1 **Strigolactones, super hormones in the fight against *Striga***

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Abstract

Strigolactones are plant hormones that control diverse aspects of plant growth, but are also exuded into soil as recruitment signals for arbuscular mycorrhizal fungi interactions. Highly damaging parasitic weeds in the Orobanchaceae family have coopted strigolactones as germination cues that indicate the presence of a host. Recent studies have established how strigolactones are actively transported within and out of plants. Key components of the strigolactone signaling system have been identified, including strigolactone receptors in angiosperms and parasites, as well as downstream targets that are polyubiquitinated and proteolyzed following strigolactone perception. The basis for protein-protein interactions among these signaling components has recently been explored. We propose several strategies to translate basic research on strigolactone transport and signaling into parasite control methods.

Introduction

Strigolactones (SLs) were first discovered in cotton root exudates as germination stimulants of *Striga lutea*, a root parasite more commonly known as witchweed [1]. *Striga* spp. and other parasitic weeds in the Orobanchaceae family cause substantial crop losses in sub-Saharan Africa, the Mediterranean, and Asia each year. Cost-effective strategies to limit the spread and detrimental effects of these weeds are much needed and continue to be the focus of intensive research. In 2008, it was shown that SLs also function as internally mobile signals that regulate branching [2,3], and they are now recognized as hormones with diverse roles in plant growth regulation (reviewed in [4]). Since then significant progress has been made toward understanding how SLs are produced[4], transported, and perceived (reviewed in [4];[5,6]). Here we highlight some of the most recent findings in SL biology and discuss their implications for parasitic weed control.

SL transport – “Up, up and away!”

Even before SLs were identified, grafting studies demonstrated that SLs could move from roots into shoots to inhibit shoot branching ([7], reviewed in [8]). SLs are also exuded from roots into the rhizosphere, promoting hyphal branching and symbiotic interactions with arbuscular mycorrhizal fungi (AMF) [9,10]. AMF supply inorganic nutrients, especially phosphate, to the host plants. Accordingly, SL biosynthesis and secretion into the soil is upregulated in a number of species in response to phosphate and/or nitrogen deficiency [11-13]. How SLs move upward and outward has been an important mystery. SLs have been reported in the xylem sap of *Arabidopsis* and tomato [12,14], but this finding is controversial as others have been unable to detect SLs in *Arabidopsis* or in the xylem sap of several species [15,16]. Exogenous SLs move slowly from roots into shoots [16], suggesting active cell-to-cell transport rather than movement through the xylem stream [6].

Reasoning that ATP-binding cassette (ABC) transporters have been implicated in plant hormone movement, and that a SL transporter gene may be expressed in roots and upregulated by phosphate-deficiency, Kretschmar *et al* identified the cellular SL

exporter PLEIOTROPIC DRUG RESISTANCE 1 (PDR1) through a candidate gene approach in petunia [17]. The *pdr1* mutant has decreased SL exudation from roots, causing reduced AMF hyphal branching and colonization, and reduced germination of the parasitic weed *Phelipanche ramosa*. *PDR1* is expressed in tissues adjacent to dormant buds, in root cortex cells (overlapping with the SL biosynthesis gene *CCD8/DAD1*), and in the unsubsized hypodermal passage cells (HPCs) where hyphal entry occurs [17,18]. Intriguingly, *PDR1* is asymmetrically localized in the cell, but its orientation is cell type-specific; *PDR1* is apically localized in undifferentiated hypodermal cells in the root tip, but is localized to the outer lateral side of HPCs in differentiated roots [18]. Together, these findings indicate that *PDR1* mediates cell-to-cell SL transport in plants and SL exudation into soil (Figure 1).

SL perception – “My strigol sense is tingling.”

The discoveries of SL-insensitive mutants with high tillering/branching phenotypes in rice, petunia, and *Arabidopsis* were instrumental to identifying the SL receptor *DWARF14(D14)/DECREASED APICAL DOMINANCE2(DAD2)* in angiosperms [19-21]. *D14* is a member of the α/β -hydrolase fold superfamily, which also includes the gibberellin receptor *GID1*. *D14* catalyzes cleavage of SLs, and the biosynthetically related compound methyl carlactonoate, at a conserved enol-ether bridge that links a butenolide moiety to the rest of the molecule [15,20,22,23]. Hydrolysis of SL is slow (~ 0.3 molecules min^{-1} for rice *D14*), but is required for SL signal transduction, as *D14/DAD2* proteins harboring mutations in the conserved Ser-His-Asp catalytic triad do not rescue *d14/dad2* mutants [20,22]. The byproducts of SL hydrolysis are not bioactive branching regulators, however, implying that *D14* is not simply an enzyme, but instead undergoes structural changes during SL binding or hydrolysis that enable signal transduction [20]. Indeed the loss-of-function allele *d14-2/seto5* affects an external surface residue that is unlikely to affect either the active site or structural stability of *D14* [24]. Further supporting the idea of allosteric regulation, SL enhances protein-protein interactions between *D14* and its signaling partners *D3/MAX2* and *D53/SMXL6/7/8* (see below). However, a comparison of crystal structures of *D14* in complex with SL or SL byproducts does not reveal any obvious changes during ligand binding or hydrolysis

[25]. Interestingly, D14 does undergo thermal destabilization in the presence of SL; this response requires an active catalytic site, and D14 destabilization is further enhanced by SL-induced interaction with D3/MAX2 [20,25,26].

A close paralog of *D14* in *Arabidopsis*, *KARRIKIN INSENSITIVE2 (KAI2)/HYPOSENSITIVE TO LIGHT (HTL)* acts as a receptor for karrikins [21,27,28]. Karrikins (KAR) are a class of butenolide compounds found in smoke that activate seed germination of many species (although not parasitic weeds in the Orobanchaceae family) [29,30]. *Arabidopsis kai2* mutants have enhanced seed dormancy and decreased responses to light in seedlings, phenotypes that are opposite to KAR growth responses [21]. In *Arabidopsis*, a species from habitats that are not considered fire-prone, KARs may fortuitously serve as analogs of an undiscovered endogenous ligand of KAI2 [30]. *Arabidopsis* KAI2 is not thought to be involved in SL perception, as *kai2* mutants have different phenotypes than SL-deficient mutants and a *KAI2* transgene does not rescue *d14*, even when expressed under control of a *D14* promoter [26]. KAI2 does confer seed and some seedling responses to the synthetic SL analog GR24, but genetic and biochemical studies have shown that this is primarily due to a stereoisomer commonly present in GR24 preparations that has a conformation not found in natural SLs [26,31-33]. Others have shown that AtKAI2 has negligible binding or hydrolytic activity on GR24 *in vitro* [22,34]. Based on these data and the logical argument that SLs would likely be a detrimental germination stimulant for autotrophic plants (i.e. SLs would trigger growth under competitive conditions), it is often postulated that KAI2 is not a SL receptor in most angiosperms. Aside from a recent study that shows RNAi-mediated suppression of D14-like/KAI2 in rice does not reduce GR24 responses in the mesocotyl, however, this idea remains untested [35].

Despite strong sequence and structural similarities, *D14* and *KAI2* have different roles in plant growth in addition to different ligand specificities [21,33,34,36]. For example, *D14* controls shoot branching and leaf senescence, whereas *KAI2* regulates seed germination, seedling growth, and the ability to form AMF interactions [21,35,37-39]. This raises obvious questions about how these receptors have evolved different

functions. Phylogenetic analyses suggest that *D14* evolved as a SL receptor in angiosperms from an ancient *KAI2* duplication. SLs are produced and perceived in basal land plants extending back to the Charophyte lineage [21,40-42]. It remains unclear whether basal land plants use a similar SL signaling system to angiosperms; one hypothesis is that *KAI2*-like proteins function as SL receptors [43]. Regardless, the discoveries made in model systems have been instrumental to understanding host perception in parasites.

As obligate parasites, *Striga* spp. cannot survive longer than several days after germination without a host. Therefore its success as a weed hinges upon decades-long physiological seed dormancy that can be broken by detecting trace amounts of SL from nearby host roots, similar to other weeds in the Orobanchaceae family. Low-coverage shotgun genome sequencing and *de novo* transcriptome assemblies were used to survey *D14* and *KAI2* genes in several parasitic Orobanchaceae species [44]. Parasite genomes contain one *D14* gene, as do most plants, but most have a significant increase in *KAI2/HTL* copy number relative to their non-parasitic relatives in the Lamiids [44]. As gene duplication can enable the evolution of new protein functions, *KAI2* was an attractive candidate for further analysis. Most of the *KAI2* paralogs were phylogenetically grouped into a parasite-specific, “divergent” clade (*KAI2d*) that has undergone faster evolution than other *KAI2* paralogs found in parasites and Lamiids. Homology modeling predicts that *KAI2d* have significantly enlarged ligand-binding pockets that are more similar to *D14* than non-parasitic *KAI2* [44]. *KAI2d* class genes from *Striga hermonthica* (ShHTL4 to ShHTL11) bind and hydrolyze natural SLs and SL analogs *in vitro* [45]. Moreover, several of these genes and two *KAI2d* from the parasite *Phelipanche aegyptiaca* confer SL-specific germination responses to *Arabidopsis kai2* mutants [44,46]. Therefore *KAI2d/HTL* proteins are SL receptors in parasitic plants that are likely to mediate host perception in seeds (Figure 1).

A crystal structure of ShHTL5 confirms an enlarged ligand-binding pocket in this SL receptor [46]. Several amino acids surrounding the ligand-binding pocket that are typically conserved have undergone extensive substitutions in *KAI2d* proteins; it

remains to be determined which residues contribute to SL-specificity [44,46]. The presence of multiple *KAI2d* in parasitic weed genomes and evidence for different SL specificities among *KAI2d*/ShHTL supports the hypothesis that *KAI2d* diversification reflects antagonistic co-evolution between hosts and parasites and may influence host range. Where and when SLs are perceived in parasite seed remains an open question, but intriguing clues are provided by the development of a SL analog, Yoshimulactone Green, which becomes fluorescent after hydrolysis. This reporter molecule suggests that *in vivo* perception of SL occurs in the radicle tip of *Striga* in two temporally separated waves [45].

SL targets – “Experimentation on mutants. It’s not unheard of.”

The current SL signaling model is analogous to other phytohormone signaling mechanisms, in particular those of gibberellin, auxin, and jasmonate, which feature ligand-activated degradation of proteins that repress hormone responses (reviewed in [5]). Both SL and KAR signaling require the F-box protein MORE AXILLARY GROWTH 2 (*MAX2*)/*DWARF3* (*D3*)/*RAMOSUS4* (*RMS4*) [2,3,47]. F-box proteins act as adapter components of Skp1-Cullin-F-box (SCF) ubiquitin E3 ligase complexes that confer specificity for the polyubiquitination of protein substrates, which are subsequently degraded by the 26S proteasome. Like *d14*, *max2* mutants display a highly branched phenotype that cannot be suppressed by exogenous SL addition or grafting of wildtype roots (reviewed in [8]). Genetic and biochemical studies in *Arabidopsis* and rice have revealed the targets of SL-induced, *MAX2*-*D14*-dependent protein degradation to be members of the SUPPRESSOR OF *MAX2* 1-LIKE (*SMXL*)/*DWARF53* (*D53*) family (Figure 2). This family was discovered independently through a screen for genetic suppressors of *max2* phenotypes at seed and seedling stages in *Arabidopsis*, and through characterization of a dominant, SL-insensitive *d53* mutant in rice that mimics *d3* tillering phenotypes [48-50]. *D53* and its orthologs in *Arabidopsis*, *SMXL6*, *SMXL7*, and *SMXL8*, are rapidly degraded following SL perception in a *D3*/*MAX2*- and *D14*-dependent manner [49-52]. The *d53* mutant protein lacks a highly conserved C-terminal motif and is no longer proteolyzed following SL perception; combined loss-of-function

mutations in *SMXL6,7,8* suppress the increased branching of *max2* and SL-deficient *ccd7/max3* mutants, as well as other *d14*-associated phenotypes. In contrast, *smax1* and *smx2* mutations suppress *kai2*-associated phenotypes of *max2*, producing constitutive KAR growth responses in seed, seedlings, and leaves [48,53]. This suggests that different D14 and KAI2 functions are mediated through negative regulation of different clades of the SMXL/D53 family.

SL promotes association of D14 with D3/MAX2 and D53/SMXL6/7/8 proteins [20,25,49,50,54]. Formation of a tripartite D3-D14-D53 complex following SL perception is anticipated, but this has not yet been demonstrated. As D14 undergoes destabilization in the presence of SL, an intriguing point of speculation is that D53 proteins – which are distantly related to the heat shock protein HSP101 – may have higher affinity for D14 in a disordered state. Residues that contribute to D14-D53/SMXL6/7/8 interactions have not been determined, but several candidate specificity-determining residues that distinguish D14 and KAI2 have been suggested from bioinformatic analysis [24]. The basis for D14-D3/MAX2 interactions, however, has recently been studied in depth through extensive site-directed mutagenesis of *D14* [25]. At least eight sites on the surface of D14 are critical for *in vitro* interactions with D3 [25], one of which is in close proximity to the Pro169 residue affected in the *Arabidopsis d14-2/seto5* mutant [24]. Intriguingly, several of these residues are well-conserved in KAI2 proteins, implying they may have functional significance for KAI2 interactions (Figure 3). However, KAI2-MAX2 interactions have not yet been demonstrated, and were not detected after GR24 treatment in *in vitro* assays [25]. Nonetheless we note that three *KAI2d/ShHTL* transgenes from *Striga hermonthica* (two of which show biochemical evidence to be SL receptors [45]), which were nonfunctional in *Arabidopsis* [44,46], have potentially disruptive amino acid substitutions at two or more of the eight positions. This could explain why these genes were inactive in *Arabidopsis*. It also implies that a second MAX2 paralog, which has coevolved with these *KAI2d* genes, may be present in the *Striga hermonthica* genome. Similar cross-species complementation assays were attempted to assess the function of KAI2 homologs in the basal land plants *Selaginella moellendorffii* and *Marchantia polymorpha* [26]. Only one gene was partially functional

in *Arabidopsis*, although others showed evidence of *in vitro* hydrolase activity, suggesting a lack of compatible interactions with *Arabidopsis* MAX2 or SMAX1/SMXL proteins.

How SMXL proteins function and are regulated is still largely unknown. A popular hypothesis is that SMXL regulates gene expression via interactions with the TOPLESS (TPL) family of transcriptional co-repressors, similar to AUX/IAA and JAZ proteins in auxin and jasmonate signaling, respectively (reviewed in [5]). SMXL proteins have a highly conserved EAR motif that is necessary for TPL interactions [50-52], but the significance of this motif for SMXL function *in vivo* has not been evaluated. Also similar to the AUX/IAA and JAZ proteins, it will be useful to identify the motif(s) of SMXL proteins that enable ligand-activated degradation. Such degrons can be used to develop degradation-based biosensors of hormone action *in vivo*, enabling dynamic and quantitative analysis of SL responses as has been done for auxin and jasmonate [55-57].

Parasitic weed control – “Knowing is half the battle.”

A broad range of strategies for controlling parasitic weeds have been explored, but the search for practical, cost-effective solutions continues (reviewed in [58]). So how can discoveries of SL transport and perception be applied to this problem? One strategy is to reduce parasite germination stimulants in the rhizosphere by either limiting SL exudation or SL production. *PDR1* and SL biosynthesis genes are therefore potential targets whose activity could be modified in crops through RNA-guided gene editing or selective breeding. Loss-of-function mutations in these genes can reduce parasite germination, but this benefit may be countered by pleiotropic effects on plant growth or AMF association that decrease crop yields. It may be possible to counter negative effects of low endogenous SLs on crop growth by modifying the affinity of D14 for SL, MAX2, or SMXL proteins, or by modifying the stability of select SMXL proteins. The SL reduction strategy has shown promise for some species, such as faba bean and rice [59,60], but negative impacts on agronomic traits may limit its utility for maize and tomato [14,61]. Modifying SL composition rather than abundance may prove a more

258 viable alternative [62,63]. A highly sensitive biological reporter of SLs has been
259 developed based on germination of *Arabidopsis* carrying a *ShHTL7* transgene. This
260 could provide an inexpensive new tool for breeding low SL-emitting crops to labs that
261 cannot work directly with noxious pests due to safety regulations [46].

262
263 Conversely, tricking parasites into germinating in the absence of a host, also known as
264 suicidal germination, is an attractive strategy because it depletes the parasite seed
265 bank. This may be accomplished through biological or chemical means. Trap crops that
266 emit SLs but cannot be parasitized are already used in intercropping or rotation [58]. It
267 may be possible to make trap crops more effective by increasing SL emission. The
268 development of chemical stimulants of parasite germination, such as SL analogs, that
269 are practical for field use has faced challenges of synthetic complexity and cost, as well
270 as compound instability in soil. However, some compounds have recently shown
271 promising success in field trials [64,65]. The identification of SL receptors in parasites
272 unlocks the door for high-throughput chemical screens, similar to one that was
273 performed with *Arabidopsis* KAI2, to identify additional parasite germination agonists
274 [66]. To conclude, with continuing advances in the field of SL biology, the scourge of
275 witchweed promises to become a much more manageable problem.

276 277 **Acknowledgements**

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References

References and recommended reading

- of special interest
- of outstanding interest

1. Cook CE, Whichard LP, Turner B, Wall ME, Egley GH: Germination of Witchweed (Striga lutea Lour.): Isolation and Properties of a Potent Stimulant. *Science* 1966, 154:1189-1190.
2. Gomez-Roldan V, Fermas S, Brewer PB, Puech-Pages V, Dun EA, Pillot JP, Letisse F, Matusova R, Danoun S, Portais JC, et al.: Strigolactone inhibition of shoot branching. *Nature* 2008, 455:189-194.
3. Umehara M, Hanada A, Yoshida S, Akiyama K, Arite T, Takeda-Kamiya N, Magome H, Kamiya Y, Shirasu K, Yoneyama K, et al.: Inhibition of shoot branching by new terpenoid plant hormones. *Nature* 2008, 455:195-200.
4. Al-Babili S, Bouwmeester HJ: Strigolactones, a Novel Carotenoid-Derived Plant Hormone. *Annual Review of Plant Biology*, Vol 66 2015, 66:161-186.
5. Morffy N, Faure L, Nelson DC: Smoke and Hormone Mirrors: Action and Evolution of Karrikin and Strigolactone Signaling. *Trends Genet* 2016, 32:176-188.
6. Borghi L, Liu GW, Emonet A, Kretschmar T, Martinoia E: The importance of strigolactone transport regulation for symbiotic signaling and shoot branching. *Planta* 2016, 243:1351-1360.
7. Foo E, Turnbull CG, Beveridge CA: Long-distance signaling and the control of branching in the rms1 mutant of pea. *Plant Physiol* 2001, 126:203-209.
8. Domagalska MA, Leyser O: Signal integration in the control of shoot branching. *Nature reviews. Molecular cell biology* 2011, 12:211-221.

9. Akiyama K, Matsuzaki K, Hayashi H: Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* 2005, 435:824-827.
 10. Besserer A, Puech-Pages V, Kiefer P, Gomez-Roldan V, Jauneau A, Roy S, Portais JC, Roux C, Becard G, Sejalón-Delmas N: Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria. *PLoS Biol* 2006, 4:e226.
 11. Lopez-Raez JA, Charnikhova T, Gomez-Roldan V, Matusova R, Kohlen W, De Vos R, Verstappen F, Puech-Pages V, Becard G, Mulder P, et al.: Tomato strigolactones are derived from carotenoids and their biosynthesis is promoted by phosphate starvation. *New Phytol* 2008, 178:863-874.
 12. Kohlen W, Charnikhova T, Liu Q, Bours R, Domagalska MA, Beguerie S, Verstappen F, Leyser O, Bouwmeester H, Ruyter-Spira C: Strigolactones are transported through the xylem and play a key role in shoot architectural response to phosphate deficiency in nonarbuscular mycorrhizal host *Arabidopsis*. *Plant physiol* 2011, 155:974-987.
 13. Yoneyama K, Xie X, Kim HI, Kisugi T, Nomura T, Sekimoto H, Yokota T: How do nitrogen and phosphorus deficiencies affect strigolactone production and exudation? *Planta* 2012, 235:1197-1207.
 14. Kohlen W, Charnikhova T, Lammers M, Pollina T, Toth P, Haider I, Pozo MJ, de Maagd RA, Ruyter-Spira C, Bouwmeester HJ, et al.: The tomato CAROTENOID CLEAVAGE DIOXYGENASE8 (SICCD8) regulates rhizosphere signaling, plant architecture and affects reproductive development through strigolactone biosynthesis. *New Phytol* 2012, 196:535-547.
 15. Abe S, Sado A, Tanaka K, Kisugi T, Asami K, Ota S, Kim HI, Yoneyama K, Xie X, Ohnishi T, et al.: Carlactone is converted to carlactonoic acid by MAX1 in *Arabidopsis* and its methyl ester can directly interact with AtD14 in vitro. *Proc Natl Acad Sci U S A* 2014, 111:18084-18089.
- This work provides new insights into the SL biosynthesis pathway, and shows that SL is not the only bioactive signal derived from the MAX pathway. The authors show that the SL precursor carlactone is converted to carlactonoic acid by the CYP450 MAX1. Its methyl ester, methyl carlactonoate, can interact directly with the SL receptor D14 and inhibit shoot branching in *Arabidopsis*.

- 338 16. Xie X, Yoneyama K, Kisugi T, Nomura T, Akiyama K, Asami T, Yoneyama K:
339 Strigolactones are transported from roots to shoots, although not through the
340 xylem. *Journal of Pesticide Science* 2015, 40:214-216.
- 341 17. Kretzschmar T, Kohlen W, Sasse J, Borghi L, Schlegel M, Bachelier JB, Reinhardt
342 D, Bours R, Bouwmeester HJ, Martinoia E: A petunia ABC protein controls
343 strigolactone-dependent symbiotic signalling and branching. *Nature* 2012,
344 483:341-344.
- 345 18. Sasse J, Simon S, Gubeli C, Liu GW, Cheng X, Frimi J, Bouwmeester H, Martinoia
346 E, Borghi L: Asymmetric Localizations of the ABC Transporter PaPDR1 Trace
347 Paths of Directional Strigolactone Transport. *Current Biology* 2015, 25:647-655.
- 348 •• This work shows how SLs are transported through plants. The authors demonstrate that the
349 petunia ABC transporter PDR1 is asymmetrically localized in different root tissues. Polar
350 localization of PDR1 mediates SL export up out of the root tip, either for lateral exudation to soil
351 or for inhibition of branching in the shoot.
- 352 19. Arite T, Umehara M, Ishikawa S, Hanada A, Maekawa M, Yamaguchi S, Kyojuka J:
353 d14, a strigolactone-insensitive mutant of rice, shows an accelerated outgrowth
354 of tillers. *Plant Cell Physiol* 2009, 50:1416-1424.
- 355 20. Hamiaux C, Drummond RS, Janssen BJ, Ledger SE, Cooney JM, Newcomb RD,
356 Snowden KC: DAD2 Is an alpha/beta Hydrolase likely to Be Involved in the
357 Perception of the Plant Branching Hormone, Strigolactone. *Current Biology* 2012,
358 22:2032-2036.
- 359 21. Waters MT, Nelson DC, Scaffidi A, Flematti GR, Sun YK, Dixon KW, Smith SM:
360 Specialisation within the DWARF14 protein family confers distinct responses to
361 karrikins and strigolactones in Arabidopsis. *Development* 2012, 139:1285-1295.
- 362 22. Zhao L, Zhou X, Wu Z, Yi W, Xu Y, Li S, Xu T, Liu Y, Chen R, Kovach A, et al.:
363 Crystal structures of two phytohormone signal-transducing α/β hydrolases:
364 karrikin-signaling KAI2 and strigolactone-signaling DWARF14. *Cell Research*
365 2013, 23:436-439.

- 366 23. Nakamura H, Xue YL, Miyakawa T, Hou F, Qin HM, Fukui K, Shi X, Ito E, Ito S, Park
367 SH, et al.: Molecular mechanism of strigolactone perception by DWARF14.
368 *Nature communications* 2013, 4:2613.
- 369 24. Chevalier F, Nieminen K, Sanchez-Ferrero JC, Rodriguez ML, Chagoyen M,
370 Hardtke CS, Cubas P: Strigolactone promotes degradation of DWARF14, an
371 alpha/beta hydrolase essential for strigolactone signaling in Arabidopsis. *Plant*
372 *Cell* 2014, 26:1134-1150.
- 373 • This work provides further support that DWARF14 (D14) functions as a SL receptor, with the
374 identification of a d14 mutant protein that has a defect in a surface residue. SL is shown to induce
375 degradation of D14 in a MAX2-dependent manner. This mechanism may limit the intensity and duration of
376 SL-signaling.
- 377 25. Zhao LH, Zhou XE, Yi W, Wu Z, Liu Y, Kang Y, Hou L, de Waal PW, Li S, Jiang Y,
378 et al.: Destabilization of strigolactone receptor DWARF14 by binding of ligand
379 and E3-ligase signaling effector DWARF3. *Cell Res* 2015, 25:1219-1236.
- 380 •• This paper provides several important insights into SL signaling. The authors obtained a missing
381 crystal structure of D14 in complex with an intact synthetic SL GR24 molecule. Contrary to prevailing
382 models, they show that D14 does not undergo structural changes during SL activation, but rather
383 undergoes destabilization that is enhanced by interaction with its signaling partner D3/MAX2. Extensive
384 mutagenesis defines D14 residues involved in SL binding and protein-protein interactions.
- 385 26. Waters MT, Scaffidi A, Moulin SL, Sun YK, Flematti GR, Smith SM: A *Selaginella*
386 *moellendorffii* Ortholog of KARRIKIN INSENSITIVE2 Functions in Arabidopsis
387 Development but Cannot Mediate Responses to Karrikins or Strigolactones.
388 *Plant Cell* 2015, 27:1925-1944.
- 389 • This work shows that KAI2 homologs in basal land plants can hydrolyze SL analogs *in vitro*.
390 Surprisingly, the authors do not find evidence that *Arabidopsis* KAI2 undergoes thermal destabilization in
391 the presence of karrikins. Partial rescue of *Arabidopsis kai2* phenotypes with a lycophyte *KAI2* suggests
392 a conserved, unknown endogenous ligand for KAI2 proteins.
- 393 27. Sun XD, Ni M: HYPOSENSITIVE TO LIGHT, an alpha/beta fold protein, acts
394 downstream of ELONGATED HYPOCOTYL 5 to regulate seedling de-etiolation.
395 *Mol Plant* 2011, 4:116-126.

- 396 28. Guo Y, Zheng Z, La Clair J, Chory J, Noel J: Smoke-derived karrikin perception by
397 the α/β -hydrolase KAI2 from Arabidopsis. *Proc Natl Acad Sci U S A* 2013.
- 398 29. Nelson DC, Flematti GR, Ghisalberti EL, Dixon KW, Smith SM: Regulation of Seed
399 Germination and Seedling Growth by Chemical Signals from Burning Vegetation.
400 *Annual Review of Plant Biology* 2012, 63:107-130.
- 401 30. Conn CE, Nelson DC: Evidence that KARRIKIN-INSENSITIVE2 (KAI2) receptors
402 may perceive an unknown signal that is not karrikin or strigolactone. *Frontiers in*
403 *Plant Science* 2015, 6.
- 404 31. Flematti GR, Scaffidi A, Waters MT, Smith SM: Stereospecificity in strigolactone
405 biosynthesis and perception. *Planta* 2016, 243:1361-1373.
- 406 32. Waters MT, Scaffidi A, Flematti G, Smith SM: Substrate-Induced Degradation of the
407 α/β -Fold Hydrolase KARRIKIN INSENSITIVE2 Requires a Functional
408 Catalytic Triad but Is Independent of MAX2. *Mol Plant* 2015, 8:814-817.
- 409 33. Scaffidi A, Waters MT, Sun YK, Skelton BW, Dixon KW, Ghisalberti EL, Flematti
410 GR, Smith SM: Strigolactone Hormones and Their Stereoisomers Signal through
411 Two Related Receptor Proteins to Induce Different Physiological Responses in
412 Arabidopsis. *Plant Physiol* 2014, 165:1221-1232.
- 413 34. Kagiya M, Hirano Y, Mori T, Kim SY, Kyojuka J, Seto Y, Yamaguchi S,
414 Hakoshima T: Structures of D14 and D14L in the strigolactone and karrikin
415 signaling pathways. *Genes to Cells* 2013, 18:147-160.
- 416 35. Kameoka H, Kyojuka J: Downregulation of rice DWARF 14 LIKE suppress
417 mesocotyl elongation via a strigolactone independent pathway in the dark. *J*
418 *Genet Genomics* 2015, 42:119-124.
- 419 36. Bythell-Douglas R, Waters MT, Scaffidi A, Flematti GR, Smith SM, Bond CS: The
420 structure of the karrikin-insensitive protein (KAI2) in Arabidopsis thaliana. *PLoS*
421 *One* 2013, 8:e54758.
- 422 37. Ueda H, Kusaba M: Strigolactone Regulates Leaf Senescence in Concert with
423 Ethylene in Arabidopsis. *Plant Physiol* 2015, 169:138-147.

- This report shows clear and compelling evidence for crosstalk between SL and ethylene, helping to explain the delayed senescence phenotype of *ore9*, the first mutant identified in *MAX2*.
38. Yamada Y, Furusawa S, Nagasaka S, Shimomura K, Yamaguchi S, Umehara M: Strigolactone signaling regulates rice leaf senescence in response to a phosphate deficiency. *Planta* 2014, 240:399-408.
 39. Gutjahr C, Gobbato E, Choi J, Riemann M, Johnston MG, Summers W, Carbonnel S, Mansfield C, Yang SY, Nadal M, et al.: Rice perception of symbiotic arbuscular mycorrhizal fungi requires the karrikin receptor complex. *Science* 2015, 350:1521-1524.
 - The authors report the first *kai2* mutant in a monocot. A surprising new role for KAI2 in mediating interactions with AMF is uncovered. Although SL is important for efficiently recruiting AMF interactions, KAI2 appears to be needed for receiving signals from germinating AMF spores or establishing competence for AMF invasion.
 40. Proust H, Hoffmann B, Xie X, Yoneyama K, Schaefer DG, Nogue F, Rameau C: Strigolactones regulate protonema branching and act as a quorum sensing-like signal in the moss *Physcomitrella patens*. *Development* 2011, 138:1531-1539.
 41. Delaux PM, Xie X, Timme RE, Puech-Pages V, Dunand C, Lecompte E, Delwiche CF, Yoneyama K, Becard G, Sejalón-Delmas N: Origin of strigolactones in the green lineage. *New Phytol* 2012, 195:857-871.
 42. Hoffmann B, Proust H, Belcram K, Labruno C, Boyer FD, Rameau C, Bonhomme S: Strigolactones inhibit caulonema elongation and cell division in the moss *Physcomitrella patens*. *PloS one* 2014, 9:e99206.
 43. Lopez-Obando M, Conn CE, Hoffmann B, Bythell-Douglas R, Nelson DC, Rameau C, Bonhomme S: Structural modelling and transcriptional responses highlight a clade of PpKAI2-LIKE genes as candidate receptors for strigolactones in *Physcomitrella patens*. *Planta* 2016.
 44. Conn CE, Bythell-Douglas R, Neumann D, Yoshida S, Whittington B, Westwood JH, Shirasu K, Bond CS, Dyer KA, Nelson DC: PLANT EVOLUTION. Convergent evolution of strigolactone perception enabled host detection in parasitic plants. *Science* 2015, 349:540-543.

- This is one of a trio of papers that establish KAI2 paralogs as SL receptors in parasite seed. The authors combine molecular evolutionary analyses, structural modeling, and functional tests through cross-species complementation to establish the basis for SL perception. They find evidence that *KAI2* genes in the Orobanchaceae acquired SL receptor functionality, convergent with D14.
45. Tsuchiya Y, Yoshimura M, Sato Y, Kuwata K, Toh S, Holbrook-Smith D, Zhang H, McCourt P, Itami K, Kinoshita T, et al.: PARASITIC PLANTS. Probing strigolactone receptors in *Striga hermonthica* with fluorescence. *Science* 2015, 349:864-868.
- The authors designed a SL-like molecule that generates green fluorescence when cleaved by a SL receptor. This fluorescent turn-on probe was used to characterize the affinities of HTL/KAI2 proteins in *Striga* for different SL variants, providing compelling biochemical evidence that these are SL receptors. Live imaging of the probe's hydrolysis by *Striga* seeds suggested two waves of SL signal perception.
46. Toh S, Holbrook-Smith D, Stogios PJ, Onopriyenko O, Lumba S, Tsuchiya Y, Savchenko A, McCourt P: Structure-function analysis identifies highly sensitive strigolactone receptors in *Striga*. *Science* 2015, 350:203-207.
- This research reports functional characterization of several SL receptors from *Striga* through cross-species complementation in *Arabidopsis*. The authors also solve the crystal structure of a SL receptor in *Striga*, which shows a substantially enlarged ligand-binding pocket. One *Striga* HTL/KAI2 transgene confers very sensitive responses to SL in *Arabidopsis* seed.
47. Nelson DC, Scaffidi A, Dun EA, Waters MT, Flematti GR, Dixon KW, Beveridge CA, Ghisalberti EL, Smith SM: F-box protein MAX2 has dual roles in karrikin and strigolactone signaling in *Arabidopsis thaliana*. *Proc Natl Acad Sci U S A* 2011, 108:8897-8902.
48. Stanga JP, Smith SM, Briggs WR, Nelson DC: SUPPRESSOR OF MORE AXILLARY GROWTH2 1 controls seed germination and seedling development in *Arabidopsis*. *Plant Physiol* 2013, 163:318-330.
49. Zhou F, Lin Q, Zhu L, Ren Y, Zhou K, Shabek N, Wu F, Mao H, Dong W, Gan L, et al.: D14-SCF(D3)-dependent degradation of D53 regulates strigolactone signalling. *Nature* 2013, 504:406-410.

- 484 50. Jiang L, Liu X, Xiong G, Liu H, Chen F, Wang L, Meng X, Liu G, Yu H, Yuan Y, et
485 al.: DWARF 53 acts as a repressor of strigolactone signalling in rice. *Nature*
486 2013, 504:401-405.
- 487 51. Soundappan I, Bennett T, Morffy N, Liang Y, Stanga JP, Abbas A, Leyser O, Nelson
488 DC: SMAX1-LIKE/D53 Family Members Enable Distinct MAX2-Dependent
489 Responses to Strigolactones and Karrikins in Arabidopsis. *Plant Cell* 2015.
- 490 •• One of a pair of recent reports showing that SL regulates shoot development in *Arabidopsis* via
491 degradation of the DWARF53-Like proteins SMXL6, 7, and 8. The *smxl6,7,8* triple mutant
492 suppresses several SL related phenotypes in *max2*, including auxin transport, PIN1
493 accumulation, *BRC1* expression, and root branching phenotypes. A division of labor between
494 SMXL clades in karrikin and SL signaling is proposed.
- 495 52. Wang L, Wang B, Jiang L, Liu X, Li X, Lu Z, Meng X, Wang Y, Smith SM, Li J:
496 Strigolactone Signaling in Arabidopsis Regulates Shoot Development by
497 Targeting D53-Like SMXL Repressor Proteins for Ubiquitination and
498 Degradation. *Plant Cell* 2015.
- 499 •• One of a pair of recent reports showing that *SMXL6, 7, and 8* are *D53* orthologs in *Arabidopsis*.
500 The authors find that the *smxl6,7,8* triple mutant suppresses the shoot branching phenotype of
501 SL-deficient and *max2* mutants. Extensive biochemical analysis demonstrates SMXL
502 ubiquitination and degradation. Evidence is provided that SMXL can suppress gene expression
503 via TOPLESS interactions in a GAL4-UAS system.
- 504 53. Stanga JP, Morffy N, Nelson DC: Functional redundancy in the control of seedling
505 growth by the karrikin signaling pathway. *Planta* 2016.
- 506 54. Umehara M, Cao M, Akiyama K, Akatsu T, Seto Y, Hanada A, Li W, Takeda-Kamiya
507 N, Morimoto Y, Yamaguchi S: Structural Requirements of Strigolactones for
508 Shoot Branching Inhibition in Rice and Arabidopsis. *Plant Cell Physiol* 2015,
509 56:1059-1072.
- 510 55. Larrieu A, Champion A, Legrand J, Lavenus J, Mast D, Brunoud G, Oh J,
511 Guyomarc'h S, Pizot M, Farmer EE, et al.: A fluorescent hormone biosensor
512 reveals the dynamics of jasmonate signalling in plants. *Nat Commun* 2015,
513 6:6043.

- 514 56. Brunoud G, Wells DM, Oliva M, Larrieu A, Mirabet V, Burrow AH, Beeckman T,
515 Kepinski S, Traas J, Bennett MJ, et al.: A novel sensor to map auxin response
516 and distribution at high spatio-temporal resolution. *Nature* 2012, 482:103-106.
- 517 57. Wend S, Dal Bosco C, Kampf MM, Ren F, Palme K, Weber W, Dovzhenko A,
518 Zurbriggen MD: A quantitative ratiometric sensor for time-resolved analysis of
519 auxin dynamics. *Sci Rep* 2013, 3:2052.
- 520 58. Fernandez-Aparicio M, Reboud X, Gibot-Leclerc S: Broomrape Weeds.
521 Underground Mechanisms of Parasitism and Associated Strategies for their
522 Control: A Review. *Front Plant Sci* 2016, 7:135.
- 523 • This is an excellent, comprehensive review of many strategies currently in development for
524 controlling parasitic weeds, and will be especially useful to the reader interested in translational
525 research.
- 526 59. Fernandez-Aparicio M, Kisugi T, Xie X, Rubiales D, Yoneyama K: Low strigolactone
527 root exudation: a novel mechanism of broomrape (*Orobanche* and *Phelipanche*
528 spp.) resistance available for faba bean breeding. *Journal of agricultural and food*
529 *chemistry* 2014, 62:7063-7071.
- 530 60. Jamil M, Rodenburg J, Charnikhova T, Bouwmeester HJ: Pre-attachment *Striga*
531 *hermonthica* resistance of New Rice for Africa (NERICA) cultivars based on low
532 strigolactone production. *New Phytol* 2011, 192:964-975.
- 533 61. Guan JC, Koch KE, Suzuki M, Wu S, Latshaw S, Petruff T, Goulet C, Klee HJ,
534 McCarty DR: Diverse roles of strigolactone signaling in maize architecture and
535 the uncoupling of a branching-specific subnetwork. *Plant Physiol* 2012,
536 160:1303-1317.
- 537 62. Yoneyama K, Arakawa R, Ishimoto K, Kim HI, Kisugi T, Xie X, Nomura T, Kanampiu
538 F, Yokota T, Ezawa T, et al.: Difference in *Striga*-susceptibility is reflected in
539 strigolactone secretion profile, but not in compatibility and host preference in
540 arbuscular mycorrhizal symbiosis in two maize cultivars. *New Phytol* 2015,
541 206:983-989.

- 542 63. Cardoso C, Charnikhova T, Jamil M, Delaux PM, Verstappen F, Amini M,
543 Laouressergues D, Ruyter-Spira C, Bouwmeester H: Differential activity of *Striga*
544 *hermonthica* seed germination stimulants and *Gigaspora rosea* hyphal branching
545 factors in rice and their contribution to underground communication. *PloS one*
546 2014, 9:e104201.
- 547 64. Samejima H, Babiker AG, Takikawa H, Sasaki M, Sugimoto Y: Practicality of the
548 suicidal germination approach for controlling *Striga hermonthica*. *Pest Manag Sci*
549 2016.
- 550 65. Zwanenburg B, Mwakaboko AS, Kannan C: Perspective of suicidal germination for
551 parasitic weeds control. *Pest Manag Sci* 2016.
- 552 66. Toh S, Holbrook-Smith D, Stokes ME, Tsuchiya Y, McCourt P: Detection of parasitic
553 plant suicide germination compounds using a high-throughput *Arabidopsis*
554 HTL/KAI2 strigolactone perception system. *Chemistry & biology* 2014, 21:988-
555 998.
- 556 67. Yang T, Zhang L, Hao H, Zhang P, Zhu H, Cheng W, Wang Y, Wang X, Wang C:
557 Nuclear-localized AtHSPR links abscisic acid-dependent salt tolerance and
558 antioxidant defense in *Arabidopsis*. *Plant J* 2015, 84:1274-1294.
- 559 68. Crooks GE, Hon G, Chandonia JM, Brenner SE: WebLogo: a sequence logo
560 generator. *Genome research* 2004, 14:1188-1190.

Figure Legends

Figure 1. SL export into the rhizosphere

Diagram of a root cross-section showing the strigolactone transporter PDR1 (green) localized to the outer lateral side of hypodermal passage cells (HPCs). HPCs (white) are not suberized (gray), enabling hyphal penetration by arbuscular mycorrhizal fungi (AMF). Strigolactones in the rhizosphere promote hyphal branching of AMF and germination of root parasitic plants such as *Striga* spp. SLs are recognized in parasite seed by KAI2d/HTL proteins. It is unknown how AMF perceive SLs. en, endodermis; ic, inner cortex; oc, outer cortex; hy, hypodermis; ep, epidermis.

Figure 2. Simplified phylogeny of the SMXL/D53 family

Diagram is based upon a previously published phylogeny [49]. SMXL family members from *Arabidopsis* (circles) and rice (squares) are shown. SMXL are divided into four clades that were present before the divergence of dicots and monocots. To date, growth control by SMAX1 and SMXL2 (red) is associated with KAI2 signaling and KAR responses, whereas growth control by the D53 and SMXL6,7,8 clade (blue) is associated with D14 signaling and SL responses (reviewed in [5]). SMXL4/AtHSPR has been implicated in stress responses [67]. It is unclear whether SMXL3, SMXL4, and SMXL5 are regulated by MAX2, as they lack a C-terminal RGKT motif that is necessary for SL-induced degradation of D53, SMXL6, and SMXL7 [49-52].

Figure 3. Critical residues for D14 interactions with D3/MAX2

Crystal structure of OsD14 in complex with GR24 (PDB 5DJ5). This structure lacks a 50 aa leader sequence found in rice D14 but not dicot D14. The affected residue in *d14-2/seto5*, a loss-of-function P169L substitution, is marked in purple [24]. Residues marked in white or yellow were identified as critical for in vitro interactions between D14 and D3/MAX2 [25]. Residues indicated in yellow, or bold below, are well conserved at equivalent positions in 17 *KAI2* transgenes that are functional in *Arabidopsis thaliana* (*AtKAI2*, *PaKAI2c*, *PaKAI2d1*, *PaKAI2d3*, *ShKAI2c*, *ShKAI2i*, *ShKAI2d1*, *ShHTL1*, *ShHTL9*, *SmKAI2a*), but are not conserved in three transgenes that are not functional in

592 *Arabidopsis* (*ShKAI2d2*, *ShHTL10*, *ShHTL11*) [26,44,46]. These substitutions might
593 affect KAI2 binding to MAX2 and therefore their function in *Arabidopsis*. Frequency plots
594 of KAI2 amino acid composition at each positional equivalent to D14 are shown [68].
595 *Striga hermonthica*, Sh; *Phelipanche aegyptiaca*, Pa; *Selaginella moellendorffii*, Sm.
596

Figure 1

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Figure 2

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Figure 14

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