Strigolactones (TTPB15) – Teaching Guide

Overview – Strigolactones (SLs) are multifunctional hormones that contribute to the control of shoot branching, and serve as signals between plants and arbuscular mycorrhizal fungi and between host plants and parasitic Striga plants. They are produced in minute quantities and so studying strigolactones has been difficult. Strigolactone research has benefited from the identification of pea, Arabidopsis and rice mutants affected in SL synthesis or signaling. Because they contribute to beneficial symbiotic interactions with mycorrhizal fungi, as well as detrimental interactions with parasitic plants, understanding how SLs are produced and act is a high priority particularly in tropical regions where Striga parasites are endemic. Progress in the past decade has been rapid, but we have much still to learn about this fascinating family of hormones.

Learning objectives
By the end of this lecture the student should be able to:
- Distinguish between a hormone, pheromone, quormone, and allelochemical
- Summarize the three seemingly independent research threads that converged on SLs
- Compare and contrast the phenotypes characteristic of SL-deficient mutants and SL-insensitive mutants
- Interpret phylogenetic studies of the family of SL receptor proteins
- Compare the proposed model for SL perception with those for other hormones
- Evaluate various approaches to the management of parasitic Striga

Study / exam questions (understanding and comprehension)
- What are three of the different processes that strigolactones are involved in?
- True or False: Arbuscular mycorrhizal fungi symbiosis is restricted to legumes and their closest relatives.
- Which of the following experiment contributed evidence that strigolactones are derived from carotenoids?
  - Carotenoids were shown to promote branching of arbuscular mycorrhizal hyphae
  - Carotenoid-deficient pea plants were shown to have decreased shoot branching
  - Carotenoid-deficient corn plants were shown to be deficient in production of the Striga germination promoter
  - Carotenoid-deficient Striga plants were shown to be germination-deficient
- Striga plants are obligate parasites, in part because of their unusual root morphology. How do Striga roots differ from other plant roots?
- The max mutants of Arabidopsis and the rms mutants of pea have been important tools in SL research. What do max and rms stand for?
- Which two are thought to contribute to plant fitness under phosphate-limited conditions?
  - Decreased synthesis of SL under low phosphate conditions prevents Striga seeds from germinating
  - Increased synthesis of SL under low phosphate conditions promotes mycorrhizal symbiosis and nutrient uptake
  - Increased synthesis of SL under low phosphate conditions restricts shoot branching, freeing up energy for root growth
  - Decreased synthesis of SL under low phosphate conditions promotes root branching to facilitate nutrient uptake.

www.plantcell.org/cgi/doi/10.1105/tpc.111.tt0411
**Discussion questions (engagement and connections)**

The figure to the left shows the results of grafts between wild-type (WT) and mutant *Arabidopsis* plants.

Based on these results, which gene, *MAX1* or *MAX2*, is likely to be involved in SL biosynthesis? **Explain** your answer.

Which gene is likely to be involved in SL perception or signaling? **Explain** your answer.

What phenotype might you predict in a plant formed from a graft of *max2* as the scion (top) and *max1* as the stock (bottom)? **Explain**.

What phenotype might you predict in plant formed from a graft of *max1* as the scion (top) and *max2* as the stock (bottom)? **Explain**.

- Compare and contrast the mechanisms for perception of auxin, jasmonates and gibberellins with our current understanding of the interactions between SLs and karrikins, DAD2/D14, and MAX2.

- Hamiaux et al (2012) ([http://www.cell.com/current-biology/retrieve/pii/S0960982212009359](http://www.cell.com/current-biology/retrieve/pii/S0960982212009359)) show that DAD2’s hydrolytic activity is important for its function. What are some of the possible explanations for this requirement? Propose two different models to explain this requirement, and describe how you would test them. Compare the explanations you came up with those discussed by the paper’s authors.

- How would you investigate the mode of perception of SLs in AM fungi? Propose two different experimental approaches.

- Companion cropping or co-cropping, such as planting rows of *Desmodium* between rows of corn, can dramatically restrict the incidence of *Striga* parasitism. How does co-cropping, and particularly *Desmodium*, confer protection from *Striga*?

- Strigolactone-deficient moss fail to show a colony-size sensitivity to colony density. This observation has led to the suggestion that SLs act like quorum sensing molecules, or quormones. Find another example of a quormone and describe how it functions. In the paper by Proust et al (2011) ([http://dev.biologists.org/content/138/8/1531.full](http://dev.biologists.org/content/138/8/1531.full)), how do the authors differentiate between SL effects within an organism versus its effects as an inter-organismal signal?
**Lecture synopsis**

**Introduction (1 - 10)**
Strigolactones are a family of small molecules that were first characterized as agents that promote germination of the seeds of tiny parasitic plants of the *Striga* genus. Much later, they were shown to promote branching of arbuscular mycorrhizal (AM) fungi, and to be involved in the regulation of shoot branching. These three lines of research thus converged, and current investigations are filling in the gaps of strigolactone synthesis and perception, and their roles as endogenous hormones, and exogenous, intra- and inter-kingdom signals.

**Synthesis and transport (11 – 24)**
Strigolactones were first purified from cotton roots in 1966 and the chemical structure was determined in 1972. Strigolactones are a small family of compounds derived from 5-deoxystrigol. A synthetic analogue, GR24, is used widely. Strigolactones are derivatives of the carotenoid pathway. Several mutants in SL biosynthesis have been identified in plants such as pea, *Arabidopsis*, rice and petunia. These mutants were identified based on the SL-deficient phenotype of increased shoot branching. Analysis of these mutants revealed three enzymes (D27, CCD7 and CCD8) that act sequentially in the plastid to produce carlactone, which itself has some SL function, and which is thought to be subject to further modification in the cytoplasm by a cytochrome P450 encoded by the MAX1 gene. The abnormal phenotypes of SL-deficient plants can be rescued by exogenous SLs. Grafting studies show that the shoot phenotype can be rescued by SLs produced in the roots, and that the mobile signal, which may include carlactone, is downstream of CCD8. An ABC transporter protein called PDR1 has been identified that seems to be required for SL exudation from roots; *pdr1* mutants show a reduced stimulation of parasitic seed germination and colonization by AM fungi.

**Perception and signaling (25 – 35)**
Other mutants with increased shoot branching have been identified that are not sensitive to exogenous SLs. These SL-insensitive mutants have revealed something about the perception of the hormone in plants. The RMS4, D3, and MAX2 genes encode an F-box protein that is necessary for SL responses. The DAD2 and D14 genes encode an α/β fold hydrolase that has recently been shown to a) specifically bind to SLs, b) hydrolyze SLs, and c) bind to the MAX2 F-box protein in an SL-dependent manner. Collectively, these results suggest a model in which the hormone interacts with a specific binding protein (D14 / DAD2), and, through interaction with an F-box protein, promote the degradation of repressor proteins such as D53. This model resembles those for auxin, jasmonate and gibberellin signaling. Interestingly, *max2* mutants are also insensitive to the action of karrikins, which are small molecules present in smoke that promote germination in some species. The karrikins bind to KAI2 (also known as D14-LIKE), a protein closely related to DAD2/ D14. Thus, SLs and karrikins are perceived by very similar receptor-like proteins, which raises the question of which signal is ancestral? Finally, although we’re beginning to understand the mode of perception of SLs in plants, we don’t yet understand how AM fungi recognize and respond to SLs.

**Strigolactones and whole-plant processes (36 – 59)**
SLs have diverse functions in plants, that include promoting secondary growth, leaf senescence, root hair elongation and primary root growth, and inhibiting axillary bud outgrowth, adventitious root formation, in moss, colony growth and chloronemata branching. Some of these effects likely are mediated through interactions with auxin and other hormones. For example, in the shoot, SLs interfere with polar auxin transport, which interferes with bud outgrowth. These developmental responses are intimately connected to growth plasticity in response to environmental conditions. Under phosphate limited conditions, plants enhance root growth at the expense of shoot growth. These responses are mediated in part by SLs, and SL synthesis is elevated under low phosphate conditions. Although best characterized in vascular plants, new research shows that SLs are present in bryophytes and even in
some green algae. In moss, SLs act as a diffusible signal that may function as a quorum sensor (quormone) to regulate growth.

**Strigolactones promote branching in arbuscular mycorrhizal fungi (60 – 65)**

In 2005, SLs were shown to promote branching in AM fungi. Interestingly, SL production increases in low phosphate conditions, which are the conditions in which the plant benefits most from symbiosis with AM fungi. Thus, SLs enhance plant survival in low nutrient conditions both through effects on plant architecture, but also by the recruitment of AM fungi as symbiotic partners.

**Strigolactones promote germination in parasitic and other plants (66 – 70)**

Exudation of SLs into the soil has a detrimental effect on plant survival because SLs promote germination in some parasitic (and other) plants.

**How can we move towards Striga-resistant crops? (71 – 81)**

In some parts of the world, crop losses to *Striga* and *Orobanche* parasitic plants are substantial. Efforts to protect crops from these detrimental parasites include pre-treating fields to promote “suicidal” germination (some of the tiny-seeded parasitic plants die if they germinate without a host to feed off of), efforts to modify the structure and function of SLs to eliminate the germination-promoting effects, and efforts to engineer plants that don’t extrude germination-promoting SLs into the soil.

**Summary and ongoing research (82 – 85)**

Strigolactones are unusual in that they have documented functions as hormones working within an organism, pheromones or quormones that signal between individuals of the same species, and as allelochemicals that signal between species. Furthermore, as allelochemicals, they contribute to the serious agricultural problem of parasitic *Striga* and its relatives. Tackling this problem means unravelling the evolutionary processes that have led to SLs’ diverse functions, to try to eliminate their “bad” effects from their beneficial ones.
Overview – strigolactones (SLs) are hormones that inhibit shoot branching. In the soil, root-extruded SLs induce branching or spore germination of arbuscular mycorrhizal (AM) fungi and germination of seeds of parasitic plants. Thus, strigolactones are both exogenous and endogenous signals. What is the connection between shoot branching, root parasitism and root symbiosis, which came first, and is it possible to separate these functions? What are the ancestral functions for SLs?

Lecture outline

11 Synthesis and transport

12 Strigolactones are a small family of compounds
13 SLs, stimulators of Striga germination, derive from the carotenoid pathway
14 Genes involved in SL biosynthesis were identified genetically
15 Proposed SL biosynthesis pathway
16 D27 encodes an Fe-binding enzyme necessary for SL synthesis
17 Rice SL biosynthesis mutants are rescued by exogenous SL
18 Arabidopsis SL biosynthesis mutants are rescued by SL
19 MAX1 encodes a P450 enzyme shown in Arabidopsis to affect shoot branching
20 SL synthesis in root or shoot is sufficient to control shoot branching
21 An intermediate between MAX4 and MAX1, action (possibly carlactone) can move within the plant and across a graft junction
22 - 23 The ABC transporter PDR1 appears to be an SL exporter, and its distribution is consistent with its roles in transport

24 Synthesis - summary

25 - 35 Perception and signaling.
1. D14 is an α/β-fold hydrolase that binds SLs
2. D3 is an F-box protein that promotes interaction with the proteasome
3. The interaction between SLs, D14 and D3 leads to degradation of target proteins including D53, SMAX and SMXL

26 -27 1. D14 is an α/β-fold hydrolase that binds SLs and cleaves SLs
2. D3, an F-box protein, promotes interaction with the proteasome protein degradation machinery

28 SL receptors D14/DAD2 are related to the KAI2 karrikin receptors
29 Parasitic Striga make highly-sensitive SL receptors
30 In the presence of SLs, DAD2/D14 interacts with D3/MAX2
31 Karrikin signals are transduced in a similar manner as SLs
32 Genetic approaches identified D53/SMXLs in SL signalling

35 Signaling summary

36 - 59 Strigolactones and whole-plant processes

37 Strigolactones regulate shoot/root branching and nutrient responses.
38 Strigolactones dampen polar auxin transport (PAT)
39 How do strigolactones inhibit bud outgrowth?
40 Axillary bud outgrowth is hormonally and environmentally responsive
41 SL effect on branching can occur via effects on polar auxin transport
42 SL effects on shoot branching can also be auxin-independent
43 - 50 Shoot branching is also regulated by nutrients, with phosphate starvation leading to reduced shoot branching; this effect is mediated by increased SL biosynthesis
51 - 55 SLs suppress adventitious development, and promote secondary stem growth, regulate shoot gravitropic, and stimulate leaf senescence. SL mutants are more sensitive to abiotic stress.
56 SLs can be found in some green algae and in non-vascular plants. What do they do in green algae?
SLs act like a quorum sensing molecule (*quormone*) that regulates moss growth.

<table>
<thead>
<tr>
<th>57 - 58</th>
<th>SLs in whole plant responses - summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 - 65</td>
<td>SLs promote branching of <em>arbuscular mycorrhizal (AM)</em> fungi. Symbiotic associations with AM fungi help plants to take up phosphate from the soil. The fungi sense SLs, which in some plant are elevated when the plant is phosphate-starved. Thus, SL production restricts shoot growth and promotes symbiosis, both of which can enhance growth in nutrient limited conditions.</td>
</tr>
<tr>
<td>66</td>
<td>Strigolactones promote <strong>germination of seeds of parasitic and other plants</strong>. In fact, SLs were first characterized as promoters of <em>Striga</em> germination.</td>
</tr>
<tr>
<td>67</td>
<td>SLs can also promote <em>Arabidopsis</em> germination in some conditions, meaning it is possible to exploit <em>Arabidopsis</em> genetics to understand SL effects on parasitic plants.</td>
</tr>
<tr>
<td>68 - 70</td>
<td><em>Striga</em> species, aka witchweeds, are parasitic plants and serious agricultural pests that attach to the roots of crop plants and withdraw nutrients from them. They are particularly devastating in nutrient-poor, tropical soils.</td>
</tr>
<tr>
<td>71 - 76</td>
<td>How can we move towards <em>Striga</em>-resistant crops? Cultural methods: Promote suicidal germination, fertilize soils to lower the amount of SLs produce by the crop plants, use nitrogen-fixing companion crops some of which produce allelochemicals to suppress <em>Striga</em> parasitism.</td>
</tr>
<tr>
<td>77 - 79</td>
<td>Structure – function studies of SLs can lead to the identification of mono-functional chemicals that for example can promote symbiosis without promoting <em>Striga</em> germination.</td>
</tr>
<tr>
<td>80 - 81</td>
<td>Can we engineer <em>Striga</em>-resistant plants? Are SL-deficient mutants <em>Striga</em> resistant? The <em>d10</em> SL-deficient rice mutant has greatly reduced <em>Striga</em> parasitism, reduced AM fungi symbiosis, and too many shoot branches, but other genes may be altered to rescue the normal shoot branching phenotype.</td>
</tr>
<tr>
<td>82 - 85</td>
<td><strong>Conclusions and future directions</strong></td>
</tr>
<tr>
<td></td>
<td>SLs have pleiotropic effects in regulating plant development, and contribute to the modulation of plant architecture in response to nutrient limitation. Because they also affect the establishment of symbiosis with AM fungi, and the germination of parasitic plant seeds, the question of their “ancestral” function is intriguing. Furthermore, SLs provide an opportunity through which address the challenge of crop losses to parasitism. The very rapid growth in our understanding of these hormones reflects their novelty and importance.</td>
</tr>
</tbody>
</table>