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REVIEW PAPER

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

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Abstract

After quickly touching upon general aspects of strigolactone 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 of considering roots and shoots separately as well as combined versus individual stress treatments; and of performing 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 maximize crop resilience to abiotic stresses.

Keywords: Abscisic acid, drought, hormone crosstalk, osmotic stress, resilience, root–shoot communication, stomatal closure, strigolactones.

Introduction

The quest for strigolactones (SLs) 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*), and *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 molecule mainly but not exclusively produced in roots. From there, these compounds would be transported to the shoot to inhibit branching, blocking cytokinin while reinforcing auxin activity on axillary buds. Such molecules were identified in 2008 as SLs (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, SLs have several demonstrated

Abbreviations: ABA, abscisic acid; ABCG, ABC transporter G family protein; ABI, ABA insensitive; AM, arbuscular mycorrhizal; CCD, carotenoid-cleavage dioxygenase; CYP, cytochrome P450; D, dwarf; DAD, decreased apical dominance; 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, 9-*cis*-epoxycarotenoid dioxygenase; NO, nitric oxide; 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; SLAC, slow anion channel-associated; SMAX, suppressor of MAX2; SMXL, SMAX-like; TPL, topless; TPR, TPL-related

functions in the rhizosphere, all favoured by the steep SL gradient around the root, which makes the presence of SLs in soil a reliable indicator of proximity to a living plant root. Indeed, SLs are rather labile molecules due to inherent instability of the enol–ether bond between rings C and D (Fig. 1), whose integrity is essential for bioactivity (see later) (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 host plant. A second, indirect positive effect on plant mineral nutrition was proven in 2005, when SLs 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 SLs on rhizobial swarming and on infection thread formation were also suggested to favour nodulation in legumes (López-Ráez *et al.*, 2017) (see Lumba *et al.*, 2017*b* for a graphical timeline of SL-related discoveries).

After the identification of the endogenous hormonal role of SLs, further pervasive effects in the host plant were assigned to this molecular family, comprising at present ~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 SLs 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 SLs (Marzec *et al.*, 2013). More recently, though, it has appeared that SLs may also be one of the endogenous molecules in acclimatization responses to water deprivation, possibly the major environmental constraint to crop productivity. This fact, given also their strong developmental effects, places SLs 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.*, 2017*a*, *b*; Makhzoum *et al.*, 2017). In this review, we provide a quick overview on the structure, synthesis, transport, and perception of SLs, 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 of considering roots and shoots separately as well as of comparing combined versus individual stress treatments, to simulate more realistic conditions; and of performing reciprocal grafting experiments to work out contributions of the various organs and long-distance signalling events and components. Finally, we discuss if and how synthetic or natural SLs, alone or in combination with crop management strategies such as grafting, may contribute to maximize crop resilience to abiotic stress.

General structure, biosynthesis, transport, and signal transduction of SLs

Structure

The term SLs was proposed in 1995 to indicate a group of terpenoid derivatives sharing a conserved lactone ring and able

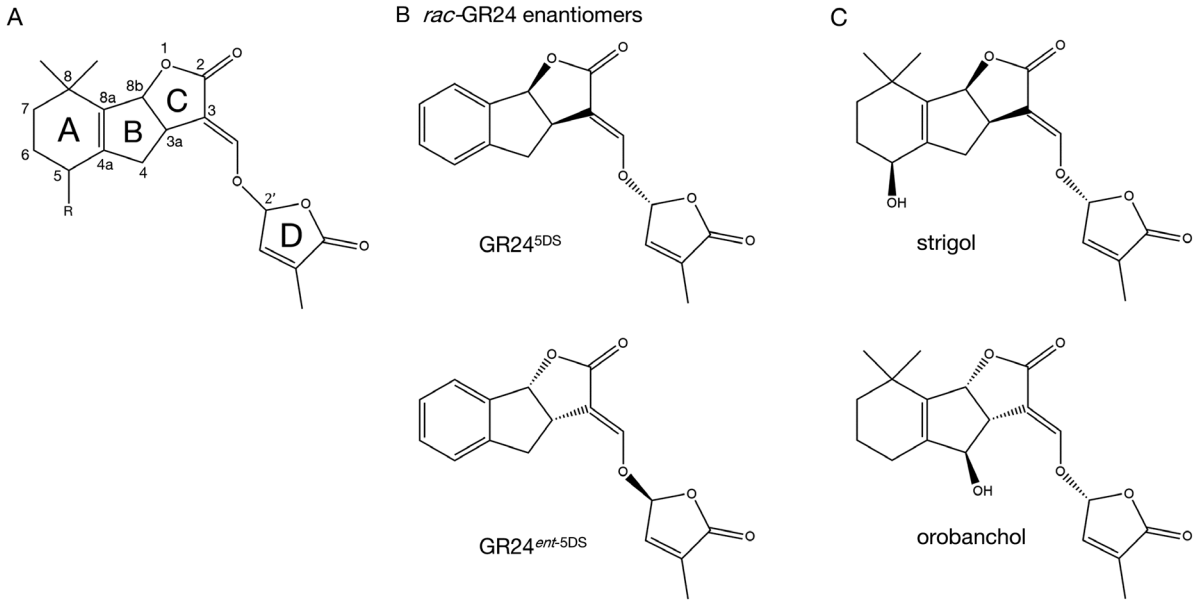

Fig. 1. Prototypal structures of natural SLs and analogues. (A) General four-ring structure (ABCD) of SLs, and relative C-atom numbering. (B) The racemic solution of GR24, the most commonly used synthetic analogue of SL, is composed of the equimolar mixture of the two enantiomers GR24^{5DS} (with the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

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, SLs analysed so far are characterized by a four-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.*, 2017*a*) (Fig. 1). Substitutions on ring A and the 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' (Fig. 1). Structure–activity relationship studies on natural and synthetic variants of SLs indicate that the bioactiphore includes the C and D rings and the connecting enol–ether bridge (Lumba *et al.*, 2017*a*), while the D ring alone is proposed to become part of the activated receptor complex (see later). Racemic (*rac*) GR24, the most commonly used synthetic analogue of SLs, 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 SLs; Fig. 1).

While the structural diversity of naturally occurring SLs has been described at least in part, its biological and ecological meaning is largely unexplained as yet. In plant species that interact with arbuscular mycorrhizal (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 SLs within a single species may induce different responses due to specificities in perception or localization has not yet been addressed experimentally. Future studies will test whether different SLs regulate different processes within a single species, but high quantities of natural SLs are hard to obtain, given that the daily production rate is very low (in the range of picomoles per plant per day) (Yoneyama *et al.*, 2010).

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 SLs the number of C atoms and the presence of a butenolide ring (Fig. 2). It is actually debated whether carlactone should be

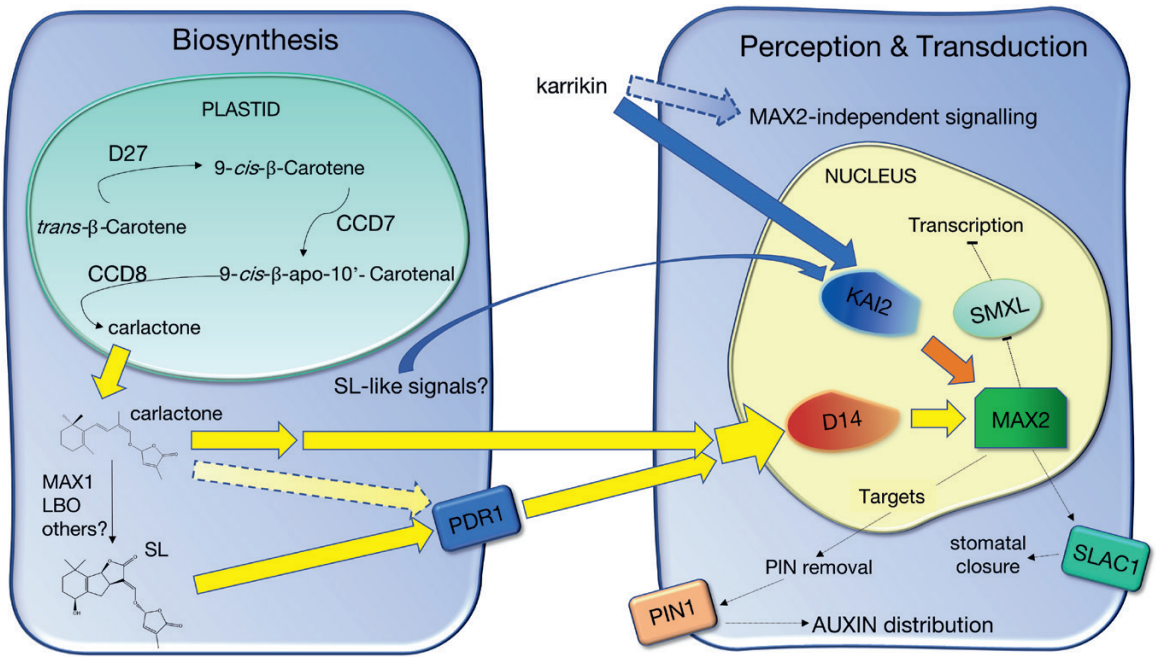

Fig. 2. Main pathways of synthesis and perception of SLs. Left-hand panel: SL biosynthesis starts in plastids where three enzymes, D27, CCD7, and CCD8, act sequentially on carotenoids to produce carlactone, a precursor of SLs. Carlactone is then transferred to the cytosol, where it is further processed in order to produce SLs. SLs and carlactone are then perceived in the same cell where they were produced (not shown) and/or transferred to other cells; while the former are probably transferred via the PDR1 protein, the transporter for carlactone has not been 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: SLs (or other carlactone derivatives) activate MAX2-dependent signal transduction after physical binding with the receptor D14. Through this pathway, SLs modulate transcription by destabilizing members of the SMXL family of transcriptional co-repressors; 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).

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 SLs, 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 sulphotransferase 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 SLs, which is obvious among species but may also be represented in the same plant by a blend of different SLs, 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 (see later), 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 the tips and the vasculature (Al-Babili and Bouwmeester, 2015). Grafting experiments and tracking of SLs and of the SL analogue GR24 showed that SLs (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, SLs may also be synthesized in stem nodes as well as along the shoot vasculature (Lopez-Obando *et al.*, 2015). Local synthesis above-ground 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, and 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 later). However, conclusive proof—beyond SL biosynthetic gene activation—that leaf tissues are, or are not, a true SL source is still lacking. Such proof will probably not come until markers [transcriptional or fluorescence resonance energy transfer (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 quantify individual SLs reliably in small tissue portions or individual cell types such as axillary buds or stomata. Rather recently, a genetically-encoded bioassay named StrigoQuant was reported, which could potentially be exploited in this sense (Samodelov *et al.*, 2016).

Transport

The ABCG protein PLEIOTROPIC DRUG RESISTANCE 1 (PDR1) of *Petunia hybrida* is the only *bona fide* SL transporter characterized thus far (Fig. 2). The defective mycorrhizal phenotype of *pdr1* mutants (Kretzschmar *et al.*, 2012) compared with the faster mycorrhization in plants overexpressing the PDR1 protein (Liu *et al.*, 2018), and the pattern of PDR1 localization (Sasse *et al.*, 2015) strongly suggest that SL transport is important for SL effects on establishment of mycorrhiza. On the other hand, SL transport contributes to inhibition of lateral bud outgrowth and to resource allocation in responses to environmental constraints, at both the root and shoot levels. This is suggested by (i) the activity profile of the *PhPDR1* promoter (in addition to in the root cortex also in elongating root hairs, leaf petioles, and at the base of lateral axils) (Liu *et al.*, 2018); (ii) the bushy shoots of *pdr1* mutants (Kretzschmar *et al.*, 2012); and (iii) the fact that petunia plants overexpressing PDR1 show increased lateral root formation and extended root hair elongation, and increased biomass under P deprivation (Liu *et al.*, 2018). There are also indications that mature leaves may transport SLs towards the stem and subtended axillary bud to join root-produced, upstream-flowing SLs (Liu *et al.*, 2018). 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.*, 2018). 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.*, 2018). Indeed, the possibility that systemic and local transport establish SL gradients 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 SLs, as demonstrated for other phytohormones such as auxin (Krupinski and Jönsson, 2010). It is worth noting also that the expression profile of *D14* (the gene encoding the SL receptor, see below) 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 so the deciphering of local versus systemic SL functions.

Perception and transduction

A remarkable amount of information has been gathered on the perception and early signal transduction mechanisms in the SL pathway (Fig. 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 sufficiently resolved or decisive enough in this respect (Lombardi *et al.*, 2017), the hydrolysed SL molecule should dock more favourably than the intact molecule in the active pocket (Gaiji *et al.*, 2012). This peculiarity would explain the very low catalytic turnover of D14-type receptors (Hamiaux *et al.*, 2012; Nakamura *et al.*, 2013; de Saint Germain *et al.*, 2016) 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 SLs (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 leitmotif 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) (Fig. 2). Genetic and biochemical data support a role for these proteins in repression of MAX2 functions, though at different developmental stages and dependent on distinct receptor/ligand pairs (Waters *et al.*, 2012). Further work in Arabidopsis points to the combined action of SMAX1-LIKE (SMXL) paralogues nos 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 was observed in the auxin and jasmonate pathway. However, non-TPR-dependent action mode(s) should not be excluded (Lumba *et al.*, 2017*b*; 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 host and in the parasitic plant (Lumba *et al.*, 2017*b*). 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 SLs) (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) (Fig. 2). The D14- and KAI2-mediated pathways therefore converge on MAX2, a crucial issue for researchers trying to disentangle the effects of SLs and KL.

Organ-specific dynamics of SL synthesis and crosstalk with ABA under single and combined abiotic stress

Do SLs contribute to shoot acclimatization under osmotic stress?

Given their inducibility by nutrient deprivation, contribution to nutritional root symbioses, and ability to shape plant morphology, SLs were quickly proposed as a molecular interface between phenotypic plasticity and a changing and often challenging environment (Liu *et al.*, 2013). Indeed, SLs contribute to root and shoot morphological and physiological responses to nutrient (N and especially P) scarcity in soil. This concept was later also tested 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; Liu *et al.*, 2015; Visentin *et al.*, 2016; Li *et al.*, 2017; Lv *et al.*, 2017). In these experiments, survival and physiological performances of SL-related mutants were severely affected when either progressively dehydrated (Ha *et al.*, 2014; Visentin *et al.*, 2016; Li *et al.*, 2017) or exposed to polyethylene glycol (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 SLs as the culprits for the *max2* phenotype, in favour of other pathways in which MAX2 would be involved. There are several apparent contrasting points between this data set 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 at *JXB* online for a detailed comparison),

and from the difficulty in pinpointing subtle phenotypes, in particular in SL biosynthetic mutants. This, in turn, might be due to leaking of the biosynthetic mutants, with residual SLs 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 for SL in stomatal closure as in Visentin *et al.* (2016) and Lv *et al.* (2017), but all three of 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 for both SLs 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 probably, the relatively weaker drought-related phenotype in SL-depleted compared with *max2* mutants is due to both pathways converging onto MAX2 being involved. The time is ripe now to work out in detail the individual contributions of the D14- and KAI2-dependent 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 signalling mutant *d14* (Ha *et al.*, 2014; Liu *et al.*, 2015; Visentin *et al.*, 2016; Li *et al.*, 2017; Lv *et al.*, 2017). Therefore, the contribution of SLs to proper guard cell functioning and acclimatization responses to water deprivation is supported enough to be included among the effects of SLs 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 later).

Current understanding of the mechanism of action of SLs in osmotic stress responses: crosstalk between the SL and ABA pathways

At the biosynthesis level

When it comes to the aetiology of such a physiological effect, a modulation of free ABA concentration seems not to be responsible in general terms, since free ABA content in Arabidopsis leaves is comparable in the wild type 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 also observed in wild-type versus *CCD7*-silenced Lotus plants under no stress, or individual osmotic or nutritional stresses (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 slightly different too: quantification in well-watered plants showed slightly more (Visentin *et al.*, 2016) or less (Torres-Vera *et al.*, 2014) concentrated free ABA in leaves of SL-depleted plants than in those of the wild type, probably depending on whether values were expressed per fresh or dry tissue weight, respectively. These slight fluctuations are indeed reasonably explained by the fact that SL-depleted and replete leaves have a different relative water content already in the absence of stress (Visentin *et al.*, 2016). In tomato suffering moderate and severe drought, however, free ABA was significantly less concentrated in *CCD7*-silenced plants than in the wild type; these values were obtained per fresh weight unit and could not be underestimated in SL-depleted plants, which are more dehydrated than corresponding wild-type controls. Less concentrated ABA may of course contribute to the poor fitness of this line under water deprivation conditions (Visentin *et al.*, 2016).

The influence of SLs on ABA concentration under stress is far less well 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 wild-type Lotus pre-treated with *rac*-GR24 are unable to increase the 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 SLs 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, SLs may relieve secondary dormancy (thermoinhibition of Arabidopsis seed germination) by lowering the 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 wild type, in both the absence and presence of drought. This effect is likely to be 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, the endogenous KAI2 ligand might also interfere with ABA levels so, once again, care should be taken in separating the effects of the two.

A positive influence of SLs on ABA synthesis in shoots is therefore documented, especially in 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 a lower ABA concentration in SL-depleted plants; indeed, transcripts of some ABA biosynthetic genes are less concentrated in leaf tissues of Arabidopsis *max2* than in those of the wild type under drought (Ha *et al.*, 2014). Additionally, *9-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 SLs 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 SLs, obtained, for example, by treatment with GR24, modulates the 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 crosstalk 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 later). This suggests that beyond the most characterized role at the crossroads 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.

At the ABA sensitivity level

Beyond the above observations, which suggest that the influence of ABA and SLs 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) 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 stomatal aperture and conductance than the wild type in the absence and presence of stress, and slower closure in response to exogenous ABA treatment (Ha *et al.*, 2014; Liu *et al.*, 2015; Visentin *et al.*, 2016; Li *et al.*, 2017; Lv *et al.*, 2017).

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; Visentin *et al.*, 2016; Lv *et al.*, 2017). 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; that is, 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 with the wild type 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.

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 disturbance; in fact, gene transcript and metabolite concentrations are quickly reduced, when local water potential has not yet dropped 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 above. 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, SLs 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 SLs 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 (wild type) tomato scions grafted to SL-depleted rootstocks displayed more concentrated transcript of SL biosynthetic genes, and higher sensitivity to endogenous and exogenous ABA compared not only with shoots of SL-depleted plants, but also with wild-type scions grafted onto wild-type rootstocks (Visentin *et al.*, 2016). The fact that root-produced SLs 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 SLs 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, due to lack of detailed structural and biosynthetic information on other possibly co-occurring molecules, the most parsimonious hypothesis at present is that stomata in such hetero-grafted plants display an ABA-hypersensitive phenotype because synthesis of SLs 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 stomatal closure in the absence of exogenous ABA in Arabidopsis (Lv *et al.*, 2017) just as it improves survival rate under drought in both wild-type 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 SLs in roots may well be a component of the systemic drought stress signal in tomato (Visentin *et al.*, 2016), in which (just as in Arabidopsis) ABA does not have a long-distance signalling function of drought stress (Holbrook *et al.*, 2002; Christmann *et al.*, 2007). Based on the above data, obtained in herbaceous dicots, a mode

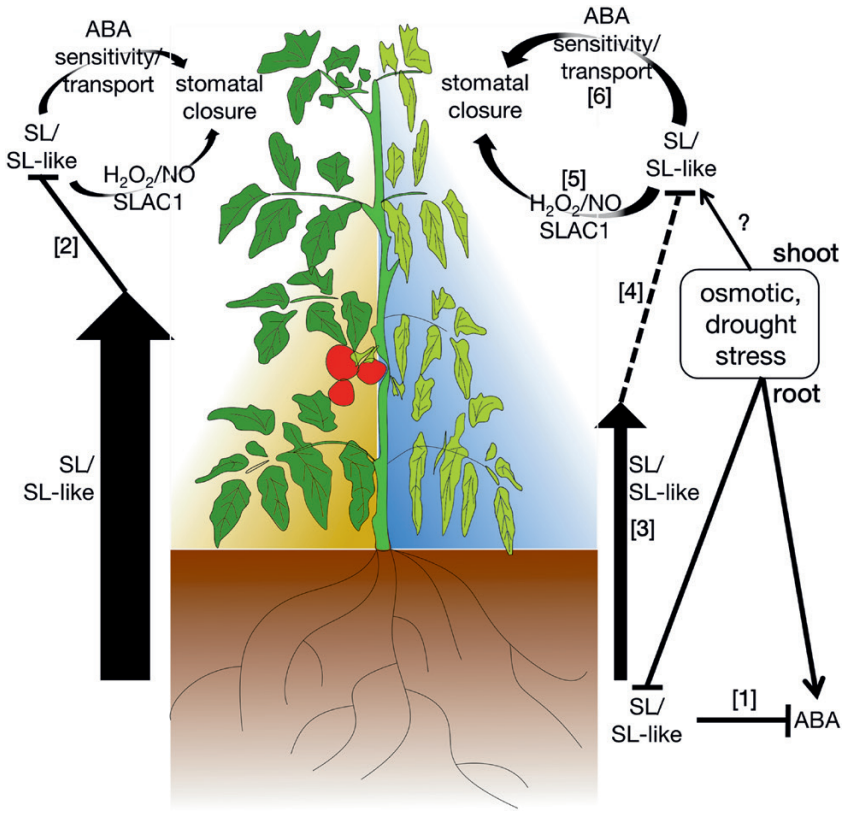

Fig. 3. Model for SL action in root–shoot communication and local signalling under drought. The main connections between SLs (or SL-like signal molecules such as SL precursors, or KL) and ABA in roots and shoots under drought stress are highlighted. SLs/SL-like molecules may have a negative effect on 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 SLs/SL-like molecules represses, by an unknown mechanism, the transcription of SL/SL-like biosynthetic genes in shoots, especially under normal conditions when more SLs are produced in the roots and probably translocated to the shoot [2] than under stress (see [1] and [3]). 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, probably increasing the metabolite levels [4] (dotted inhibition arrow indicates lower repression than in [2]). Shoot-produced SLs/SL-like molecules may induce SLAC1-dependent stomatal closure directly, by triggering the production of H_2O_2 and nitric oxide (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 gene 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), Visentin *et al.* (2016), Li *et al.* (2017), and Lv *et al.* (2017).

of action in osmotic stress responses for SLs and/or SL-like molecules such as SL intermediates, or KL can be proposed (Fig. 3). Such a model places a drop in SL synthesis at the root level above the dynamic concentration adjustment of SLs (and/or of SL-like molecules) throughout the plant. As a direct or indirect (i.e. mediated by a second messenger) consequence of such a drop, synthesis of SLs and/or SL-like molecules would be induced in shoots, namely in leaves, for the immediate and positive purpose of making stomatal closure more efficient. How this effect is achieved, and through which mediators, is not yet understood. As an obvious path to take, the possibility that the ABA transport, perception, and/or signalling machinery is primed by SLs or SL-like molecules should be explored, with emphasis on the post-transcriptional levels of regulation. However, at least in Arabidopsis, all ABA signalling components investigated were found not to be required for the effect of *rac*-GR24 on stomatal closure, which was instead dependent on *MAX2*, *D14*, *SLOW ANION CHANNEL-ASSOCIATED1* (*SLAC1*), and an ABA-independent H_2O_2/nitric oxide burst at the guard cell level (Lv *et al.*, 2017) (Fig. 3). These results unveil an interesting, completely novel link between SLs or SL-like molecules and SLAC1 activity, and open up a new avenue of investigation in SL biology. However, they cannot explain why stomata of SL-related mutants in Lotus, tomato, and Arabidopsis are hyposensitive to exogenous ABA in feeding experiments (Ha *et al.*, 2014; Liu *et al.*, 2015; Visentin *et al.*, 2016; Lv *et al.*, 2017). A possible key to reconciliation of these apparent discrepancies is that given the low background of stomatal reactivity they cause, mutations compromising endogenous SL synthesis or perception are able to unveil a contribution of SL-dependent priming of ABA signalling/transport to stomata during ABA feeding experiments. During *rac*-GR24 feeding experiments instead, the effects of ABA-independent, direct SLAC1 stimulation by exogenous SLs may be strong enough to mask milder ABA-dependent stimulation. In other words, while the effect of ABA on stomatal closure is at least partially dependent on endogenous SLs, *rac*-GR24's effects on the same feature are largely ABA independent. Clearly, this signalling module is not the only ABA-independent response to SLs or SL-like molecules: *max2* and *kai2* Arabidopsis mutants were reported to dismantle their photosynthetic machinery more slowly, and switch on anthocyanin synthesis less efficiently than the wild type, in an ABA-independent way (Ha *et al.*, 2014; Li *et al.*, 2017)—two features that, once again, may worsen performances under stress. It must be noted here that *rac*-GR24-triggered flavonoid synthesis was shown to be dependent on both D14 and KAI2 in Arabidopsis roots (Walton *et al.*, 2016).

Perspectives on abiotic stress relief and practical applications of SLs in agriculture

Modern agriculture continually requires 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 satisfactory results in protection against biotic stresses such as bacterial or fungal diseases, and weed plant infestation. On the other hand, the same solutions cannot produce 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 of these signal molecules are already present as phytoregulators or biofertilizers 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.). SLs could also provoke 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 (Screpanti *et al.*, 2016*a*; Makhzoum *et al.*, 2017). 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 because of the early discovery of SLs as potent seed germination stimulants for *Striga*, *Phelipanche*, and *Orobanche* seeds (Yoneyama *et al.*, 2010; Screpanti *et al.*, 2016*b*). 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': SLs 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 SLs were associated with 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 (Suty-Heinze and Vors, 2008, 2009; Dahmen *et al.*, 2011) as a mitigation strategy against combined stresses. Put simply, marginal soils could be amended with exogenous SLs 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 (i.e. of the juvenile to reproductive phase transition) are very interesting endogenous effects in a perspective of crop management practices, and could possibly also be 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 SLs 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 biofertilizers in plant protection is the chemical instability of natural SLs in aqueous solution which, particularly at alkaline pH, rather rapidly hydrolyse by producing an ABC-formyl lactone and 5-hydroxybutenolide (Akiyama *et al.*, 2010). In addition to this restriction, the mass production of natural SLs is also technically and economically challenging at present. In fact, ~20 different natural SLs 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 SLs 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 SLs, yet showing bioactivity comparable with that of the natural compounds, were developed (Prandi and Cardinale, 2014). 'Synthetic SLs' can be classified into two main categories: analogues, whose structure is very similar to that of natural SLs 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 a lot of effort has been devoted by organic chemists, biochemists, and modellers to the design of 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 a 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 host 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 ealier in this review, treatment with exogenous *rac*-GR24 was shown to increase stomatal reactivity in tomato and Arabidopsis (Visentin *et al.*, 2016; Lv *et al.*, 2017) and performances under drought in SL-depleted and wild-type, but not in SL-insensitive, Arabidopsis (Ha *et al.*, 2014). Notwithstanding the caveats on the use of racemic mixtures in proof-of-concept experiments (see earlier), and taking into account that the non-natural enantiomer in the racemic mixture probably contributes to the effect through KAI2, this ability of synthetic molecules to confer drought resistance by foliar nebulization opens up interesting scenarios. Synthetic SL derivatives were indeed proven to relieve drought of maize under field conditions, and were 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 indeed clear 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 with 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 SLs be foreseen. For example, if SLs are delivered to leaves of dicot plants under combined osmotic and nutritional stress (by both of which SLs may be induced in leaves), it is likely that the effects on stress resilience will be positive; but not necessarily so if treatments were targeted to the roots (in which, during combined stress, the SL decrease triggered by osmotic stress will over-ride the increase induced by P deprivation) (see above). Additionally, since SLs 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 context, but is still lacking for any realistic stress combinations.

It must also be noted that a potentially exploitable effect on stomatal conductance could be obtained in wild-type 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 up 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 with wild-type shoots grafted onto wild-type 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 the SL- (or SL-like) related toolbox more efficiently. 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 produce enough SLs to allow for regular colonization by AM fungi (see, for example, Vogel *et al.* 2010), identifying a balance point between contrasting ecological needs.

Thus, the many features of SL bioactivity make these compounds potentially interesting for agronomic applications against abiotic stress: soil treatment to improve beneficial symbiosis with AM fungi and Rhizobium, foliar nebulization,

and grafting contrasting genotypes for SL production to increase drought resistance seem to be the most promising strategies at present. On the other hand, the road to market uptake for any SL-based product is inevitably long: chemical instability in water solution, difficulties in the isolation of such low concentration natural metabolites, the economic burden of productive scale-up and of the registration of synthetic molecules are the biggest challenges to tackle. Nonetheless, if enrichment strategies and protocols can be optimized to allow for the development of a natural SL-enriched biostimulant, a decrease in the industrial costs (due in particular to the registration and certification load) could be achieved. A biostimulant can be defined as a (mix of) substance(s) and/or microorganisms that, when applied to plants or the rhizosphere, stimulates natural processes to enhance/benefit crop yield and quality, also by enhancing resilience to and recovery from abiotic stress, including drought (Van Oosten *et al.*, 2017). The positive influence of biostimulants is dependent on plant species, cultivars, climatic conditions, dose, origin, and time of application, but their use is fully compatible with both conventional and organic agriculture. New, SL-enriched biostimulant formulations could ideally be developed and tested for proof-of-concept with the long-term goal of integrating them into the set of most effective crop management practices and tools that prevent and mitigate the effect of abiotic stress. In Europe, biostimulants can be currently placed on the market either under the national regulations on fertilizers, or under the European pesticides law, which combines both supranational and national provisions for introducing plant protection products (PPPs) to the market (EC regulation No 1107/2009). However, a Fertilizer Proposal covering biostimulants as 'fertilizing products' (i.e. distinct from fertilizers *sensu strictu*, but also from PPPs) is currently under discussion by the EC; its goal is to amend the 2009 Regulation on PPPs, to exclude biostimulants explicitly. This currently leaves biostimulants in a regulatory limbo, which is thought to be over shortly. Were biostimulants to be registered for commercialization under less demanding regulations than PPPs, natural SL-enriched versions might become as or more attractive than synthetic SLs for certain applications.

Main open questions and conclusions

Many open questions of course persist, both at the basic understanding level and on the feasibility of practical applications of fundamental knowledge. Namely, main avenues of research will have to provide further details on 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 SLs accumulate in stressed compared with unstressed leaves is still waiting to be conclusively proven or disproven; it is indeed possible that SL synthesis in droughted leaves is highly localized (e.g. in guard cells; and anyway enough to escape detection in whole-leaf analyses), and/or that different metabolites from those known, such as KL, are co-responsible for the observed phenotypes. Towards this goal, readouts of SL activity are needed, but are 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 exploit fully the theoretical potential of SLs in modern agriculture.

Supplementary data

Supplementary data are available at *JXB* online.

Table S1. Comparative table of main results in Ha *et al.* (2014) and Bu *et al.* (2014).

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