Strigolactones: mediators of osmotic stress responses with a potential for agrochemical manipulation of crop resilience

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Highlight

We review the role and regulation of strigolactones during osmotic stress, namely on organ-specific dynamics of synthesis and interaction with abscisic acid and on their potential for crop protection.

Abstract

After quickly touching upon general aspects of strigolactones biology and functions, including structure, synthesis and perception, this review focuses on the role and regulation of the strigolactone pathway during osmotic stress, in light of the most recent research developments. We discuss available data on organ-specific dynamics of strigolactone synthesis and interaction with abscisic acid in the acclimatization response, with emphasis on the ecophysiological implications of the effects on the stomatal closure process. We highlight the importance to consider roots and shoots separately as well as combined *vs* individual stress treatments; and to perform reciprocal grafting experiments to work out organ contributions and long-distance signalling events and components under more realistic conditions. Finally, we elaborate on the question of if and how synthetic or natural strigolactones, alone or in combination with crop management strategies such as grafting, hold potential to maximise crop resilience to abiotic stresses.

Key words

Abscisic acid, Drought, Hormone cross-talk, Osmotic stress, Resilience, Root-shoot communication, Stomata closure, Strigolactones

Abbreviations

ABA: Abscisic Acid

ABCG: ABC Transporter G Family Protein

ABI: ABA Insensitive

AM: Arbuscular Mycorrhizal

CCD: Carotenoid-Cleavage Dioxygenase

D: Dwarf

DAD: Decreased Apical Dominance

HAB: Hypersensitive to ABA

HTD: High Tillering and Dwarf

IPA: Ideal Plant Architecture

KAI: Karrikin Insensitive

KL: KAI2 Ligand

LBO: Lateral Branching Oxidoreductase

LGS: Low Germination Stimulant

MAX: More Axillary Growth

N: Nitrogen

NCED: Nine-*Cis*-Epoxycarotenoid Dioxygenase

ORA: Octadecanoid-Responsive AP2/ERF-domain transcription factor

P: Phosphate

PDR: Pleiotropic Drug Resistance

PIN: PIN-formed

PPP: Plant Protection Product

RMS: Ramosus

SL: Strigolactone(s)

SLAC: Slow Anion Channel-Associated

SMAX: Suppressor of MAX2

SMXL: SMAX-Like

TPL: Topless

TPR: TPL-related

1. Introduction

The quest for Strigolactones (SL) as endogenous regulators of plant development started when mutants affected in shoot development, displaying stunted and bushy phenotypes, were identified in a number of model species: *Oryza sativa*, rice (*d*, *dwarf*; or *htd*, *high tillering and dwarf* mutants), *Petunia hybrida*, petunia (*dad*, *decreased apical dominance*), *Arabidopsis thaliana*, Arabidopsis (*max*, *more axillary growth*), *Pisum sativum*, pea (*rms*, *ramosus*) (Waters *et al.*, 2017). These phenotypes were quickly shown not to be due to mutations in any known developmental pathway, and to be related to a novel kind of mobile signal molecules mainly but not exclusively produced in roots. From there, these compounds would be transported to the shoot to inhibit branching, contrasting cytokinin while reinforcing auxin activity on axillary buds. Such molecules were identified in 2008 as SL (Gomez-Roldan *et al.*, 2008; Umehara *et al.*, 2008), a family of lactone derivatives of carotenoids, produced in roots and exuded in soil, first detected in 1966 (Cook *et al.*, 1966) and identified a few years later (Cook *et al.*, 1972). Besides their endogenous role in the control of shoot branching, SL have several demonstrated functions in the rhizosphere, all favoured by the steep SL gradient around the root, which makes the presence of SL in soil a reliable indicator of proximity to a living plant root. Indeed, SL are rather labile molecules due to inherent instability of the enol-ether bond between ring C and D (Figure 1), whose integrity is essential for bioactivity (§ 2.1) (Al-Babili and Bouwmeester, 2015). Such exogenous signalling roles include stimulation of seed germination in parasitic plants belonging to the genera *Striga* and *Orobanche* (some former species of which now belong to the genus *Phelipanche*) – an obviously detrimental outcome for the producing plant. A second, indirect positive effect on plant mineral nutrition was proven in 2005, when SL exuded in soil were shown to trigger hyphal branching in arbuscular mycorrhizal (AM) fungi, thus increasing the chances of contact between the symbionts (Akiyama *et al.*, 2005). More recently, stimulating effects of SL on rhizobial swarming and on infection thread formation were also suggested to favour nodulation in legumes (Lopez-Raez *et al.*, 2017) (see (Lumba *et al.*, 2017b) for a graphical timeline of SL-related discoveries).

After the identification of the endogenous hormonal role of SL, further pervasive effects in the producing plant were assigned to this molecular family, comprising at present about 20 described molecular structures (Al-Babili and Bouwmeester, 2015). Reproduction (including flower and seed setting in several species), senescence, and secondary growth are all seemingly promoted by SL to various extents (especially based on the defects of SL-depleted or insensitive plants) (Brewer *et al.*, 2013). Also, their involvement in abiotic stress responses was highlighted by the initial observation of their inducibility by N and especially P deprivation; and later, by phenotypic comparison of mutant plants under nutritional stress. These studies proved that part of the molecular and morphological responses needed for acclimatization to a nutritionally poor environment are indeed

mediated by SL (Marzec *et al.*, 2013). More recently though, it has appeared that SL may also be one of the endogenous molecular workings in acclimatization responses to water deprivation, possibly the major environmental constraint to crop productivity. This fact, given also their strong developmental effects, places SL in an optimal position to act as an integration hub between environmental stimuli and endogenous cues, favouring proper resource allocation decisions by the plant (Liu *et al.*, 2013).

The above-mentioned general aspects of SL biology and functions are covered in detail by other reviews (Al-Babili and Bouwmeester, 2015; Lumba *et al.*, 2017a; Lumba *et al.*, 2017b; Makhzoum *et al.*, 2017). In this review, we provide a quick overview on structure, synthesis, transport, and perception of SL, and we focus thereafter on the role and regulation of the SL pathway during osmotic stress. We discuss available data on organ-specific dynamics of SL synthesis and interaction with abscisic acid (ABA) in the response process, highlighting the importance to consider roots and shoots separately as well as to compare combined *vs* individual stress treatments, to simulate more realistic conditions; and to perform reciprocal grafting experiments to work out organ contributions and long-distance signalling events and components. Finally, we discuss if and how synthetic or natural SL, alone or in combination with crop management strategies such as grafting, may contribute to maximise crop resilience to abiotic stress.

2. General structure, biosynthesis, transport and signal transduction of SL

2.1 Structure

The term SL was proposed in 1995 to indicate a group of terpenoid derivatives sharing a conserved lactone ring and able to induce seed germination in *Striga hermontica*, a holoparasitic plant that, together with other *Orobanchaceae*, imposes huge yield losses in several crops worldwide (Fernandez-Aparicio *et al.*, 2011). Most, though not all, SL analysed so far are characterized by a 4-ring structure, in which the AB and C rings are condensed in a tricyclic lactone, while ring D is a butenolide bound to ring C by an enol ether bridge (Al-Babili and Bouwmeester, 2015; Lumba *et al.*, 2017a) (Figure 1). Substitutions on ring A and stereochemistry of the B-C junction make up most of the diversity within the family, with β- and α-oriented C rings being typical of strigol- and orobanchol-like compounds, respectively; while both subgroups share the *R* orientation of C-2' (Figure 1). Structure-activity relationship studies on natural and synthetic variants of SL indicate that the bioactiphore includes the C and D rings and the connecting enol-ether bridge (Lumba *et al.*, 2017a), while the D ring alone is proposed to become part of the activated receptor complex (*vide infra*, § 2.4). Racemic (*rac*) GR24, the most commonly used synthetic analogue of SL, is composed of

the equimolar mixture of the two enantiomers GR24^{5DS} (with the same stereochemistry as strigol) and GR24$^{ent\text{-}5DS}$ (with stereochemistry at 2'*S* not occurring in natural SL; Figure 1).

While it the structural diversity of naturally occurring SL has been described at least in part, its biological and ecological meaning is largely unexplained yet. In plant species that interact with AM fungi or parasitic plants, co-evolution with the guest, be it friend or foe, might justify the drive to diversification of molecular signals. However, there is no proof that such diversity is only targeted to rhizosphere partners. Indeed, the possibility that multiple endogenous SL within a single species may induce different responses due to specificities in perception or localization has not been addressed experimentally yet. Future studies will test whether different SL regulate different processes within a single species, but high quantities of natural SL are hard to obtain, given that the daily production rate is very low (in the picomoles/plant/day range) (Yoneyama *et al.*, 2010).

2.2 Biosynthesis

A combination of pharmacological and forward genetic strategies reconstructed a basic SL-biosynthetic module highly conserved across species, and composed of the plastid-localized, iron-binding carotenoid isomerase named D27 in rice; of carotenoid cleavage dioxygenase 7 (CCD7) (Arabidopsis MAX3, rice D17/HTD1, pea RMS5, and petunia DAD3); and of CCD8 (Arabidopsis MAX4, rice D10, pea RMS1, and petunia DAD1) (Al-Babili and Bouwmeester, 2015). These three enzymes act sequentially to produce carlactone, a compound sharing with SL the number of C atoms and the presence of a butenolide ring (Figure 2). It is actually debated whether carlactone should be considered a true ("canonical") SL or not, given the lack of B and C rings; nonetheless, its identification as a product of the concerted action of D27, CCD7 and CCD8 solved the core SL-synthesis pathway, providing the missing molecular link between linear carotenoids and tricyclic SL, and pointing to CCD8 as an unusual CCD able to perform multiple operations on its substrate (Bruno *et al.*, 2017).

The subsequent steps leading to the mature SL structures are less clearly defined, and might vary substantially in different species. The cytochrome P450 MAX1 in Arabidopsis converts carlactone to carlactonoic acid, which undergoes further methylation by an unknown methyltransferase (Abe *et al.*, 2014; Seto *et al.*, 2014). The resulting methyl carlactonoate needs further oxygenation by an oxidase such as LBO (Lateral Branching Oxidoreductase) to become bioactive (Brewer *et al.*, 2016). In rice instead, one of the four functional MAX1 orthologues (Os900) acts as a carlactone oxidase, catalysing the formation of the condensed B and C rings to give 4-deoxyorobanchol. Os1400, another MAX1 paralogue, can then form orobanchol from 4-deoxyorobanchol (Zhang *et al.*, 2014). In sorghum, functional loss of the putative sulfotransferase LOW GERMINATION STIMULANT1 (LGS1) converts the dominant SL in root exudates from 5-deoxystrigol to orobanchol, via an unknown mechanism (Gobena *et al.*, 2017).

Therefore, our current understanding of the SL biosynthetic pathway indicates that the natural diversity of SL, which is obvious among species but may be also represented in the same plant by a blend of different SL, originates mainly from the action of modifying enzymes downstream of the core set formed by D27, CCD7, CCD8 and MAX1. These late-acting enzymes are proving hard to identify, possibly because their expression patterns do not necessarily overlap if intermediates are mobile (*vide infra*), and/or because the corresponding mutants have weak phenotypes, and/or because enzyme redundancy masks their molecular, physiological or morphological defects totally or in part (Al-Babili and Bouwmeester, 2015).

In spite of the analytical difficulties due to the very low concentrations, evidence collected so far indicates that SL synthesis is highest in roots, especially tips and vasculature (Al-Babili and Bouwmeester, 2015). Grafting experiments and tracking of SL and of the SL analogue GR24 showed that SL (or their precursors) move from the root to the shoot (Domagalska and Leyser, 2011; Kohlen *et al.*, 2011; Sasse *et al.*, 2015; Xie *et al.*, 2015). However, SL may also be synthesized in stem nodes as well as along the shoot vasculature (Lopez-Obando *et al.*, 2015). Local synthesis aboveground is sufficient for SL-dependent shoot phenotypes, as shown by grafting experiments (Foo *et al.*, 2001; Sorefan *et al.*, 2003; Visentin *et al.*, 2016). SL synthesis in shoots, possibly in leaves, was also proposed to be important for the regulation of guard cell sensitivity to ABA and for proper response to water deprivation (Visentin *et al.*, 2016) (see § 3). However, conclusive proof - beyond SL-biosynthetic gene activation - that leaf tissues are, or not, a true SL source is still missing. Such proof will likely not come until markers (transcriptional or FRET-based for example, as for ABA) (Jones, 2016) are described, that could be used to localize SL synthesis/activity at or close to the single-cell level; and/or until methods are developed to reliably quantify individual SL in small tissue portions or individual cell types such as axillary buds or stomata.

2.3 Transport

The ABCG protein Pleiotropic Drug Resistance1 (PDR1) of *Petunia hybrida* is the only *bona fide* SL transporter characterized thus far (Figure 2). The defective mycorrhizal phenotype of *pdr1* mutants (Kretzschmar *et al.*, 2012) compared to the faster mycorrhization in plants over-expressing the PDR1 protein (Liu *et al.*, 2017), and the pattern of PDR1 localization (Sasse *et al.*, 2015) strongly suggest that SL transport is important for SL effects on mycorrhiza establishment. On the other hand, SL transport contributes to inhibition of lateral bud outgrowth and to resource allocation in responses to environmental constraints, both at the root and shoot levels. This is suggested by 1) the activity profile of the *PhPDR1* promoter (besides root cortex also in elongating root hairs, leaf petioles and at the base of lateral axils) (Liu *et al.*, 2017); 2) the bushy shoots of *pdr1* mutants (Kretzschmar *et al.*, 2012); 3) the fact that petunia plants over-expressing PDR1 show increased lateral root formation and extended root hair elongation. There are also indications that mature

leaves may transport SL towards the stem and subtended axillary bud to join root-produced, upstream-flowing SL (Liu *et al.*, 2017). This route seems to be relevant for leaf senescence regulation, which is partly SL-dependent (Ueda and Kusaba, 2015) and is increased in PDR1-overexpressing plants (Liu *et al.*, 2017). It is thus becoming increasingly clear that the SL source/sink map may be more complicated than initially postulated (*i.e.* following a main root-to-shoot concentration gradient), due to a new leaf-to-stem SL transport route that is important to regulate SL levels in leaves and stems (Liu *et al.*, 2017). Indeed, the possibility that systemic and local transport establish SL gradients both throughout the plant and/or between adjoining tissues is certainly worth exploring. It is possible that local peaks of synthesis and distribution and the resulting local gradient(s), rather than absolute hormone concentrations, are important determinants of the physiological output of SL, as demonstrated for other phytohormones such as auxin (Krupinski and Jonsson, 2010). It is worth noticing also that the expression profile of *D14* (the gene encoding the SL receptor, see § 2.4) is poorly overlapping with that of the core biosynthetic enzymes in Arabidopsis (Chevalier *et al.*, 2014); and that the D14 protein itself was recently proven to act as an intercellular signal molecule, travelling in the phloem to fine-tune and specify the location of SL perception (Kameoka *et al.*, 2016). Of course, the fact that both the SL signal and the receptor are mobile complicates the interpretation of mutant phenotypes, and even more, the deciphering of local *vs* systemic SL functions.

2.4 Perception and transduction

A remarkable amount of information has been gathered on the perception and early signal transduction mechanisms in the SL pathway (Figure 2). The SL receptor proteins in vascular plants are called D14-type receptors after the first characterized member of the clade, D14 in rice (Arite *et al.*, 2009). These proteins are members of the α/β hydrolase-fold superfamily, and cleave the SL molecule generating a tricyclic ABC and a D-ring moiety (Hamiaux *et al.*, 2012). At this point the D ring, or a derivative thereof, is proposed to be trapped and covalently bound within the catalytic pocket (de Saint Germain *et al.*, 2016; Yao *et al.*, 2016). Even though available crystallographic data are not resolving nor decisive enough in this respect (Lombardi *et al.*, 2017), the hydrolysed SL molecule should dock more favourably than the intact one in the active pocket (Gaiji *et al.*, 2012). This peculiarity would explain the very low catalytic turnover of D14-type receptors (de Saint Germain *et al.*, 2016; Hamiaux *et al.*, 2012; Nakamura *et al.*, 2013) and suggests that hydrolytic activity is needed for signal transduction events and/or to de-sensitize the cell in subsequent SL perception events, by lowering the number of available receptor pockets. As D14 itself is actively degraded after physical interaction with SL (Chevalier *et al.*, 2014; Hu *et al.*, 2017), SL perception indeed entails destruction both at the metabolite (Smith and Waters, 2012) and at the receptor level.

Pervasive changes in the 3-D structure of D14 are triggered by the interaction with protein partners (Nakamura *et al.*, 2013; Zhao *et al.*, 2013), prominently the F-box protein MAX2 (Bythell-Douglas *et al.*, 2017). F-box proteins are a leitmotiv in phytohormone biology: as promiscuous adaptors recruiting protein targets for ubiquitination and degradation by the proteasome, they suit perfectly the function of specifically and quickly relieving constitutive response repression (Santner and Estelle, 2010). The direct targets of MAX2 certainly include members of the SUPPRESSOR OF MAX2 1 (SMAX1) and D53 protein families (Jiang *et al.*, 2013; Zhou *et al.*, 2013) (Figure 2). Genetic and biochemical data support for these proteins a repressive role of MAX2 functions, though at different developmental stages and in dependence of distinct receptor/ligand pairs (Waters *et al.*, 2012). Further work in Arabidopsis points to the combined action of SMAX1-LIKE (SMXL) paralogues no. 6, 7 and 8 in branching promotion, *i.e.* as D53 orthologues (Soundappan *et al.*, 2015). These proteins may act through interaction with TOPLESS (TPL)/TOPLESS-RELATED (TPR) proteins, analogously to what observed in the auxin and jasmonate pathway. However, non-TPR-dependent action mode(s) should not be excluded (Lumba et al., 2017b; Waters et al., 2017). Indeed recently, IDEAL PLANT ARCHITECTURE1 (IPA1) has been shown to be one of the long-sought transcription factors repressed by D53 in rice (Song *et al.*, 2017).

Much interesting research has been done on the molecular evolution of SL perception, both in the producing and in the parasitic plant (Lumba *et al.*, 2017b). D14-type SL receptors seem to have generated by gradual neo-functionalization of KARRIKIN INSENSITIVE2 (KAI2) paralogues in higher plants (Bythell-Douglas *et al.*, 2017). KAI2, a close homologue of D14-type proteins, functions as a receptor for karrikins (smoke-derived compounds that stimulate seed germination and share some structural features with SL) (Smith and Li, 2014; Waters *et al.*, 2017). The primary function of KAI2 may be in the recognition of an uncharacterized, endogenous SL-like signal named KL (for KAI2-Ligand), and in the transduction of the KL signal by interaction with MAX2 (Conn and Nelson, 2016) (Figure 2). The D14 and KAI2-mediated pathways therefore converge on MAX2, a crucial issue for researchers trying to disentangle the effects of SL and KL.

3. Organ-specific dynamics of SL synthesis and cross-talk with ABA under single and combined abiotic stress

3.1 Do SL contribute to shoot acclimatization under osmotic stress?

Given their inducibility by nutrient deprivation, contribution to nutritional root symbioses, and ability to shape plant morphology, SL were quickly proposed as a molecular interface between phenotypic plasticity and a changing and often challenging environment (Liu *et al.*, 2013). Indeed, SL contribute to root and shoot morphological and physiological responses to nutrient (N and especially P) scarcity in soil. This concept was later tested also for other abiotic stresses. SL-

deficient or insensitive *Arabidopsis thaliana*, *Lotus japonicus* and *Solanum lycopersicum* are hypersensitive to osmotic stress and respond less to endogenous and exogenous ABA, which strongly suggests that SL synthesis and perception are important for acclimatization (Ha *et al.*, 2014; Li *et al.*, 2017; Liu *et al.*, 2015; Lv *et al.*, 2017; Visentin *et al.*, 2016). In these experiments, survival and physiological performances of SL-related mutants were severely affected when either progressively dehydrated (Ha *et al.*, 2014; Li *et al.*, 2017; Visentin *et al.*, 2016) or exposed to PEG at the root level (Liu *et al.*, 2015).

It must be noted here that one controversial study in Arabidopsis (Bu *et al.*, 2014) reports that signalling (*max2*) but not biosynthetic (*max1*, *max3* and *max4*) mutants are hypersensitive to stress. This led these authors to absolve SL as culprit for the *max2* phenotype, in favour of other pathways in which MAX2 would be involved. There are several apparent contrasting points between this dataset and that of Ha *et al.* (2014), which call for careful reassessment of ABA-related phenotypes especially at the early developmental stages for Arabidopsis SL mutants. The observed discrepancies may derive from differences in the experimental design (see Table S1 for a detailed comparison), and from the difficulty of pinpointing subtle phenotypes, in particular in SL-biosynthetic mutants. This, in turn, might be due to leaking of the biosynthetic mutants, with residual SL being produced at a sufficient level to confound results. Another possibility is that MAX2 might take part in additional pathways also contributing to drought resilience, making the *max2* phenotype more severe than that of biosynthetic mutants: in this context, one rather obvious possibility is that KL, the thus far unidentified endogenous KAI2 ligand, may contribute to the observed phenotype (Li *et al.*, 2017), and do so to variable extents in different species. Given our current understanding of signalling for SL-related molecules, one way to sort this point out would be to test the effects of the pure GR24 enantiomers, to assess if the reported KAI2-dependent activity of the 2'*S* enantiomer ($GR24^{ent\text{-}5DS}$) in Arabidopsis might possibly extend to other species and conditions (Scaffidi *et al.*, 2014; Waters *et al.*, 2017), and how this would relate to drought resilience. On this point, it must be noted that the stress-relieving effect of *rac*-GR24 treatment in Ha *et al.* (2014) is consistent with a positive role of SL in stomatal closure as in Visentin *et al.* (2016) and Lv *et al.* (2017), but all three these works cannot exclude a contribution by $GR24^{ent\text{-}5DS}$. Additionally, *d14* and *kai2* mutants should be included in the panel of analysed lines - if available for the species under study. In two very recent articles this was done for Arabidopsis, supporting a role both for SL and KL in drought responses, including stomatal closure (Li *et al.*, 2017; Lv *et al.*, 2017). So, both KAI2- and D14-dependent signalling pathways seem to contribute additively to acclimatization, given the drought-sensitive phenotype of single and double *kai2*/*d14* mutants (Li *et al.*, 2017). These data confirm that most likely, the relatively stronger drought-related phenotype in SL-depleted *vs max2* mutants is due to the two pathways converging onto MAX2 – the D14- and

KAI2-dependent ones- being both involved. The time is ripe now to work out in detail the individual contributions of the two pathways; the identification of KL would represent, in this sense among many others, a major leap forward.

Notwithstanding these *caveats* and still open questions, the fact that guard cells in SL-depleted plants are hypersensitive to stress and hyposensitive to ABA was confirmed in three different eudicot species by independent groups with a combination of different eco-physiological approaches, including the analyses of SL-depleted plants and now, also of the signaling mutant *d14* (Ha *et al.*, 2014; Li *et al.*, 2017; Liu *et al.*, 2015; Lv *et al.*, 2017; Visentin *et al.*, 2016). Therefore, SL contribution to proper guard cell functioning and acclimatization responses to water deprivation is supported enough to be included among the effects of SL as phytohormones. Expression data for SL-biosynthetic genes upon treatments such as drought, salinity and osmotic stress (Ha *et al.*, 2014; Lv *et al.*, 2017; Visentin *et al.*, 2016), as well as transcript enrichment for *D14* and *MAX2* in the stomatal cell lineage (Lv *et al.*, 2017) are also consistent with this picture (see § 3.3).

3.2 Current understanding of SL mechanism of action in osmotic stress responses: cross-talk between the SL and ABA pathways

3.2.1 At the biosynthesis level

When it comes to the aetiology of such physiological effect, a modulation of free ABA concentration seems not to be blamed in general terms, since free ABA content in Arabidopsis leaves is comparable in WT and *max2* mutants (Bu *et al.*, 2014), even though stomata are consistently more open in the latter genotype (Bu *et al.*, 2014; Ha *et al.*, 2014). Whole-leaf analyses of course do not rule out that the modulation of ABA biosynthesis, catabolism, and transport could lead to transient and/or very localized accumulation of ABA in a specific tissue, ultimately contributing to the observed phenotypes. Invariant free ABA was observed also in WT *vs* *CCD7*-silenced Lotus plants under no stress, or individual osmotic or nutritional stress (P deprivation); however when both stresses were applied together, lower free ABA was recorded in leaves of SL-depleted plants (Liu *et al.*, 2015). The situation in tomato is yet slightly different: quantification in well-watered plants showed slightly more (Visentin *et al.*, 2016) or less (Torres-Vera *et al.*, 2013) concentrated free ABA in leaves of SL-depleted plants than WT, likely depending on whether values were expressed per fresh or dry tissue weight unit, respectively. These slight fluctuations are indeed reasonably explained by the fact that SL-depleted and replete leaves have different relative water content already in the absence of stress (Visentin *et al.*, 2016). In tomato suffering moderate and severe drought though, free ABA was significantly less concentrated in *CCD7*-silenced plants than in WT; these values were obtained per fresh weight unit and could not be underestimated in SL-depleted plants, which are more dehydrated than corresponding WT controls. Less concentrated

ABA may of course contribute to the poor fitness of this line under water deprivation conditions (Visentin *et al.*, 2016).

SL influence on ABA concentration under stress is far less documented at the root level. While no data exist for Arabidopsis, the profile of free ABA concentrations in roots of SL-depleted tomato and Lotus roughly reflects what happens in shoots (Liu *et al.*, 2015; Visentin *et al.*, 2016). Additionally, roots of WT Lotus pre-treated with *rac*-GR24 are unable to increase free ABA concentration in response to subsequent PEG-induced osmotic stress. This observation suggests that - at least in Lotus - there might also be some root-specific negative effect of SL on ABA synthesis under drought (Liu *et al.*, 2015); and/or that once again, the non-natural enantiomer in the *rac*-GR24 used for treatment might be responsible for the effect. A very similar situation is observed in seeds of parasitic plants, in which GR24 is thought to stimulate germination also by accelerating ABA degradation via the ABA-8′ hydroxylase *PrCYP707A1* (Lechat *et al.*, 2012). Analogously, SL may relieve secondary dormancy, *i.e.* thermoinhibition of Arabidopsis seed germination, by lowering ABA concentration (Toh *et al.*, 2012). These examples highlight once again how, depending on the examined organ and conditions, the SL and ABA pathways might be wired differently. It might be worth mentioning here that free ABA concentrations are higher in *kai2* mutants of Arabidopsis than in the WT, both in the absence and presence of drought. This effect is likely due to compromised activity of ABA-8′-hydroxylase enzymes (such as AtCYP707A3), given the lower transcript levels in the *kai2* background (Li *et al.*, 2017). Therefore, also the endogenous KAI2 ligand might interfere with ABA levels so once again, care should be taken in separating the effects of the two.

A positive influence of SL on ABA synthesis in shoots is therefore documented, especially but not limited to shoots under drought, although there seem to be species-specific differences in amplitude. The overall prevailing trend in leaves is for lower ABA concentration in SL-depleted plants; indeed, transcripts of some ABA biosynthetic genes are less concentrated in leaf tissues of Arabidopsis *max2* than WT under drought (Ha *et al.*, 2014). Additionally, *Nine-Cis-Epoxycarotenoid Dioxygenase3* (*NCED3*), *Cytochrome P450 707A3*, *ABCG22*, *ABA Insensitive1* (*ABI1*), and *Hypersensitive to ABA1* (*HAB1*) are all less transcribed in response to drought when *MAX2* is mutated (Bu *et al.*, 2014). This picture is unsupportive of the initial hypothesis that SL and ABA might be influencing each other's levels by merely competing for the same precursor substrate (i.e. carotenoids). It is still not known whether excess SL, obtained for example by treatment with GR24, modulates free ABA content in shoot tissues. On the other hand, the reverse effect - i.e. of genetically reduced ABA content on endogenous SL concentration - was explored in tomato, leading to the conclusion that the overall trend was for a positive correlation between ABA levels and SL synthesis in the roots; correlations were not explored in the shoot, in which both the SL-biosynthetic gene transcripts and final metabolites are undetectable under normal conditions

(López-Ráez *et al.*, 2010). However, ABA treatment induces *MAX3* and *MAX4* transcript accumulation in Arabidopsis leaves (Ha *et al.*, 2014). One potential candidate regulator of both ABA and SL levels in Arabidopsis is ORA47 (Octadecanoid-Responsive AP2/ERF-domain transcription factor47) (Chen *et al.*, 2016), a transcriptional regulator involved in the cross-talk and integration of several phytohormones, prominently of jasmonic acid and ABA. Its chromatin occupancy profile includes, among others, the promoters of biosynthetic and signalling genes in the ABA pathway, and of *MAX3* and *MAX4*. Occupancy is higher-than-background only under normal but not drought conditions in leaves (Chen *et al.*, 2016), when transcripts of these genes accumulate (see § 3.3). This suggests that beyond the most characterized role at the cross-road of ABA and jasmonic acid, ORA47 may act as a transcriptional repressor and integration hub for the SL and ABA pathways as well. This hypothesis is worth investigating and if indeed demonstrated, may define ORA47 as the first molecular link in the SL-ABA crosstalk, namely under drought.

3.2.2. At the ABA-sensitivity level

Beyond the above observations, which suggest that the influence of ABA and SL on their mutual concentrations may be more or less intimate in different species and organs, a combination of eco-physiological measurements (including leaf temperature, stomatal conductance and water potential) all pointed to increased stomatal conductance as a primary reason for higher sensitivity to water deprivation in SL-biosynthetic or signalling mutants. Lower guard cell sensitivity to endogenous and exogenous ABA is identified as another contributing factor to this phenotype. Indeed, SL-depleted and insensitive plants have higher-than-WT stomatal aperture and conductance in the absence and presence of stress, and slower closure in response to exogenous ABA treatment (Ha *et al.*, 2014; Li *et al.*, 2017; Liu *et al.*, 2015; Lv *et al.*, 2017; Visentin *et al.*, 2016).

As expected for positive regulators of acclimatization responses, ABA, drought and/or osmotic stress enhance transcript accumulation for SL biosynthetic genes in leaves (Ha *et al.*, 2014; Lv *et al.*, 2017; Visentin *et al.*, 2016). However, and unexpectedly perhaps, SL-related gene expression and metabolite levels drop in the roots of non-mycorrhizal Lotus (Liu *et al.*, 2015), lettuce and tomato (Ruiz-Lozano *et al.*, 2016; Visentin *et al.*, 2016) undergoing drought. It must be noted that in Lotus, the drought-induced SL repression is independent of nutrient availability, *i.e.* if osmotic stress and P scarcity are applied together, the drought response profile will prevail, and SL synthesis will be inhibited (Liu *et al.*, 2015). These results indicate that the dynamics of SL synthesis are different in different organs, which reinforces the need to separate above- and below-ground organs when addressing issues related to systemic signalling under stress; and that the outcome of combined stresses might not be easily predictable based on single-stress effects. These observations might also explain why roots of SL-depleted and insensitive Arabidopsis plants grow comparably to the WT, in the presence of high mannitol and NaCl (Ha *et al.*, 2014). In fact, if osmotic stress represses

SL synthesis in Arabidopsis roots (which is still to be demonstrated) as it does in lettuce, Lotus and tomato, any genetic defect in SL metabolism or signalling will be less likely to cause a detectable root-related phenotype under these conditions.

3.3 Local and systemic effects of SL and SL-like molecules on stomatal conductance: a parsimonious, preliminary model

The inhibition of SL synthesis and possibly transport in dicot roots under osmotic stress is unlikely to be due to mere metabolic suffering; in fact, gene transcript and metabolite concentrations are quickly reduced, when local water potential has not dropped yet as a consequence of low water availability (Liu *et al.*, 2015; Visentin *et al.*, 2016). Rather, a local consequence of this drop may be the de-repression of ABA synthesis, as mentioned in § 3.2.1. This possibility however is so far suggested only by a pharmacological approach in Lotus, and awaits confirmation in other species and by using the SL enantiomer GR24^{5DS} before it can be generalized to any extent. Whatever the local effect, SL and/or SL precursors travel shootward (Akiyama *et al.*, 2010; Domagalska and Leyser, 2011; Kohlen *et al.*, 2011; Sasse *et al.*, 2015). Therefore, the possibility that a drastically diminished flow of SL or SL-like molecules from the roots may carry precise information to the shoots, could not be excluded. A reductionist approach (mimicking in the absence of stress the SL gradient observed under drought) was taken to disentangle the inherent complexity of the hypothesized interactions *in situ*. SL-replete (WT) tomato scions grafted to SL-depleted rootstocks displayed more concentrated transcript of SL-biosynthetic genes, and higher sensitivity to endogenous and exogenous ABA not only compared to shoots of SL-depleted plants, but also to WT scions grafted onto WT rootstocks (Visentin *et al.*, 2016). The fact that root-produced SL negatively feed back on the SL biosynthetic pathway in above-ground organs had been already proposed in other species, based on similarly hetero-grafted plants (Johnson *et al.*, 2006). Although SL remain stably under the analytical detection threshold in these leaf tissues, as they do under drought (Visentin *et al.*, 2016) and osmotic/salt stress (Lv *et al.*, 2017); and in lack of detailed structural and biosynthetic information on other possibly concurring molecules, the most parsimonious hypothesis at present is that stomata in such hetero-grafted plants display a ABA-hypersensitive phenotype because synthesis of SL or SL-like molecules is enhanced in leaves (as supported by gene expression data). Notably, *rac*-GR24 is sufficient to increase the speed of stomatal closure in response to exogenous ABA in tomato (Visentin *et al.*, 2016), and to trigger stomata closure in the absence of exogenous ABA in Arabidopsis (Lv *et al.*, 2017) just as it improves survival rate under drought both in WT and SL-depleted, but not SL-insensitive *max2* Arabidopsis (Ha *et al.*, 2014). Additionally, as *MAX2* and *D14* transcripts are more concentrated in the stomatal lineage than in other leaf tissues, SL perception may be specifically enhanced in guard cells (Lv *et al.*, 2017). In this context, low SL in roots may well be a component of the systemic drought stress

461 signal in tomato (Visentin *et al.*, 2016), in which (just as in Arabidopsis) ABA does not cover a long-
462 distance signalling function of drought stress (Christmann *et al.*, 2007; Holbrook *et al.*, 2002). Based
463 on the above data, obtained in herbaceous dicots, a mode of action in osmotic stress responses for
464 SL and/or SL-like molecules such as SL intermediates, or KL can be proposed (Figure 3). Such model
465 places a drop in SL synthesis at the root level above the dynamic concentration adjustment of SL
466 (and/or, of SL-like molecules) throughout the plant. As a direct or indirect (*i.e.* mediated by a second
467 messenger) consequence of such drop, synthesis of SL and/or SL-like molecules would be induced
468 in shoots, namely in leaves, to the immediate and positive purpose of making stomatal closure
469 more efficient. How this effect is achieved, and through which mediators, is not yet understood. As
470 an obvious path to beat, the possibility that the ABA transport, perception and/or signalling
471 machinery is primed by SL or SL-like molecules should be explored, with emphasis on the post-
472 transcriptional levels of regulation. However at least in Arabidopsis, all ABA signalling components
473 investigated were found not to be required for the effect of *rac*-GR24 on stomatal closure, which
474 was instead dependent on *MAX2*, *D14*, *SLOW ANION CHANNEL-ASSOCIATED1* (*SLAC1*) and an
475 ABA-independent H_2O_2/NO burst at the guard cell level (Lv *et al.*, 2017) (Figure 3). These results
476 unveil an interesting, completely novel link between SL or SL-like molecules and SLAC1 activity,
477 and open a new avenue of investigation in SL biology. However, they cannot explain why stomata
478 of SL-related mutants in Lotus, tomato and Arabidopsis are hyposensitive to exogenous ABA in
479 feeding experiments (Ha *et al.*, 2014; Liu *et al.*, 2015; Lv *et al.*, 2017; Visentin *et al.*, 2016). A possible
480 reconciliation key for these apparent discrepancies is that given the low background of stomata
481 reactivity they cause, mutations compromising endogenous SL synthesis or perception are able to
482 unveil a contribution of SL-dependent priming of ABA signalling/transport to stomata during ABA
483 feeding experiments. During *rac*-GR24 feeding experiments instead, the effects of ABA-
484 independent, direct SLAC1 stimulation by exogenous SL may be strong enough to mask milder
485 ABA-dependent ones. In other words, while the effect of ABA on stomatal closure is at least
486 partially dependent on endogenous SL, *rac*-GR24 effects on the same feature are largely ABA
487 independent. Clearly, this signalling module is not the only ABA-independent response to SL or SL-
488 like molecules: *max2* and *kai2* Arabidopsis mutants were reported to dismantle their photosynthetic
489 machinery more slowly, and switch on anthocyanin synthesis less efficiently than the WT, in an
490 ABA-independent way (Ha *et al.*, 2014; Li *et al.*, 2017) – two features that, once again, may worsen
491 performances under stress. It must be noted here that *rac*-GR24-triggered flavonoid synthesis was
492 shown to be dependent both on D14 and KAI2 in Arabidopsis roots (Walton *et al.*, 2016).
493
494 **4. Perspectives on abiotic stress relief and practical applications of SL in agriculture**

Modern agriculture requests continue, more and more specific interventions during the growth season in order to manage a wide range of biotic and abiotic challenges; and thus, innovative crop protection solutions must be continuously developed. In the last years, traditional breeding has been associated with the use of a new generation of agrochemical compounds. These give satisfying results in protection against biotic stresses such as bacterial or fungal diseases, and weed plant infestation. On the other hand, the same solutions cannot warrant adequate results against abiotic stresses such as water or nutrient deficiency. Generally, plants acclimate to adverse conditions by exploiting signal molecules that in turn, will modulate several genetic and metabolic pathways. Many among these signal molecules are already present as phytoregulators or biofertilisers in the catalogue of agrochemical companies, with a prominent role played by phytohormones (gibberellins to stimulate seed germination and fruit ripening, auxins to promote flower and fruit development etc.). SL as well could raise a similar interest by the agro-technical market thanks to their already characterized activity both as signal molecules in the rhizosphere and as endogenous hormones (Makhzoum *et al.*, 2017; Screpanti *et al.*, 2016a). The potential for application in the control of parasitic weeds has been the first to be investigated, both because of the huge market impact of these pathogens, and of the early discovery of SL as potent seed germination stimulants for *Striga*, *Phelipanche* and *Orobanche* seeds (Screpanti *et al.*, 2016b; Yoneyama *et al.*, 2010). Seed banks of parasitic species in these genera infest not only Asia and Africa but also the Mediterranean and Black Sea regions (Zwanenburg *et al.*, 2016), causing huge yield losses in commercial crops by hampering host growth and life-cycle completion through subtraction of water and nutrients from the phloem in colonized roots (Parker, 2009). The proposed SL-based control strategy is named "suicidal germination": SL are delivered to the parasitic seed-infested soils in the absence of a host crop, in order to lead germinated seeds to death. The strategy is covered in detail elsewhere (Fernandez-Aparicio *et al.*, 2011; Zwanenburg *et al.*, 2016). Similarly, as soon as SL were associated to the stimulation of hyphal branching in AM fungi, their soil application in combination with other compounds such as elicitors of defence responses or fungicides was promptly patented (Dahmen *et al.*, 2011; Suty-Heinze and Vors, 2008, 2009) as a mitigation strategy against combined stresses. Simplifying, marginal soils could be amended with exogenous SL and AM fungi (and/or Rhizobia where appropriate, given the effects on swarming discovered later), in order to increase the chances of successful host colonization and thus, of improving plant mineral nutrition. Analogously, plastic remodelling of root/shoot morphology and modulation of developmental progression (namely, of the juvenile to reproductive phase transition) are very interesting endogenous effects in a perspective of crop management practices, and could be possibly also achieved by targeted delivery to the site of action, in order to reduce the amount of active principle required. The latter strategy would of course be sustainable only in high-profitability

crops, and needs careful evaluation of goals and formulations on a case-by-case basis; for example, mere spraying with exogenous SL is known, at least in certain model plants, not to inhibit shoot branching (Gomez-Roldan *et al.*, 2008; Umehara *et al.*, 2008).

Unfortunately, a key limit for the use of these potential biofertilisers in plant protection is the chemical instability of natural SL in aqueous solution, which especially at alkaline pH, rather rapidly hydrolyse by producing an ABC-formyl lactone and 5-hydroxybutenolide (Akiyama *et al.*, 2010). In addition to this restriction, also the mass production of natural SL is at present technically and economically challenging. In fact, about 20 different natural SL have been isolated and characterized so far, but their concentration in plant-derived samples such as root exudates is very low (Al-Babili and Bouwmeester, 2015). Complete chemical synthesis has been achieved, but besides the low yield, it is labour- and time-consuming (Brooks *et al.*, 1985; Shoji *et al.*, 2009). Therefore, the task of obtaining large quantities of natural SL from plants or through organic synthesis is still daunting and/or not economically viable for the agrochemical market – certainly so for commodity crops, on which mark-ups are generally low. For these reasons, synthetic molecules with a simpler chemical structure than natural SL, yet showing comparable bioactivity to the natural compounds were developed (Prandi and Cardinale, 2014). "Synthetic SL" can be classified into two main categories: analogues, whose structure is very similar to natural SL though easier to synthesize *in vitro*; and mimics, whose structure is much simpler. Both will retain all or a subset of SL-like bioactivity features. With regard to the latter point, it must be noted that quite some effort has been devoted by organic chemists, biochemists and modellers to design molecular structures retaining SL-like bioactivity towards only a subset of target organisms or organs, if applicable (Prandi and Cardinale, 2014). For example, the mimic molecule named 4-BD (4-Br debranone) is not active as germination stimulant of parasitic seeds; thus, a 4-BD-based weed-avoidance strategy can be envisaged, that couples SL-deficient plants (to prevent seed-bank stimulation by natural SL exudation in the rhizosphere) and 4-BD (to compensate for possible unwanted phenotypic effects of SL deficiency in the producing plant, without contributing to weed infestation) (Fukui *et al.*, 2013). A similar strategy was also proposed based on other analogues that retain their bioactivity on plant morphology, but induce very little germination of parasitic weeds (Boyer *et al.*, 2014).

More recently, as described in § 3, treatment with exogenous *rac*-GR24 was shown to increase stomata reactivity in tomato and Arabidopsis (Lv *et al.*, 2017; Visentin *et al.*, 2016) and performances under drought in SL-depleted and WT, while not in SL-insensitive Arabidopsis (Ha *et al.*, 2014). Notwithstanding the *caveats* on the use of racemic mixtures in proof-of-concept experiments (see § 3.1), and taking into account that the non-natural enantiomer in the racemic mixture likely contributes to the effect through KAI2, this ability of synthetic molecules to confer drought resistance by foliar nebulization opens interesting scenarios. Synthetic SL derivatives were

indeed proven to relieve drought of maize under field conditions, and patented in this respect (Davidson *et al.*, 2015; Lumbroso and De Mesmaeker, 2017); foliar application would bypass most instability issues for molecules delivered in soil. This highlights how available SL analogues/mimics and karrikins could serve as a blueprint for the development of future agrochemicals aimed at controlling plant water use and improving yield under water stress conditions, just like ABA agonists (Helander *et al.*, 2016). While it is clear indeed that ABA is a central regulator of plant water use, the fact that *rac*-GR24 acts mostly ABA-independently on stomatal closure might allow for efficient control of water losses, without stimulating the full array of ABA responses (Ha *et al.*, 2014; Lv *et al.*, 2017). On the other hand, different stresses may be associated to non-overlapping SL profiles in different organs (see for example, osmotic stress and P deprivation); therefore, what outcome combined stress might have in terms of metabolite profile, must be determined experimentally. Only after such data are available might the effect of treatment with exogenous SL be foreseen. For example, if SL are delivered to leaves of dicot plants under combined osmotic and nutritional stress (by both of which SL may be induced in leaves), it is likely that the effects on stress resilience will be positive; not necessarily so if treatments were targeted to the roots (in which, during combined stress, the SL decrease triggered by osmotic stress will override the increase induced by P deprivation) (see § 3). Additionally, since SL in soil may stimulate parasitic seed germination, foliar application may be safer than soil delivery if the risk of weed infestation is not zero in any given field. Wet testing is needed in this sense, but still missing for any realistic stress combinations.

It must be noted as well that a potentially exploitable effect on stomatal conductance could be obtained in WT shoots of tomato plants grafted onto SL-depleted rootstocks (Visentin *et al.*, 2016). This result, besides providing mechanistic insights in SL-dependent root-to-shoot communication, opens the possibility to develop efficient drought resistance strategies for graftable plants, in which SL dynamics under drought mirror what happens in tomato. The use of SL-depleted (possibly non-transgenic) rootstocks for SL-replete scions leads to higher water use efficiency and better performances under stress thanks to the demonstrated increase of ABA sensitivity in such scions compared to WT shoots grafted onto WT roots (Visentin *et al.*, 2016); and this, without using any natural or synthetic chemical endowed with SL-like activity. Additionally, the possibility cannot be excluded that natural variants exist among tomato accessions and wild relatives, which are more resilient than cultivated genotypes because they exploit more efficiently the SL- (or SL-like) related toolbox. In this sense, collections could be screened looking for genotypes displaying the most effective root/shoot activation profile of the SL or SL-like pathways, under normal and stress conditions. It must be noted in this regard that rootstocks in which SL production is knocked down (yet not completely out) may also induce less germination in seed banks of parasitic weeds, and yet

599 produce enough SL to allow for regular colonization by AM fungi (see for example (Vogel *et al.*,
600 2010), identifying a balance point between contrasting ecological needs.
601
602 Thus, the many features of SL bioactivity make them potentially interesting for agronomic
603 applications against abiotic stress: soil treatment to improve beneficial symbiosis with AM fungi
604 and Rhizobium, foliar nebulization and grafting contrasting genotypes for SL production to increase
605 drought resistance seem to be the most promising strategies at present. On the other hand, the
606 road to market uptake for any SL-based product is inevitably long: chemical instability in water
607 solution, difficulties in the isolation of such low-concentration natural metabolites, the economic
608 burden of productive scale-up and registration of synthetic molecules are the biggest challenges to
609 tackle. Nonetheless, if enrichment strategies and protocols can be optimized to allow for the
610 development of a natural SL-enriched biostimulant, a decrease of the industrial costs (due in
611 particular to the registration and certification load) could be achieved. A biostimulant can be
612 defined as a (mix of) substance(s) and/or microorganisms that, when applied to plants or the
613 rhizosphere, stimulates natural processes to enhance/benefit crop yield and quality, also by
614 enhancing resilience to and recovery from abiotic stress, drought included (Van Oosten *et al.*, 2017).
615 The positive influence of biostimulants is dependent on plant species, cultivars, climatic conditions,
616 dose, origin and time of application, but their use is fully compatible with both conventional and
617 organic agriculture. New, SL-enriched biostimulant formulations could be ideally developed and
618 tested for proof-of-concept, to the long-term goal of integrating them into the set of most effective
619 crop management practices and tools that prevent and mitigate the effect of abiotic stress. In
620 Europe, biostimulants can be currently placed on the market either under the national regulations
621 on fertilisers, or under the European pesticides law, which combines both supranational and
622 national provisions for introducing plant protection products (PPPs) on the market (EC regulation
623 No 1107/2009). However, a Fertiliser Proposal covering biostimulants as "fertilising products" (*i.e.*
624 distinct from fertilisers *sensu strictu*, but also from PPPs) is currently under discussion by the EC; its
625 goal is to amend the 2009 Regulation on PPPs, to explicitly exclude biostimulants. This currently
626 leaves biostimulants in a regulatory limbo, which is thought to be over shortly. Were biostimulants
627 to be registered for commercialization under less demanding regulations than PPPs, natural SL-
628 enriched versions might become as or more attractive than synthetic SL for certain applications.
629
630 **5. Main open questions and conclusions**
631 Many open questions of course persist, both at the basic understanding level and on the feasibility
632 of practical applications of fundamental knowledge. Namely, main avenues of research will have to
633 give further details in the molecular underpinnings of SL effects on stomatal closure, explaining the

reasons for the ABA-dependent share of guard cell activity impairment in SL mutants. The fact that SL accumulate in stressed *vs* unstressed leaves is still awaiting to be conclusively proven or disproven; it is indeed possible that SL synthesis in droughted leaves is highly localized (for example, in guard cells; and anyway enough to escape detection in whole-leaf analyses), and/or that different metabolites than the known ones, such as KL, are co-responsible for the observed phenotypes. To this goal, readouts of SL activity are needed, but yet to be developed, which are both sensitive, quantitative and at high spatial resolution (ideally, at the single-cell level); and knowledge on the elusive KL is to be acquired. Finally, the actual mitigation effects of SL-based management strategies on abiotic stress consequences in realistic field (open or protected) situations must be explored soon by the academic community, if we are to fully exploit the theoretical potential of SL in modern agriculture.

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Bibliography

Abe S, Sado A, Tanaka K, et al. 2014. Carlactone is converted to carlactonoic acid by MAX1 in Arabidopsis and its methyl ester can directly interact with AtD14 *in vitro*. Proceedings of the National Academy of Sciences of the United States of America **111**, 18084-18089.
Akiyama K, Matsuzaki K, Hayashi H. 2005. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature **435**, 824-827.
Akiyama K, Ogasawara S, Ito S, Hayashi H. 2010. Structural requirements of strigolactones for hyphal branching in AM fungi. Plant & Cell Physiology **51**, 1104-1117.
Al-Babili S, Bouwmeester HJ. 2015. Strigolactones, a novel carotenoid-derived plant hormone. Annual Review of Plant Biology **66**, 161-186.
Arite T, Umehara M, Ishikawa S, Hanada A, Maekawa M, Yamaguchi S, Kyozuka J. 2009. *d14*, a strigolactone-insensitive mutant of rice, shows an accelerated outgrowth of tillers. Plant & Cell Physiology **50**, 1416-1424.
Boyer FD, de Saint Germain A, Pouvreau JB, et al. 2014. New strigolactone analogs as plant hormones with low activities in the rhizosphere. Molecular Plant **7**, 675-690.
Brewer PB, Koltai H, Beveridge CA. 2013. Diverse roles of strigolactones in plant development. Molecular Plant **6**, 18-28.
Brewer PB, Yoneyama K, Filardo F, et al. 2016. LATERAL BRANCHING OXIDOREDUCTASE acts in the final stages of strigolactone biosynthesis in Arabidopsis. Proceedings of the National Academy of Sciences of the United States of America **113**, 6301-6306.
Brooks DW, Bevinakatti HS, Krennedy E, Hathaway J. 1985. Practical total synthesis of +/- strigol. Journal of Organic Chemistry **50**, 628-632.
Bruno M, Vermathen M, Alder A, Wust F, Schaub P, van der Steen R, Beyer P, Ghisla S, Al-Babili S. 2017. Insights into the formation of carlactone from in-depth analysis of the CCD8-catalyzed reactions. FEBS Letters **591**, 792-800.
Bu Q, Lv T, Shen H, et al. 2014. Regulation of drought tolerance by the F-box protein MAX2 in Arabidopsis. Plant Physiology **164**, 424-439.
Bythell-Douglas R, Rothfels CJ, Stevenson DWD, Graham SW, Wong GK, Nelson DC, Bennett T. 2017. Evolution of strigolactone receptors by gradual neo-functionalization of KAI2 paralogues. BMC Biology **15**, 52.
Chen HY, Hsieh EJ, Cheng MC, Chen CY, Hwang SY, Lin TP. 2016. ORA47 (octadecanoid-responsive AP2/ERF-domain transcription factor 47) regulates jasmonic acid and abscisic acid biosynthesis and signaling through binding to a novel *cis*-element. New Phytologist **211**, 599-613.
Chevalier F, Nieminen K, Sanchez-Ferrero JC, Rodriguez ML, Chagoyen M, Hardtke CS, Cubas P. 2014. Strigolactone promotes degradation of DWARF14, an alpha/beta hydrolase essential for strigolactone signaling in Arabidopsis. Plant Cell **26**, 1134-1150.
Christmann A, Weiler EW, Steudle E, Grill E. 2007. A hydraulic signal in root-to-shoot signalling of water shortage. Plant Journal **52**, 167-174.
Conn CE, Nelson DC. 2016. Evidence that KARRIKIN-INSENSITIVE2 (KAI2) receptors may perceive an unknown signal that is not karrikin or strigolactone. Frontiers in Plant Science **6**, 1219.
Cook CE, Whichard LP, Turner B, Wall ME, Egley GH. 1966. Germination of witchweed (*Striga lutea* Lour.): isolation and properties of a potent stimulant. Science **154**, 1189-1190.
Cook CE, Whichard LPW, M.E., Egley GH, Coggan P, Luhan PA, McPhail AT. 1972. Germination stimulants II: The structure of strigol—a potent seed stimulant for witchweed (*Striga lutea* Lour). Journal of the American Chemical Society **94**, 6198-6199.

Dahmen P, Portz D, Spica G, Vors JP. 2011. Compositions comprising a strigolactone compound and a chito-oligosaccharide compound for enhanced plant growth and yield. WO 2010/125065A9.
Davidson EA, Bayer TS, Windram O, Hleba Y. 2015. Formulations de strigolactone et leurs utilisations. WO 2015061764 A1.
de Saint Germain A, Clave G, Badet-Denisot MA, et al. 2016. An histidine covalent receptor and butenolide complex mediates strigolactone perception. Nature Chemical Biology **12**, 787-794.
Domagalska MA, Leyser O. 2011. Signal integration in the control of shoot branching. Nature Reviews: Molecular Cell Biology **12**, 211-221.
Fernandez-Aparicio M, Westwood J, Rubiales D. 2011. Agronomic, breeding, and biotechnological approaches to parasitic plant management through manipulation of germination stimulant levels in agricultural soils. Botany **89**, 813–826.
Foo E, Turnbull CG, Beveridge CA. 2001. Long-distance signaling and the control of branching in the *rms1* mutant of pea. Plant Physiology **126**, 203-209.
Fukui K, Ito S, Asami T. 2013. Selective mimics of strigolactone actions and their potential use for controlling damage caused by root parasitic weeds. Molecular Plant **6**, 88-99.
Gaiji N, Cardinale F, Prandi C, Bonfante P, Ranghino G. 2012. The computational-based structure of Dwarf14 provides evidence for its role as potential strigolactone receptor in plants. BMC Research Notes **5**, 307.
Gobena D, Shimels M, Rich PJ, Ruyter-Spira C, Bouwmeester H, Kanuganti S, Mengiste T, Ejeta G. 2017. Mutation in sorghum *LOW GERMINATION STIMULANT 1* alters strigolactones and causes *Striga* resistance. Proceedings of the National Academy of Sciences of the United States of America **114**, 4471-4476.
Gomez-Roldan V, Fermas S, Brewer PB, et al. 2008. Strigolactone inhibition of shoot branching. Nature **455**, 189-194.
Ha CV, Leyva-Gonzalez MA, Osakabe Y, et al. 2014. Positive regulatory role of strigolactone in plant responses to drought and salt stress. Proceedings of the National Academy of Sciences of the United States of America **111**, 851-856.
Hamiaux C, Drummond RS, Janssen BJ, Ledger SE, Cooney JM, Newcomb RD, Snowden KC. 2012. DAD2 is an alpha/beta hydrolase likely to be involved in the perception of the plant branching hormone, strigolactone. Current Biology **22**, 2032-2036.
Helander JD, Vaidya AS, Cutler SR. 2016. Chemical manipulation of plant water use. Bioorganic and Medicinal Chemistry **24**, 493-500.
Holbrook NM, Shashidhar VR, James RA, Munns R. 2002. Stomatal control in tomato with ABA-deficient roots: response of grafted plants to soil drying. Journal of Experimental Botany **53**, 1503-1514.
Hu Q, Yajun He Y, Wang L, et al. 2017. DWARF14, a receptor covalently linked with the active form of strigolactones, undergoes strigolactone-dependent degradation in rice. Frontiers in Plant Science **in press**.
Jiang L, Liu X, Xiong G, et al. 2013. DWARF 53 acts as a repressor of strigolactone signalling in rice. Nature **504**, 401-405.
Johnson X, Brcich T, Dun EA, Goussot M, Haurogne K, Beveridge CA, Rameau C. 2006. Branching genes are conserved across species. Genes controlling a novel signal in pea are coregulated by other long-distance signals. Plant Physiology **142**, 1014-1026.
Jones AM. 2016. A new look at stress: abscisic acid patterns and dynamics at high-resolution. New Phytologist **210**, 38-44.

Kameoka H, Dun EA, Lopez-Obando M, Brewer PB, de Saint Germain A, Rameau C, Beveridge CA, Kyozuka J. 2016. Phloem transport of the receptor DWARF14 protein is required for full function of strigolactones. Plant Physiology **172**, 1844-1852.
Kohlen W, Charnikhova T, Liu Q, et al. 2011. Strigolactones are transported through the xylem and play a key role in shoot architectural response to phosphate deficiency in nonarbuscular mycorrhizal host Arabidopsis. Plant Physiology **155**, 974-987.
Kretzschmar T, Kohlen W, Sasse J, et al. 2012. A petunia ABC protein controls strigolactone-dependent symbiotic signalling and branching. Nature **483**, 341-344.
Krupinski P, Jonsson H. 2010. Modeling auxin-regulated development. Cold Spring Harbor Perspectives in Biology **2**, a001560.
Lechat MM, Pouvreau JB, Peron T, et al. 2012. *PrCYP707A1*, an ABA catabolic gene, is a key component of *Phelipanche ramosa* seed germination in response to the strigolactone analogue GR24. Journal of Experimental Botany **63**, 5311-5322.
Li W, Nguyen KH, Chu HD, et al. 2017. The karrikin receptor KAI2 promotes drought resistance in *Arabidopsis thaliana*. PLoS Genetics **13**, e1007076.
Liu G, Pfeifer J, de Brito Francisco R, et al. 2017. Changes in the allocation of endogenous strigolactone improve plant biomass production on phosphate-poor soils. New Phytologist **in press**.
Liu J, He H, Vitali M, et al. 2015. Osmotic stress represses strigolactone biosynthesis in *Lotus japonicus* roots: exploring the interaction between strigolactones and ABA under abiotic stress. Planta **241**, 1435-1451.
Liu J, Lovisolo C, Schubert A, Cardinale F. 2013. Signaling role of strigolactones at the interface between plants, (micro)organisms, and a changing environment. Journal of Plant Interactions **8**, 17-33.
Lombardi C, Artuso E, Grandi E, Lolli M, Spirakys F, Priola E, Prandi C. 2017. Recent advances in the synthesis of analogues of phytohormones strigolactones with ring-closing metathesis as a key step. Organic & Biomolecular Chemistry **in press**.
Lopez-Obando M, Ligerot Y, Bonhomme S, Boyer FD, Rameau C. 2015. Strigolactone biosynthesis and signaling in plant development. Development **142**, 3615-3619.
López-Ráez JA, Kohlen W, Charnikhova T, et al. 2010. Does abscisic acid affect strigolactone biosynthesis? New Phytologist **187**, 343-354.
Lopez-Raez JA, Shirasu K, Foo E. 2017. Strigolactones in plant Interactions with beneficial and detrimental organisms: the Yin and Yang. Trends in Plant Science **22**, 527-537.
Lumba S, Bunsick M, McCourt P. 2017a. Chemical genetics and strigolactone perception. F1000 Research **6**, 975.
Lumba S, Holbrook-Smith D, McCourt P. 2017b. The perception of strigolactones in vascular plants. Nature Chemical Biology **13**, 599-606.
Lumbroso AFJC, De Mesmaeker A. 2017. Plant growth regulator compounds. WO 2017025427 A1.
Lv S, Zhang Y, Li C, et al. 2017. Strigolactone-triggered stomatal closure requires hydrogen peroxide synthesis and nitric oxide production in an abscisic acid-independent manner. New Phytologist **in press**.
Makhzoum A, Yousefzadi M, Malik S, Gantet P, Tremouillaux-Guiller J. 2017. Strigolactone biology: genes, functional genomics, epigenetics and applications. Critical Reviews in Biotechnology **37**, 151-162.
Marzec M, Muszynska A, Gruszka D. 2013. The role of strigolactones in nutrient-stress responses in plants. International Journal of Molecular Sciences **14**, 9286-9304.

Nakamura H, Xue YL, Miyakawa T, et al. 2013. Molecular mechanism of strigolactone perception by DWARF14. Nature Communications **4**, 2613.
Parker C. 2009. Observations on the current status of *Orobanche* and *Striga* problems worldwide. Pest Management Science **65**, 453-459.
Prandi C, Cardinale F. 2014. Strigolactones: a new class of plant hormones with multifaceted roles. *eLS 2014* 10.1002/9780470015902.a0023754: John Wiley & Sons Ltd: Chichester.
Ruiz-Lozano JM, Aroca R, Zamarreno AM, Molina S, Andreo-Jimenez B, Porcel R, Garcia-Mina JM, Ruyter-Spira C, Lopez-Raez JA. 2016. Arbuscular mycorrhizal symbiosis induces strigolactone biosynthesis under drought and improves drought tolerance in lettuce and tomato. Plant, Cell & Environment **39**, 441-452.
Santner A, Estelle M. 2010. The ubiquitin-proteasome system regulates plant hormone signaling. Plant Journal **61**, 1029-1040.
Sasse J, Simon S, Gubeli C, Liu GW, Cheng X, Friml J, Bouwmeester H, Martinoia E, Borghi L. 2015. Asymmetric localizations of the ABC transporter PhPDR1 trace paths of directional strigolactone transport. Current Biology **25**, 647-655.
Scaffidi A, Waters MT, Sun YK, Skelton BW, Dixon KW, Ghisalberti EL, Flematti GR, Smith SM. 2014. Strigolactone hormones and their stereoisomers signal through two related receptor proteins to induce different physiological responses in Arabidopsis. Plant Physiology **165**, 1221-1232.
Screpanti C, Fonne-Pfister R, Lumbroso A, Rendine S, Lachia M, De Mesmaeker A. 2016a. Strigolactone derivatives for potential crop enhancement applications. Bioorganic and Medicinal Chemistry Letters **26**, 2392-2400.
Screpanti C, Yoneyama K, Bouwmeester HJ. 2016b. Strigolactones and parasitic weed management 50 years after the discovery of the first natural strigolactone strigol: status and outlook. Pest Management Science **72**, 2013-2015.
Seto Y, Sado A, Asami K, Hanada A, Umehara M, Akiyama K, Yamaguchi S. 2014. Carlactone is an endogenous biosynthetic precursor for strigolactones. Proceedings of the National Academy of Sciences of the United States of America **111**, 1640-1645.
Shoji M, Suzuki E, Ueda M. 2009. Total synthesis of (+/-)-5-deoxystrigol via reductive carbon-carbon bond formation. Journal of Organic Chemistry **74**, 3966-3969.
Smith SM, Li J. 2014. Signalling and responses to strigolactones and karrikins. Current Opinion in Plant Biology **21**, 23-29.
Smith SM, Waters MT. 2012. Strigolactones: destruction-dependent perception? Current Biology **22**, 924-927.
Song X, Lu Z, Yu H, et al. 2017. IPA1 functions as a downstream transcription factor repressed by D53 in strigolactone signaling in rice. Cell Research **27**, 1128-1141.
Sorefan K, Booker J, Haurogne K, et al. 2003. *MAX4* and *RMS1* are orthologous dioxygenase-like genes that regulate shoot branching in Arabidopsis and pea. Genes and Development **17**, 1469-1474.
Soundappan I, Bennett T, Morffy N, Liang Y, Stanga JP, Abbas A, Leyser O, Nelson DC. 2015. SMAX1-LIKE/D53 family members enable distinct MAX2-dependent responses to strigolactones and karrikins in Arabidopsis. Plant Cell **27**, 3143-3159.
Suty-Heinze A, Vors JP. 2008. Pesticidal composition comprising a strigolactone derivative and a fungicide compound. WO 2008/152092A2.
Suty-Heinze A, Vors JP. 2009. Pesticidal composition comprising a strigolactone derivative and a fungicide compound. WO 2008/152092A3.

Toh S, Kamiya Y, Kawakami N, Nambara E, McCourt P, Tsuchiya Y. 2012. Thermoinhibition uncovers a role for strigolactones in Arabidopsis seed germination. Plant and Cell Physiology **53**, 107-117.
Torres-Vera R, Garcia JM, Pozo MJ, López-Ráez JA. 2013. Do strigolactones contribute to plant defence? Molecular Plant Pathology **15**, 211-216.
Ueda H, Kusaba M. 2015. Strigolactone regulates leaf senescence in concert with ethylene in Arabidopsis. Plant Physiology **169**, 138-147.
Umehara M, Hanada A, Yoshida S, et al. 2008. Inhibition of shoot branching by new terpenoid plant hormones. Nature **455**, 195-200.
Van Oosten MJ, Pepe O, De Pascale S, Silletti S, Maggio A. 2017. The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants. Chemical and Biological Technologies in Agriculture **4**, 5.
Visentin I, Vitali M, Ferrero M, et al. 2016. Low levels of strigolactones in roots as a component of the systemic signal of drought stress in tomato. New Phytologist **212**, 954-963.
Vogel JT, Walter MH, Giavalisco P, et al. 2010. SlCCD7 controls strigolactone biosynthesis, shoot branching and mycorrhiza-induced apocarotenoid formation in tomato. Plant Journal **61**, 300-311.
Walton A, Stes E, Goeminne G, et al. 2016. The response of the root proteome to the synthetic strigolactone GR24 in Arabidopsis. Molecular and Cellular Proteomics **15**, 2744-2755.
Waters MT, Gutjahr C, Bennett T, Nelson DC. 2017. Strigolactone signaling and evolution. Annual Review of Plant Biology **68**, 291-322.
Waters MT, Nelson DC, Scaffidi A, Flematti GR, Sun YK, Dixon KW, Smith SM. 2012. Specialisation within the DWARF14 protein family confers distinct responses to karrikins and strigolactones in Arabidopsis. Development **139**, 1285-1295.
Xie X, Yoneyama K, Kisugi T, Nomura T, Akiyama K, Asami T, Yoneyama K. 2015. Strigolactones are transported from roots to shoots, although not through the xylem. Journal of Pesticide Science **40**, 214-216.
Yao R, Ming Z, Yan L, et al. 2016. DWARF14 is a non-canonical hormone receptor for strigolactone. Nature **536**, 469-473.
Yoneyama K, Awad AA, Xie X, Takeuchi Y. 2010. Strigolactones as germination stimulants for root parasitic plants. Plant & Cell Physiology **51**, 1095-1103.
Zhang Y, van Dijk ADJ, Scaffidi A, et al. 2014. Rice cytochrome P450 MAX1 homologs catalyze distinct steps in strigolactone biosynthesis. Nature Chemical Biology **10**, 1028–1033.
Zhao LH, Zhou XE, Wu ZS, et al. 2013. Crystal structures of two phytohormone signal-transducing alpha/beta hydrolases: karrikin-signaling KAI2 and strigolactone-signaling DWARF14. Cell Research **23**, 436-439.
Zhou F, Lin Q, Zhu L, et al. 2013. D14-SCF(D3)-dependent degradation of D53 regulates strigolactone signalling. Nature **504**, 406-410.
Zwanenburg B, Mwakaboko AS, Kannan C. 2016. Suicidal germination for parasitic weed control. Pest Management Science **72**, 2016-2025.

Fig.1. Prototypal structures of natural SL and analogues. (A) General four-ring structure (ABCD) of SL, and relative C-atom numbering. **(B)** The racemic solution of GR24, the most commonly used synthetic analogue of SL, is composed of the equimolar mixture of the two enantiomers $GR24^{5DS}$ (with the same stereochemistry as strigol) and $GR24^{ent\text{-}5DS}$. **(C)** Molecular structures of strigol and orobanchol, two naturally occurring SL characterized by β- and α-orientations of the C ring, respectively. They are representatives of the two main molecular types of natural SL; both share the *R* configuration at the C-2′ of ring D.

Fig. 2. Main synthesis and perception avenues of SL. Left-hand panel: SL biosynthesis starts in plastids where three enzymes, D27, CCD7 and CCD8, act sequentially on carotenoids to produce carlactone, a precursor of SL. Carlactone is then transferred to the cytosol, where it is further processed in order to produce SL. SL and carlactone are then perceived in the same cell where they were produced (not shown) and/or transferred to other cells; while the first are probably transferred via the PDR1 protein, the transporter for carlactone is not identified yet (dotted arrow). It is also not known if some steps of the SL biosynthetic pathway are shared by other SL-like molecules. Right-hand panel: SL (or, other carlactone derivatives) activate MAX2-dependent signal transduction after physical binding with the receptor D14. Through this pathway, SL modulate transcription by destabilizing members of the SMXL family of transcriptional corepressors; induce stomatal closure by influencing the activity of the ion channel SLAC1; and influence auxin distribution by promoting the removal of PIN-FORMED (PIN) transporters. MAX2 is also a component of the KAI2-triggered transduction cascade. The ligands to this receptor are thought to be an endogenous, putative SL-like signal molecule (KL) and karrikins (which are also suspected to activate a MAX2-independent signalling pathway; dotted arrow).

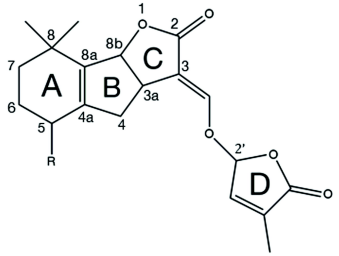
Fig. 3. Model for SL action in root-shoot communication and local signalling under drought. The main connections between SL (or SL-like signal molecules such as SL precursors, or KL) and ABA in roots and shoots under drought stress are highlighted. SL/SL-like molecules may have a negative effect on osmotic stress-induced ABA levels in roots, as indicated by *rac*-GR24 treatment in *Lotus japonicus*. This suggests that a drop in SL/SL-like synthesis in this organ under osmotic stress may be required (but not sufficient) to let ABA levels rise [1]. The shootward flow of SL/SL-like molecules represses by an unknown mechanism the transcription of SL/SL-like biosynthetic genes in shoots, especially under normal conditions when more SL are produced in the roots and likely translocated to the shoot [2] than under stress (*vide infra*). SL/SL-like synthesis is inhibited in roots under osmotic/drought stress and, as a positive consequence for acclimatization, shootward SL/SL-like flow is decreased [3]. The transcription of SL/SL-like biosynthetic genes is thus de-repressed in

shoots, likely increasing the metabolite levels [4] (dotted inhibition arrow indicates lower repression than in [2]). Shoot-produced SL/SL-like molecules may induce SLAC1-dependent stomatal closure directly, by triggering the production of H_2O_2 and NO in guard cells [5]; moreover, they could also impact stomatal closure more indirectly, by positively regulating ABA sensitivity in guard cells [6]. It is not known whether osmotic/drought stress can increase SL/SL-like biosynthetic genes transcription in shoots independently of SL-related signals from the roots [?]. Adapted from: Visentin *et al*. (2016) based on data by Liu *et al*. (2015); Li *et al*. (2017); Lv *et al*. (2017); Visentin *et al*. (2016).

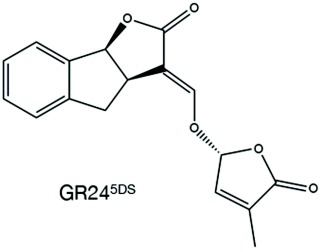
Supplementary Information

Table S1. Comparative table of main results in Ha *et al*. (2014) and Bu *et al*. (2014). "*Lower*", "*higher*" and "*equal*" are intended in comparison with the WT genotype; *n.a.*, not assessed.

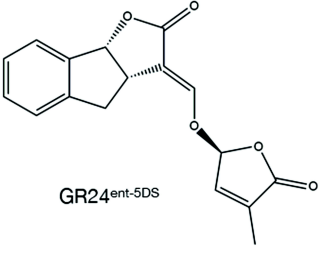
A



B *rac*-GR24 enantiomers

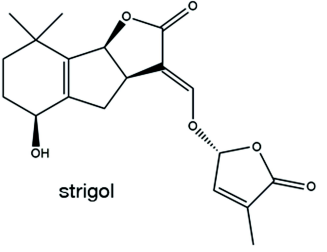


GR24^{5DS}

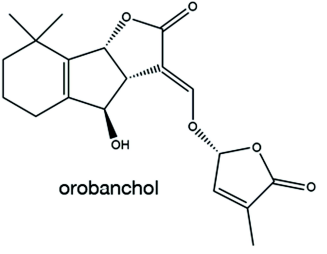


GR24$^{ent\text{-}5DS}$

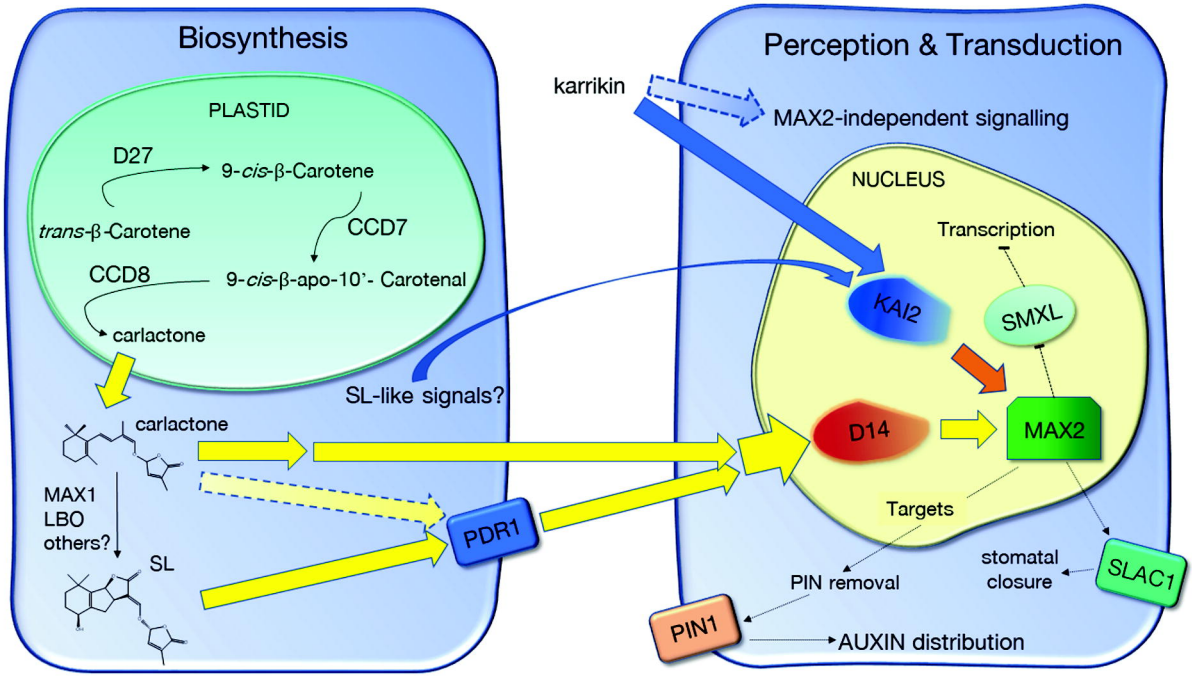
C

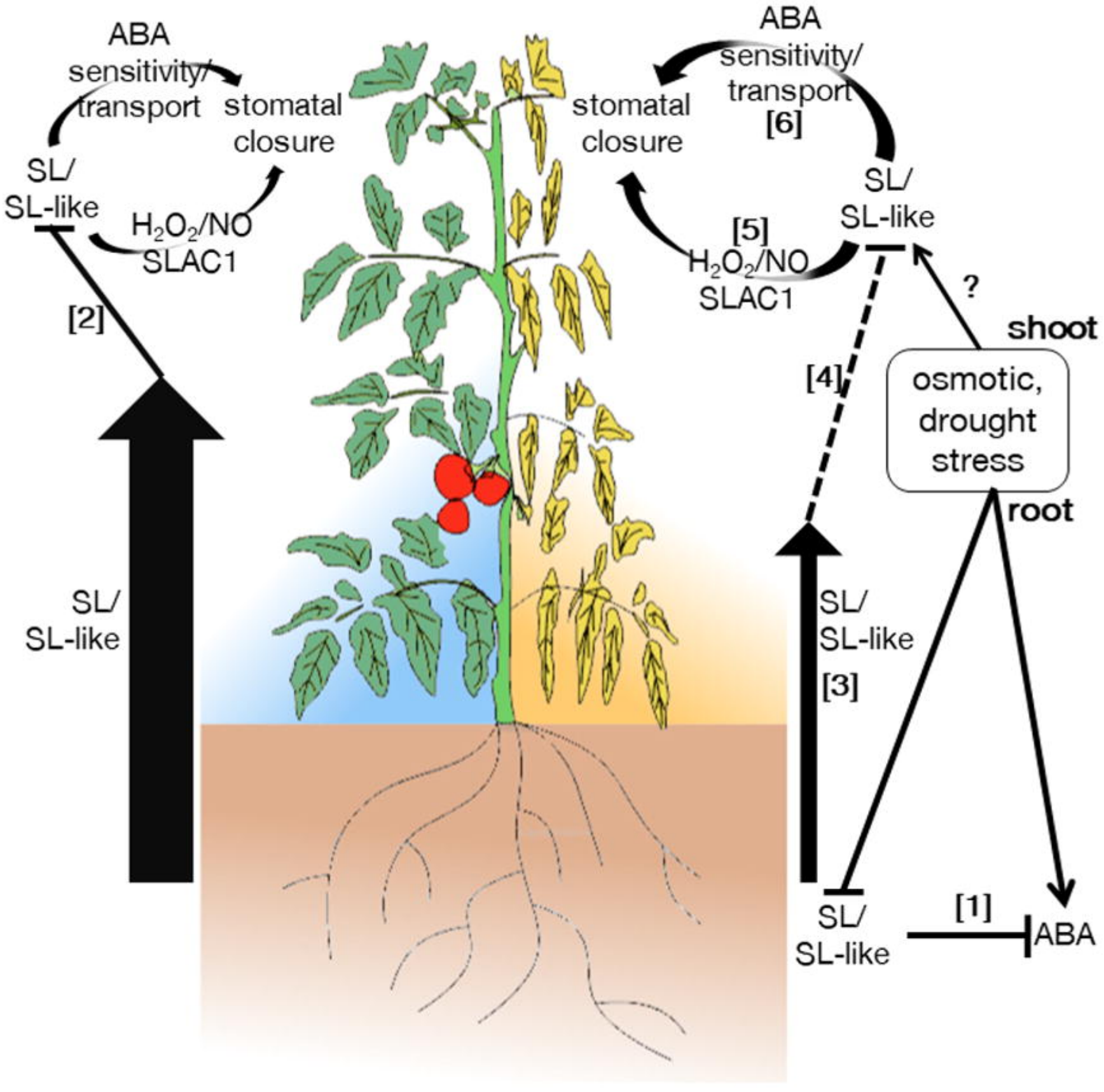


strigol



orobanchol


Biosynthesis
PLASTID
D27
9-cis-β-Carotene
trans-β-Carotene
CCD7
CCD8
9-cis-β-apo-10'- Carotenal
carlactone
SL-like signals?
carlactone
MAX1
LBO
others?
SL
PDR1
Perception & Transduction
karrikin
MAX2-independent signalling
NUCLEUS
Transcription
KAI2
SMXL
D14
MAX2
Targets
PIN removal
stomatal
closure
SLAC1
PIN1
AUXIN distribution


ABA
sensitivity/
transport
stomatal
closure
SL/
SL-like
H_2O_2/NO
SLAC1
[2]
SL/
SL-like
ABA
sensitivity/
transport
[6]
stomatal
closure
SL/
SL-like
[5]
H_2O_2/NO
SLAC1
?
shoot
[4]
osmotic,
drought
stress
root
SL/
SL-like
[3]
SL/
SL-like
[1]
ABA