Structural diversity in the strigolactones

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Abstract

Strigolactones (SLs) are a class of signalling molecules secreted by the roots of plants into the rhizosphere. On the one hand, they serve as the signal for recruiting arbuscular mycorrhizal fungi which have a symbiotic relationship with plants. On the other hand, they are also host detection signals for the non-symbiotic, pathogenic, root parasitic plants, which use the SLs as germination stimulants. Finally, recently the SLs were discovered to be a new class of plant hormones that regulate processes such as branching/tillering and root architecture. Intriguingly, >25 different SLs have already been discovered that all have the so-called D-ring but otherwise display many differences in structure and functional groups. In this review, we will critically discuss the structural diversity in the SLs. How are they synthesized in plants; how has this structural diversity possibly evolved; what is the biological relevance of this diversity; and what does this imply for the perception of the SLs by receptors in the plant itself and in other organisms? Finally, we conclude that only little is known about the biological significance of this structural diversity, and we will give an outlook on how to elucidate their importance further.

Keywords: Biological relevance, biosynthesis, evolution, perception, receptor, strigolactones, structural diversity.

Introduction

Already in the first half of the previous century scientists had observed that there is a compound or compounds present in the root exudates of clover, maize, sorghum, and linseed that induce(s) the germination of seeds of parasitic plants (Saunders, 1933; Brown et al., 1949, 1951). In the 1960s, the first germination stimulant for witchweed (Striga lutea Lour.) named strigol was isolated from the root exudates of cotton (Gossypium hirsutum L.) (Cook et al., 1966, 1972). Since then, >20 different molecules with similar properties to strigol, collectively called the strigolactones (SLs) (see below), have been identified in the plant kingdom. In this review, we will discuss the structural diversity of the SLs in the different plant species and what is known about their biosynthesis. We discuss how this structural diversity has possibly evolved and what the biological relevance may be. Finally, we discuss what this structural diversity implies for the perception of these molecules in plants and the other organisms that plants communicate with through the SLs. Furthermore, we will give an opinion on how to elucidate further the biological significance of this structural diversity.

Structural diversity in the strigolactones

As described above, the first germination stimulant, strigol, was discovered in the root exudate of cotton. Cotton is not a host of this Striga species (even though it does induce germination of its seeds), but strigol was later also identified in true Striga hosts such as sorghum [Sorghum bicolor (L.) Moench], maize (Zea mays L.), and proso millet (Panicum miliaceum L.) (Siame et al., 1993). Apparently, not only host plant species but also non-host species produce these germination stimulants. At around the same time, two germination stimulants that are chemically closely related to strigol were discovered in the root exudates of sorghum [Sorghum bicolor (L.)
cv. Haygrazer—sorgolactone (Hauck et al., 1992)—and in the root exudate of cowpea [Vigna unguiculata cv (Walp)]—alectrol (which was later identified as orobanchyl acetate; Xie et al., 2008b; Ueno et al., 2015). In 1995, Butler suggested to call all these strigol-related compounds ‘strigolactones’ (Butler, 1995). Three years later, the first germination stimulant of the broomrape, Orobanche minor Smith, was isolated from the root exudate of red clover (Trifolium pratense L.) and named orobanchol (Yokota et al., 1998). This discovery demonstrated that both Striga and Orobanche spp. utilize SLs produced by their host as germination cues to ensure they germinate in the presence of a host root.

In 2008, a novel SL, sorgomol (Fig. 1), a germination stimulant for parasitic plants Striga hermonthica and O. minor, was isolated from Sorghum bicolor (Table 1) (Xie et al., 2008a). Fabacol and fabacyl acetate (Fig. 1), which stimulate germination of O. minor, were originally purified from the root exudates of pea (Pisum sativum L.) (Table 1; Xie et al., 2009). Strigone (Fig. 1) that exhibited a highly potent activity on S. hermonthica was isolated from root exudates of the herb, and non-host, Houttuynia cordata (Kisugi et al., 2013). Highly intriguing and surprising was the discovery of new, so-called non-canonical, SLs that lack the so far consistent ABCD-ring structure (Table 1). In Arabidopsis, SL-like 1 (or methyl carlactonate, MeCLA) was reported (Fig. 1; Table 1) (Abe et al., 2014; Seto and Yamaguchi, 2014). Another SL-like molecule, called heliolactone (Fig. 1), was isolated from the root exudate of sunflower (Table 1) (Ueno et al., 2014). A non-canonical SL, zealactone (called methyl zealactonoate by Xie et al., 2017) (Fig. 1), was isolated from maize root exudate (Table 1) and was shown to stimulate S. hermonthica germination (Charnikhova et al., 2017). Another non-canonical SL, avenaol (Fig. 1), was isolated from the root exudate of black oats (Avena strigosa Schreb.) (Table 1) (Kim et al., 2014). Avenaol is a potent stimulant for Phelipanche ramosa seeds, but has only a weak effect on the germination of S. hermonthica and O. minor (Kim et al., 2014).

So far, all plant species examined have been shown to exude mixtures of several SLs. Different species have different SL profiles, and sometimes the profile differs between different cultivars within the same species. Furthermore, the amounts and ratios of SLs may vary with different growth conditions and developmental stages (Yoneyama et al., 2009).

**Strigolactones are not only germination stimulants**

An intriguing question that emerged upon the discovery of strigol was why plants would secrete SLs if these compounds would only have a negative consequence for the host. It took 50 years, until 2005, to answer that question, when Kohki...
Table 1. The distribution of strigolactones in different plant species

<table>
<thead>
<tr>
<th>Strigolactones</th>
<th>Plant species</th>
<th>Arabidopsis</th>
<th>Tomato</th>
<th>Rice</th>
<th>Tobacco</th>
<th>Sorghum</th>
<th>Maize</th>
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a Orobanchoyl, orobanchyl acetate, and 5-deoxystrigol have been reported in the root exudate of Arabidopsis (Kohlen et al., 2010), but they have not been confirmed by others.
b Orobanchoyl acetate, 7-oxo orobanchyl acetate, and 7-hydroxyorobanchyl acetate have been reported in petunia (Yoneyama et al., 2011), but it has not been confirmed by others.
c Structures are unidentified; classifications are tentative.
Akiyama reported that SLs induce hyphal branching in arbuscular mycorrhizal (AM) fungi and hence promote the symbiosis of plants with these fungi (Akiyama et al., 2005). AM fungi can engage in a symbiotic interaction with the majority of land plants, and supply water and nutrients, particularly phosphate, which they efficiently obtain from the soil, to the plants, in return for photoassimilates. This symbiotic relationship is postulated to be >460 million years old, dating back to when plants moved from water to land. The important role that these fungi play in the acquisition of phosphate (and to a lesser extent nitrogen) from the soil explains why phosphate starvation (and to a lesser extent nitrogen) elevate(s) the SL level so markedly in the root exudates of many plant species (Yoneyama et al., 2007a, b, 2008; López-Ráez et al., 2008; Umehara et al., 2008; Jamil et al., 2011).

Three years after the discovery of the role of SLs in symbiosis, another role for these molecules was discovered. The production of SLs was significantly reduced in a series of mutants with a highly branched/tillering phenotype, which demonstrated that SLs act as a new plant hormone that regulates the above-ground plant architecture (Gomez-Roldan et al., 2008; Umehara et al., 2008). The up-regulation of SL production under conditions of limited phosphate availability mentioned above was later shown also to be key in the reduction of branching/tillering of plants under low phosphate conditions (Umehara et al., 2010; Kohlen et al., 2011). Further studies discovered that SLs also regulate other aspects of plant development including root architecture, secondary stem growth, and leaf senescence (Ruyter-Spira et al., 2011; Kapulnik and Koltai 2014; Yamada et al., 2014; Sun et al., 2015).

Strigolactone biosynthesis

The naturally occurring SLs can be divided into strigol- and orobanchol-type SLs, which only differ in the stereochemistry of the C-ring [(3aR,8bS) in strigol and (3aS,8bR) in orobanchol] while the D-ring is always R configured (Figs 1, 2). Together these two types of SLs have been termed ‘canonical strigolactones’ because they exhibit the A-, B-, C-, and D-rings that originally were used to define the SLs (Butler, 1995). It logically appears that the strigol-type SLs are derived from 5-deoxystrigol (5DS); so far there is no direct biosynthetic evidence for this even though it has been reported that 5DS is bioconverted to sorgomol by sorghum (Motonami et al., 2013) (Fig. 2), while orobanchol-type SLs are derived from 4-deoxyorobanchol (Zhang et al., 2014) (Fig. 2). In contrast, there are also SL-like compounds that do not have the canonical A-, B-, and/or C-part and are therefore termed ‘non-canonical SLs’ (Fig. 2). However, in all cases, the butenolide (D-ring) is connected to the rest of the molecule through an enol ether bridge (Alder et al., 2012) (Figs 1, 2). In our view, it would be better to revise the definition of an SL to ‘a carotenoid-derived molecule with a butenolide D-ring’ such that it includes both the canonical and non-canonical SLs. In canonical SLs, the AB-rings

![Diagram of Strigolactone Biosynthesis](https://academic.oup.com/jxb/article-abstract/69/9/2219/4924111)
can be modified by demethylation, hydroxylation, epoxidation, acetylation, and ketolation (Bhattacharya et al., 2009; Al-Babili and Bouwmeester, 2015). Together all these reactions give rise to the structural diversification we see in the canonical SLs (Figs 1, 2).

Plants often produce a blend of different SLs (Table 1). Both canonical and non-canonical SLs are produced in some plants such as Arabidopsis (Kohlen et al., 2011; Abe et al., 2014), maize (Awad et al., 2006; Yoneyama et al., 2015; Charnikhova et al., 2017), and populus (Xie, 2016) (Table 1). Some species, such as tomato, petunia, pea, and populus, only have the orobanchol-type SLs (Table 1), and some species such as tobacco (Xie et al., 2007) and sorghum (Hauck et al., 1993; Siame et al., 1993; Mohamed et al., 2016; Gobena et al., 2017) produce both types of canonical SLs (Table 1). The SL blend can be quite complex. Tomato root exudate, for example, contains orobanchol, solanacol, hydroxysolacol, oxoorobanchol, orobanchyl acetate, and didehydro-orobanchol isomers (Table 1; Kohlen et al., 2013). Also tobacco has a complex SL composition, not only consisting of various SLs but also with both types: orobanchol-type SLs such as 4-deoxyorobanchol (4DO), orobanchol, orobanchyl acetate, solanacol, and solanacetyl acetate, and strigol-type SLs such as 5DS, 4α-hydroxy-5DS, and 4α-acetoxy-5DS (Table 1) (Xie et al., 2007, 2013).

The elucidation of SL biosynthesis started with the discovery that root exudates of carotenoid biosynthesis mutants in maize, and of wild-type maize seedlings treated with the carotenoid biosynthesis inhibitor furidione, induced lower germination of Striga (Matusova et al., 2005), which suggested that the SLs are derived from a carotenoid. The large structural diversity in the SLs suggests the involvement of many genes in their biosynthesis. Nevertheless, just a handful of SL biosynthetic genes have been identified, mostly using genetics, particularly from the core SL pathway (Table 2). Mutants with a highly branched phenotype in Arabidopsis showed that MORE AXILLARY GROWTH 1 (MAX1), MORE AXILLARY GROWTH 3 (MAX3), and MORE AXILLARY GROWTH 4 (MAX4) are essential for the biosynthesis of a shoot branching inhibition signal (Booker et al., 2004, 2005; Gomez-Roldan et al., 2008; Umehara et al., 2008). The orthologues of MAX3 and MAX4 are also characterized in other species, such as rice, DWARF17 (D17) (Zou et al., 2006) and DWARF10 (D10) (Arite et al., 2007); pea, RAMOSOUS 5 (RMS5) (Johnson et al., 2006) and RAMOSOUS 1 (RMS1) (Sorefan et al., 2003); and petunia, DECREASED APICAL DOMINANCE 3 (DAD3) (Drummond et al., 2009) and DECREASED APICAL DOMINANCE 1 (DADI) (Snowden et al., 2005). MAX3 and MAX4 encode CAROTENOID CLEAVAGE DIOXYGENASEs 7 and 8 (CCD7 and CCD8), respectively. Consistent with the postulated carotenoid origin of the SLs, it was shown in pea and rice that these two CCDs are required to produce SLs and the shoot branching-inhibiting signal, which may be an SL, an SL precursor, or a compound derived from any of these (Gomez-Roldan et al., 2008; Umehara et al., 2008). As a third enzyme required for SL biosynthesis, DWARF 27 (D27) was identified, an iron-containing protein with at that time unknown catalytic function (Lin et al., 2009). Later it was shown that D27, an all-trans/9-cis-β-carotene isomerase, catalyses the first dedicated step in SL biosynthesis by forming the substrate for the next enzyme in the pathway, CCD7 (Alder et al., 2012). In land plants, there is evidence for the presence of other D27-like proteins (Waters et al., 2012). For example, in the Arabidopsis genome, there are three genes encoding proteins that show superficial similarity to the rice D27. To date, all CCD7 genes identified are single copy (Vallabhaneni et al., 2010). In contrast, CCD8 has four, two, and six copies in rice, maize, and sorghum, respectively. These copies of CCD8 are divided into group CCD8a and group CCD8b based on their separate clustering in a phylogenetic tree. Intriguingly, CCD8b is not present in Arabidopsis. CCD8a was shown to

Table 2. Genes related to strigolactone biosynthesis and signalling in model plants

<table>
<thead>
<tr>
<th>Arabidopsis thaliana</th>
<th>Oryza sativa</th>
<th>Petunia hybrida</th>
<th>Pisum sativum</th>
<th>Function of the proteins</th>
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<tr>
<td><strong>SL biosynthesis</strong></td>
<td>D1D27 (At1g03350)</td>
<td>OsCCD7/D17/HTD1 (Os04g0550600)</td>
<td>DAD3</td>
<td>Carotenoid isomerase</td>
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<td>MAX3 (At2g44990)</td>
<td>OsCCD8/D10 (Os01g0746400)</td>
<td>DAD1</td>
<td>Carotenoid cleavage dioxygenase</td>
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<td>MAX4 (At4g32810)</td>
<td>OsMAX1 (Os01g0700900, Os01g0701400, Os01g0701500)</td>
<td>RMS1</td>
<td>Carotenoid cleavage dioxygenase</td>
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<td>MAX1 (At2g26170)</td>
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<td>RMS5</td>
<td>Cytochrome P450 enzyme; catalytic activity varies</td>
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<td>LBO (At3g21420)</td>
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<td>2-Oxoglutarate- and Fe(II)-dependent dioxygenase and acting downstream of MAX1 on MeCLA</td>
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<td><strong>SL and KAR signalling</strong></td>
<td>D14/D88/HTD2 (Os03g0203200)</td>
<td>DAD2</td>
<td>α/β-Fold hydrolase and receptor for SL</td>
<td></td>
</tr>
<tr>
<td>MAX2 (At2g43260)</td>
<td>D3 (Os06g0154200)</td>
<td>PhMAX2A, PhMAX2B</td>
<td>α/β-Fold hydrolase and receptor component of SCF complex</td>
<td></td>
</tr>
<tr>
<td>KAI2 (At4g37470)</td>
<td>D14L (Os03g0437600)</td>
<td>RMS4</td>
<td>α/β-Fold hydrolase and receptor for KAR signaling</td>
<td></td>
</tr>
<tr>
<td>SMXL6 (At1g07200), SMXL7</td>
<td>D63 (Os11g0104300, DS3-LIKE)</td>
<td>RMS3</td>
<td>Targets of SL signalling and involved in regulating transcription</td>
<td></td>
</tr>
<tr>
<td>(At2g29970), SMXL8</td>
<td>(Os12g0104300)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
convert 10′-apo-β-carotenal into 13′-apo-β-carotenal. The enzymatic role of the CCD8b group remains elusive.

Together, D27, CCD7, and CCD8 form the core pathway of SL biosynthesis which results in the formation of carlactone (CL) (Alder et al., 2012). By now it seems that CL is the common intermediate in the biosynthesis of all SLs discovered so far (Al-Babili and Bouwmeester, 2015; Charnikhova et al., 2017). Alder et al. (2012) also speculated that MAX1 would catalyse the next step in the pathway, namely the oxidation of CL, to a next at that time unknown pathway intermediate. Indeed, it was demonstrated that in rice, one MAX1 homologue, CL oxidase (Os900), catalyses the hydroxylation of MeCLA at C3, further oxidation and stereoselective conversion of CL into 4DO (Zhang et al., 2014) (Fig. 2). Subsequently, another MAX1 homologue, 4DO hydroxylase (Os1400), catalyses the hydroxylation of 4DO to orobanchol (Fig. 2). Orobanchol seems to be a central intermediate in SL biosynthesis, and it has been postulated that further modification of orobanchol can result in the formation of fabacol (Fig. 2) (Xie et al., 2009), orobanchyl acetate (Fig. 2; Ueno et al., 2011b), solanacol (Fig. 2) (Chen et al., 2010), and so on (Fig. 2). In the parallel biosynthetic pathway of the strigol-type SLs, 5DS is postulated to be the precursor for strigol and other strigol-type SLs (Al-Babili and Bouwmeester, 2015) such as sorgomol (Xie et al., 2008a) and strigol (Fig. 2). These can be further modified into other strigol-type SLs, such as strigone (Kisugi et al., 2013) and strigyl acetate (Sato et al., 2005) from strigol, and sorgolactone (Xie et al., 2008a) from sorgomol (Fig. 2).

In Arabidopsis, MAX1 catalyses quite a different reaction, the oxidation of CL to carlactonoic acid (CLA) (Abe et al., 2014). It was shown that CLA can be converted by an as yet unknown methyl transferase into the so-called SL-like or methyl carlactonoate (MeCLA) (Abe et al., 2014; Seto and Yamaguchi, 2014). Later, studies showed that an oxoglutarate-dependent dioxygenase, called LATERAL BRANCHING OXIDOREDUCTASE (LBO), facilitates additional processing, converting MeCLA into an unknown SL-like product (MeCLA+16 Da) (Brewer et al., 2016) (Fig. 2). Recently, in maize, it was proposed that MeCLA can be converted into zealactone (named methyl zealactonoate by Xie et al., 2017) through hydroxylation of MeCLA at C3, further oxidation (to form heliolactone), double bond epoxidation, and ring cleavage (Charnikhova et al., 2017) (Fig. 2). Heliolactone, an SL initially isolated from sunflower and closely related to zealactone (Ueno et al., 2014; Charnikhova et al., 2017), was previously also postulated to be derived from MeCLA (Ueno et al., 2014; Fig. 2). In conclusion, although a number of enzymatic steps after CL have now been elucidated, our knowledge of how the large structural diversification in the canonical and non-canonical SLs arises is still very limited.

What evolutionary pressure has caused the structural diversity in the strigolactones?

The evolutionary mechanisms underlying the production of so many different SLs are unknown, but it is likely that the multiple roles of SLs as a signalling molecule in rhizosphere communication and as endogenous plant hormones have contributed to the diversification. For many plant species, the root parasitic plants—which take water, assimilates, and nutrients from their host—are disastrous enemies that should be avoided. AM fungi, on the contrary, have a symbiotic interaction with the majority of land plant species, which is beneficial. When plants are exposed to phosphate shortage, they produce and release more SLs into the rhizosphere to stimulate hyphal branching and thus root colonization by the AM fungi that will subsequently help the plant to obtain water and nutrients from the soil. On the one hand, there is a selective pressure for exudation of high amounts of biologically active SLs to induce this hyphal branching effectively in the AM fungi. On the other hand, however, to reduce infection by root parasitic plants, there is a selection pressure to produce SLs that are not or are less active with regard to the parasitic plant seed germination-inducing activity. Involvement of SLs in additional benign or noxious signalling relationships in the rhizosphere—for which indications are available in the literature (Schlemper et al., 2017)—may further contribute to this selection pressure on the creation of biological specificity through structural changes.

Changes in structure also require concomitant changes in the receptors of these signals. For the role as a plant hormone this may be less of an issue as plants could selectively transport SLs to the rhizosphere, independently from the transport to the shoot. This would separate the internal need for SLs to regulate development from the high risk of releasing them into the rhizosphere, which can potentially attract enemies, and/or from unnecessary secretion when the plant is already colonized by AM fungi.

From an evolutionary viewpoint, it is beneficial for a host plant to produce an SL with low parasitic plant germination stimulatory activity, but with high AM fungi hyphal branching stimulatory activity. Recently, an example of this was reported in sorghum. Mutant alleles at the LGS1 (LOW GERMINATION STIMULANT 1) locus result in reduced 5DS and enhanced orobanchol levels in sorghum root exudate which drastically reduces Striga germination stimulant activity but does not negatively affect AM fungi colonization (Gobena et al., 2017). On the other hand, in the parasitic plants there is a strong selection pressure to adapt their perception to the changes in the structure of the signalling molecule. The enormous amounts of seeds produced by these parasitic plants form a perfect reservoir for genetic variation and a rapid expansion of a certain genotype. This antagonistic co-evolution of host and parasites may function as a driver for structural diversification in the SLs of the host plant species.

Biological relevance of structural diversity

The above speculation about the driving force of structural diversification in the SLs only makes sense if there are indications for a biological relevance of this process. There are sufficient indications that there is biological specificity for these structural variants in the processes they control. Some
SLs have a high activity in one biological process but much lower in another, and vice versa. Indeed, there are large differences in the efficiency of different SLs to induce hyphal branching in the AM fungus Gigaspora margarita (Fig. 3). For example, orobanchol and 4α-hydroxy-5DS will induce hyphal branching at only 1 pg per disc, making them effective inducers of hyphal branching in this AM fungus, while strigol and sorgomol are relatively weak inducers with a minimum required amount of 100 pg per disc (Akiyama et al., 2010). Parasitic plant species also vary considerably in their germination responses to different host root exudates and SLs (Fernández-Aparicio et al., 2009, 2010). In O. minor, for example, 10 pM 4α-hydroxy-5DS and orobanchol induce 80% and 86% germination, respectively (Kim et al., 2010) (Fig. 3), while in the same concentration strigol only induces 24% germination. In S. hermonthica, 0.1 µM 5DS induced 50.9% germination, while 4α-hydroxy-5DS and 4α-acetoxy-5DS in the same concentration only induced 26.5% and 10.9% germination, respectively (Ueno et al., 2011a) (Fig. 3). Intriguingly, this shows that the same SL displays quite different germination-inducing activity in different parasitic plants. 4α-Hydroxy-5DS is a low inducer of germination in Striga but a high inducer of germination in Orobanche seeds. Orobanchol and 4α-hydroxy-5DS are also strong inducers of hyphal branching in the AM fungus, G. margarita (Fig. 3). That this is relevant in the field is clear from the study on sorghum described above, where a difference in the SL profile between genotypes results in Striga resistance without negatively affecting AM colonization (Gobena et al., 2017). Also in the inhibition of shoot branching, different SLs exhibit different activities. According to a study by Boyer et al. (2012), orobanchyl acetate and 5DS are relatively active in inhibiting shoot branching, while strigol and orobanchol showed less activity. From the above, we can conclude that different biological processes often have a different SL specificity.

The perception of strigolactones

The different specificities for different SLs in different biological processes, such as plant development, hyphal branching of AM fungi, and germination of parasitic plants, suggests that the receptor involved in the recognition of the SLs is highly specific. It has indeed been demonstrated that the

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Fig. 3. The biological activity of different strigolactones. SLs are 5-deoxystrigol (5DS) (a), 2′-epi-5DS (b), orobanchol (c), 4α-hydroxy-5DS (d), strigol (e), sorgomol (f), orobanchyl acetate (g), 4α-acetoxy-5DS (h), 7-oxoorobanchyl acetate (i), and strigyl acetate (j), respectively. The black columns represent the minimum effective concentration (MEC) of SLs for hyphal branching-inducing activity in Gigaspora margarita (Akiyama et al., 2010); the dark grey columns represent germination-stimulating activity of the strigolactones on Orobanche minor seeds (Kim et al., 2010); the light grey columns represent the germination-stimulating activity of the strigolactones on Striga hermonthica seeds (Ueno et al., 2011a); the white columns represent the node 4 bud outgrowth inhibition activity of the strigolactones in garden pea (Pisum sativum) (Boyer et al., 2012); the concentration of the stigolactones used in Orobanche, Striga, and bud outgrowth assays are 10 pM, 0.1 µM, and 100 nM, respectively. The names of the SLs given above represent the revised stereochemistry (Xie et al., 2013; Zwanenburg and Pospíšil 2013; Čavar et al., 2015). In Akiyama et al. (2010) a, b, c, d, e, f, g, and i are named (+)-5DS, (+)-2′-epi-5DS, (+)-orobanchol, (+)-2′-epi-orobanchol, (+)-strigol, (+)-sorgomol, (+)-orobanchyl acetate, and (+)-7-oxoorobanchyl acetate, respectively. In Ueno et al. (2011a), d and h are called orobanchol and orobanchyl acetate, respectively. In Kim et al. (2010), c and d are called 2-epi-orobanchol and orobanchol, respectively.
Similar to other plant hormones, SL signal transduction is based on hormone-activated proteolysis (Morffy et al., 2016). In this mechanism, the F-box component, first discovered in Arabidopsis and termed MAX2 (see Table 2 for orthologues in other species), of the SCF (Skp1–Cullin–F-box) complex targets a specific protein for the polyubiquitination and degradation by the 26S proteasome (Yan et al., 2013; Seto and Yamaguchi, 2014; Yao et al., 2016; Li et al., 2017). The perception of the SLs occurs through the α/β-hydrolase fold superfamily protein D14, first identified in rice (for orthologues in other species, see Table 2) (Hamiaux et al., 2012; Nakamura et al., 2013; Zhao et al., 2013; Al-Babili and Bouwmeester, 2015; de Saint Germain et al., 2016; Flematti et al., 2016; Waters et al., 2017). D14 functions as both an enzyme and a receptor (Snowden and Janssen 2016; Yao et al., 2016). It has a conserved catalytic triad (Ser-His-Asp) which is required for the hydrolysis of the SL ligand and its signalling function (Al-Babili and Bouwmeester, 2015; Waters et al., 2017). A conformational change in D14 upon binding and hydrolysis of the SL ligand allows recruitment of MAX2, which can then target proteins for proteasomal degradation (Al-Babili and Bouwmeester, 2015; Flematti et al., 2016; Waters et al., 2017). Target proteins are D53, that was first identified in rice (Jiang et al., 2013; Zhou et al., 2013), and SMXL6/7/8 [identified as proteolytic targets of SL signaling in Arabidopsis (Wang et al., 2015); for orthologues in other species, see Table 2 (Hamiaux et al., 2012; Soundappan et al., 2015)]. As a result, D53/SMXLs are ubiquitinated and degraded; at the same time the receptor D14 is degraded and the SL ligand hydrolysed (Jiang et al., 2013; Wang et al., 2015; Yao et al., 2016, 2018). Upon the degradation of D53 and the SMXLs that are repressors of SL signalling (Wang et al., 2015; Waters et al., 2017), expression of the initially repressed genes is activated, which results in the physiological changes incurred by the SLs.

Parasitic plant species such as S. hermonthica and P. aegyptiaca also express a seemingly functional D14, the SL receptor (Das et al., 2015; Y. Zhang, C. Ruyter-Spira, H. Bouwmeester, et al., unpublished results), and MAX2 (Liu et al., 2014). Intriguingly, however, the perception of SLs in the seeds of these parasitic plants proceeds differently from that in all other higher plants. Just like other higher plants, parasitic plants, such as S. hermonthica and P. aegyptiaca, have a KA12 (KARIKIN INSENSITIVE 2) or HYPOSENSITIVE TO LIGHT (HTL) which is a parologue of D14 and was discovered in a mutant screen for the induction of germination of Arabidopsis by karrikin (KAR) (Nelson et al., 2011). KARs are a class of molecules produced by burning vegetation that stimulate the germination of seeds of species pioneering after a fire has destroyed the vegetation (Flematti et al., 2009; Nelson et al., 2012), and structurally display some resemblance to the SLs (Flematti et al., 2004). Like D14, KA12 requires the presence of MAX2, in order to be functional. Surprisingly, S. hermonthica and P. aegyptiaca contain a whole family of KA12-like proteins, HTLs/KA12s, of which a subclass (class KA12d) have evolved to bind SLs as their ligand rather than KARs (Tsuchiya et al., 2015). With a series of elegant experiments they—and not the normal SL receptor D14—were proven to be responsible for the SL-induced seed germination in parasitic plants (Conn et al., 2015; Tsuchiya et al., 2015; Flematti et al., 2016). There are 11 HTL/KA12 homologues in S. hermonthica. Using chemical and structural biology, these 11 receptors were clustered into groups with different chemical responsiveness (Conn et al., 2015). The class KA12d that includes ShHTL4 to ShHTL9 is most sensitive to a number of natural SLs. ShHTL7 responds to picomolar concentrations of 5DS, 4DO, and sorgolactone, but responded to strigol in the nanomolar range (Toh et al., 2015). The crystal structure of this highly sensitive SL receptor revealed a larger binding pocket than that of the original KA12 receptor (Toh et al., 2015; Yao et al., 2017). ShHTLs seem to have evolved differential SL binding affinities through modulation of their active site architecture (Yao et al., 2017). Striga receptors with this larger and modified active site architecture had higher sensitivities to SLs compared with that of Arabidopsis HTL, and these evolutionary changes seem to explain their increased SL sensitivity (Toh et al., 2015). With the exception of ShHTL7, the class KA12d ShHTLs are differentially sensitive to different SLs (Toh et al., 2015). That is to say, the different receptors of the Striga parasitic plants have different specificity for different SLs.

For unknown reasons, different host plant species produce different blends of SLs, which are characteristic for that plant species. From an evolutionary point of view, it could be that these plant species evolved new SLs that induce less germination in the seeds of the parasitic plants. However, the SL receptors in parasitic plants, the HTLs, appear to have evolved rapidly, possibly allowing the parasites again to recognize the new SLs to continue parasitism of that host. Thus, it seems that the diversity in the receptors in the parasites is a result of the antagonistic co-evolution of the host plants producing new SLs and of the parasites to adapt their receptor to new structural SLs.

As there seems to be no easily recognizable D14/KA12 receptor in AM fungi, the discovery of the SL receptor in the AM fungi remains a challenge. Genome and transcriptome sequencing approaches in symbiotic AM fungi will be crucial to assist in the elucidation of their SL perception mechanism. From the biological activity of SLs on AM fungal hyphal branching, we know that different SLs exhibit differences in their stimulating effect. That implies that AM fungi also exhibit SL specificity and thus that there must be a specific receptor. This could also imply that there is host specificity in AM fungi, based on differences in the SL profile, although so far there is no evidence for this. If host specificity does exist, this could help the elucidation of the perception mechanisms of the SLs in AM fungi just as it did in the parasitic plants. The presence of multiple copies of the elusive AM fungi SL
receptor would provide additional evidence for co-evolution of structural diversity in the SLs and specificity in the corresponding receptors in friends and enemies.

**Perspectives**

*In vitro* assays have begun to shed light on the relevance of the structural diversity in the SLs, and in a number of cases their importance was also demonstrated *in planta* (Gobena et al., 2017). However, for a rigorous evaluation of the biological importance of the different SLs, we need to be able to perform *in planta* studies with genotypes differing only in the SL blend that they produce. Mutants and transgenic lines with deletions of single or multiple enzymes will be the material of choice to use in such studies. However, until now, only the core SL biosynthetic pathway is known, and most of the enzymes responsible for the structural diversification of the SLs are still unknown. Elucidating those unknown diversification enzymes by forward genetics approaches will be challenging, as mutations in those enzymes probably only have weak phenotypes owing to functional redundancy of the different SLs. Transcriptomics and reverse genetics could be an alternative option to elucidate fully the biosynthetic pathway of the SLs. The discovery of LBO in Arabidopsis is an example of this approach (Brewer et al., 2016). With new genes to hand, using TILLING (Targeting Induced Local Lesions IN Genome) or genetic modification, we can change the composition of the SLs *in planta* and study the biological consequences, such as changes in the recruitment of AM fungi or the infection with parasitic plants. It will also be exciting to use those modified plants to study the rhizosphere microbiome, which could lead to the discovery of new relationships between SLs and other microorganisms.

Elucidation of the role of the HTL receptors in the generation of host-specific races of root parasitic species, and if and how the expression of these receptors are regulated, would increase our understanding of how parasites rapidly evolve new host specificities and become virulent agricultural weeds. A better understanding of how host specificity is mediated by SLs and how their receptors are regulated would also help to develop selective herbicides for parasitic weeds such as SL analogues and antagonists that highly specifically trigger or inhibit, respectively, the germination of parasitic plant seedlings (Johnson et al., 1976; Babiker and Hamdoun, 1982; Kgosi et al., 2012; Holbrook-Smith et al., 2016; Lumbroso et al., 2016; Samejima et al., 2016; Zwanenburg et al., 2016). The use of such analogues will help to eliminate the seeds from the field and reduce parasitic plant infestation. Engineering the SL profile of crop plants can also be an attractive target for breeders who want to breed parasitic plant-resistant varieties of crops. This could possibly be achieved using existing natural variation (such as was done in sorghum; Gobena et al., 2017), through the selection of mutants in target genes using TILLING, or through the use of genetic modification. Since there is a potential possibility that the parasites will adapt to recognize the new structural SLs of resistant varieties, farmers and breeders need to take measures to prevent that by stacking several resistance mechanisms and/or cultural measures.

Finally, in the future, more and more new SLs will be identified, further complicating the elucidation of the complete picture of the biological relevance of the structural diversity in the SLs. The elucidation of the biosynthesis of this diversity, the possibility to modify it, and a rigorous understanding of the perception in the host as well as in other organisms should help us understand how plants and their partners try to balance the positive and negative effects of their chemical communication.

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**References**


Hamiaux C, Drummond RS, Janssen BJ, Ledger SE, Cooney JM, Newcomb RD, Snowden KC. 2012. DAD2 is an α/β hydrolase likely to be involved in the perception of the plant branching hormone, strigolactone. Current Biology 22, 2032–2036.


of 5-deoxystrigol, the host recognition signal for arbuscular mycorrhizal fungi and root parasites. Planta 227, 125–132.


