Novel insights into strigolactone distribution and signalling
Alexandre de Saint Germain¹, Sandrine Bonhomme¹, François-Didier Boyer¹,² and Catherine Rameau¹

Strigolactones (SLs), a group of small carotenoid-derived molecules, were first known for their function in the rhizosphere in both symbiotic and parasitic interactions. Most of the progress for deciphering SL biosynthesis and signalling pathways comes from the use of high branching mutants identified in several species demonstrating that SLs also play a hormonal role in plant development. How SLs are perceived by the different organisms on which they show bioactivity is a current major challenge for the growing SL research community. These molecules very likely predate the colonization of land by plants and represent a fascinating example of signalling molecules involved in key innovations during plant evolution.

Addresses
¹Institut Jean-Pierre Bourgin, INRA UMR1318 INRA-AgroParisTech, F-78000 Versailles, France
²Centre de Recherche de Gif, Institut de Chimie des Substances Naturelles, UPR2301 CNRS, F-91198 Gif-sur-Yvette, France

Corresponding author: Rameau, Catherine (Catherine.Rameau@versailles.inra.fr)

Diversity of SLs and bioactivity in different models
The structural core of SLs is a tricyclic lactone (ABC rings) with various substitution patterns on AB rings. It is connected via an enol ether bridge to an invariable αβ-unsaturated furanone moiety (D-ring). To date, at least 19 naturally occurring SLs (Figure 2aI and aII) have been identified and characterized in root exudates of various land plants [7,8]. They can be separated into two families according to a recent structure revision [9], that is, one in which the stereochemistry in the BCD part is the same as (+)-strigol, and the other in which the stereochemistry in the BCD part is the same as (−)-orobanchol. The roles of SL stereochemistry and their various chemical decorations need to be determined in the future. The recent discovery of the intermediate carlactone (Figure 2aIII) [5**], was a major breakthrough in understanding the SL biosynthesis, suggesting that the BC rings are formed after the D ring to give 5-deoxystrigol via putative(s) cytochrome P450 enzyme(s), possibly MAX1 (Figure 1). Further hydroxylation, epoxidation/oxidation and methyl transfers or acetylation would lead to hydroxy-SLs or acetylated-SLs.

Introduction
SL research has increased markedly since their discovery as a plant hormone in 2008 for their role in inhibiting shoot branching [1,2]. During the last five years, novel hormonal functions have been demonstrated [3], a likely receptor has been identified [4**] and important progress has been made in deciphering the biosynthesis pathway from carotenoids with the identification of the intermediate carlactone ([5**] and Figure 1). SLs exuded by the host root stimulate hyphal growth of the obligate biotrophic arbuscular mycorrhizal (AM) fungi [6]. Arbuscular mycorrhizal symbiosis occurring in more than 80% of land plants, should play in the future a determinant role in agriculture to reduce abiotic stresses. Later during evolution, the obligate parasitic plants, Striga (witchweeds) and Orobanche (broomrapes), causing devastating yield reductions in several major crops, used SLs to develop a system inducing seed germination only when seeds were in close proximity to the host root [7]. Consequently current research on these interkingdom signalling molecules will have a significant impact on both fundamental and applied perspectives.

In this review, structural diversity of natural SLs will be examined and structural requirements for SL bioactivity for the three major functions (hormonal, symbiotic and parasitic interactions) will be compared. Current knowledge of SL reception for branching control will be presented with the first report on structural data to determine how SLs interact with the receptor. Recent studies on the origin of SLs during plant evolution will be given and hypotheses on alternative SL biosynthesis and signalling pathways discussed.
not for parasitic seeds and for branching (Figure 2b) [13–15]. Few studies have been described for the hormonal function of SLs [1,2,15,16]. Through an SAR study in pea, we established that the presence of a Michael acceptor and a methylbutenolide or a dimethylbutenolide motif in the same molecule is essential [17*]. We demonstrated that SLs show potent activity for the control of shoot branching in a structure-dependent manner but with low specificity [17*,18]. The differences in bioactivity observed in the different systems suggest that they use distinct modes of perception. Biochemical and structural studies with proteins belonging to the α/β-hydrolase superfamily have started to provide insights into SL reception for its function on shoot architecture.

**SL reception, a novel mechanism among plant hormones?**

Studies of branching mutants and analogy with other plant hormone receptors indicated that the F-box protein MAX2 from *Arabidopsis* and the α/β-hydrolase D14 from rice [19] were good candidates for the SL receptor [20]. D14 protein was also a possible activating enzyme to transform SL into a bioactive molecule. Characterization of DAD2, the petunia orthologue of D14, showed that this α/β-hydrolase presented both enzymatic and receptor activities and suggested a novel mechanism-of-action of SL on its target protein [4**,21]. X-ray crystallography studies of DAD2, AtD14 and OsD14 [22,23*] confirmed that they belong to the α/β hydrolase fold family. Their structure forms a hydrophobic pocket containing the conserved Ser–His–Asp catalytic triad (Figure 3a). The interaction between the protein and the synthetic SL GR24 was confirmed by differential scanning fluorimetry (DSF) for DAD2 [4**] and by isothermal titration calorimetry (ITC) for OsD14 [23*]. Surprisingly, the binding of GR24 to the DAD2 protein destabilized it, whereas conventional hormone binding to its receptor stabilizes the protein. In a yeast two-hybrid assay, GR24 was shown to trigger an interaction between DAD2 and the petunia F-box protein, PhMAX2A [4**]. The structure of D14 is very similar to that of the gibberellins (GA) GID1 receptor. GID1 undergoes a conformational change when GAs bind to it, resulting in closure of the N-terminal lid over the binding pocket and interaction of the lid with the GA negative regulator DELLA [25]. For SL, the function of the lid appears different, with higher conformational stability in D14, even in the absence of ligand [24].

D14 exhibits weak binding affinity for GR24, low for a receptor ($K_d = 0.36 \mu M$ vs. 5 μM for gibberellin A4 with OsGID1) [22,25] indicating that a co-factor or co-receptor may be involved for increasing binding affinity. In addition to binding SLs, both DAD2 and OsD14 catalyze hydrolysis of GR24, however with slow enzymatic activity [4**].

In vitro studies showed that both rice and *Arabidopsis* D14 hydrolysed the GR24 into ABC-ring and D-ring products [23*] (Figure 3b); in petunia, neither of the two hydrolytic products, the ABC-ring product and an unidentified compound retained any SL activity [4**].

Most, if not all, SLs have been identified in root exudates and extracts. Studies for SL identification in shoot tissue...
are very rare, very likely because SL levels are very low in shoot. Grafting experiments early indicated that SLs principally produced in roots, but also in the stem, are transported upward via the xylem to the aerial shoot [26]. This hypothesis is supported by the detection of orobanchol in Arabidopsis and tomato xylem sap [27,28]. Interestingly, other SLs, abundant in root extracts, were not detected in xylem sap suggesting preferential loading in the xylem possibly involving the ABC transporter, PDR1, identified in petunia, which may act as a SL exporter [29*]. Metabolism studies with labelled SLs, identification of derived molecules and synthesis for testing their bioactivity are nevertheless still lacking.

Nonetheless, the recent biochemical and structural studies suggest a novel mechanism of hormone reception and signalling among plant hormones (Figure 3) where the enzymatic activity would be essential to the signalling pathway [23*]. A similar perception mechanism is described for karrikins, smoke derived compounds structurally...
4 Cell signalling and gene regulation 2013

![Figure 3](image)

A model of SL signalling pathway through SL hydrolysis. (a) The proposed SL receptor D14 is an α/β-hydrolase with both binding and enzymatic activities. Structure of D14 is typical of α/β-hydrolase family with 2 domains: a core α/β-hydrolase fold with 7-stranded β-sheets surrounded by 7 α helices and a lid of 4 α helices that covers the core domain linked by 2 loops (not shown). The hydrophobic active site pocket contains the conserved serine (S), aspartate (D), histidine (H) catalytic triad essential for D14 enzymatic and binding activities. Structural change of D14 following SL binding or hydrolysis would trigger MAX2 F-box protein binding and subsequent degradation via the proteasome of SL-signalling repressors still to be identified. How MAX2 binds D14 is still not understood. (b) Current proposed model for GR24 hydrolysis by D14 with nucleophilic attack of the D-ring and formation of an intermediate. The hydroxyl group of the serine residue (S97) of the catalytic triad attacks the SL butenolide ring on position C5′ leading to the transitory opening of the D ring and the release of product 1. A transient intermediate product is generated, covalently linked to the protein by the serine and rapidly converted in a hydroxyl butenolide (product 2).

related to SL and involved in seed germination [30]. Karrikin and SL signalling pathways share the F-box protein MAX2. However, a D14-like (KAI2) protein, not able to bind GR24 because of a smaller binding pocket, is proposed to bind and hydrolyse karrikin [22]. Although the moss Physcomitrella patens synthesizes and responds to SLs [31*], its genome does not contain a true canonical D14 gene, but rather several D14-like sequences. D14 and D14-like sequences probably derived from a common ancestor, but at present it is not clear at what point during land plant evolution this duplication occurred [32*]. Other recent findings question the conservation of biosynthesis and signalling pathways between vascular and non-vascular plants.

**When did strigolactones appear, and for what purpose?**

Recently, SL production was reported not only in Bryophytes [31*] but also in charales [33*], a group of green algae (among Charophytes) considered the closest to land plants (Embryophytes) [34] (Figure 4). Extracts or exudates from other groups of green algae (one group of chlorophytes and two other groups of charophytes) do not induce germination of parasitic weeds seeds [33*], which
is so far the most sensitive method to detect SLs. Consequently, SLs were likely to be present before land colonization.

Besides SLs detection, searches for SL-related genes (or transcripts) have been performed in green lineage. *D27* (Figure 1) homologues are present in the genome of all species from the green lineage, including those that do not synthesize SLs [33*]. However, in green algae, moss and Selaginella (Lycophyta) at least, *D27* homologues are non-canonical (*D27-like*) and may catalyse different reactions or have other functions in these species [35]. In contrast, genes encoding enzymes catalysing further SL synthesis steps (Carotenoid Cleavage Dioxygenase genes *CCD7* and *CCD8*, Figure 1) have only been found in Embryophytes (including moss), and not in Charales, for which complete genome sequence is however lacking [33*]. As the liverwort *Marchantia polymorpha* lacks the *CCD8* gene, it has been suggested that an alternative (more ancient) *CCD8*-independent SL-biosynthesis pathway would operate in this basal Embryophyte and in Charales [33*]. It could explain why SLs are still detected in null *ccd8* mutants [31*,36]. Moreover, the *MAX1* gene (Figure 1), encoding a cytochrome P450 [37] is present in all Embryophytes except the moss *P. patens* and the liverwort *M. polymorpha*, and absent from algae genomes ([31*,38], Bowman JL, pers. comm.). As *P. patens* produces complex decorated SLs (Figure 2 and
6 Cell signalling and gene regulation 2013

[31*] another P450 may insure MAX1 function, or the last SL synthesis steps are different in moss, again highlighting flexibility in the SL synthesis pathways [35].

The perception mechanism likely differs between vascular and non-vascular plants. Only D14-like sequences have been identified in non-vascular plants, and although MAX2 homologues have been found in all sequenced Embryophyte genomes, genetic and physiology data indicate that the moss MAX2 homologue might not be involved in the SL response in P. patens (our unpublished results). Genome sequencing and SL quantification data in other land plants (e.g. hornworts) and algae groups are needed for a better understanding of the SL pathway evolution. The recent finding of SLs and related genes in Charales indicates that these molecules, by favouring anchorage ability of rhizoids before the apparition of roots, would have first had a hormonal function on plant architecture [3,33*].

Conclusion

Although an understanding of SL signalling has been rapidly increasing, major questions still remain to be answered. In particular, the mechanism by which the probable SL receptor D14 recognizes the MAX2 F-box protein is not yet known and the putative repressors of SL signalling have still not been identified. These repressors are supposed to be targeted for degradation via the 26S proteasome. Discovering receptors for SL in different organisms, vascular and non-vascular plants, parasitic plants and fungi will have significant ecological and agronomical implications. Particularly, the design of novel SL analogues with specific bioactivity will be of major interest, for example, analogues with similar bioactivity as natural SLs for shoot branching and low bioactivity on parasitic plants [16,17].

Acknowledgements

The authors thank Rosemary and Jean-Marie Beau for careful reading of the manuscript, Junko Koyuzuka for the rice picture and the Agence Nationale de la Recherche (contract ANR-12-BSV6-004-01) for financial support.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


The authors clearly demonstrate by comparison with natural SLs and synthetic standards the presence of two chemical families of SLs based on stereochimical differences.


The authors reported here the first SL structure–activity relationship studies for inhibition of bud outgrowth based on simple bioassays on pea.


Evolution of strigolactone signalling de Saint Germain et al.


The authors obtained the crystal structure of the rice D14 α/β-hydrolase bound to a GR24 degradation intermediate.


This is the first report of SL occurrence in a non-vascular plant. The results suggest a specific role for SL in moss community structure, by regulating plant size according to individual density. Such function is completely novel for a plant hormone.


Waters et al. demonstrate that the Arabidopsis α/β hydrolase AtD14 is involved in strigolactone response whereas the paralogue AtD14-like/KA2 is required for karrakin response.


Through a detailed search of SL content/activity and SL-related genes occurrence in many different species of the green lineage, the authors showed that SL were already present in some green algae (Charales) and suggested that SL primary role was hormonal rather than to promote AM symbiosis.


