homologous D53 protein was identified in rice through a dominant SL-insensitive *d53* mutant that shares *d3* and *d14* phenotypes, including high tillering and increased SL production (91, 198). Subsequent work in *Arabidopsis* has shown that triple loss-of-function mutants of the *D53* co-orthologs *SMAX1-LIKE6* (*SMXL6*), *SMXL7*, and *SMXL8* completely suppress all tested SL-related aspects of the *max2* phenotype (155, 175). Conversely, *smax1* suppresses all tested KAR/KL-associated phenotypes of *max2*, causing growth effects that mimic KAR responses (155, 157). *SMXL2* also contributes to growth control of *Arabidopsis* seedlings with *SMAX1* (156). D53 and its SMXL7 orthologs physically interact with D14 in an SL-enhanced manner and are rapidly degraded upon *rac*-GR24 treatment in a D14- and MAX2/D3-dependent manner (91, 107, 155, 169, 175, 198). It has been reported that D53 and SMXL6, -7, and -8 can interact directly with MAX2, but current evidence suggests that any such interactions are much weaker than those with D14 (107, 175). Small deletions in a conserved C-terminal Arg-Gly-Lys-Thr motif present in D53, SMXL6, and SMXL7 prevent their polyubiquitination and degradation, causing phenocopy of the *d14* phenotype (91, 107, 155, 175, 198). These dominant d53 isoforms maintain SL-induced interactions with D14. Altogether, these observations are consistent with SMXL7/D53 proteins being bona fide targets of SCF^{MAX2} in response to SL signaling. Although biochemical evidence is currently lacking, genetic evidence and homology to SMXL7/D53 suggest that SMAX1 is the target of SCF^{MAX2} in response to KAR/KL signaling.

Several other proteins have also been suggested to be targets of MAX2, including the brassinosteroid target protein BRI1-EMS SUPPRESSOR1 (BES1) and the DELLA family of GRAS transcriptional regulators, which are targets of gibberellin signaling (125, 176). However, neither loss-of-function nor stabilized isoforms of BES1 and DELLA proteins result in phenocopy of *d14* in any examined shoot phenotype, and *bes1* mutants are fully SL sensitive in a branching assay (15). Furthermore, unlike SMXL6 and SMXL7, a GFP-DELLA translational fusion shows no response to *rac*-GR24 treatment in either the shoot or the root (15).

SMXL/D53 function remains poorly understood, but substantial attention has been given to the hypothesis that SMXL/D53 proteins are transcriptional regulators (91, 155, 175). This idea is based on a C-terminal ETHYLENE RESPONSE FACTOR–associated amphiphilic repression (EAR) motif that is conserved throughout the SMXL/D53 family in both angiosperms and basal land plants (14). In plants, EAR motifs are most well known as domains that enable interactions with the TOPLESS (TPL)/TOPLESS-RELATED (TPR) family of transcriptional corepressors (161). Both Aux/IAA and jasmonate ZIM-domain (JAZ) proteins, which are the proteolytic targets of auxin and jasmonate signaling, respectively, contain EAR motifs and mediate transcriptional repression through interaction with TPR proteins. It has therefore been suggested that SMXL/D53 proteins act in a similar manner. Bimolecular fluorescence complementation assays have demonstrated EAR-motif-dependent interactions between SMAX1 and SMXL7 with TPR2 in vivo (155), and coimmunoprecipitation with transiently expressed SMXL6, -7, and -8 and TPR2 in *Arabidopsis* protoplasts also shows EAR-motif-dependent interactions (175). SMXL6, -7, and -8 suppress gene expression in a *GAL4–upstream activation sequence* (*UAS*) reporter assay in an EAR-motif-dependent manner; this is enhanced by coexpression of TPR2 (175). These data support the possibility that SMXL works with TPL/TPR proteins, but the highly pleiotropic nature of *tpl/tpr* mutants will make it difficult to investigate their role in SL signaling (37, 111). It remains possible that proteins other than TPL/TPR with C-terminal LisH-motif (CTLH) domains may associate with SMXL through the EAR motif (14).

Other observations challenge the EAR-mediated transcriptional repressor hypothesis for SMXL/D53 function. First, transcriptional changes caused by *rac*-GR24 treatment appear to be quite modest, and KAR treatments also have limited effects on transcription (116, 127). Second, SMXL7 variants lacking the EAR motif can restore many shoot phenotypes of *smxl6 smxl7 smxl8 max2* quadruple mutants to a *max2*-like state, indicating that the EAR motif is not required for all SMXL7 functions (107). Intriguingly, although SMXL7 function in some developmental responses (e.g., shoot branching) is EAR dependent, other responses (e.g., leaf growth) seem to be EAR independent (107). Third, *rac*-GR24 can rapidly influence distribution of the auxin efflux carrier PIN-FORMED1 (PIN1) even when translation is blocked by cycloheximide, suggesting that transcriptional changes would be irrelevant for this response (152). Therefore, nontranscriptional modes of SMXL/D53 action must also be kept under consideration.

STRIGOLACTONE ROLES IN PLANT DEVELOPMENT

Many roles have been proposed for SLs in the regulation of plant development. In the next few sections, we discuss some of these processes in detail, but first it is important to distinguish which have the strongest evidence for SL involvement (**Figure 3**). Experiments that rely on *max2* and/or *rac*-GR24 treatments can have confounded results, because *max2* has defects in both SL and KAR/KL signaling and different components of *rac*-GR24 can activate both pathways (147, 178). The gold standard for ascribing a role to SLs is therefore to include analyses of SL biosynthesis and/or *d14* mutants, or to test the effect of enantiomerically pure 2′*R* SLs (62). For example, SLs have been implicated in promoting photomorphogenesis in *Arabidopsis* (90, 166, 181), but this conclusion was based on *max2* phenotypes and the application of exogenous *rac*-GR24. Examination of SL-deficient *Arabidopsis* mutants suggests that endogenous SLs have no such function, at least under standard growth conditions (129, 151, 178). Instead, the effects of *rac*-GR24 and MAX2 on photomorphogenesis likely result from KAI2-mediated signaling (147).

Similar reasoning can apply to the proposed role of SLs in promoting seed germination in *Arabidopsis* (151, 165), which is also a *KAI2*- rather than *D14*-regulated process (147, 178). Interestingly, in rice, *d14* and SL-biosynthesis mutants demonstrate that SL regulates seedling

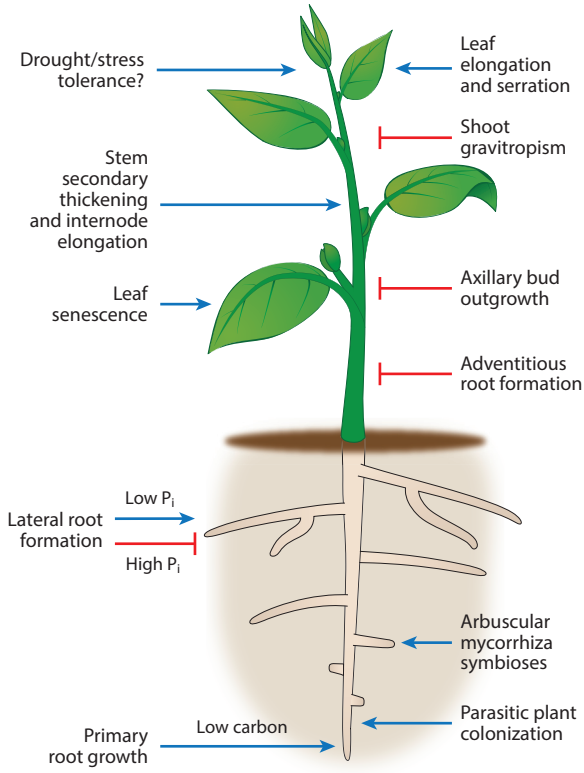


Figure 3

The roles and effects of SLs in plant development. SLs promote (*blue arrows*) or inhibit (*red bars*) many different plant growth processes (2a, 143a, 153a, 167a, 186a, and others discussed in the main text). These roles have been confirmed in multiple species and/or with SL-deficient mutants. The effects of SLs on leaf development are complex and species specific. In *Arabidopsis*, SLs generally promote expansion of the leaf blade and petiole along the proximo-distal axis, promoting a more open rosette architecture (38, 146, 155). In *Medicago*, SLs enhance leaf serration but do not dramatically affect overall leaf shape (106). The role of SLs in drought and abiotic stress tolerance has not been fully resolved (34, 80). Abbreviations: P_i, inorganic phosphate; SL, strigolactone. Adapted from Reference 153 with permission.

development by inhibiting mesocotyl elongation in the dark (85). However, *d3* mutants show a much stronger phenotype, consistent with an additional contribution from KAI2/D14L-dependent signaling (77, 96). SLs are also thought to play a role in tolerance to abiotic stresses, such as drought; however, this conclusion is again based on studies in *Arabidopsis* that have not made use of *d14*, *kai2*, or natural SL stereoisomers, and existing publications in this area are contradictory (34, 80). Extended experiments with additional mutants will help to resolve the current ambiguities. In general, however, if the phenotype of *max2* is stronger than or different from that of SL-deficient mutants or *d14*, other pathways beyond SLs likely contribute to the process in question.

Strigolactone Signaling in the Regulation of Shoot Branching

The best-characterized role of SLs in plant development is in the regulation of shoot branching by the canonical D14-MAX2-D53 signaling mechanism (91, 155, 175, 198). However, there is no

consensus on signaling events downstream of SMXL/D53 degradation (14). Two main models have been proposed (**Figure 4**). One posits that SLs regulate transcription of the TCP-domain transcription factor *BRANCHED1* (*BRC1*) (54), and the other posits that SLs regulate protein levels of the auxin efflux carrier PIN1 at the plasma membrane (43, 152). Broadly speaking, these models of SL action mirror the two main models of shoot branching regulation: the direct-action (or second-messenger) model and the canalization model (50).

The direct-action model proposes that hormones synthesized primarily in the root—SLs and cytokinin—are transported to the shoot through the xylem and affect branching directly in the bud (32, 54). Auxin produced by active shoot apices also inhibits outgrowth of dormant buds (apical dominance), but it acts indirectly (reviewed in 50). A long-standing idea has therefore been that there are second messengers for auxin that relay the auxin signal into the bud. Because auxin regulates the synthesis of both SLs and cytokinin in the stem and both hormones are proposed to act directly in the bud, they are plausible second messengers for auxin (50). Recent work has suggested that cytokinin is not a relevant target of auxin in apical dominance (122), but it remains possible that SLs act as a second messenger. In the direct-action model, both SLs and cytokinin act by regulating transcription in buds of *BRC1* (54), a putative regulatory nexus for shoot branching control (2) (**Figure 4**). In support of this, *brc1* mutants in both *Arabidopsis* and pea have increased shoot branching levels and appear to be insensitive to treatment with SL (2, 28). *BRC1* expression is strongly reduced in the *max2* mutant and strongly increased in the *smxl6 smxl7 smxl8 max2* quadruple mutant, and is thus correlated with the branching phenotypes of these lines (155). Furthermore, treatment of pea with *rac*-GR24 rapidly upregulates *BRC1* expression independently of new protein synthesis, suggesting that *BRC1* is a direct target of SL signaling (54).

The direct-action model is a straightforward and appealing explanation for the effect of SL on branching, but it is challenged by several observations. First, there are qualitative differences in branching between *brc1* and SL mutants in *Arabidopsis* and pea, and these mutations have additive effects on branching in *Arabidopsis* (28, 39). Thus, some proportion of the branching increase in SL mutants cannot be explained by loss of *BRC1* activity. Second, expression of *BRC1* orthologs in grass species—*TEOSINTE BRANCHED1* (*TB1*) in maize and *FINE CULM1* (*FC1*) in rice—is not *rac*-GR24 responsive and is not reduced in SL mutants (7, 74, 119). Furthermore, *TB1* expression in domesticated maize is constitutively elevated but does not prevent branching phenotypes when SL biosynthesis or signaling is disrupted (74). These data indicate that *BRC1* orthologs in grasses are unlikely to be transcriptional targets of SL signaling. There may be other bud growth regulatory genes targeted by SLs that could support the direct-action model, but if so, they remain elusive. Therefore, at present, the direct-action model does not provide a complete explanation for the regulation of shoot branching in flowering plants.

The canalization model, which is derived from the eponymous model of vascular patterning (reviewed in 14), provides an alternative framework for shoot branching control that includes a mechanism for coordinating growth between branches (50). In this model, buds are auxin sources that can grow out only if they can form a canalized auxin transport link to the main stem, allowing auxin export (139) (**Figure 4**). The number of buds that can export auxin is determined by the auxin sink strength of the stem, which is determined principally by the ability to transport auxin rootward. Buds are therefore in competition with each other to export auxin, and the strongest auxin sources tend to be the buds that grow out, because they are most likely to canalize to the stem. Note that it is the relative—and not absolute—strengths of auxin sources and sinks that are important in determining outcomes, along with both the history and dynamics of the system.

The phenomenon of canalization is well supported in the context of vascular patterning by both experimental observations and mathematical modeling, and to a lesser extent in the context of shoot branching (10, 139, 145, 148, 152). Mechanistically, canalization is not well understood,

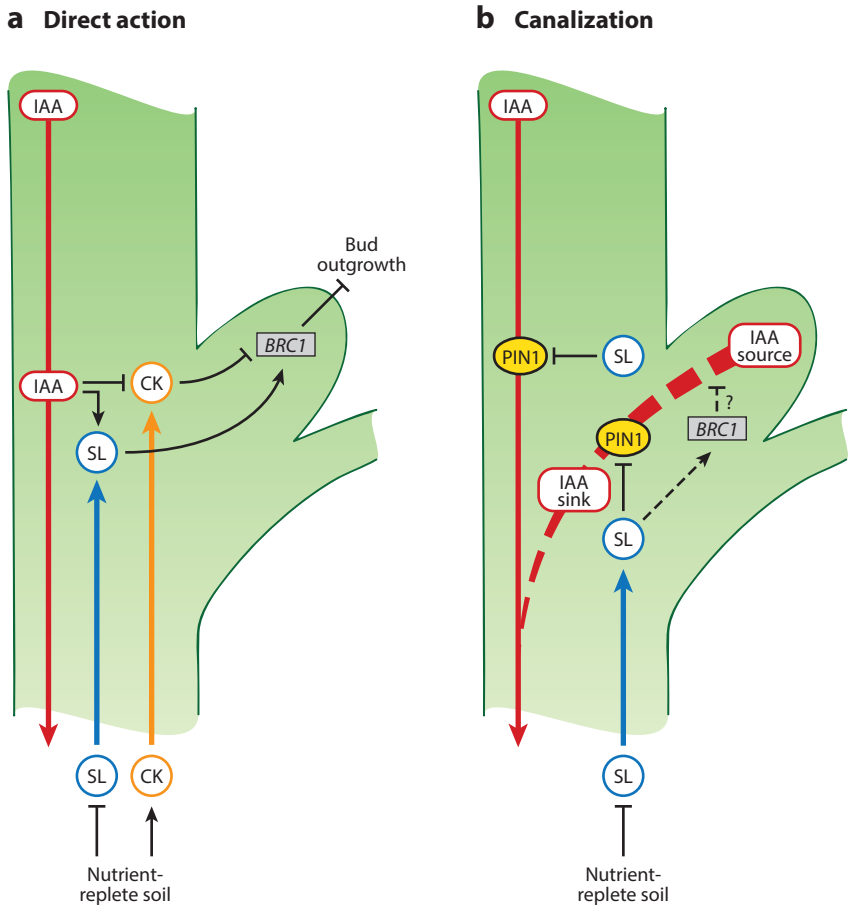


Figure 4

Two models for the regulation of shoot branching. (*a*) The direct-action model. In this model, root-derived SLs and CK promote and inhibit, respectively, the expression of transcription factors that regulate bud outgrowth, such as *BRC1*. Local levels of auxin (IAA) may affect the activity and/or production of SLs and CK in nearby tissues, thus modulating hormonal activity in the bud. (*b*) The canalization model. In this model, bud outgrowth relies on the capacity of the bud to export auxin from a high concentration (the source) toward a lower concentration in the main stem (the sink). Such coordinated export requires the subcellular and supracellular mobilization of the PIN1 transporter. SLs from the root inhibit PIN1 activity both in the bud and in the main stem, thus affecting both source and sink strength and reducing the capacity of the bud for auxin export. In some species, SL may also affect bud outgrowth via the promotion of *BRC1* expression (*dashed lines*). In both models—which are not mutually exclusive—the availability of soil nutrients influences the production of hormones produced in the root, providing a mechanism for coordinating relative shoot and root growth. Abbreviations: *BRC1*, *BRANCHED1*; CK, cytokinin; IAA, indole-3-acetic acid; PIN1, PIN-FORMED1; SL, strigolactone.

but it is thought to be driven by the self-organizing behavior of members of the PIN family of auxin efflux carriers, which can somehow acquire supracellular patterns of subcellular localization (12).

Under the canalization model, SLs increase competition between buds by promoting removal of PIN1 from the plasma membrane across the shoot system in both stems and buds. This theoretically results in reduced sink strength in the stem, reducing the number of buds that can

grow while making it harder for each bud to export its auxin (139). Physiological and genetic data support regulation of PIN1 by SLs: *rac*-GR24 reduces PIN1 levels at the basal plasma membrane of xylem parenchyma cells in as little as 30 min, and SL-biosynthesis, *d14*, and *max2* mutants have increased PIN1 accumulation at the plasma membrane (15, 16, 43, 152). In a *max2* background, loss of SMXL6, -7, and -8 completely suppresses PIN1 accumulation. Conversely, stabilization of SMLX7 is sufficient to increase PIN1 levels in the stem (107, 155). However, the relevance of the SL effect on PIN1 for shoot branching regulation has been controversial. Physiological experiments have provided evidence both for and against the relevance of this effect (16, 31, 32, 43, 152). For instance, Brewer et al. (32) argue against the importance of this effect because *rac*-GR24 can inhibit bud outgrowth even in plants with strongly inhibited auxin transport. Although striking, this observation is nevertheless difficult to interpret because canalization depends on relative differences in auxin transport. Genetic tools that allow specific reduction of PIN1 levels in the stem—thus avoiding pleiotropic effects—or that block the SL effect on PIN1 levels could be key to resolving the importance of the SL effect on PIN1 and auxin transport in shoot branching responses.

The poor mechanistic understanding of the canalization model has hampered experiments to test it, yielding ambiguous data that both support and contradict it (13, 32, 152). This has led to a polarizing debate over the last decade about the regulation of shoot branching and the role of SLs therein. However, it is important to recognize that these models are not mutually exclusive. Given the available data, a hybrid model in which both direct and canalization-like effects regulate branching deserves consideration. Understanding how SLs and SMXLs regulate BRC1 and PIN1 in eudicots will be essential next steps to resolve this issue. Shoot branching is a complex process, and it would not be surprising if multiple interacting regulatory mechanisms have aggregated over its long evolutionary history. Indeed, significant insights into the mechanism of SL action could likely be gained by understanding how SL regulation of branching has evolved across the land plants.

Strigolactone Signaling in the Regulation of Root Architecture

In addition to their roles in the shoot, SLs have been implicated in the control of root system architecture. The best evidence comes from rice SL-biosynthesis (*d10*, *d17*, and *d27*) and SL-perception (*d3* and *d14*) mutants, which have considerably shorter crown roots and fewer root meristem cells than wild-type plants (8). Furthermore, a barley *d14* mutant has shorter seminal roots and increased lateral root density (115). Unambiguous effects of SL on *Arabidopsis* root development, which have been identified through analysis of SL-biosynthesis mutants, are currently limited to a mild stimulation of primary root growth and meristem cell number and suppression of adventitious rooting (141, 143).

Other SL roles in *Arabidopsis* are less clear, because many experiments have tested only the effects of *max2* and *rac*-GR24. Treatment with *rac*-GR24 causes a significant MAX2-dependent inhibition of lateral root development in *Arabidopsis*; consistent with this, *max2* mutants have strongly increased lateral root density (97, 143). Recent work has suggested that the effects of MAX2 and *rac*-GR24 are mediated at the lateral root priming and emergence stages (92). Suppression of lateral root formation by *rac*-GR24 can be restored in *max2* mutants by transgenic *MAX2* expression under the control of an endodermis-specific *SCARECROW* (*SCR*) promoter, which is consistent with the important role of the endodermis in lateral root initiation and emergence (104, 172). However, as discussed above, these experiments cannot discriminate the effects on root growth that are due to SL signaling from those that are due to KL signaling. *Arabidopsis* SL-biosynthesis mutants show either no change in lateral root density phenotype or a small

increase in density that is not comparable in magnitude to the effect of *max2* (97, 143). This suggests that the effects of *rac*-GR24 and MAX2 on lateral root density are not related solely to SL signaling, but root phenotypes of *Arabidopsis d14* and *kai2* remain to be reported.

Studies of downstream signaling components are intriguing in this respect. Loss of SMXL6, -7, and -8 completely suppresses the *max2* lateral root density phenotype, but loss of SMAX1 has no effect (155). These data suggest either that SL signaling is more significant for lateral root density than expected from SL-biosynthesis mutants or that SL-independent signaling can trigger degradation of SMXL6, -7, and -8 via MAX2. KAI2-mediated degradation of SMXL6, -7, and -8 in roots would contradict the close signaling associations between KAI2-SMAX1 and D14-SMXL6/7/8 that have been observed to date (155). Resolving these possibilities will require a more detailed examination of the physiological and genetic basis for the effects of SL and KL on lateral root development, including assessment of the contributions of *SMXL2* and other *SMXL* genes to root growth.

Kapulnik et al. (97, 98) have also suggested that SLs may promote root hair elongation, based on the ability of *rac*-GR24 to do so in *Arabidopsis* and tomato. However, SL-biosynthesis and *max2* mutants show no consistent effect on root hair length under published growth conditions (reviewed in 117). Thus, at the moment, it is not possible to consider this a bona fide effect of endogenous SLs on root development. Nevertheless, the root hair elongation response to exogenous *rac*-GR24 is *MAX2* dependent and is restored in *max2* mutants upon endodermis-specific *MAX2* expression, revealing a non-cell-autonomous function of MAX2 (104).

Downstream of core SL/KL signaling mechanisms, work has focused on changes in auxin distribution or homeostasis as a possible basis for root development responses to *max2* and *rac*-GR24, by analogy with events in the shoot. The effect of *rac*-GR24 on lateral root formation depends on auxin levels in the root: At low auxin concentrations, *rac*-GR24 inhibits lateral root formation, but at high auxin concentrations, it does the opposite (143). The simplest explanation for these effects is that there is an optimum auxin concentration for lateral root formation, and by altering auxin transport, *rac*-GR24 treatment can either move the root away from or toward that optimum, depending on ambient auxin levels (143). This is consistent with the observation that SL signaling plays a role in root system responses to phosphate starvation (8, 118, 143) that involve changes in root system sensitivity to auxin (136). Ruyter-Spira et al. (143) reported that a prolonged *rac*-GR24 treatment reduces PIN1, PIN3, and PIN7 levels in the root meristem, but a shorter treatment does not (152), implying that this is a long-term physiological response. Pandya-Kumar et al. (132) also suggested that PIN2 abundance in root epidermal cells may increase in response to *rac*-GR24 treatment.

More recently, the effects of *rac*-GR24 on lateral root density have been ascribed to a cytokinin-auxin feedback loop involving the cytokinin signaling components *ARABIDOPSIS HISTIDINE KINASE3* (*AHK3*), *ARABIDOPSIS RESPONSE REGULATOR1* (*ARR1*), and *ARR12* and the Aux/IAA gene *SHORT HYPOCOTYL2* (*SHY2*) (92), which is similar to the loop that controls the position of the transition zone in the primary root meristem (49). Jiang et al. (92) additionally suggested that transcriptional downregulation of *PIN1* in the mature root may account for the effect of *max2* and *rac*-GR24 on lateral root formation near the shoot-root junction. It is also notable that *rac*-GR24 and its purified constituent stereoisomers induce the accumulation of certain flavonols in *Arabidopsis* roots. This *rac*-GR24 response is *MAX2* dependent but can be activated through either *KAI2* or *D14* (174). The effects of flavonols are somewhat enigmatic, but Peer et al. (135) reported that they inhibit auxin transport in some tissues. Further work will be needed to cleanly delineate SL effects on roots from other MAX2-dependent signaling pathways and to understand the crosstalk between SLs and other hormones in the root.

STRIGOLACTONE SIGNALING IN SYMBIOTIC INTERACTIONS

SLs are important signals in symbiotic interactions of roots with AM fungi, which improve plant nutrient uptake (24). AM symbioses are widespread in the plant kingdom, but a few families, such as the Brassicaceae, have lost the ability to form them (29, 47). Hence, the role of SLs in AM symbiosis has been studied in the AM host plants tomato, petunia, and rice and in the legumes pea, *Lotus japonicus*, and *Medicago truncatula*, which also have the capacity for nodulation.

Arbuscular Mycorrhiza

To establish AM symbiosis, the fungus first attaches to the root surface with a hyphal structure called the hyphopodium. It then colonizes the cortical cells inside the roots by forming highly branched hyphal structures known as arbuscules, which release mineral nutrients, especially phosphate and nitrogen, to the host (78, 89). SL effects on AM fungi occur prior to root colonization. SLs exuded from roots into the rhizosphere induce a suite of fungal responses, such as spore germination, hyphal growth, hyphal branching, respiratory activity, mitosis, expression of effector genes, and release of chitotetraose and pentaose, which in turn trigger symbiotic responses in the plant (3, 18, 19, 71, 162). The AM fungus *Gigaspora margarita*, which displays a particularly conspicuous hyphal branching response, can be activated by a variety of SL molecules, but both the intact tricyclic lactone and the methylated D ring are required for a response. The enol-ether bond is less critical and can be replaced by alkoxy or imido esters (4). Carlactonoic acid also triggers hyphal branching, whereas carlactone is less active. Structure-function studies using different synthetic carlactone derivatives revealed that BC ring formation is dispensable (121). The fungal receptor for SLs must be highly sensitive to suitable SLs because hyphal branching can be induced by *rac*-GR24 concentrations as low as 10 nM (19). The receptor is not known, but it likely differs from the plant receptor because the structural requirements for SLs to induce hyphal branching are different from those to suppress shoot branching, at least in *G. margarita* (27, 76). Furthermore, the sequenced genome of another AM fungus, *Rhizophagus irregularis*, does not appear to contain clear homologs of either D14 or MAX2 (162). Although AM fungi are not amenable to classical genetics, GR24 inhibits growth and increases hyphal branching of the genetically tractable fungal pathogen *Botrytis cinerea*, which may open an avenue for identifying fungal SL receptors (11).

SLs are not essential for AM colonization, but their importance is illustrated by SL-biosynthesis and SL-exudation mutants of pea, tomato, petunia, and rice, which are colonized at much lower levels than wild-type plants (73, 79, 101, 103, 105, 173, 192). In petunia, SL exudation into the rhizosphere is mediated by the ABC transporter PLEIOTROPIC DRUG RESISTANCE1 (PDR1) (105). GFP fusions of PDR1 localize to the outer membrane of hypodermal passage cells (144). These cells lack Casparian strips and may therefore be particularly well suited for SL export. The localization of PDR1 in AM host species that lack a hypodermal Casparian strip awaits further investigation.

Several environmental factors influence SL production and interactions with AM fungi. Under conditions that favor AM colonization—such as phosphate and nitrogen starvation—the expression of SL-biosynthesis genes and *PDR1* increases, leading to increased SL exudation (66, 101, 105, 113, 171, 190, 191). In turn, an established AM symbiosis appears to reduce parasitic weed germination and thus probably the production of SLs, possibly because the fungus improves the plant's phosphate status (114, 160). In drought-stressed tomato, however, AM symbiosis triggers an increase in SL accumulation (142), suggesting an interaction of AM and abiotic stress signaling in the regulation of SL biosynthesis. A low red:far-red light ratio or mutation of the *PHYTOCHROME B* gene of *L. japonicus* reduces root colonization, expression of SL-biosynthesis genes, and SL

exudation (123). However, the direct contribution of SLs to the reduced AM colonization has not been examined.

Rice *d3* and pea *ramosus 4* (*rms4*) mutants display a strong reduction in AM colonization, despite having increased or normal SL exudation (66, 192). This observation is especially surprising, however, because rice *d14* mutants are colonized at even higher levels than wild-type plants, probably because of increased SL exudation (77, 192). It was recently found that the *d3*/*rms4* phenotype does not result from a lack of SL perception, but rather from defective KAI2 signaling. The rice *d14l* mutant blocks AM colonization prior to hyphopodium formation and lacks transcriptional responses to exudates of germinating fungal spores (77). This has the exciting implication that the spore exudates may contain ligands that activate KAI2/D14L, or that KAI2/D14L-mediated signaling indirectly regulates the ability of roots to respond to the exudates.

Nodulation

Legumes can form an additional root symbiosis with nitrogen-fixing rhizobia. Root nodule symbiosis evolved long after AM symbiosis and recruited some of the plant genetic components required for AM development (133). The hallmarks of root nodule symbiosis establishment include the development of nodules (which derive from plant cell divisions similar to those that occur in lateral root initiation) and the intracellular accommodation of rhizobia inside infection threads. Infection threads are subcellular structures that form in root hairs and guide bacteria through several cell layers before they are released into symbiosomes (131). SL-biosynthesis genes are transcriptionally activated in root hairs containing infection threads and in nodule primordia of *M. truncatula*, suggesting that SL production increases during bacterial infection and several stages of nodule development (30, 171).

However, the role of SLs in nodulation is still unclear because of conflicting data and the focus on quantifying only nodule numbers instead of including other features of the symbiosis. For example, RNA interference of *D27* in *M. truncatula* hairy roots has no effect on nodulation despite strong suppression of *D27* transcripts (171). However, compared with the phenotypes of other SL-deficient mutants, the shoot branching phenotypes of rice and *Arabidopsis d27* mutants are relatively weak, perhaps because of nonenzymatic isomerization of β-carotene (108, 177). If SL biosynthesis can partially bypass D27, then this might account for the lack of effect of *D27* suppression on nodulation in *M. truncatula*. Supporting a role for SLs in promoting nodulation, a pea *rms1*/*ccd8* mutant has reduced nodulation that is restored to wild-type nodule numbers by *rac*-GR24 application (64, 65). Furthermore, application of low *rac*-GR24 concentrations to wild-type *M. truncatula* increases nodulation (44). However, higher *rac*-GR24 concentrations reduce nodule numbers; this is likely due to activation of ethylene biosynthesis, which suppresses nodulation, because nodulation of the ethylene-insensitive *sickle* (*skl*) mutant is resistant to GR24 (44). To further complicate matters, the *rms4*/*max2* mutant in pea has more nodules than wild-type plants, although they are smaller (66). Therefore, multiple MAX2-dependent pathways may contribute to different aspects of rhizobial symbiosis.

EVOLUTION OF STRIGOLACTONE SIGNALING PATHWAYS

Distribution of Strigolactone Production

The biosynthesis of SLs is evolutionarily ancient. Algae within the Charales, a sister group of the land plants, produce detectable amounts of sorgolactone, and *Chara corallina* responds to exogenous GR24 with an increase in rhizoid elongation (48). Interestingly, other charophyte

algae—namely the Coleochaetales and the Zygnematales—do not produce any bioactive extracts or identifiable SLs, nor do they respond to exogenous SL treatments (48). Thus, SL production and response may provide an informative character that can help resolve the debate about the origins of land plants (25). Regardless, the production of SLs in streptophyte lineages prior to the emergence of AM symbioses on land suggests that the primary function of SLs was hormonal (**Figure 5**).

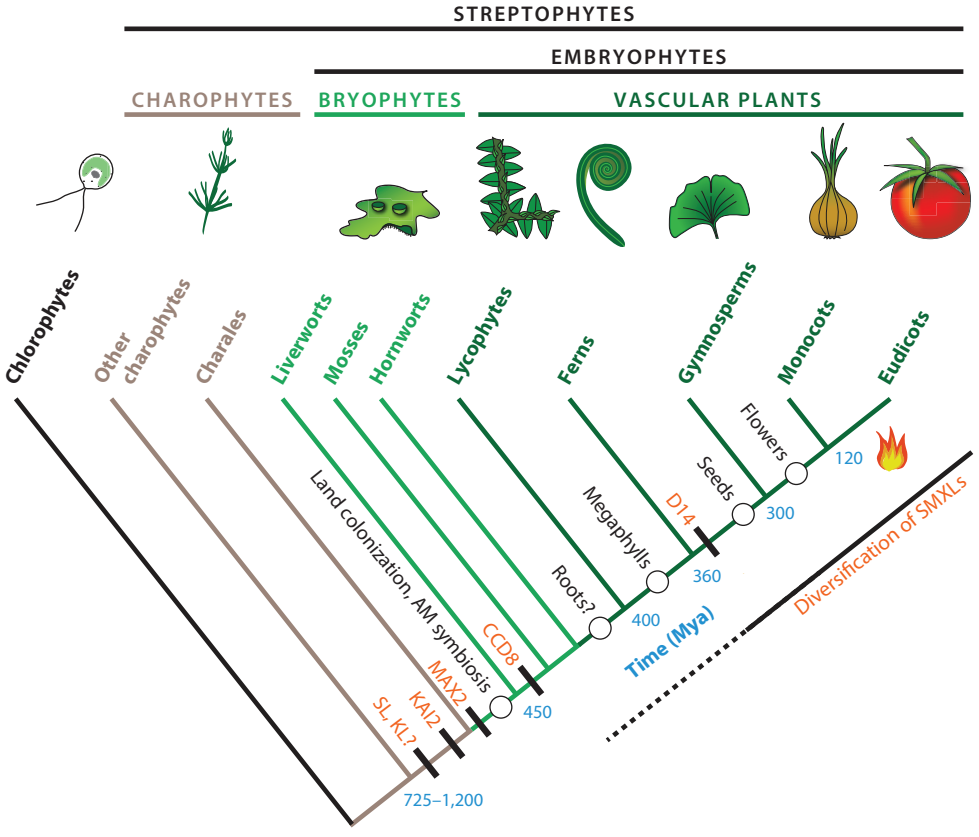


Figure 5

Evolutionary time line of SL-related signaling components and functions. Approximate time points are shown in blue; orange text indicates the emergence of key SL-related innovations. SLs are produced by members of the sister group of the land plants, the Charales, but not by other charophyte algae, suggesting that SLs originated 725–1,200 Mya, when the streptophytes emerged within the chlorophytes (48). Homologs of *KAI2*, but not of *MAX2*, in *Nitella* spp. (Charales) suggest that the SL receptor emerged at the same time as the SLs; however, the ligand specificity of such KAI2 homologs has not been established, and a KL may also have emerged at a similar time. The presence of SLs and the absence of a *CCD8* homolog in the liverwort *Marchantia polymorpha* suggest that basal land plants have a noncanonical pathway for SL production (48). *D14* likely emerged via gene duplication in the ancestor of seed plants (48, 178). Coupled with an expansion in the SMXL family, this provided opportunities for neofunctionalization, such as the ability to recognize and respond to karrikins from fire, which became a prevalent feature during the Cretaceous, 65–145 Mya (82). Abbreviations: AM, arbuscular mycorrhiza; CCD8, CAROTENOID CLEAVAGE DIOXYGENASE8; D14, DWARF14; KAI2, KARRIKIN-INSENSITIVE2; KL, endogenous KAI2 ligand; MAX2, MORE AXILLARY GROWTH2; SL, strigolactone; SMXL, SMAX1-LIKE.

Among the basal land plants, both the liverwort *Marchantia polymorpha* and the moss *Physcomitrella patens* produce SLs, including orobanchol and 5-deoxystrigol (48, 138). Deletion of *CCD8* in *P. patens* induces SL deficiency and provides compelling evidence for a developmental function of SLs in moss (138). *Ppccd8* mutants have increased caulonemal cell elongation and fail to restrict growth toward neighboring colonies, suggesting that SLs suppress filament extension and thus overall plant size (83, 138). GR24 treatment rescues these defects, supporting the conclusion that SL biosynthesis and perception have a hormonal role in basal land plants. Curiously, deletion of *Ppccd8* does not abolish all measurable SLs, as 7-oxoorobanchol acetate and strigol levels are unaltered (138). Coupled with the lack of a clear *CCD8* ortholog in *Marchantia* or *Nitella*, this finding suggests an evolutionarily ancient, *CCD8*-independent route for SL biosynthesis (48). There is no evidence for such a pathway in angiosperms, as SLs are not detectable in rice or pea *ccd8* mutants (73, 170).

Origins of Signaling Components

D14 orthologs have been found only in seed plant genomes (48, 178), raising an obvious question: If basal land plants synthesize and respond to SLs but do not have a D14 protein, what is the receptor? All land plants and at least one *Nitella* species contain at least one *KAI2* ortholog (48, 178), suggesting that *D14* evolved from a *KAI2* paralog in the ancestral lineage leading to seed plants (**Figure 5**). Consistent with this interpretation, a *KAI2* ortholog from the lycophyte *Selaginella moellendorffii*, *SmKAI2a*, can complement some phenotypes of *Arabidopsis kai2* but not those of *d14* (180). Because *SmKAI2a* does not confer responses to KARs, carlactone, or GR24 stereoisomers, and several *KAI2*-dependent functions in *Arabidopsis* and rice do not involve SLs, it is possible that a KL is present in both *Arabidopsis* and basal land plants (41, 96, 178). This finding further implies that the developmental functions of KAI2 proteins predate those of D14.

Given this evolutionary history, a KAI2 homolog is a reasonable candidate for an SL receptor in early land plants. *P. patens* has 11 putatively functional *KAI2* homologs that form two distinct clades (48, 112, 178). The first clade comprises proteins with a ligand-binding pocket predicted to be similar to that of AtKAI2. These proteins probably do not recognize KARs, because *P. patens* does not have detectable growth responses to KAR_1 (83). The second clade comprises proteins with larger ligand-binding pockets that could potentially accommodate an SL molecule (112). Reverse genetic analysis is needed to determine their function.

MAX2, which is essential for both SL and KAR/KL signaling in angiosperms, has not yet been identified in algal genomes but is present in *P. patens* (38, 45, 48). The distribution of SMXL proteins among land plants is much less well understood, in part because most of the SMXL sequence is poorly conserved. The *P. patens* genome contains at least three *SMXL* homologs (198). Thus, *MAX2* and *SMXL* may have evolved after SL-biosynthesis and *KAI2* genes had emerged, suggesting a MAX2-independent SL signaling system may be present in Charales. A role for *MAX2* and *SMXL* in SL signaling in extant bryophytes has not been established, but these genes may constitute an SL signaling mechanism similar to that in angiosperms. The recruitment of *MAX2* and *SMXL* into the SL signaling process might have coincided with the evolution of more complex plant structures or as a means of interacting with land microbes. Nevertheless, the diversity of *SMXL* genes in sampled angiosperms suggests an evolutionary trend of expansion in the *SMXL* family, potentially corresponding to an increase in signaling outputs and functions.

Strigolactone Receptor Evolution in Parasitic Weeds

Several species of obligate root parasites in the Orobanchaceae family are weeds that cause extensive crop losses throughout sub-Saharan Africa, Asia, and the Mediterranean. Witchweeds

(*Striga* spp.), broomrapes (*Orobanche* and *Phelipanche* spp.), and *Alectra* spp. can cause complete crop failure and are estimated to affect 100 million smallholder farmers in sub-Saharan Africa alone. These weeds spread easily, and infestations are difficult to eliminate owing to the plants' high fecundity, small seed size, and long seed dormancy, which can last for over a decade. Because of their small seed size, the emerging obligate parasite seedlings have limited nutrient stores and must attach to a host within several days of germination to survive (17, 183). Consequently, a key adaptation of obligate parasitic weeds is the ability to delay germination until they detect a nearby host root, which they do by sensing chemicals present in root exudates, such as SLs. Parasite germination has served as a highly sensitive bioassay for host-derived germination stimulants, enabling the first identification of SLs (42). In addition to the ~20 known canonical SLs, related host-derived compounds, such as dehydrocostus lactone, heliolactone, avenaol, and peagol, can activate germination of some parasites (56, 93, 99, 168).

Because most studies of the Orobanchaceae have focused on agricultural weeds, comparatively little is known about other obligate parasites in this family. However, several examples of highly specific host-triggered germination responses have been described, and some weeds show at least general preferences for a stereochemical class of SLs (57, 60). *Striga gesnerioides*, for example, has positive germination responses to a few orobanchol-type SLs and is inhibited by strigol-type SLs (130). Host compatibility is determined by the ability of the parasite to penetrate the host root and maintain a viable haustorial attachment, which may be possible only with a limited set of species (188, 193). Variation in the types and amounts of each SL exuded by different species may have provided a basis for evolutionary selection of parasites with selective germination responses to a compatible host's chemical fingerprint. In turn, selective pressures from parasite or fungal interactions may have driven diversification of SL profiles in hosts.

How parasite seeds sense SLs has been a long-standing mystery, but the recent characterization of the SL signaling pathway in model species laid a foundation to solve this problem. In several parasitic weed genomes, *KAI2/HTL* has undergone extensive gene duplication, whereas *D14* appears to be maintained as a single gene copy. *KAI2/HTL* paralogs in the Orobanchaceae can be divided into three groups that have undergone different rates of evolutionary selection. One *KAI2* clade (*KAI2d*) is present only in parasites, contains most of the *KAI2* paralogs in a parasite genome, and has undergone the fastest rate of evolution (40). Moreover, KAI2d proteins are predicted or known to have enlarged ligand-binding pockets that are substantially different from those of AtKAI2. At least eight *KAI2d* transgenes from two parasitic species confer SL-specific germination responses to *Arabidopsis kai2* mutants (40, 163). Biochemical characterization of KAI2d/HTL from *S. hermonthica* indicated that these proteins can hydrolyze SLs and have diversified SL affinities (167). Therefore, KAI2d likely provides the basis for detection of host-derived SLs (**Figure 6**). A profluorescent SL analog, Yoshimulactone Green Double (YLGW), has been used to investigate the spatiotemporal dynamics of SL perception in parasite seeds. SLs are putatively recognized in two phases: A transient initial burst of YLGW hydrolysis in the radicle tip is followed by a sustained period of hydrolysis in the elongating root (167).

These discoveries demonstrate that SL perception can evolve through neofunctionalization of KAI2 paralogs; independent occurrences of this evolutionary process may explain the origins of D14 in seed-bearing plants and SL perception in moss (40, 112). Intriguingly, representatives from the other two groups of *KAI2* paralogs in parasites and nonparasitic relatives in the Lamiales appear to perceive altogether different ligands. Functional assays with transgenic *Arabidopsis* have suggested that highly conserved KAI2c proteins may recognize KL, whereas less conserved KAI2i proteins recognize KARs (40, 41, 186). If *Arabidopsis KAI2*, which has a capacity for both KL and KAR responses, is representative of angiosperm *KAI2*, then these paralogs evolved

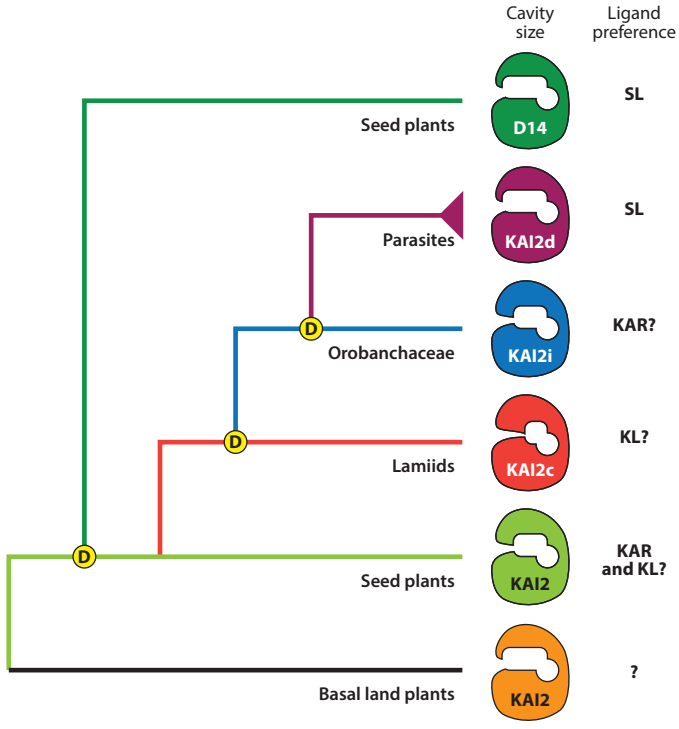


Figure 6

Model for KAI2 evolution in root parasites. Prior to the emergence of seed plants, a gene duplication event (D) gave rise to D14 and KAI2 proteins with different ligand preferences and developmental functions. A probable duplication of *KAI2* in the lamiid lineage gave rise to two clades, KAI2c and KAI2i. Further extensive duplication of KAI2i in parasitic species of the Orobanchaceae yielded the divergent KAI2d clade. Homology modeling and transgenic complementation experiments indicate that members of KAI2c, KAI2i, and KAI2d clades have differing ligand-binding cavity sizes that correlate with a change in ligand preference (40). Most pertinently, KAI2d has converged upon D14 to perceive SLs (40, 163, 167). It is also possible that KAI2c, which has a relatively small cavity compared with KAI2i and the ancestral KAI2 found in non-lamiid seed plants, has retained a specific function to perceive KL (40, 41). Abbreviations: D14, DWARF14; KAI2, KARRIKIN-INSENSITIVE2; KAR, karrikin; KL, endogenous KAI2 ligand; SL, strigolactone.

subfunctionalized ligand preferences following *KAI2* duplication. It will be interesting to determine whether a similar pattern of *KAI2* evolution independently led to KAR-responsive germination in diverse fire-following species.

TRANSLATIONAL APPLICATIONS FOR PARASITIC WEED CONTROL

Parasitic weed control is a high-value target for many researchers in the SL field. As for other pests, achieving lasting, effective control will likely require an integrated management program that incorporates multiple techniques (59). The substantial recent progress in understanding how SLs are made, exported, and recognized has laid a foundation for translational work to reduce crop losses to parasites. Many groups have pursued chemical approaches to the problem, through the development of SL antagonists, or mimics that trigger suicidal germination (84, 124, 149, 164). Below, we propose several biological routes that might be taken.

Stealth Crops

One way to reduce parasite attack is to reduce the amount of SLs exuded into the rhizosphere that can trigger parasite germination. This may be accomplished through breeding or selection for low SL emitters, which would be limited by the genetic diversity in available germplasms, or through targeted mutagenesis of genes involved in SL biosynthesis or transport. Low SL production has shown promise for preattachment resistance to parasites in rice, maize, fava bean, pea, and tomato (51, 58, 88, 134, 189). A potential weakness to this approach is that SL levels that are too low can have undesirable effects on agronomic traits (see **Figure 2**) or AM symbiosis, which may reduce the net benefits of parasite resistance (74, 103). A more difficult but sophisticated approach for targeted mutagenesis could involve introducing regulatory mutations that eliminate expression in select tissues (e.g., preventing expression of *PDR1* orthologs in hypodermal passage cells) or mutations of SL signaling components that enhance sensitivity to endogenous SLs, potentially preserving SL responses while triggering feedback inhibition of SL biosynthesis. Improved knowledge of how SL biosynthesis is transcriptionally regulated and influenced by mineral nutrient deprivation will also provide new avenues for selectively modulating SL levels.

An alternative to reducing SL exudation is to switch SL types. Even parasitic weeds with a broad host range, such as *S. hermonthica*, can exhibit orders-of-magnitude differences in sensitivity to different SLs as germination stimulants. *S. hermonthica* prefers strigol-type SLs (e.g., 5-deoxystrigol and strigol) over orobanchol-type SLs (e.g., 4-deoxyorobanchol and orobanchol), whereas *S. gesnerioides* has the opposite preferences (36, 130, 167). Zhang et al. (194) proposed that the carlactone oxidase in rice determines the B-C ring stereochemistry of SLs; therefore, it may be possible to convert SL production in sorghum from strigol-type SLs to orobanchol-type SLs by introducing carlactone oxidase from rice, making it less detectable by *S. hermonthica*. As additional enzymes are identified that are responsible for diversification of SL structures (e.g., hydroxylation of the ABC ring), it may become possible to use breeding or overexpression to shunt SL biosynthesis into less stable SLs or SLs that are not as readily recognized by parasites (36, 87). The utility of this approach will depend on the standing variation for SL recognition in parasitic weed populations and whether it has unanticipated impacts on associations with AM fungi.

Trap Crops

Parasites may respond to germination cues from plants that they are unable to establish or maintain attachments to following germination. These nonhosts can be used as trap crops that trigger suicidal germination of parasites and work well as rotation crops or as intercrops with susceptible crops (59). The added benefit of trap crops is that the parasite seed bank can be gradually depleted. It may be possible to create more effective trap crops by reversing the strategies described above—that is, by increasing SL production and exudation or switching to more active SL types. This biological approach could provide an alternative to chemical SL agonists that is more practical for low-income, smallholder farmers with limited access to agricultural infrastructure.

Gene Drive

A more controversial option made possible by the emergence of CRISPR/Cas9 genome-editing technology is the development of an RNA-guided gene drive to create male bias (55). In this scenario, a gene required for female reproduction (e.g., carpel or ovule formation) would be

targeted for editing by CRISPR/Cas9. Several highly conserved sites within the gene would need to be simultaneously targeted to reduce the possibility of resistance arising in the population, and the construct would be designed to integrate the CRISPR/Cas9 cassette at the target locus through homology-directed repair. A similar mutagenic chain reaction was highly successful in *Drosophila* (69). However, this approach can work only for outcrossing parasite species and would still take many generations to cause a population crash, a period that would be further extended by the longevity of the parasite seed bank and any inefficiencies in the frequency of homology-directed repair. Obvious ethical and ecological implications would also need to be considered (55).

OUTSTANDING QUESTIONS

Many important mysteries of SL signaling remain to be solved. For example, the functions of SMXL proteins remain unknown, as does the chain of downstream events that lead to physiological changes. Identifying the direct downstream targets of SMXLs—whether they are genetic loci or proteins—will be essential. Current data suggest that specific interactions occur between KAI2, D14, and different clades of the SMXL family, and the molecular basis of such pairings and the degree to which the interactions are flexible will give insights into the evolutionary history of the mirrored KAI2 and D14 signaling mechanisms. Comprehensive investigations are needed to determine which *MAX2*-dependent growth responses are truly SL regulated and to define the developmental roles of each *SMXL* gene. The mechanistic basis by which *KAI2* and *MAX2* facilitate AM symbiosis is unclear but will be vital for understanding a symbiotic interaction of major agricultural significance. Finally, the identification of KAI2 ligands—whether endogenous or perhaps derived from AM fungi—could unlock the function of the ancestral pathway that gave rise to SL signaling.

SUMMARY POINTS

1. Strigolactones (SLs) are carotenoid-derived plant hormones that regulate diverse aspects of development, such as shoot branching and host-symbiont interactions.
2. D14, a receptor protein with enzymatic activity, hydrolyzes the SL ligand and changes shape to transduce the hormone signal.
3. Perception of SLs triggers proteolytic degradation of the SMXL/D53 family of growth-regulating proteins, which ultimately brings about physiological change.
4. There are two broad models of how SL inhibits shoot branching: the direct-action model and the canalization model. Neither model is completely resolved.
5. Under nutrient deprivation, increased exudation of SL from host roots enhances symbiosis with arbuscular mycorrhizal fungi. However, SLs are not essential for colonization by these fungi.
6. Despite important differences in the SL perception mechanisms of basal land plants and seed-bearing plants, SLs are present in basal land plants and most likely originated in charophyte algae.
7. Rapidly improving knowledge of SLs is inspiring new ways to translate basic research on SLs into beneficial agricultural outcomes.

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