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Mechanistic insights into strigolactone biosynthesis, signaling and regulation during plant growth and development

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Abstract

Strigolactones (SLs) constitute a group of carotenoid-derived phytohormones with butenolide moieties. These hormones are involved in various functions, including regulation of secondary growth, shoot branching and hypocotyl elongation, and stimulation of seed germination. SLs also control hyphal branching of arbuscular mycorrhizal (AM) fungi, and mediate responses to both abiotic and biotic cues. Most of these functions stem from the interplay of SLs with other hormones, enabling plants to appropriately respond to changing environmental conditions. This dynamic interplay provides opportunities for phytohormones to modulate and augment one another. In this article, we review our current mechanistic understanding of SL biosynthesis, receptors and signaling. We also highlight recent advances regarding the interaction of SLs with other hormones during developmental processes and stress conditions.

Keywords: Carotenoid-derived phytohormone; butenolide moieties; Phytohormone crosstalk; Strigolactone biosynthesis; Strigolactone receptors; Strigolactone signaling

Introduction

Strigolactones (SLs) comprise a novel class of phytohormones first discovered as germination inducers of various parasitic plant species (Cook et al. 1966; Kohlen et al. 2011). Their name originates from their role in stimulating *Striga* (parasitic witchweeds) germination, and from their characteristic lactone ring structure. The first isolated *Striga* seed germination inducers were strigyl acetate and strigol from *Gossypium hirsutum* L. (Cook et al. 1966). Retrospectively, SLs were first indicated as phytohormones through their presence as unknown graft-transmissible signals that suppressed *Pisum sativum* shoot branching (Beveridge et al. 1994). Signal-deficient mutants showed a hyper branching phenotype that was independent of known phytohormones, like cytokinins and auxins (Koltai 2014).

Two research groups then independently identified SLs as new phytohormones regulating the shoot branching phenotypes (Gomez-Roldan et al. 2008; Umehara et al. 2008). Plant shoot branching is inhibited by endogenous SL production or exogenous SL application in these hyper branching mutants (Umehara et al. 2008) (Fig. **1**). Root and shoot extracts of various species, including *Arabidopsis*, contain various types, combinations and levels of SL molecules (Goldwasser et al. 2008; Koltai and Beveridge 2013; Kapulnik and Koltai. 2014; Saeed et al. 2017; Bürger and Chory 2020). To regulate shoot branching, root-derived SLs are mainly transported to shoots through the xylem (Kohlen et al. 2011; Borghi et al. 2016). Since the discovery of SLs as phytohormones, extensive research has revealed novel insights about their diversity, biosynthesis and signaling. Because of their important roles in plant growth and development, SLs can potentially be used for crop improvement. For example, mutating the SL biosynthetic gene *HTD1/D17* increases rice yields, which contributed to the “Green Revolution” since the 1960s (Wang et al. 2020a).

SLs are characterized by their butenolide moieties – lactones with a 4-C heterocyclic ring structure (Omoarelojie et al. 2019). These hormones are at the forefront of plant science research because of their diverse biological roles, ranging from growth and development to interactions with other organisms (Agusti et al. 2011; Cook et al. 1966; Toh et al. 2012; Domagalska and Leyser 2011). The synthetic SL analog GR24 is an important tool in investigating the functions of SLs in plant physiology (Arite et al. 2009). It has been most useful in species without known SL biosynthetic/signaling mutants and its application reverses SL biosynthetic but not signaling mutant phenotypes (Gomez-Roldan et al. 2008; Umehara et al. 2008).

Although initially considered to be detrimental to plants since they enhanced parasitic plant germination (Cook et al. 1966), SLs were later considered beneficial since they also mediate arbuscular mycorrhizal (AM) fungal colonization (Akiyama et al. 2005; Besserer et al. 2006). Moreover, they initiate AM fungal hyphal branching even before host root infection (Akiyama et al. 2005). SLs also interact with rhizobia and affect nodule formation in leguminous plants, reflecting their diverse roles in biotic interactions (Foo et al. 2014). Apart from their functions in regulating plant symbiotic relationships, SLs may mediate defences against pathogens (Torres-Vera et al. 2014).

In addition, SLs can effectively alleviate various abiotic stresses (Fig. 1), such as salt and drought stresses (Ma et al. 2017; Van Ha et al. 2014; Lu et al. 2019). In *Arabidopsis thaliana*, SLs can regulate adaptive responses, such as stress-induced changes in stomatal density and closure (Van Ha et al. 2014). In their study, SL-deficient plants were hypersensitive to such stresses (Van Ha et al. 2014). Exogenous SL application rescued drought-sensitive mutant phenotypes, while it augmented the drought tolerance of wild type (WT) plants (Van Ha et al. 2014).

>>>>>**Insert Fig. 1. here**<<<<<

Other hormones interact with SLs to regulate various physiological processes, enabling plants to respond to changing environmental factors, such as nutrient availability, shading and temperature (Cheng et al. 2013). For example, auxins work together with SLs to control shoot branching patterns (Hayward et al. 2009, Bennett et al. 2016, Ligerot et al. 2017). SLs and abscisic acid (ABA) work together during abiotic stresses (Ren et al. 2018). Moreover, ethylene and SLs act antagonistically to control hypocotyl growth (Yu et al. 2013).

Strigolactone biosynthesis: From humble pigment beginnings

SLs and SL-like compounds have a conserved lactone structure consisting of three rings (ABC-rings) connected through an enol ether bridge with a fourth methyl butenolide or furanone moiety (D-ring) (Al-Babili and Bouwmeester 2015; Yoneyama et al. 2018). The region connecting the core (ABC) with the D-ring acts as the bioactiphore (Zwanenburg et al. 2009). Endogenous SLs are classified into two main types (strigol and orobanchol type) based on whether the C ring is α- or β-oriented (Cui. 2014). Strigol and orobanchol are canonical SLs as both have A, B, C, and D-rings (Butler. 1995); around 23 types of canonical SLs have been characterized in root exudates (Xie et al. 2010). Certain SL-like compounds are considered non-canonical, because they lack the A, B and/or C-ring; however, they still possess the D-ring bonded to the rest of the molecule (Alder et al. 2012; Boyer et al. 2014; Waters et al. 2017). Non-canonical SLs include certain synthetic and natural compounds like methyl carlactonoate (MeCLA), avenaol and Yoshimulactone Green (Abe et al. 2014; Kim et al. 2014; Tsuchiya et al. 2015). The structural diversity in canonical SLs stems from various AB ring system modifications, including epoxidation, hydroxylation,

98 ketolation and oxidation (Bhattacharya et al. 2009). This wide structural diversity involves many
99 SL biosynthetic genes (Saeed et al. 2017), homologs of which have been found in algae and
100 bryophytes (Delaux et al. 2012).

101 Several studies have elucidated the molecular mechanism of SL biosynthesis. The
102 involvement of the carotenoid pathway was reported using fluridone, an inhibitor of carotenoid
103 biosynthesis (Matusova et al. 2005). SL biosynthesis has also been investigated using certain
104 carotenoid catabolic mutants (Matusova et al. 2005), and different branching mutants such as *P.*
105 *sativum ramosus* (*rms*) mutants (Johnson et al. 2006; Beveridge et al. 1994), *Arabidopsis max*
106 (*more axillary growth*) mutants (Sorefan et al. 2003) and *Petunia decreased apical dominance*
107 (*dad1, dad2, dad3*) mutants (Snowden et al. 2005). Gene cloning, reciprocal grafting experiments
108 and mutant analysis implied that SLs are synthesized from carotenoids and are transported
109 acropetally (Ongaro et al. 2008).

110 SL biosynthesis initially occurs in the chloroplasts (Alder et al. 2012; Saeed et al. 2017)
111 involving DWARF27 (D27/β-carotene isomerase), which requires iron as a cofactor (Lin et al.
112 2009). D27 catalyses β-carotene isomerization by acting on its 9^{th} chemical bond, changing its
113 configuration from trans-β-carotene into 9-cis-β-carotene (C-40) (Alder et al. 2012). These
114 carotenoids have a 40-carbon skeleton with an extended conjugated double bond system (Moise
115 et al. 2014). Downstream of D27, carotenoid cleavage dioxygenases (CCDs) convert carotenoids
116 into apocarotenoids (Auldridge et al. 2006; Waters et al., 2012a; Hou et al. 2016), which are then
117 modified by other CCD enzymes (Alder et al. 2008). Oxidation of various carotenoid precursors,
118 resulting in specific double bond breakage, yields various compounds like ABA, SLs and retinal
119 (a conjugated chromophore) (Felemban et al. 2019). The *Arabidopsis* genome encodes about nine
120 different CCDs (*CCD1-9*), five of which are 9-cis-epoxycarotenoid cleavage dioxygenase

(NCEDs) involved in ABA biosynthesis (Tan et al. 2003). In addition, various enzymes encoded by *MAX* genes (*MAX1, MAX3 and MAX4*) regulate SL biosynthesis in *Arabidopsis* (Ruyter-Spira et al. 2013). ABA itself may also regulate SL biosynthesis, because ABA-deficient maize (*vp14*) and tomato (*notabilis*) mutants showed lower seed germination (Matusova et al. 2005).

In molecular detail, CCD-catalysed SL biosynthesis produces intermediates that are further oxidized by cytochrome P450s (Matusova et al. 2005). Two known CCDs (CCD7 and CCD8) act progressively in the pathway; CCD7 is encoded by *MAX3* and its orthologs *RMS5* and *D17/HTD1* (Booker et al. 2004), whereas CCD8 is encoded by *MAX4* and its orthologs *RMS1*, *D10* and *DAD1* (Arite et al. 2007). 9-cis-β-carotene is converted by CCD7 into 9-cis-β-apo-10-carotenal (C-27) and β ionone (C-13) (Waters et al. 2012a). 9-cis-β-apo-10-carotenal is then converted by CCD8 into Carlactone (CL), a possible mobile intermediate containing two rings (A and D) along with the enol ether bridge and an SL-like carbon skeleton (Alder et al. 2012; Seto et al. 2014). CL is produced by intra-molecular rearrangement of 9-cis-β-apo-10-carotenal, which suggests that each β-carotene molecule produces a single SL molecule (Alder et al. 2012; Seto et al. 2014). CL has similar properties as SLs, such as stimulating seed germination of *Striga hermonthica*, and is a putative intermediate during the biosynthesis of other SLs (Alder et al. 2012). Seto and colleagues (2014) used ^{13}C-labeled CL to detect its conversion into SLs *in vivo*. Conversion of exogenous CL into SL has been reported in rice, suggesting that CL is the precursor of endogenous SLs (Seto et al. 2014). Remarkably, Baz et al. (2018) reported that a new product 3-OH-carlactone is formed *in vitro* from 9-cis-3-OH-β-apo-10′-carotenal by the action of D27, CCD7 and CCD8. They also showed 3-OH-carlactone formation *in planta* by expressing rice and *Arabidopsis* CL biosynthetic genes in *Nicotiana benthamiana* leaves (Baz et al. 2018).

CL is subsequently transported into the cytoplasm for further processing (Al-Babili and Bouwmeester 2015). CL (with a complete D ring) acts as the common precursor of all SLs; however, it needs further modifications since it lacks the B and C rings (Alder et al. 2012). CL is then converted into carlactonoic acid (CLA) by the cytochrome P450 monooxygenase enzyme MAX1 in *Arabidopsis* (Abe et al. 2014; Zhang et al. 2014). Booker et al. (2005) demonstrated the role of MAX1 (CYP711A1) in CLA synthesis, by reciprocal grafting experiments in *A. thaliana.* In these experiments, the excessive branching phenotype of *max4* (*ccd8*) mutant scions were eventually reversed by grafting with wild type *MAX1* root stocks (Booker et al. 2005). The conversion of CL into CLA *in vitro* using recombinant MAX1 protein inside yeast microsomes further clarified the function of *MAX1* (Abe et al. 2014). MAX1 catalyses back-to-back oxidation of CL at C-19, first forming 19-hydroxy-CL and then CLA (Abe et al. 2014). CLA has been reported to accumulate in *Arabidopsis* roots, including those in *atd14* and *max2* mutants (Abe et al. 2014). Endogenous CLA has also been reported in rice plants, and exogenous CLA is converted into SLs using the *d10-2* rice mutant (Abe et al. 2014). When provided with ^{13}C-labelled CLA, *d10-2* mutant root exudates subsequently accumulated ^{13}C-labelled 5-deoxystrigol and orobanchol (Abe et al. 2014). In *Arabidopsis*, CLA is similarly converted into 5-deoxystrigol and 4-deoxyorobanchol (4DO) (Abe et al. 2014). 5-deoxystrigol is the simplest SL as it lacks hydroxyl, acetyloxyl and other oxygen-containing substituents (Awad et al. 2006; Yoneyama et al. 2008). It is found in both monocots (Awad et al. 2006) and dicots (Yoneyama et al. 2008), indicating it as the precursor of all SLs. 5-deoxystrigol then undergoes either allylic hydroxylation (to strigol or orobanchol) or homoallylic hydroxylation (to sorgomol) (Rani et al. 2008: Xie et al. 2010). Further modification of sorgomol – oxidation of its hydroxymethyl group followed by decarboxylation – results in the formation of sorgolactone (Xie et al. 2010). CLA can also undergo methylation

(through an unknown methyl transferase enzyme) and be converted into the methyl ester MeCLA (SL-LIKE1) (Seto et al. 2014). Interestingly, the conversion of CLA into MeCLA is *MAX1*-independent as confirmed by *Arabidopsis* mutant analyses (Abe et al. 2014). Another enzyme LBO (Lateral Branching Oxidoreductase) acts downstream of MAX1 to convert MeCLA into the recently identified hydroxymethyl carlactonoate involved in shoot branching (Brewer et al. 2016; Yoneyama et al. 2020)

Recently, a carotenoid-derived molecule zaxinone has been shown to negatively regulate SL (4-deoxyorobanchol) biosynthesis in rice under phosphate (Pi) limiting conditions (Wang et al. 2019). This was confirmed by increased SL content in *zaxinone synthase* (*zas*) mutant seedlings under Pi stress and enhanced *Striga* germination stimulation potential of *zas* root exudates (Wang et al. 2019). This was similarly observed in tomato root exudates under Pi-deficient conditions (Lopez-Raez et al. 2008). Enhanced seed germination vigour coincided with increased SL levels, which then decreased upon phosphate restoration (Lopez-Raez et al. 2008).

Strigolactone signaling cascade: A tale of binding, derepression and hydrolysis

Phytohormone perception relies on a well-defined receptor system. Just like jasmonate, auxin and gibberellin signaling (Schwechheimer and Willige. 2009: Dharmasiri et al. 2005; Katsir et al. 2008), SL signaling involves polyubiquitination and proteasomal degradation. The SL signaling cascade involves three important components: (1) an α/β fold hydrolase called D14 in rice (Arite et al. 2009), (2) an F-box leucine-rich protein called MAX2/D3 (Stirnberg et al. 2002; Johnson et al. 2006) and (3) a repressor protein called D53 belonging to the SMAX1-like (SMXL) protein family (Jiang et al. 2013; Stanga et al. 2013). The SL receptor protein D14 is activated after ligand

188 binding, leading to its interaction with other molecules to form a signaling complex; hormonal
189 signal transduction is followed by subsequent hydrolysis of the bound SL, deactivating the
190 hormone (Marzec et al. 2016).

191 Various SL-insensitive mutants were analysed to identify different SL signaling
192 components (Seto et al. 2014). AtD14/D14/DAD2 are the orthologous SL receptors in *A. thaliana*,
193 *Oryza sativa* and *Petunia*, respectively (Waters et al. 2012b; Arite et al. 2009; Hamiaux et al.
194 2012); gene mutations result in a SL-specific phenotype that is not reversed by GR24 treatment
195 (Arite et al. 2009). These gene orthologs encode proteins similar to the soluble gibberellic acid
196 (GA) receptor GID1 (GIBBERELLIN-INSENSITIVE DWARF1) (Ueguchi-Tanaka et al. 2005).
197 These receptor proteins have a conserved catalytic triad consisting of Ser, His, and Asp (Zhao et
198 al. 2013). GR24 undergoes hydrolysis, most probably due to catalytic triad activity (Kagiyama et
199 al. 2013). The *Petunia* receptor DAD2 loses its catalytic activity with a Ser-to-Ala substitution
200 (DAD2:S96A) in the triad (Hamiaux et al. 2012), leading to loss of receptor interaction with the
201 F-box protein, thereby suppressing shoot branching (Hamiaux et al. 2012; Marzec et al. 2016).
202 GR24 undergoes very slow hydrolysis with DAD2, but the *dad2* mutant phenotype is not reversed
203 by the resulting products (Zhao et al. 2013). This confirms DAD2 involvement in SL signaling,
204 with the hydrolytic process being more important than the end products (Seto and Yamaguchi
205 2014).

206 In rice, the SL hormone-D14 receptor interaction results in SL cleavage and subsequent
207 production of a "covalently linked intermediate molecule" (CLIM) bound to D14 (Bythell-Douglas
208 et al. 2017). Unlike other phytohormones, SL signaling depends upon hormone degradation. In
209 detail, binding of D14 with SL leads to nucleophilic attack, resulting in SL ligand dissociation into
210 two molecules: (1) the ABC ring portion called ABC-formyltricycliclactone (ABC-FTL) and (2)

211 the remaining part with the D-ring called hydroxymethylbutenolide (HMB) (Nakamura et al.
212 2013). ABC-FTL is released while HMB remains covalently attached to the D14 receptor; this
213 HMB-D14 intermediate is called CLIM (Yao et al. 2016). This reaction changes the D14
214 conformation, allowing it to interact with downstream signaling components (Marzec et al. 2019).

215 SL signaling proceeds from the interaction between the receptor D14 and F-box leucine-
216 rich protein MAX2/D3/RMS4 (orthologs in *A. thaliana*, *Oryza sativa* and *Petunia*, respectively)
217 (Hamiaux et al. 2012). MAX2 forms a part of the Skp–Cullin–F-box containing (SCF) E3 ubiquitin
218 ligase complex (Hamiaux et al. 2012; Zheng et al. 2014; Zhao et al. 2014). Mutations in these
219 orthologs lead to SL insensitivity, confirming their crucial role in SL signaling (Marzec et al.
220 2016).

221 This SCF complex targets the D53 and D53-like SMXL repressor proteins for proteasomal
222 degradation (Jiang et al. 2013; Zhou et al. 2013; Bennett et al. 2016). In *Arabidopsis*, *SMXL6-8*
223 have been proposed to be *D53* orthologs, as they regulate shoot branching and other SL-controlled
224 processes (Soundappan et al. 2015; Bennett et al. 2016; Ligerot et al. 2017). Due to its EAR motifs,
225 D53 is expected to interact with TOPLESS-related (TPR) transcriptional corepressor proteins
226 (Smith and Li. 2014). This D53-TPR complex may then repress SL target gene expression (Smith
227 and Li. 2014). The D53 repressor also interacts with the D14 receptor; upon GR24 treatment, D53
228 undergoes SCF complex-directed degradation (Smith and Li. 2014). The ligand-induced
229 conformational change in D14 allows the receptor to recruit SMXL7 into the SCF complex (Liang
230 et al. 2016). SMXL7 functions both transcriptionally and non-transcriptionally, but the molecular
231 events after its degradation have not been clearly elucidated (Waters et al. 2017; Bythell-Douglas
232 et al. 2017). In *O. sativa*, the major regulator of plant architecture *Ideal Plant Architecture 1* (*IPA1*)
233 acts downstream of the D53 repressor, regulating SL-induced gene expression (Song et al. 2017).

IPA1 is repressed by D53 *in vitro* and *in vivo*, which represses its transcriptional activation function (Song et al. 2017).

Several engrossing hypotheses have been proposed to explain the evolution of ligand and signaling specificity by D14 and D14-like receptor proteins. In parasitic plants, D14-like proteins – closely related to D14 proteins – act as receptors of host-exuded SLs, representing a case of convergent evolution (Tsuchiya et al. 2015; Conn and Nelson. 2015). These subfamilies of D14-like proteins also include sub functionalized proteins that respond to other ligands, such as karrikins and other D-lactone-containing compounds (Waters et al. 2012b; Saeed et al. 2017). Perception of both SLs and karrikins also require the MAX2 F-box protein (Zhao et al. 2015). However, it is unknown how MAX2 discriminates between the two pathways to generate different responses, because F-box proteins tend to be indiscriminate when recruiting target proteins (Nelson et al. 2011; Nakamura et al. 2013). Wang et al. (2020b) proposed that in *Arabidopsis*, both SL and karrikin signaling pathways converge at SMXL2, as it acts as their common target for polyubiquitination and degradation in a D14- or KAI2-dependent manner.

Different lines of evidence support the model that SL signal transduction occurs as a result of SL binding/hydrolysis-induced conformational changes in the D14 receptor. For example, thermal destabilization of the D14 receptor is initiated by GR24, which depends on an intact D14 catalytic triad (Waters et al. 2015). GR24 also promotes the physical interaction between MAX2/D3 and D14, with MAX2/D3 further destabilizing the D14 receptor (Waters et al. 2017; Zhao et al. 2014). Interestingly, D14-D3 association in *O. sativa* is a bit more responsive to 2′R stereoisomers of SL analogs compared to 2′S stereoisomers (Zhao et al. 2015). Furthermore, there are no major structural differences between D14 and apo-D14, when associated with 5-hydroxy-3-methylbutenolide, 2, 4, 4, trihydroxy-3-methyl-3-butenal or SL (Nakamura et al. 2013).

Recently, several modes of SL-D14 interaction have been determined, but it is unclear how D14 functions with D3 in ubiquitinating the D53 repressor. D3 has a C-terminal α-helix that exists in either engaged or dislodged forms (Shabek et al. 2018). The engaged form enables D14 and D3 binding with a hydrolysed SL intermediate, while the dislodged form recognizes unmodified D14 and prevents its enzymatic activity (Shabek et al. 2018). The D3 α-helix helps D14 in recruiting D53 in a SL-dependent manner, which then activates the hydrolase (Shabek et al. 2018). The self-induced D14 degradation by SLs (through MAX2) limits their own signaling through a negative feedback loop (Chevalier et al. 2014; Koltai 2014).

Controversially, this CLIM model has been challenged by various experimental evidence. CLIM cannot be accommodated in the D14 active site due to its very small electron density; instead, iodine (I) in the crystallization reagents is suspected to bind the active site (Carlsson et al. 2018). D14-mediated SL hydrolysis is also too sluggish after SL treatment, in sharp contrast to the rapid degradation of target proteins (D53/SMXLs) (Seto et al. 2019). Therefore, the rapid response of SLs cannot be entirely explained by this CLIM model. Instead, it has been recently reported that binding of a complete SL molecule, not a hydrolysed one, initiates the active D14 receptor signaling; D14 then hydrolyses SL molecules only after completing the pathway (Seto et al. 2019). Kinetic analysis of the AtD14-catalysed hydrolysis of 5-deoxystrigol detected two hydrolytic products, ABC-FTL and HMB, as described earlier (Hamiaux et al. 2012). The K_{cat}, K_m and V_{max} values were found to be $0.12 min^{-1}$, 4.9μM and 4.0nmol/min/mg protein, respectively (Seto et al., 2019). In addition, 3,6′-dihydroGR24, which has a single bond instead of a double bond in the enol ether bridge, is not hydrolysed by the SL receptors in rice and *Arabidopsis* (Umehara et al. 2015). Furthermore, D14 catalytic activity is quite low for debranones (SL analogs without the enol-ether

bridge), but these analogs interestingly yield the same results as GR24 (Scaffidi et al. 2014). These observations raise questions about the role of hydrolysis (by D14) in SL signaling.

Therefore, D14 has a dual function and a new mechanism of SL signaling perception could be proposed (Yao et al. 2016). In molecular detail, the D14 conformational change enlarges the catalytic pocket, allowing SL movement into this pocket and then closing the helical lid domain (Shabek et al. 2018). When a SL molecule binds to the D14 receptor protein, D14 initially attains an unstable conformation due to interruption in the catalytic triad formation (Yao et al. 2016). In this changed conformation, the D14 receptor interacts with other components to carry out the SL signaling cascade (**Fig. 2**). After activation, D14 (through the surface of its rearranged lid domain) interacts with the F-box protein MAX2/D3 and then D53/SMXL repressor binding occurs around the region of the Asp loop (Seto et al. 2019). After D53/SMXL degradation, the D14 catalytic triad is again reconstructed, which performs the important hydrolysis step, resulting in SL deactivation (Seto et al. 2019). This hormonal degradation mechanism is also found in other hormonal pathways (like GA) and is very important for hormone homeostasis (Yamaguchi. 2008).

>>>>>**Insert Fig. 2. here**<<<<<

The evolution of the SL signaling mechanism provides informative insights. It is believed that the initial role of SLs was AM fungal recruitment to facilitate more efficient nutrient uptake; this symbiotic association was present in land plants about 360-450 million years ago (Waldie et al. 2014; Simon et al. 1993). Remarkably, SLs are found in algae and SL application results in rhizoid elongation – a response also reported in liverworts and mosses belonging to bryophytes (Delaux et al. 2012); however, it is most probably independent of MAX2 (Waldie et al. 2014). In

301 higher plants, MAX2-independent SL signaling has also been reported. Minute GR24
302 concentrations can inhibit root growth in the *max2* mutant (Shinohara et al. 2013). In charophytes,
303 a D14 member is more closely related to the KARRIKIN INSENSITIVE2 (KAI2) receptor than
304 to canonical D14 proteins (Waldie et al. 2014; Waters et al. 2012b). It might be possible that SLs
305 use this receptor instead of MAX2 to initiate their response (Waldie et al. 2014). The *D14* and
306 *MAX2* gene clades arose quickly when land plants emerged, with *D14* probably appearing due to
307 duplication in the clade, while another duplication within *D14* resulted in the evolution of the D14-
308 LIKE2 group (Waters et al. 2012b; Waldie et al. 2014). These duplication events correlate with
309 varying functions as land plants diversified. D53 protein evolution also follows a similar pattern.
310 The *D53*-like genes in mosses have higher similarity to *SMAX1* than to *D53/SMAXL7* clade; these
311 clades were then subjected to further duplications (Zhou et al. 2013). Intriguingly, the entry of
312 MAX2 into the SL pathway has not been fully elucidated. It is postulated that MAX2 was initially
313 involved in AM colonization only and its role in SL signaling evolved later (Challis et al. 2013);
314 this is supported by the *d3* rice mutant which cannot be colonized by AM fungi (Waldie et al.
315 2014).

316

317 **Strigolactone receptors: Highly conserved in diverse plant species**

318 The SL receptors have a conserved α/β hydrolase functional domain (Bennett and Leyser 2014),
319 which was first identified in the SL-insensitive *O. sativa d14* mutant (Arite et al. 2009). Orthologs
320 were eventually identified in *Petunia* (Hamiaux et al. 2012), pea (de Saint Germain et al. 2016)
321 and *Arabidopsis* (Waters et al. 2012b). According to Arite et al. (2009), *D14* homologs are found
322 in diverse plant clades, such as *Marchantia polymorpha* (bryophytes), *Selaginella*

323 *moellendorfi* (pteridophytes) and gymnosperms. These homologs belong to the D14-like
324 subfamily, whereas angiosperm genes are grouped into the D14 subfamily of the α/β-hydrolase
325 superfamily (Arite et al. 2009). Proteins of these subfamilies similarly possess a conserved
326 catalytic triad, a nucleophilic residue and an acidic residue, but have quite different sequences
327 (Nardini and Dijkstra. 1999; Arite et al. 2009). The α/β hydrolase superfamily also includes the
328 acetylcholinesterase (AChE) enzyme (responsible for acetylcholine metabolism) and the inactive
329 gibberellic acid receptor (Holmquist et al. 2000).

330 D14 (without any prefix corresponds to the *O. sativa* receptor) acts as a receptor as well as
331 an enzyme, differentiating it from other plant hormone receptors (Hamiaux et al. 2012). It has a
332 α/β hydrolase functional domain containing the Ser-His-Asp catalytic triad, forming its ligand
333 binding pocket, and 4 α helices forming its cap (Kagiyama et al. 2013). It consists of 318 amino
334 acids, and a homolog called *D14*-like is also reported in the rice genome (Arite et al. 2009. The
335 rate of SL hydrolysis *in vitro* is as low as ~0.3 molecules per minute, suggesting that bioactive SL-
336 derived signal production is not its primary function (Snowden and Janssen. 2016). Consistent
337 with this, neither the intermediate molecule 2,4,4-trihydroxy-3-methyl-3-butenal nor the end
338 products of SL hydrolysis (tricyclic lactone and HMB) act as signals for shoot branching
339 suppression (Waters et al. 2017).

340 The SL receptor in *A. thaliana* (AtD14) is evolutionarily conserved (Waters et al. 2012b;
341 Arite et al. 2009); just like the rice D14 receptor, it consists of a catalytic triad and possesses both
342 receptor and enzyme functions (Hamiaux et al. 2012). The structure of the AtD14-D3-ASK1
343 complex showed a portion of the hormone covalently bonded with the receptor through two amino
344 acids in the triad (Yao et al. 2016). When the receptor conformation changes, an α helix domain
345 increases in length, while another α helix domain unfolds and forms a loop (Yao et al. 2016). Four

α helix domains form the lid of the receptor, which probably functions in destabilizing the SL receptor upon hormone attachment (Zhao et al. 2015; Snowden and Janssen 2016). The enzymatic active site also decreases in volume resulting in closure (**Fig. 3**). Therefore, this indicates that D-ring separation is difficult without complex dissociation, and could explain the sluggish enzyme activity (Snowden and Janssen 2016). In *Arabidopsis*, the AtD14L/KAI2 protein is 51% identical and 75.9% similar to AtD14, but is instead involved in karrikin signaling; unsurprisingly, AtD14L and AtD14 belong to different phylogenetic clades (Waters et al. 2012b).

>>>>>**Insert Fig. 3. here**<<<<<

The *Petunia* D14 receptor ortholog is DAD2 (Simons et al. 2007). Hamiaux et al. (2012) solved its structure by X-ray crystallography and its lid consists of 4 α helices, connected by a β hairpin to the core. A strongly hydrophobic cavity between the lid and the core can easily accommodate known SLs (Hamiaux et al. 2012). The authors further reported that when GR24 is present, DAD2 interacts with the F-box protein PhMAX2A (the *Petunia* MAX2 ortholog). GR24 then undergoes hydrolysis upon DAD2 interaction, but mutations in the catalytic triad leads to loss of enzymatic activity and failure to interact with PhMAX2A (Hamiaux et al. 2012). The prolific branching phenotype of *dad2* mutants has also been observed in *dad1* (*CCD8*) and *dad3* (*CCD7*) biosynthetic mutants (Napoli et al. 1996). DAD2 locally controls shoot branching, as confirmed by grafting and genetic studies (Simons et al. 2007; Hamiaux et al. 2012). The branching phenotype of biosynthetic mutants is reversed by grafting with wild type root stocks; however, this reversion does not occur in *dad2* mutants, suggesting that *DAD2* is not involved in SL biosynthesis (Simons et al. 2007).

368 The SL receptor in *Hordeum vulgare* (barley) is encoded by the *HvD14* gene, which
369 consists of a 1055-bp coding sequence with two exons (Marzec et al. 2016). The approximately
370 303-amino acid HvD14 protein also contains the conserved α/β-hydrolase domain between amino
371 acids 57 and 295 (Kagiyama et al. 2013). Unsurprisingly, it has great structural similarity, high
372 sequence conservation, and comparable secondary domains to the rice D14 ortholog ((Marzec et
373 al. 2016). In *hvd14.d* mutants, the Gly at position 193 is substituted by Glu (Marzec et al. 2015);
374 this residue is present in the αD2 α-helical domain, which constitutes the cap surrounding the
375 active site along with αD1, αD3 and αD4 (Kagiyama et al. 2013).

376 Zheng et al. (2016) reported that the woody perennial plant *Populus trichocarpa* has two
377 highly identical (91.7%) and similar (95.9%) homologs *PtD14a* and *PtD14b*. They showed that
378 *PtD14a* is 79% identical and 89.1% similar to *AtD14*, while *PtD14b* is 77.5% identical and 89.1%
379 similar to *AtD14* (Zheng et al. 2016). The crucial Ser-His-Asp catalytic triad is conserved in both
380 PtD14 homologs at positions 96, 246 and 217 (Zheng et al. 2016). In terms of gene expression,
381 *PtD14a* transcript levels are higher compared to *PtD14b*, with very low co-expression between
382 them (Zheng et al. 2016).

383 The probable SL receptors in parasitic weeds were more difficult to identify, because the
384 phenotypes could not be dissected genetically (Toh et al. 2015; Tsuchiya et al. 2015).
385 Subsequently, a group of α/β-hydrolases ShKAI2s/ShHTLs (*S. hermonthica* KARRIKIN
386 INSENSITIVE2/ HYPO-SENSITIVE TO LIGHT) were discovered to be involved in SL
387 hydrolysis and SL-induced seed germination; these hydrolases are *D14* paralogs that act as SL
388 receptors (Conn et al. 2015b; Toh et al. 2015; Yao et al. 2017). Among them, ShHTL7 serves as
389 the most active SL receptor in *Striga* (Conn et al. 2015b; Yao et al. 2017). During CLIM formation,

ShHTL7 undergoes a conformational change (like AtD14) to transduce signaling through its interaction with MAX2/ShMAX2 (Yao et al. 2017).

Strigolactone-phytohormone crosstalk: Dynamic interplay for effective plant physiology

Different hormonal signaling pathways interact with one another, affecting their respective signaling components (Huot et al. 2014). These dynamic interactions regulate hormonal biosynthesis, response and transport, thereby helping plants control their morphology and adapt to changing environmental conditions (Cheng et al. 2013). These challenging conditions include severe nutritional deficiency, abiotic stress factors (i.e. salinity, heat, cold, drought and light stress), and harmful biotic invasions (i.e. pathogens and pests). Phytohormone crosstalk facilitates appropriate and tunable plant responses to these conditions by controlling nutrient distribution and by modulating growth, developmental and defence processes. Plant stress responses are primarily regulated by jasmonic acid (JA), ABA and salicylic acid (SA), whereas plant growth/developmental processes are mainly governed by auxins, gibberellins and cytokinins (Huot et al. 2014). SLs interact with other hormones in order to exert their impact (Saeed et al. 2017; Torres-Vera et al. 2014).

Strigolactones and auxins

SLs inhibit shoot branching by regulating auxin transport. Compared to wild type plants, *A. thaliana max* mutants show increased auxin transport due to increased *PIN1/3/4/6* gene transcription (Bennett et al. 2006; Lin et al. 2009). Treating *Arabidopsis max* mutants and rice *dwarf* mutants with an auxin transport inhibitor, N-1-naphthylphtalamic acid, causes inhibition of bud outgrowth (Cheng et al. 2013; Lin et al. 2009). Crawford et al. (2010) reported that treatment

412 with basal GR24 levels reduces auxin transport basipetally, as well as PIN1 accumulation in xylem
413 parenchyma cell membranes. These observations persist in biosynthetic *max1* mutants but not
414 signaling *max2* mutants, indicating that SLs slow down polar auxin transport stream in a MAX2-
415 dependent manner (Crawford et al. 2010).

416 Studies of auxin and *max* mutants showed that SLs directly affect secondary growth
417 activity, independent of auxin stacking (Agusti et al. 2011), by affecting interfascicular cambium
418 activity (Ruyter-Spira et al. 2011). Based on a quantitative study, *max* mutants have a 30%
419 decrease in interfascicular cambium-derived tissues, concomitant with lower expression levels of
420 cambium- and cell cycle-related genes (Agusti et al. 2011). SLs regulate auxin content in the
421 primary root tip, because the primary root lengths of SL biosynthetic and signaling mutants are
422 shorter compared to wild type plants (Ruyter-Spira et al. 2011). GR24 application rescues this
423 short root phenotype in SL-deficient mutants, but not in SL-insensitive *max2* mutants (Ruyter-
424 Spira et al. 2011). SLs inhibit auxin efflux by controlling PIN activity, leading to auxin
425 accumulation inside the primary root meristem cells and ultimately resulting in increased primary
426 root length (Ruyter-Spira et al. 2011). SL-auxin interaction controls root development by adjusting
427 or regulating intercellular auxin flow, auxin sensitivity and shoot-to-root transport (Mayzlish-Gati
428 et al. 2012; Omoarelojie et al. 2019). SLs also control lateral root formation by adjusting the
429 essential auxin gradient (Omoarelojie et al. 2019). Furthermore, SL-auxin interaction regulates
430 root hair elongation, whereby SLs increase intracellular auxin concentration by hindering auxin
431 efflux (Kotlai et al. 2010). Ligerot et al. (2017) suggested that a feedback loop exists in the auxin-
432 SL crosstalk. Auxins upregulate SL biosynthesis in an *RMS2*- (encodes PsAFB4/5 auxin receptor)
433 dependent manner, while SLs downregulate auxin levels in an RMS3- and RMS4-dependent
434 manner by downregulating auxin biosynthetic gene expression (Ligerot et al. 2017).

P_i deficiency leads to increased levels of RSL4, an auxin-related transcription factor that promotes root hair elongation (Omoarelojie et al. 2019; Datta et al. 2015). In contrast to auxins, SLs inhibit adventitious root (AR) formation in *Arabidopsis* and pea (Datta et al. 2015). AR inhibition was even evident with high auxin concentration, suggesting that suppression of AR formation is not due to low auxin levels (Rasmussen et al. 2012). Auxins and SLs also play a crucial role during mycorrhization; auxins are associated with arbuscule formation, whereas SLs are associated with presymbiotic fungal growth (Guillotin et al. 2017). The authors further found that auxin content increases in roots colonized by AM fungi, and exogenous auxin application promotes the colonization process. An auxin-related gene *Sl-IAA27* positively controls mycorrhization by regulating SL biosynthesis via NSPI (transcription factor of the *D27* and *MAX1* genes) (Guillotin et al. 2017).

Strigolactones and cytokinins

Cytokinins are adenine-derived plant hormones that stimulate cytokinesis and influence various processes, like enhancing shoot growth, limiting root growth, and influencing axillary shoot branching (Aloni et al. 2006; Werner et al. 2001). In *P. sativum* and *A. thaliana*, branching mutants with increased SLs have reduced cytokinin concentrations in the xylem sap (Morris et al. 2001; Foo et al. 2007). Decreased cytokinin sensitivity has also been reported in the buds of SL-insensitive plants (El-Showk et al. 2013). Dun et al. (2012) reported that the SL-insensitive and SL-deficient *P. sativum rms* mutants (*rms4 and rms1*) have increased expression of the cytokinin biosynthetic gene *PsIPT1* in shoot nodes and internodes. Interestingly, the *rms1* mutant was more sensitive to low cytokinin levels compared to wild type, when applied to the buds or supplied through the vasculature (Dun et al. 2012). The authors further found that bud outgrowth is higher in *rms1* mutants than wild type plants after applying low cytokinin levels, suggesting that SLs and

458 cytokinins play antagonistic roles. Exogenous GR24/ cytokinin application weakened the effect of
459 cytokinins in *rms1* mutants but not in *rms4* mutants, implying that SL-cytokinin interaction
460 converges at *RAMOSUS4 (RMS4)* (Dun et al. 2012). The cytokinin-SL antagonism is due to
461 PsBRC1, a common target of both hormones (El-Showk et al. 2013); its gene expression
462 negatively correlates with bud growth (Dun et al. 2012). Additionally, *PsBRC1* gene expression is
463 enhanced by GR24 but reduced by cytokinins – a trend that persists even with cycloheximide
464 (ribosomal translation inhibitor) treatment, suggesting that new protein synthesis is not required
465 for this regulation (Dun et al. 2012). Both SLs and cytokinins act as negative regulators of lateral
466 root development; the cytokinin receptors ARR1, ARR12 and AHK3 are associated with GR24-
467 induced reduction of lateral development (Ruyter-Spira et al. 2011; Jiang et al. 2015). Genetic
468 studies show that GR24-regulated lateral development is influenced by PIN1- and PIN7-mediated
469 auxin polar transport; cytokinin treatment downregulates *PIN1/PIN3/PIN5* but upregulates *PIN7*
470 expression (Jiang et al. 2015). Moreover, the *A. thaliana max2* mutants show low cytokinin
471 catabolic gene expression (*CKX1, 2, 3, 5*), reflecting the negative relationship between cytokinins
472 and SLs (Banerjee et al. 2018). In *O. sativa*, Duan et al. (2019) observed enhanced cytokinin levels
473 in shoot bases of *d53* mutants.

474 Some evidence suggests that SLs and cytokinins play important roles during drought
475 adaptation (Nishiyama et al. 2011). Analyses of cytokinin-depleted *Arabidopsis* mutants (*CKX*-
476 overexpressor), as well as signaling mutants (*arr1, 10, 12*), indicated that cytokinin signaling
477 negatively regulates drought acclimation (Nguyen et al. 2016). Drought tolerance mechanisms in
478 these mutants involve amplified stomatal closure, increased root-to-shoot ratio, enhanced cell
479 membrane integrity, and increased ABA hypersensitivity (Nishiyama et al. 2011). Due to the
480 undesirable role of cytokinins in drought tolerance, cytokinin biosynthesis and signaling in *A.*

thaliana are suppressed during drought (Cortleven et al. 2019). Drought-induced cytokinin suppression occurs through the ABA-induced transcription factor AtMYB2, and members of the ABA-activated Sucrose Nonfermenting 1 (SNF1)-Related Protein Kinase 2 family (Cortleven et al. 2019). In contrast to cytokinins, SLs positively regulate resilience to water stress conditions, as shown in studies of *Arabidopsis max1* mutants and *CCD7*-silenced tomato mutants (Visentin et al. 2016; Zhang et al. 2014). Additionally, SLs decrease stomatal density (Van Ha et al. 2014) and stomatal opening during drought (Zhang et al. 2018). The *max* mutants also show decreased response to ABA (Van Ha et al. 2014). Overall, these observations clearly indicate the contrasting roles of SLs and cytokinins under drought stress conditions (Li et al. 2019).

Strigolactones and gibberellins

The phytohormones SLs and gibberellins (GAs) may interact during their perception and signaling, acting together during plant growth and development (Marzec 2017). Remarkably, SL biosynthesis can be regulated by GAs (Ito et al. 2017). GAs are involved in flowering, seed production, leaf morphology and shoot/root growth (Claeys et al. 2014). Various studies have indicated that SL and GA signaling are very similar. Rice semi dwarf mutants in *GIBBERELLIN OXIDASE 5*, *6* and *9* exhibit an extra-branched shoot phenotype similar to SL mutants (Marzec 2017). GAs control tiller number through the action of ORYZA SATIVA HOMEOBOX1 (osHB1) and TEOSINTE BRANCHED1 (osTB1) transcription factors (Lo et al. 2008). SLs promote the interaction between the D14 receptor and SLENDER1 (SLR1), a negative regulator of GA signaling (Nakamura et al. 2013). SLR1 degradation occurs in an SL-dependent manner, which parallels the GA signaling pathway, where the GID1 receptor binds GA to promote interaction between GID1 and DELLA proteins, eventually leading to DELLA degradation via the 26S proteasome (Marzec. 2017). Additionally, gene expression databases show that GA_3 treatment decreases SL biosynthetic gene

504 expression in *O. sativa* (Ito et al. 2017). The interaction between SLs and GAs in *A. thaliana* is
505 inconclusive; microarray data showed varying SL biosynthetic gene expression profiles upon GA_3
506 treatment (Marzec et al. 2015). In *O. sativa,* Zou et al. (2019) found that SL biosynthetic and
507 signaling mutants exhibit dwarfism that is rescued by GA treatment. Interestingly, these mutants
508 have less bioactive GA and decreased GA sensitivity (Zou et al. 2019). This ultimately leads to
509 reduced shoot length by downregulating genes involved in cell division and elongation (Zou et al.
510 2019).

511 ***Strigolactones and abscisic acid***

512 ABA is regarded as a universal stress hormone since it regulates various abiotic stress responses.
513 Like ABA, SLs are apocarotenoid hormones so it is possible that they could also act as stress
514 hormones. Tomato ABA mutants have low SL biosynthetic gene expression, including *LeCCD7*
515 and *LeCCD8,* reflecting the close harmonization between SL and ABA anabolic pathways
516 (Banerjee et al. 2018). SL-deficient *Arabidopsis* mutants have downregulated ABA import genes,
517 like *ABCG22* and *ABCG40,* resulting in ABA hyposensitivity (Van Ha et al. 2014). It has also
518 been reported that mycorrhizal plants exposed to abiotic stresses have greater SL and ABA levels
519 (Ruiz-Lozano et al. 2016). GR24 application decreased the expression of *LjNCED2* in *Lotus*
520 *japonicus,* which in turn inhibited ABA accumulation during osmotic stress (Liu et al. 2015).
521 Additionally, SL-ABA interaction is demonstrated by SLs controlling ABA-induced stomatal
522 sensitivity (Van Ha et al. 2014). SLs promote seed germination under high temperature conditions
523 by regulating both ABA and GAs in parasitic and non-parasitic seeds (Mostofa et al. 2018).
524 Furthermore, SL biosynthetic and signaling genes in *Sesbania cannabina* are upregulated by ABA
525 to cope with salt stress, while SL biosynthetic inhibitor treatment induced partial salt tolerance

(Ren et al. 2018). Studies using ABA-deficient tomato mutants and CCD/NCED inhibitors suggest that SL regulates ABA biosynthesis through an unknown mechanism (López-Ráez et al. 2010).

Strigolactones and ethylene

Certain plant growth and developmental processes involve both SL and ethylene signaling, including seed germination, leaf senescence, root hair elongation and hypocotyl growth (Ueda and Kusaba 2015; Cheng et al. 2013; Kapulnik et al. 2011). During light treatment, SLs upregulate *HY5* expression in a MAX2-dependent fashion, inhibiting hypocotyl elongation (Jia et al. 2014). In contrast, ethylene promotes hypocotyl elongation by augmenting HY5 degradation via COP1 (Yu et al. 2013). These show the antagonistic roles of these two hormones in regulating hypocotyl growth. SL-mediated root hair elongation also depends on ethylene signaling, since ethylene signaling mutants (like *At-etr*) have reduced GR24 sensitivity (Kapulnik et al. 2011). Abolishing ethylene production totally eliminates SL-mediated root hair elongation, while GR24 enhances ethylene biosynthetic gene *ACS2* transcription (Kapulnik et al. 2011). Moreover, SLs stimulate ethylene biosynthesis in *Striga* seeds prior to germination (Sugimoto et al. 2003). During leaf senescence, SLs activate senescence signals mediated by ethylene (Ueda and Kusaba 2015).

Strigolactones and salicylic acid

SA is involved in plant defence responses against various pathogens, as well as tolerance to abiotic stresses (Askari and Ehsanzadeh 2015; Prodhan et al. 2018; Omoarelojie. 2019). SA-mediated stress tolerance is mainly due to changes in the plant's reactive oxygen species status (Omoarelojie. 2019). In terms of crosstalk, SA interacts with SLs during plant-fungal symbioses (Rozpadek et al. 2018). GR24 treatment results in SA build-up, whereas *max2* mutants have decreased SA concentrations, suggesting that SLs are involved in plant defences by inducing SA production

(Rozpądek et al. 2018; Omoarelojie, 2019). In wheat, foliar application of SLs and SA synergistically results in lower electrolyte leakage, higher relative leaf water content and enhanced antioxidant enzyme activities during drought stress (Sedaghat et al. 2017).

Strigolactones and Jasmonic acid

Jasmonates are involved in secondary metabolism, wounding responses and plant-pathogen/insect interactions (Yan et al. 2007; Yan and Xie. 2015). JA concentration and JA-dependent *PIN11* gene expression are reduced in the tomato SL biosynthetic mutant *Sl-ccd8* (Torres-Vera et al. 2014). Because *PIN11* provides resistance in *Solanum lycopersicum* against *Botrytis cinerea* (Torres-Vera et al. 2014), these observations hint at a possible interplay between these two hormones during disease resistance. Although there is no direct evidence depicting SL-JA interaction, both are involved together in several processes, like plant-microbe interactions, mesocotyl elongation and senescence; thus, their crosstalk cannot be totally ruled out (Omoarelojie. 2019). For example, Lahari et al. (2019) reported that SLs induce root-knot nematode infection in rice roots by inhibiting the JA pathway. Remarkably, SL biosynthetic mutants were less prone to infection by the root-knot nematode *Meloidogyne graminicola* (Lahari et al. 2019).

Strigolactones and Karrikins

Karrikins (from 'karrik' meaning smoke) or KARs are smoke-derived signals produced by burning vegetation; they form through the combustion of carbohydrates (Flematti et al. 2011). Although not produced *in planta*, they can stimulate germination of dormant seeds (De Cuyper et al. 2017) – an effect attributed to the butenolide pyran moiety (Flematti et al. 2007). Unlike SLs, however, KARs do not induce the germination of parasitic weeds (Conn et al. 2015b). Although they have different sources and effects on plant growth and development, SLs and KARs share highly similar

570 signaling mechanisms, which could be due to their shared butenolide structure (Morffy et al. 2016).
571 The KAI2 receptor of KARs work in the same manner as the D14 receptor of SLs (Morffy et al.
572 2016). Because KAI2 and D14 are paralogs, they share the F-box protein MAX2 during signaling
573 (De Cuyper et al. 2017). Structurally, the KAI2 receptor catalytic pocket is smaller than that of the
574 D14 receptor, which hints at the binding of smaller cognate molecules (Guo et al. 2013).
575 Phylogenetic studies have shown that KAI2 was present in basal land plants instead of D14
576 orthologs, suggesting that *KAI2* is ancestral and that *D14* probably evolved due to *KAI2* duplication
577 (Waters et al. 2012b).

578 The application of KAR_1, KAR_2 as well as *rac*-GR24 inhibit hypocotyl elongation in
579 *Arabidopsis*, with *rac*-GR24 having greater impact than KARs (Nelson et al. 2010; De-Cuyper et
580 al. 2017). This observation is supported by *max2* mutant plants that have longer hypocotyls
581 (Stirnberg et al. 2002), a phenotype shared by mutant *kai2* seedlings (Waters et al. 2012b). In
582 contrast, KAR_1 and *rac*-GR24 have antagonistic effects on cotyledon growth – karrikin promotes
583 growth while *rac*-GR24 negatively impacts cotyledon growth (De Cuyper et al. 2017). Mutations
584 in *KAI2* and *MAX2* cause skewing of *A. thaliana* roots, but this response is independent of SL
585 perception by the D14-MAX2 complex (Swarbreck et al. 2019). Scaffidi et al. (2014) cautioned
586 about using racemic mixtures of chemically synthesized SLs, as well as their analogs like GR24,
587 since they can activate responses that are different from natural counterparts.

588 As reported by Liu et al. (2019), both SLs and KARs shape the morphology of the
589 exodermis. They revealed that SLs positively regulate the number of hypodermal passage cells
590 (HPC), but *d14* mutants surprisingly have higher HPCs (Liu et al. 2019). They further noted that,
591 in contrast to *d14, max2* mutants have decreased HPC numbers (Liu et al. 2019). In *Petunia*, *KAI2*

mutation also reduces HPC numbers, indicating the critical importance of the dimeric KAI2/MAX2 receptor in controlling this process (Liu et al. 2019).

Strigolactones and Nitric oxide

There is evidence that SLs and nitric oxide (NO) possibly interact during various stress responses and developmental processes. Their interplay has mostly been studied in root systems; results suggest that NO negatively and positively regulates root SL biosynthesis and signaling, respectively, in a nutrient-dependent manner (Bharti and Bhatla. 2015). NO can modify proteins involved in SL biosynthesis and signaling, with *Arabidopsis max1-1* and *max2-1* mutants having increased NO levels in their root tips (Kolbert. 2019). These observations highlight the possible negative impact of SLs on NO biosynthesis; however, exogenous SL application increased NO production, contradicting earlier genetic studies (Kolbert. 2019). GR24 treatment results in decreased NO concentration in lateral roots but increased NO concentrations in primary root tips (Bharti and Bhatla. 2015). Furthermore, SLs and NO act as positive regulators of meristem activity thereby enhancing root elongation (Sun et al. 2016). Endogenous NO does not influence SL biosynthesis, while exogenous NO upregulates the expression of SL signaling but not biosynthetic genes in *O. sativa* (Sun et al. 2016). In addition, exogenous SLs promote accumulation of guard cell H_2O_2 and NO, leading to SLOW ANION CHANNEL-ASSOCIATED 1-mediated stomatal closure (Lv et al. 2017).

Conclusion and future prospects

SLs regulate plant growth, development and stress tolerance via close crosstalk with other hormones. Mechanistically, SLs elicit their response by regulating hormone content, transport and

delivery between diverse plant organs and within plant tissues, and also by interacting with other hormone signaling cascades. Plant responses are governed by synergistic as well as antagonistic interactions of SLs with other phytohormones. Based on various physiological and molecular studies, SLs are essential for plant responses to stressful environmental conditions. Due to their utmost importance, continued research is needed to more lucidly understand the SL biosynthetic pathway, SL signaling crosstalk with other hormones, and mechanisms by which SLs regulate different stress responses, growth processes and developmental programs. Although we have gained significant insights in understanding SL hormonal interplay at various levels of regulation, critical knowledge gaps still need to be addressed at both cellular and molecular levels. Certain functions of SLs have yet to be discovered, while further investigating the SL repressor D53 could reveal its involvement in other processes. On a translational level, studying SL hormones could help produce crop varieties with better nutrient allocation under limiting conditions. Long-term research programs could focus on developing more resilient crops, through genetic manipulation of SL quantity and response. Moreover, whether the SL receptor enzymatic activity is required for downstream SL signaling and function still needs to be elucidated. Because protein-protein interactions during SL signaling are unique, further research is required to fully understand SL crosstalk with other hormone pathways. To gain better insights and solve pressing biological problems, the next decade opens a lot of research opportunities in the exciting field of strigolactone hormone biology.

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Conflicts of interest

The authors declare that the submitted work was not carried out in the presence of any personal, professional or financial relationships that could potentially be constructed as a conflict of interest.

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Figure legends:

Fig. 1. Diverse roles of SLs in overall plant growth, development and resilience.

Fig. 2. The SL biosynthetic pathway showing key enzymes and intermediates.

Fig. 3. The SL signaling mechanism showing receptor complex formation and protein modifications.

Fig. 1

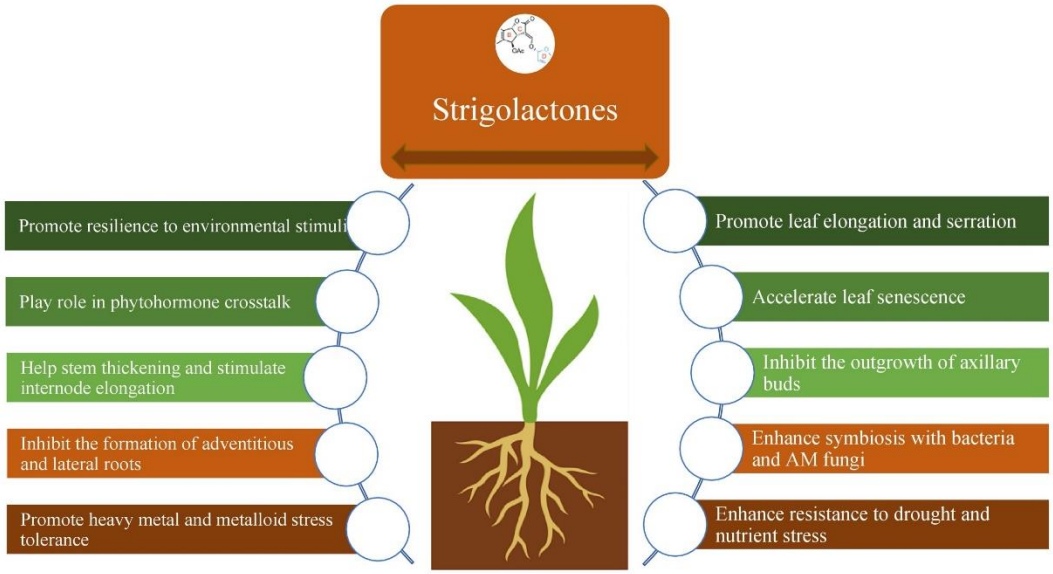

Strigolactones
Promote resilience to environmental stimuli
Play role in phytohormone crosstalk
Help stem thickening and stimulate internode elongation
Inhibit the formation of adventitious and lateral roots
Promote heavy metal and metalloid stress tolerance
Promote leaf elongation and serration
Accelerate leaf senescence
Inhibit the outgrowth of axillary buds
Enhance symbiosis with bacteria and AM fungi
Enhance resistance to drought and nutrient stress

Fig. 2

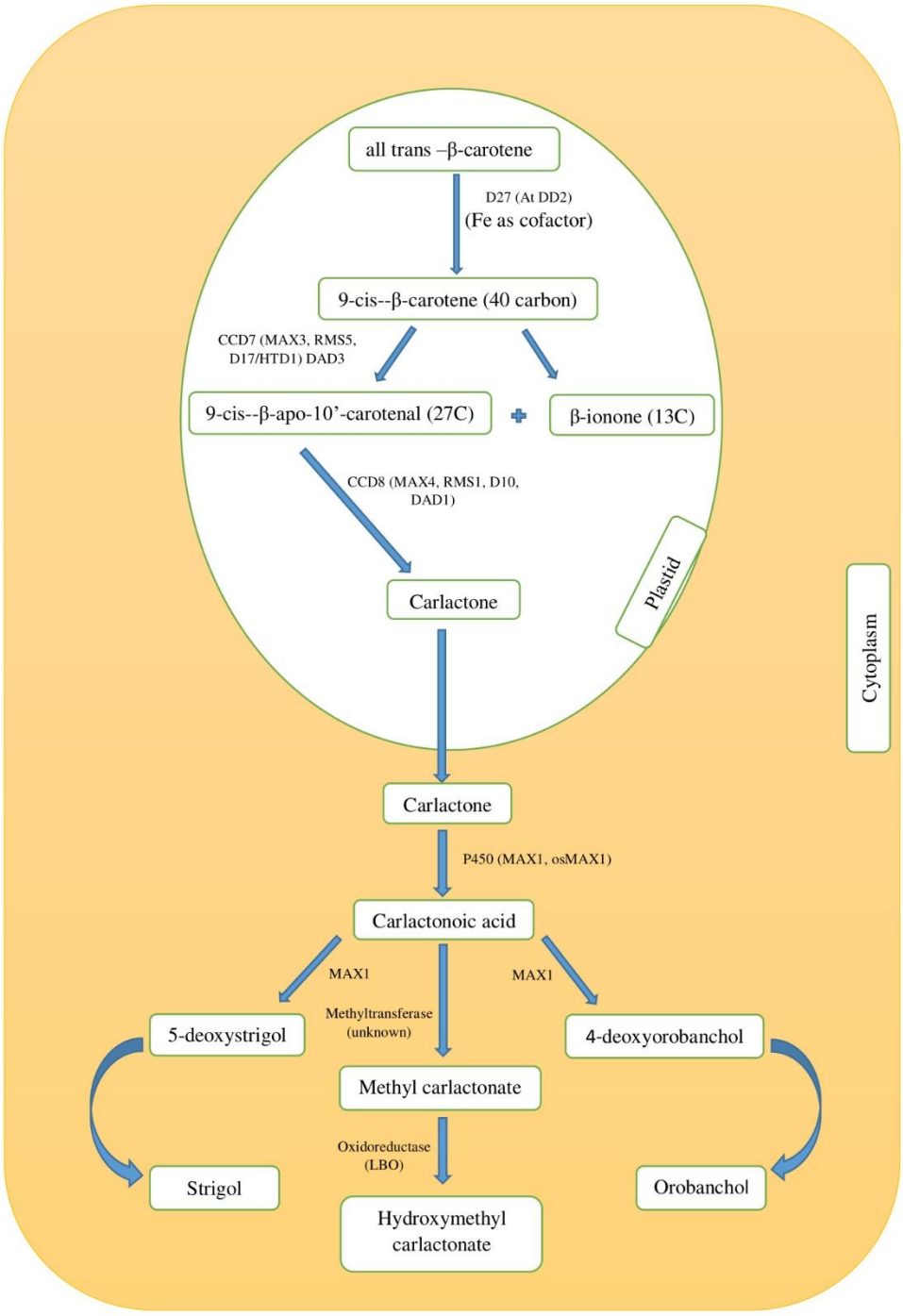
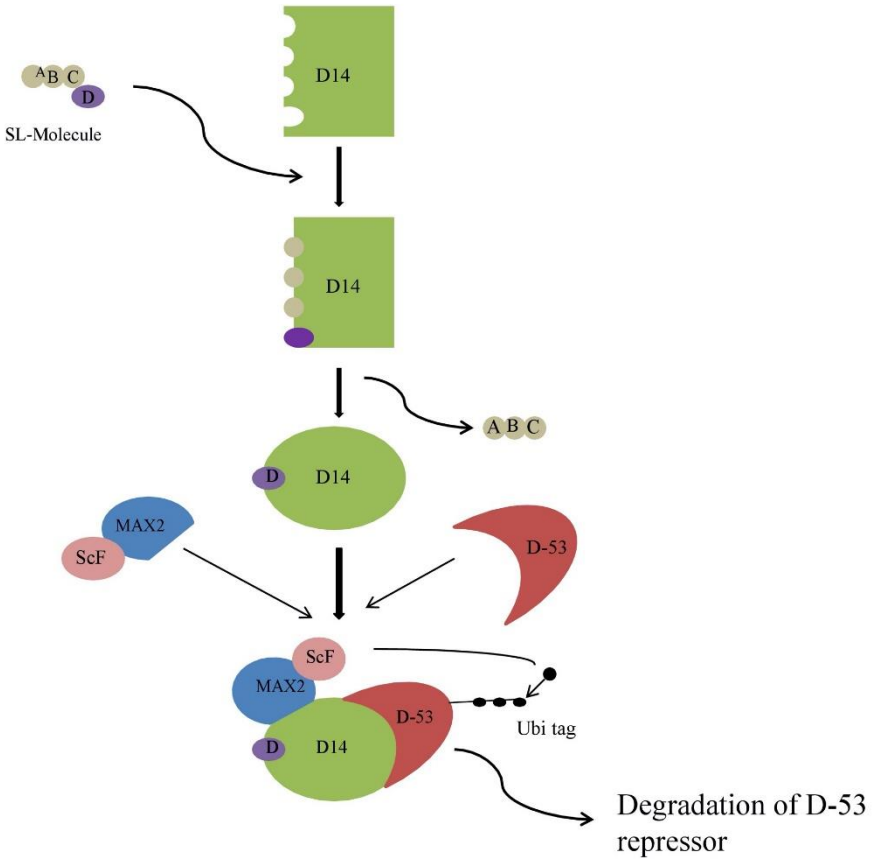

all trans –β-carotene
D27 (At DD2)
(Fe as cofactor)
9-cis--β-carotene (40 carbon)
CCD7 (MAX3, RMS5, D17/HTD1) DAD3
9-cis--β-apo-10'-carotenal (27C)
β-ionone (13C)
CCD8 (MAX4, RMS1, D10, DAD1)
Carlactone
Plastid
Cytoplasm
Carlactone
P450 (MAX1, osMAX1)
Carlactonoic acid
MAX1
MAX1
Methyltransferase (unknown)
5-deoxystrigol
4-deoxyorobanchol
Methyl carlactonate
Oxidoreductase (LBO)
Strigol
Orobanchol
Hydroxymethyl carlactonate

Fig. 3


A B C
D
SL-Molecule
D14
D14
A B C
D
D14
MAX2
ScF
D-53
ScF
MAX2
D-53
Ubi tag
D
D14
Degradation of D-53
repressor