Strigolactones Stimulate Internode Elongation Independently of Gibberellins[1][C][W]

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Strigolactone (SL) mutants in diverse species show reduced stature in addition to their extensive branching. Here, we show that this dwarfism in pea (*Pisum sativum*) is not attributable to the strong branching of the mutants. The continuous supply of the synthetic SL GR24 via the root system using hydroponics can restore internode length of the SL-deficient *rms1* mutant but not of the SL-response *rms4* mutant, indicating that SLs stimulate internode elongation via *RMS4*. Cytological analysis of internode epidermal cells indicates that SLs control cell number but not cell length, suggesting that SL may affect stem elongation by stimulating cell division. Consequently, SLs can repress (in axillary buds) or promote (in the stem) cell division in a tissue-dependent manner. Because gibberellins (GAs) increase internode length by affecting both cell division and cell length, we tested if SLs stimulate internode elongation by affecting GA metabolism or signaling. Genetic analyses using SL-deficient and GA-deficient or *DELLA*-deficient double mutants, together with molecular and physiological approaches, suggest that SLs act independently from GAs to stimulate internode elongation.

In plants, a single hormone can regulate multiple and diverse aspects of plant growth and development (Vogler and Kuhlemeier, 2003; Davies, 2010). Consequently, most developmental processes such as secondary growth, lateral root development, or hypocotyl elongation are influenced by multiple hormones that interact and integrate environmental factors to optimize plant growth and reproduction (Davies, 2010). Important questions in plant biology relate to how these different plant hormones interact to regulate particular processes and the relative roles of each plant hormone according to environmental conditions, developmental stages, and tissues (Nemhauser et al., 2006; Kuppusamy et al., 2009).

Plant height is a major target in plant breeding for improving seed yield, biomass, and standing ability. Most plant hormones have been shown to affect this trait, with auxin (Cleland, 2010), GA (Ingram et al., 1986; Hedden, 2003), and brassinosteroid (BR; Clouse and Sasse, 1998; Jager et al., 2007) promoting internode elongation and with abscisic acid, ethylene (Ross and Reid, 1986), and jasmonic acid (Heinrich et al., 2013) having an inhibitory effect (Davies, 2010; Ross et al., 2011). GA is one of the key determinants of plant height, as suggested by the different strategies designed to reduce plant height in several crops. Genetically manipulating GA biosynthesis (e.g. rice [*Oryza sativa*] gene *Semi dwarf1*) or GA signaling pathways (e.g. wheat [*Triticum aestivum*] *Reduced height*) to generate dwarf and semidwarf high-yield varieties in rice and wheat was particularly successful during the "Green Revolution" (Peng et al., 1999; Sasaki et al., 2002; Hedden, 2003; Salamini, 2003). In pea (*Pisum sativum*), the stem length gene *LE*, encoding a GA 3-oxidase (*PsGA3ox1*; Lester et al., 1997; Martin et al., 1997), controls internode length and is involved in plant height variation. The *le-1* allele is found in most dwarf pea cultivars and generates one of the seven traits studied by Mendel (Ellis et al., 2011; Reid and Ross, 2011). Genetic studies have convincingly demonstrated that BR is also a major endogenous regulator of internode elongation (Clouse and Sasse, 1998; Ross et al., 2011). Based on several lines of evidence, including the rapid growth response during gravitropism and phototropism, auxin is also a major factor controlling internode elongation (Cleland, 2010).

Different signals may affect separate components of a given process or have distinct modes of action. In contrast to hypocotyl elongation, in which only cell elongation is concerned (Gendreau et al., 1997), internode elongation is the result of both cell division and cell elongation, and hormones have different effects on

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these two mechanisms. Whereas GA affects both cell division and cell elongation, auxin and BR mostly control cell elongation. Molecular mechanisms involved in interactions among hormone signaling pathways are being deciphered, and different classes of cross regulation of a given process by multiple hormones can be described (Kuppusamy et al., 2009). One of the most common schemes of cross regulation is the modulation of the metabolism of one hormone by another. For instance, an important mechanism by which auxin stimulates internode elongation is the increase of bioactive GA levels (Ross et al., 2000, 2003). Additionally, elevated levels of jasmonic acid, a stress-related hormone, can inhibit internode elongation by repressing transcript levels of GA biosynthesis genes (Heinrich et al., 2013). Genetic studies combined with physiology (hormone-level quantifications, hormone response) are very powerful to identify cross talk or cross regulation (Kuppusamy et al., 2009) of a given process by multiple hormones. For the control of internode elongation, pea, with its simple architecture, has been intensively used for such analyses (Ross et al., 2011). In pea stems, auxin increases transcript levels of the *LE* gene (*PsGA3ox1*) and down-regulates transcript levels of the *SLENDER* (*SLN*) GA catabolism gene (encoding a GA deactivation 2-oxidase [*PsGA2ox1*]; Lester et al., 1999), leading to increased levels of GA_1, the main bioactive GA in pea (Lester et al., 1999; O'Neill and Ross, 2002; Ross et al., 2011). This ability of auxin to promote or maintain levels of bioactive GA in pea stems is also observed in other tissues (roots, pod) and in other species, such as Arabidopsis (*Arabidopsis thaliana*; Frigerio et al., 2006), barley (*Hordeum vulgare*; Wolbang et al., 2004), and tobacco (*Nicotiana tabacum*; Wolbang and Ross, 2001), with some changes in the specific GA metabolism gene that is regulated (Reid et al., 2011).

The growth repressor DELLA proteins are major players in GA signaling and are rapidly degraded in response to GA treatment (Silverstone et al., 2001; Jiang and Fu, 2007). In Arabidopsis, it was shown that genes encoding the GA biosynthetic enzymes GA3ox1 and GA20ox2 are direct DELLA targets, indicating the involvement of DELLA proteins in the feedback regulation of GA biosynthesis by GA treatment (Zentella et al., 2007). Whereas five DELLA genes are present in Arabidopsis (*GIBBERELLIN INSENSITIVE*, *REPRESSOR OF ga1-3* [*RGA*], *RGA-LIKE1* [*RGL1*], *RGL2*, and *RGL3*; Olszewski et al., 2002), the pea genome contains only two DELLA genes, *LA* and *CRY* (Weston et al., 2008). In pea, it was shown that auxin regulation of GA metabolism genes was DELLA independent, as indole-3-acetic acid (IAA) regulation of GA genes was also observed in the slender *la cry-s* line, a DELLA-deficient background (O'Neill et al., 2010; Ross et al., 2011).

In 2008, the role of strigolactones (SLs) in the control of shoot branching was demonstrated (Gomez-Roldan et al., 2008; Umehara et al., 2008). SL-deficient and SL-response mutants identified in pea (*ramosus* [*rms*]), rice (*dwarf* [*d*]), Arabidopsis (*more axillary growth* [*max*]) and petunia (*Petunia hybrida*; *decreased apical dominance* [*dad*]) display increased shoot branching (Ongaro and Leyser, 2008; Beveridge et al., 2009; McSteen, 2009). SLs are carotenoid-derived compounds that were already known for their roles in symbiotic and parasitic interactions in the rhizosphere (Akiyama et al., 2005; López-Ráez et al., 2009). Mutant-based approaches have identified several genes of the SL biosynthesis and signaling pathways (Beveridge and Kyozuka, 2010). The chloroplastic initial steps of the SL biosynthesis pathway involve D27 (Lin et al., 2009), a β-carotene isomerase, modifying all-trans-β-carotene into 9-cis-β-carotene (Alder et al., 2012) and two carotenoid cleavage dioxygenases, CCD7 (RMS5/D17/MAX3/DAD3; Booker et al., 2004; Johnson et al., 2006; Zou et al., 2006; Drummond et al., 2009) and CCD8 (RMS1/D10/MAX4/DAD1; Sorefan et al., 2003; Snowden et al., 2005; Arite et al., 2007), to produce the SL intermediate carlactone (Alder et al., 2012). Another putative SL biosynthesis enzyme has been identified, the cytochrome P450 MAX1 (Booker et al., 2005), acting very likely after carlactone formation; however, its biochemical function is not known yet.

Similar to the GA signaling pathway, the SL signaling pathway involves an F-box protein (RMS4/D3/MAX2; Stirnberg et al., 2002, 2007; Ishikawa et al., 2005; Johnson et al., 2006) and an α/β-fold hydrolase (D14/DAD2; Arite et al., 2009; Gao et al., 2009; Liu et al., 2009; Waters et al., 2012). In contrast to the GA receptor GIBBERELLIN-INSENSITIVE DWARF1, which has lost the enzymatic activity of the members of the α/β-fold hydrolase superfamily, DAD2 retains hydrolytic activity, leading to products without SL-like activity (Hamiaux et al., 2012). Direct binding of the synthetic SL, GR24, with the petunia DAD2 or the rice D14 protein (Hamiaux et al., 2012; Kagiyama et al., 2013) pinpoints the DAD2/D14 α/β-fold hydrolase as the best candidate for the SL receptor. In Arabidopsis and pea, the SL response involves the TCP (for TB1, CYCLOIDEA, and PCF domain) transcription factor BRANCHED1 (BRC1/PsBRC1; Aguilar-Martínez et al., 2007; Braun et al., 2012), homologous to the maize (*Zea mays*) TEOSINTE BRANCHED1 (TB1; Hubbard et al., 2002; Doebley et al., 2006). Branching of pea or Arabidopsis *brc1* mutant plants is not repressed by treatment with the synthetic SL, GR24 (Brewer et al., 2009; Braun et al., 2012); therefore, these mutants are considered as SL-response mutants.

Since the discovery that SLs represent a novel class of plant hormones, several functions of SLs other than the control of branching have been identified through the examination of SL-deficient and SL-response mutants and by examining the effects of treatments with the synthetic SL GR24. Developmental processes such as lateral root formation, adventitious root formation, root hair elongation, and interfascicular cambium development have been shown to be regulated by SL (Agusti et al., 2011; Kapulnik et al., 2011; Ruyter-Spira et al., 2011; Rasmussen et al., 2012). The use of SL and auxin signaling mutants suggests that SLs act

predominantly downstream of auxin to stimulate secondary growth (Agusti et al., 2011), whereas for lateral root formation, SLs would modulate auxin levels and/or signaling and ethylene synthesis for root hair elongation (Koltai, 2011). Whether SLs act directly or in coordination with other hormones to regulate these processes will need further investigation. Consequently, it appears that, as for other plant hormones, SLs are involved in many aspects of plant development.

Another phenotype of the SL-deficient and SL-response mutants observed in various species (pea, Arabidopsis, rice, petunia, tomato [*Solanum lycopersicum*], and *Lotus japonicus*) is their shorter stature in comparison with the wild type (Beveridge et al., 1996; Napoli, 1996; Stirnberg et al., 2002; Ishikawa et al., 2005; Zou et al., 2006; Kohlen et al., 2012; Liu et al., 2013). Previously, it was shown in rice that this reduced plant height was in part a consequence of the increased branching, as manual removal of tillers in the rice *htd1/d17* SL-deficient mutant led to an increase in overall plant height (Zou et al., 2006). However, the overall plant height was not completely restored to that of comparative wild-type plants, and this incomplete restoration could have been due to the diversion of energy from the main stem to the tillers prior to their removal or to their SL deficiency. Umehara et al. (2008) have shown that SLs supplied in the hydroponics medium can substantially restore the height of SL-deficient rice mutants. Here, we used the pea model to investigate the role of SL in the regulation of internode elongation. We set out to investigate the origin of the dwarfism of SL-deficient and SL-response mutants in pea, to confirm the role of SL on internode elongation, and to analyze whether SL controls cell elongation and/or cell division. A possible interaction with GA was tested by evaluating two types of putative SL action: the effect of SLs on levels of the main bioactive GA in pea, GA_1, and their possible influence on GA signaling, in particular on DELLA proteins. Overall, our results indicate that SL and GA act independently to stimulate internode elongation in pea.

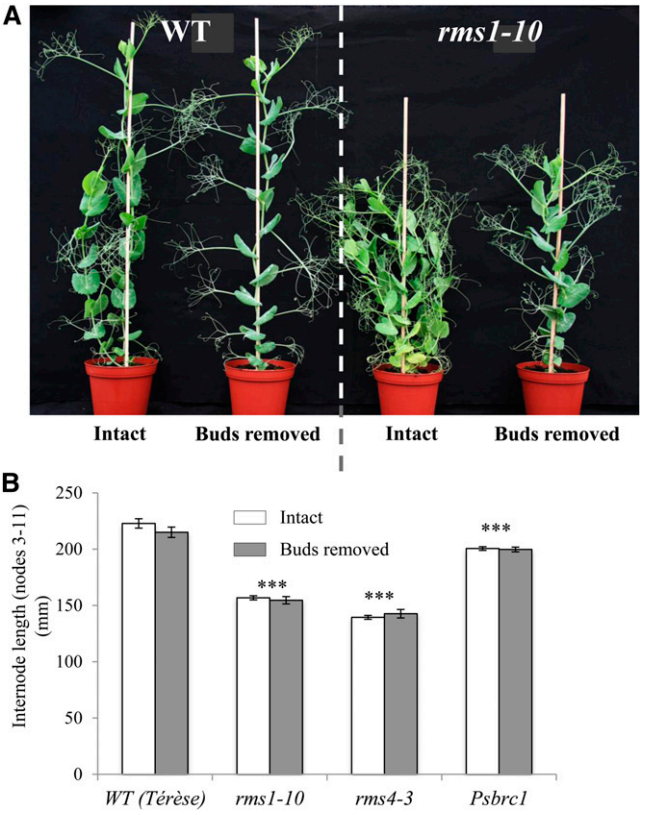

Figure 1. Reduced height of the *rms* SL mutants is not caused by their strong shoot branching. A, Phenotypes of wild-type (WT; cv Térèse) and *rms1-10* plants, intact or with axillary buds removed. B, Internode lengths between node 3 and node 11 were measured when plants were 30 d old. Axillary buds were manually removed every 2 or 3 d. Data are means ± SE ($n = 7–8$). Asterisks denote significant differences from the wild type (***$P < 0.001$, Student's t test). [See online article for color version of this figure.]

RESULTS

Reduced Internode Length of Pea SL Mutants Is Not Caused by Their Increased Shoot Branching

To investigate whether the reduced height of the pea SL-related mutants is due to their high branching, we determined the impact of manual bud removal on internode lengths of wild-type, *rms1*, *rms4*, and *Psbrc1* plants. As reported previously, on a tall background, the *rms1* SL-deficient and *rms4* SL-insensitive mutants exhibited not only a high-branching phenotype but also a relative dwarf phenotype (Beveridge et al., 1996, 1997b). Internode length of the *rms1-10* SL-deficient mutant and the *rms4-3* SL-response mutant was reduced to 70% and 63%, respectively, of the dwarf wild-type line cv Térèse (Fig. 1). In contrast, *Psbrc1*, which is also unable to respond to SL, showed a significant reduction in internode length but was far less affected than *rms1* and *rms4* (90% of the wild type; Braun et al., 2012; Fig. 1B). For all genotypes, bud removal had no significant impact on internode length (Fig. 1). Similarly, further examination of the effect of shoot branching on stem elongation revealed that bud outgrowth inhibited by exogenous treatment of the synthetic SL GR24 directly to all axillary buds of SL-deficient *rms1-1* mutant plants (Dun et al., 2013) did not lead to a significant increase in plant height (Fig. 2). Our results suggest that SLs may affect internode elongation independently of their function in bud outgrowth inhibition and that the reduced stature of the SL-related mutants is not simply a consequence of their increased branching phenotype. Manual removal of axillary buds along the stem, however, did induce a significant increase in stem diameter and leaf size in all genotypes tested, indicating that this response is SL independent and is possibly due to reallocation of resources (Supplemental Fig. S1).

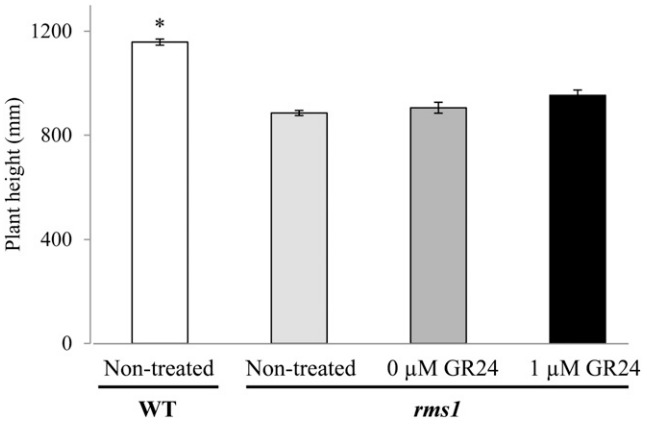

Figure 2. Inhibiting bud growth by directly treating buds with GR24 does not restore reduced *rms1* plant height. Axillary buds of wild-type (WT) or *rms1-1* plants (cv Parvus) were either left untreated (non-treated) or treated with 0 or 1 μM GR24 when the leaf was first open and retreated the following day. Bud lengths (Dun et al., 2013) and plant height were measured when the plants were 35 d old. Data are means ± SE (n = 14–16). The asterisk denotes a significant difference from the *rms1* 0 μM GR24-treated plant (*$P < 0.05$, Student's t test).

SL Promotes Internode Elongation in *rms1* But Not in *rms4* When Supplied to the Roots

To confirm that SL promotes internode elongation, GR24 (3 μM) was continuously supplied to the roots of wild-type, *rms1*, and *rms4* plants via hydroponics. After 16 d of GR24 treatment, total branch length was significantly reduced for *rms1* but not for the wild type or *rms4* (Fig. 3A). Generally, the strong basal branching at nodes 1 and 2 of dwarf pea lines (including the wild-type cv Térèse) is not inhibited by GR24 treatment using hydroponics (Boyer et al., 2012; Supplemental Fig. S1). The wild-type line displays branching only at these nodes, whereas *rms1* and *rms4* produce branches at basal and upper nodes. Upper branches in this dwarf background are inhibited by GR24 treatment in the SL-deficient *rms1* mutant but not in the *rms4* SL-response mutant. GR24 treatment caused a significant increase in internode length for the *rms1* SL-deficient mutant but not for the *rms4* SL-response mutant (Fig. 3B). No significant effect of GR24 on internode length was observed for the wild type (Fig. 3B).

To determine if SL can function similarly to GAs in promoting main stem internode elongation in pea, we compared the response of *rms1* SL-deficient mutant plants with treatment of the shoot tip with or without GR24 or GA_3. GA_3 applied to the shoot tip caused significant increases in length of all internodes except the first two nodes that were already elongated at the time of treatment. In contrast, GR24 at a dose more than 10 times greater than what is effective at axillary bud inhibition (10 μM) had no significant effect whatever the node and even on internodes that were newly forming in the shoot tip at the time of treatment (Fig. 4). These data suggest that, unlike GA, GR24 may not function in the shoot tip to regulate internode elongation.

Mutations Affecting SL (*rms1*) and GA (*le*) Levels Have Additive Effects on Branching and Internode Elongation

The *LE* gene (*PsGA3ox1*) encodes a GA 3-oxidase that is able to convert GA_{20} to the bioactive GA_1 (Lester et al., 1997; Martin et al., 1997). The enzyme encoded by the *le-1* allele shows reduced activity, resulting in a 10- to 20-fold reduction of GA_1 levels in elongating internodes of dwarf *le-1* mutant plants compared with tall *LE* plants (Ross et al., 1992). We have previously shown that the *rms1* mutation reduces plant height in different genetic backgrounds (Figs. 1 and 2; Beveridge et al., 1997b). Notably, a similar reduction is also observed in the *rms1-10* line, obtained from the dwarf wild-type line cv Térèse, which contains the *le-1* mutation affecting GA_1 levels.

To compare the effects of SL and GA on internode elongation and to investigate if GA or SL impact on each other's response, we used a series of four lines deficient in GA_1 and/or SLs (*le* or LE and *rms1* or

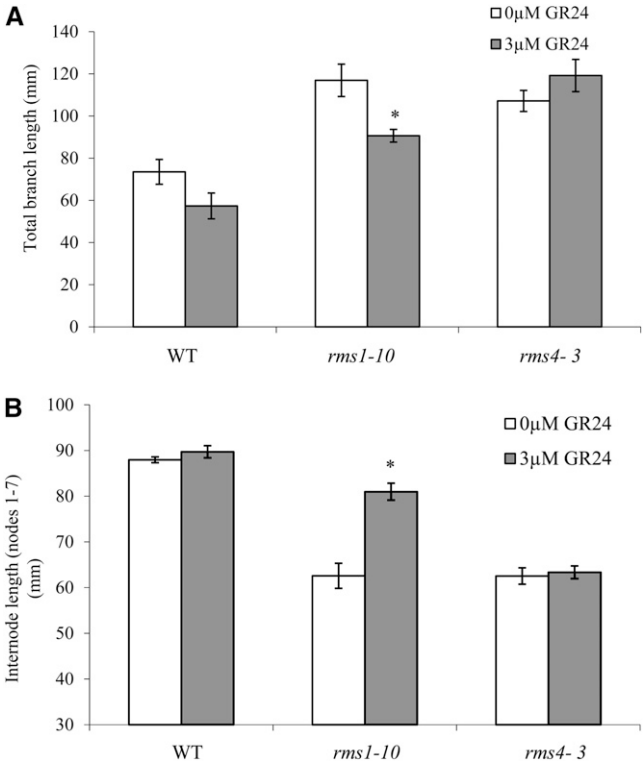

Figure 3. SL supplied via hydroponics increases the internode length of *rms1* SL-deficient mutant plants but not of *rms4* SL-response mutant plants. Six-day-old wild-type (WT), *rms1-10*, and *rms4-3* plants (cv Térèse) were supplied with 0 or 3 μM GR24 via hydroponics for 16 d, after which the sum of all lateral buds and branches at nodes 1 to 5 (A) and the internode length between nodes 1 and 7 (B) were measured. Data are means ± SE (n = 7–8). Asterisks denote significant effects of GR24-treated versus control-treated plants (*$P < 0.05$, Student's t test).

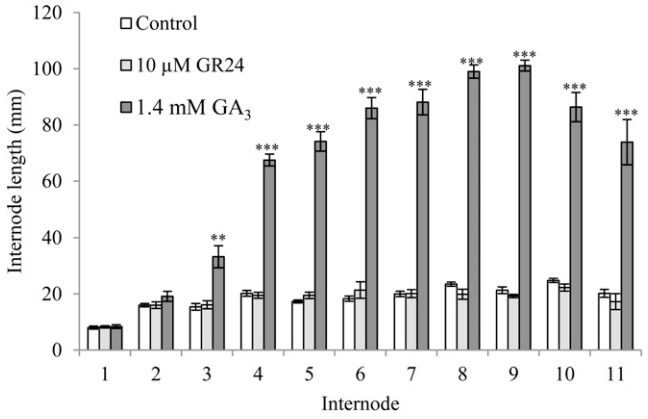

Figure 4. SL treatment to the shoot tip does not affect the growth of the main stem. The main shoot apex of 7-d-old *rms1-11* plants (cv Térèse) with 2.5 expanded leaves was treated on two consecutive days with 0 or 10 μM GR24 or 1.4 mM GA_3. Internode lengths were measured 23 d after treatment. Data are means ± SE (n = 7–10). Asterisks denote significant differences from the control (**$P < 0.01$, ***$P < 0.001$, Student's t test).

RMS1) in the cv Térèse background (Fig. 5A). Branching and internode lengths of 15-d-old plants were measured. A deficiency in GA_1 levels caused by the *le-1* allele gave rise to a 71% decrease in internode length in an *RMS1* background and 69% in an *rms1* background. The *rms1* mutation led to a significant reduction in internode length: 23% in an *LE* background and 17% in an *le-1* background (Fig. 5, A and B). Clearly, the effect of SLs on internode elongation is not as strong as the effect of GA. However, SL does significantly stimulate internode elongation irrespective of the level of GA, and vice versa. Importantly, *le-1 rms1* double mutant plants are shorter in stature than single *le-1* and *rms1* mutants, suggesting that phenotypes are additive and that SL and GA control internode elongation independently (Fig. 5, A and B).

The effect of GA on branching is suggested by the common use of the *le-1* allele in pea crops to reduce internode length and increase basal branching. In rice, reducing endogenous GA levels by overexpression of GA2ox resulted in reduced stem elongation and increased tillering (Lo et al., 2008). We often quantify branching by measuring branch length; such a measure is strongly dependent on internode length. As such, here, we quantified branching of the four lines by the ratio of total branch length to main stem length (from nodes 1 to 5). Using this ratio as a measure of branching, the double *le-1 rms1* mutant was more branched than single *le-1* and *rms1* mutants (Fig. 5C). We also estimated branching by the number of buds or branches with a size greater than 1.5 mm (Fig. 5D). Using this estimation, the double *le-1 rms1* mutant also displayed more branch/bud outgrowth than single *le-1* and *rms1* mutants. These additive results in double mutant plants indicate that *le-1* and *rms1* mutations independently induce branching and, hence, that SL and GA control branching independently.

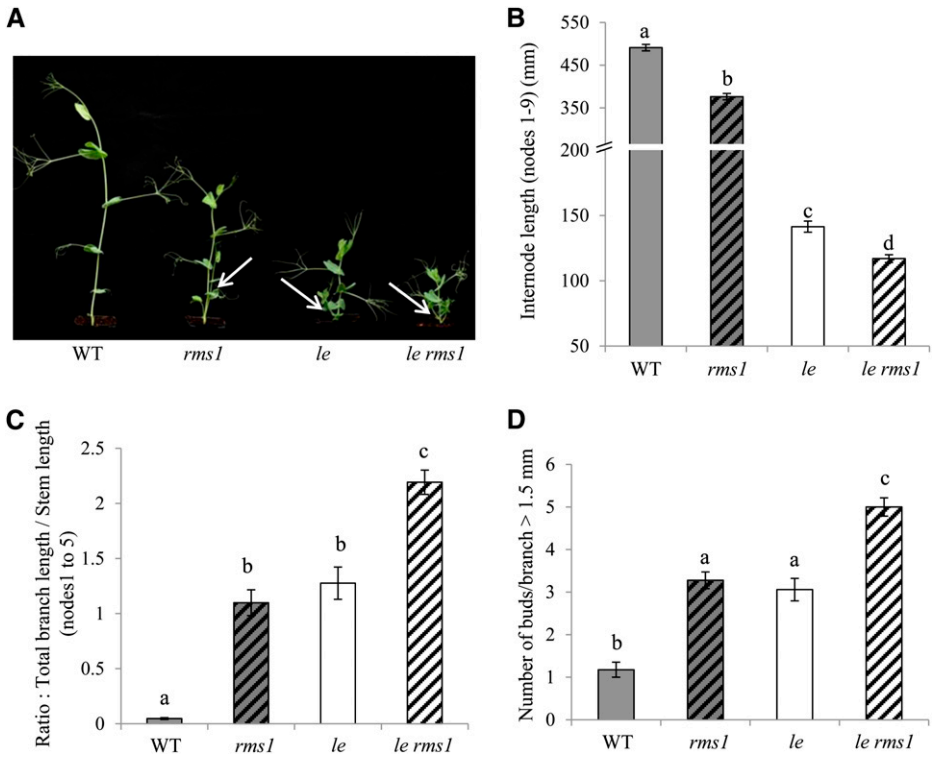

Figure 5. The effect of the *rms1-10* and *le* mutations on both internode length and branching is additive. A, Fifteen-day-old wild-type (WT), *rms1*, *le*, and *le rms1* plants. White arrows indicate the basal branching. B, Internode length between nodes 1 and 9. Data are means ± SE (n = 18). Values with different lowercase letters are significantly different from one another (ANOVA, $P < 0.01$). C, Ratio of total branch length to total stem length (n = 18). Values with different lowercase letters are significantly different from one another (ANOVA, $P < 0.001$). D, Number of buds or branches from nodes 1 to 5 with a length greater than 1.5 mm (n = 18). Values with different lowercase letters are significantly different from one another (ANOVA, $P < 0.001$). [See online article for color version of this figure.]

SLs Stimulate Stem Growth by Increasing Cell Number But Not Cell Length

To investigate whether SLs increase cell number and/or cell length to control the height of the plant, and to compare the effect of SLs with GA, we quantified epidermal cell size at a given elongated internode (internode 4) of 25-d-old wild-type, *le-1*, *rms1*, and *le-1 rms1* plants using scanning electronic microscopy (Fig. 6A). The number of cells was estimated for each plant by the ratio of internode length to cell length. Irrespective of the *LE* genotype, *rms1* significantly affected cell number but not cell length (Fig. 6, B and C). Consistent with what is known for GA, cell length and number were both strongly decreased in GA-deficient *le-1* plants in comparison with *LE* plants (Fig. 6, A, B, and D; Reid, 1983). These results indicate that SLs affect cell division rather than cell elongation to stimulate internode elongation. Both *le-1* and *rms1* mutations caused a significant increase in cell width (Supplemental Fig. S2).

GA_1 Content Is Not Correlated with the Dwarf Phenotype of the *rms* Mutants

To investigate in more detail a possible interaction between SL and GA in the control of internode elongation, we determined whether SL affects the levels of GA_1, the main bioactive GA in pea. GA_1 levels were quantified from elongating internodes of the tall line cv Torsdag (LE) and the corresponding SL mutant lines *rms1* and *rms4*. There was a small, although statistically significant ($P < 0.05$), reduction in GA_1 content in the SL-response mutant *rms4* compared with the wild type (Fig. 7). This reduction is unlikely to be physiologically significant, given the log/linear relationship between GA content and elongation in this species (Ross et al., 2011). GA_1 levels in *rms1* internodes were not significantly different from those of the wild type. To investigate further whether SLs are involved in the regulation of GA, transcript levels of genes involved in GA biosynthesis (*PsGA20ox1* and *PsGA3ox1* [*LE*]) and catabolism (*PsGA2ox1* [*SLN*] and *PsGA2ox2*) were analyzed in expanding stem tissue 24 h after GR24 treatment (3 μM) via the root system. Transcript levels of the *RMS5*/*PsCCD7* SL biosynthesis gene, known to be feedback down-regulated by SL in epicotyl, were analyzed as a positive control for GR24 treatment in the epicotyl (Supplemental Fig. S3). As expected, *RMS5* was highly expressed in the *rms1* and *rms4* mutant epicotyls in comparison with the wild type, and the GR24 treatment caused a strong down-regulation of *RMS5* transcript level in the *rms1* SL-deficient mutant, while there was no effect of GR24 on *RMS5* expression in the epicotyl of the *rms4* SL-response mutant (Supplemental Fig. S3). Transcript levels of the four genes controlling GA levels were not significantly affected by SL treatment in expanding stem tissue of any genotype (Supplemental Figs. S4

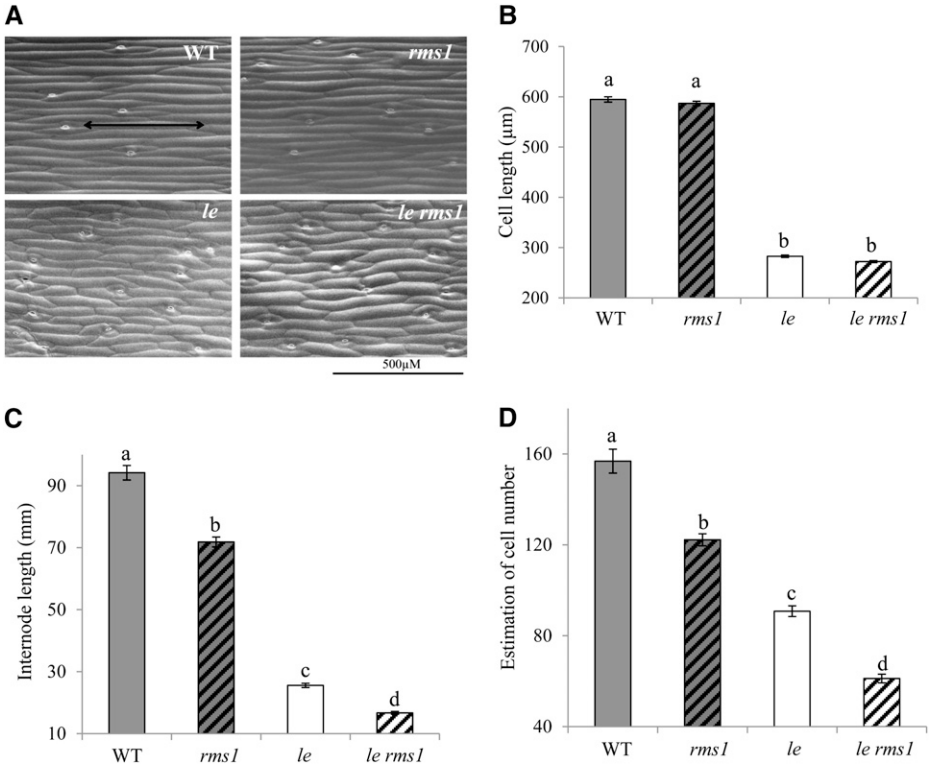

Figure 6. SLs regulate internode elongation by affecting cell number. A, Epidermal cells of internode 4 of the wild type (WT; top left), *rms1* (top right), *le* (bottom left), and *le rms1* (bottom right) in scanning electronic microscopy. B, Epidermal cell length (μm) in internode 4 of the wild type, *rms1*, *le*, and *le rms1*. Data are means ± SE (n = 7–8 plants with about 100 cells per plant). Values with different lowercase letters are significantly different from one another (ANOVA, $P < 0.05$). C, Internode 4 length (mm) of the wild type, *rms1*, *le*, and *le rms1*. Values with different lowercase letters are significantly different from one another (ANOVA, $P < 0.01$). D, Estimation of cell number in internode 4 of the wild type, *rms1*, *le*, and *le rms*. Values with different lowercase letters are significantly different from one another (ANOVA, $P < 0.001$).

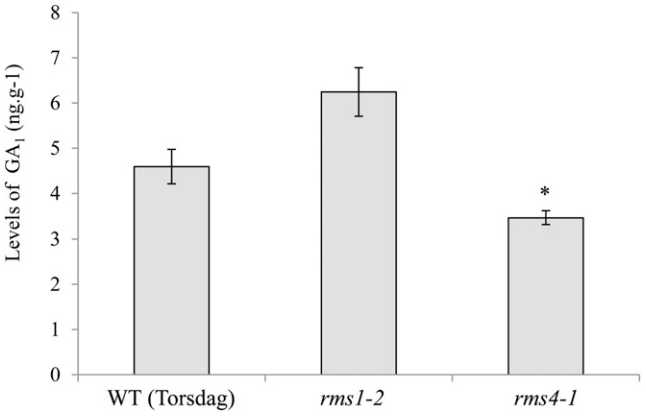

Figure 7. GA_1 levels in expanding stem tissue of wild-type (WT; Torsdag), *rms1-2*, and *rms4-1* 15-d-old plants. Data are means ± SE ($n = 3$). The asterisk denotes a significant difference from wild-type plants (*$P < 0.05$, Student's *t* test).

and S5). These results support the hypothesis that the SL and GA pathways do not interact in the control of internode elongation. However, significant increases in expression of the GA biosynthesis gene *PsGA3ox1* and the GA deactivation genes *PsGA2ox1* and *PsGA2ox2* were observed in the SL mutants relative to the wild type (Supplemental Figs. S4B and S5). Accordingly, a slight decrease in *PsGA3ox1* transcript levels (although not significant) was observed after GR24 treatment in *rms1* but not in *rms4*. Elevated transcript levels of the two GA deactivation genes in the *rms4* SL-response mutant might explain the reduced levels of GA_1 in this mutant (Fig. 7; Supplemental Fig. S5).

Hence, the dwarf phenotype of both *rms* SL mutants cannot be explained by low levels of GA_1 in expanding tissue. This strengthens the previous genetic data (Fig. 5) that the action of SL on internode elongation is independent of GA biosynthesis.

SLs Regulate Internode Elongation in a DELLA-Deficient Background

To determine if the reduction of internode length due to SL deficiency requires functional DELLA proteins, we compared the internode length of SL-deficient and nondeficient lines in a background with no functional DELLA proteins (*la cry-s*; O'Neill et al., 2010). The double mutant *la cry-s* shows a slender phenotype with very long internodes, particularly the basal internodes. If SL and DELLA proteins act in the same pathway to control internode elongation, the dwarf phenotype caused by the *rms* mutations should not be observed in a genetic background where DELLA proteins are nonfunctional.

A strong variation in internode length between *RMS1* F3 families and *rms1* F3 families was observed, despite all these families being fixed for *la cry-s* and *le-1* (as both lines, cv Térèse and HL178, contain the *le-1* allele). *RMS1* families had globally longer internodes (mean ± SE = 53.1 ± 1.59 mm) than the *rms1* families (mean ± SE = 45.4 ± 1.12 mm; Student's *t* test, $P < 0.01$; Fig. 8). In each of the three F3 families segregating for *RMS1/rms1*, a strong and significant effect of the *rms1* mutation was observed on internode length (Student's *t* test, $P < 0.01$ for family 6 and $P < 0.001$ for families 7 and 8; Fig. 8). These results indicate that functional DELLA proteins are not needed for SL to regulate internode elongation.

DELLA Proteins Are Not Degraded by SL in the Root Tip of Arabidopsis

Because pea is not amenable to transformation, we used the Arabidopsis transgenic line carrying the RGA promoter::GFP-RGA fusion (Silverstone et al., 2001) to test if SL application affects the levels of the DELLA protein. RGA is one of the five DELLA proteins present in Arabidopsis. Mutations at the *RGA* locus suppress the dwarf phenotype of the GA-deficient *ga1-3* mutant (Silverstone et al., 1997). The GFP-RGA fusion protein, localized to the nucleus in transgenic Arabidopsis seedlings, is rapidly degraded by GA treatment (Silverstone et al., 2001). The Arabidopsis *max* SL mutants have also been described to have reduced stature (Stirnberg et al., 2002; Booker et al., 2004), so we surmised that any effect of SL on DELLA proteins would also be conserved between pea and Arabidopsis.

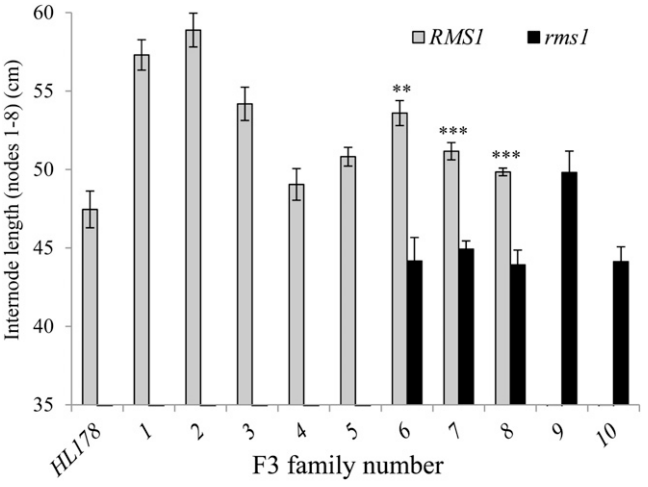

Figure 8. SLs control internode elongation in a *della* background. Internode length between nodes 1 and 8 were measured in the *della* line HL178 (*la cry-s*) and in F3 *della* families fixed for *la cry-s* and fixed for *RMS1* (1–5) or *rms1* (9 and 10) or segregating for *RMS1/rms1* (6–8). For families 6 to 8, the gray bars correspond to a mixture of *RMS1/RMS1* and *RMS1/rms1* plants ($n = 11$–28 except for *rms1* F3 6–8, for which $n = 6$–8). Asterisks denote significant differences from corresponding *rms1* plants (**$P < 0.01$, ***$P < 0.001$, Student's *t* test).

The *pRGA*::*GFP-RGA* line was used to follow the levels and the localization of the GFP-RGA fusion protein after GR24 treatment in root tips of Arabidopsis seedlings. Root tips of transgenic plants expressing *pRGA*::*GFP-RGA* were treated with water or GA_3 (100 μM) as negative and positive controls, respectively, or with GR24 (10 μM) and observed 1 and 2.5 h after treatment using confocal microscopy (Fig. 9). GFP signal in the nuclei decreased slightly after 2.5 h in water but was maintained in the nuclei, while the GA_3 treatment induced a strong decrease of GFP fluorescence in the nuclei. No degradation of GFP-RGA in the nuclei was observed 1 or 2.5 h after the GR24 treatment (Fig. 9) or after 4 h (data not shown). These results are in accordance with the results shown above and altogether suggest that SLs do not interact with the GA signaling pathway.

Dose Response of GA Application on the Wild Type, *rms1*, and *rms4*

To test if the SL-related mutants are affected in GA response, a dose-response experiment was performed where GA_3 was applied to expanding stipules at node 3 of 8-d-old plants and the length of internode 4 was measured 8 d after application. Both mutants responded to the application of GA_3, and 2.5 μg of GA_3 restored the internode length of the mutants to the wild-type control. However, GA application did not remove the difference in internode elongation between the mutants and the wild type (Fig. 10).

DISCUSSION

SLs Stimulate Stem Growth Independently from Its Effect on Branching

Pea grafting experiments have demonstrated that the reduced plant height observed in *rms1* SL-deficient plants is partly restored by wild-type rootstocks (Beveridge et al., 1997b). This could be a consequence of the majority of branching in the *rms1* shoot being inhibited by the wild-type rootstock or due to a supply of SL from the wild-type rootstock to the SL-deficient shoot. More recently, Umehara et al. (2008) demonstrated that the decreased plant height of *d10* SL-deficient mutant rice plants was restored to that of the wild type when grown with GR24 in the hydroponic medium, indicating a possible role for SL in the main stem, but again this was associated with reduced branching.

Here, we show that depleted SL levels are more directly responsible for the reduced height of the pea *rms1* SL-deficient mutant. Indeed, inhibiting the outgrowth of the buds along the *rms1* mutant stem by applying the synthetic SL, GR24, specifically to the axillary buds led to only a minimal and insignificant increase in plant height, as was also observed when all buds along the stem were manually removed (Figs.

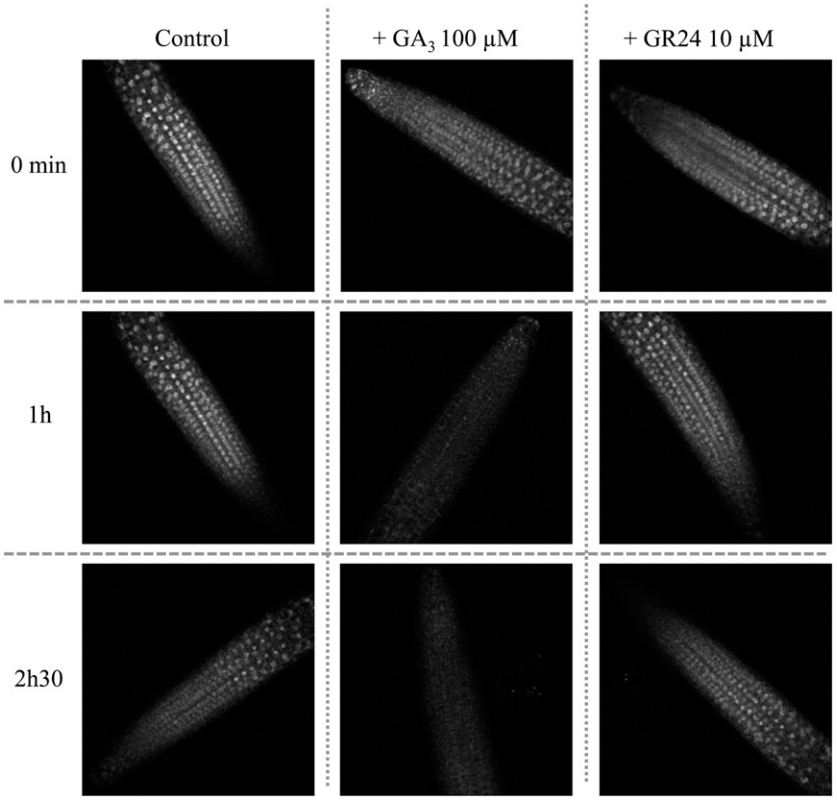

Figure 9. GA_3 but not GR24 treatment affects the expression of the GFP-RGA protein in root apex of Arabidopsis. Roots of transgenic plants (*rga/ga1-3* background) expressing the pRGA::*GFP-RGA* fusion were observed using confocal laser microscopy. Shown are three-dimensional projections of the fluorescence images of root tips treated with control solution (left column), 100 μM GA_3 (middle column), or 10 μM GR24 (right column) at different times as indicated.

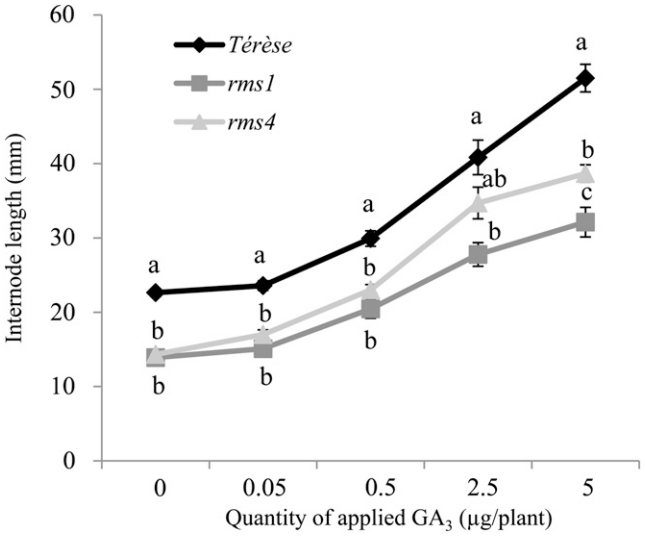

Figure 10. SL-related mutants respond to GA_3. Length of internode 4 of wild-type (diamonds), *rms1* (squares), and *rms4* (triangles) plants after treatment with various quantities of GA_3 (μg plant^{-1}). GA_3 was applied on stipules at node 3 when the plants were 8 d old. Measurements were made 8 d after treatment. Data are means ± SE (n = 8–12). Values with different lowercase letters are significantly different from one another (ANOVA, $P < 0.05$).

1 and 2). By contrast, the removal of axillary buds in rice led to a significant but partial restoration of reduced plant height in the *htd1/d17/Osccd7* SL-deficient mutant but had no significant effect on plant height in the wild type (Zou et al., 2006). This difference between pea and rice could be attributed to the huge difference of tiller numbers between the rice *htd1* mutant and the wild type (36 and one tillers, respectively); these tillers may act as a particularly strong sink; hence, their removal may substantially increase resources available to the main shoot for growth. In maize, a SL-independent action of the TB1 transcription factor to inhibit branching has been demonstrated, and, in contrast to other species, including rice, the branching of the *Zmccd8* mutant is particularly mild relative to *Tb1*. It is proposed that the dwarf phenotype of *Zmccd8* is not the result of competition for resources between the main stem and tillers, as the *Tb1* mutant displaying high branching is not affected in plant height (Guan et al., 2012). Similarly, in pea, the *Psbrc1* mutant is taller than *rms* mutants, although this comparison is less informative given that *Psbrc1* in pea has generally reduced branching compared with *rms* mutants (Braun et al., 2012).

As shown previously in rice (Umehara et al., 2008), continuously supplying GR24 to pea roots via hydroponics led to a significant increase of internode elongation in *rms1* SL-deficient plants but not in *rms4* SL-response mutant plants (Fig. 3B). This supports a role of SL in controlling plant height via an RMS4-dependent mechanism. This mechanism may be independent of the competition for resources between growing branches and the main stem, since branch suppression by bud removal or direct application of SL to buds of pea has only minor effects on stem length. While we did not achieve a complete restoration of internode elongation in *rms1*, this may be related to the dose, duration, or starting point of GR24 treatment. Therefore, we suggest that, in addition to regulating shoot branching (Gomez-Roldan et al., 2008; Umehara et al., 2008), secondary growth (Agusti et al., 2011), and various aspects of root development (Koltai, 2011; Brewer et al., 2013), SLs also contribute to the regulation of internode elongation in the main shoot.

This function does not seem to occur via *PsBRC1*, a gene mostly expressed in axillary buds, as the pea *Psbrc1* mutant is not as reduced in plant height as the SL pea mutants (Fig. 1; Braun et al., 2012). The shade-avoidance response of the *brc1* mutant in Arabidopsis further demonstrates the lack of *BRC1* influence over stem elongation. In response to simulated shade (light conditions enriched in far-red light), wild-type Arabidopsis plants exhibit strongly reduced branching, but the Arabidopsis *brc1* mutant was partially insensitive, showing little reduction of branching (Gonzalez-Grandio et al., 2013). In contrast, when considering other shade-avoidance responses, including stem elongation, *brc1* mutants responded similarly to the wild type, suggesting that *BRC1* is involved in the control of branching but not of stem elongation in response to light enriched in far-red light (Gonzalez-Grandio et al., 2013). Whether SL biosynthesis and/or MAX2 are also regulated by these light conditions will need further investigation (Finlayson et al., 2010). Altogether, these observations add further support to the notion that SL can influence primary stem growth independently of its effect on axillary bud outgrowth. Nevertheless, it is still unclear why *Psbrc1* showed reduced plant height that was not changed when buds were manually removed together with a reduced stem width in both intact plants and plants with buds removed. Novel functions for BRC1 are being discovered for this transcription factor, such as the regulation of flowering transition in axillary buds of Arabidopsis (Niwa et al., 2013), and further investigations are needed to have a good understanding of the mutant phenotype.

Tissue Specificity of SL Action on Cell Division

Among the three major plant hormones promoting stem elongation (IAA, GA, and BR), only GA has been shown to have an important effect on cell division, in addition to affecting cell elongation (Fig. 5; Achard et al., 2009; Lee et al., 2012). In pea, the length of epidermal cells in the internode is similar between *rms1* SL-deficient mutant plants and wild-type plants. Similarly, in rice, no difference in cell size was observed in the culm of the wild type and the *htd1/Osccd7* SL-deficient mutant (Zou et al., 2005). This suggests that SL does not act on cell elongation but rather stimulates cell division to regulate internode elongation. This

effect of SLs on cell division to increase plant height is in accordance with their effect on stimulating cambium-like cell divisions, as observed in the inflorescence stem of Arabidopsis and in the stem of other plants, including *Eucalyptus* species (Agusti et al., 2011). By contrast, it was shown that SLs repress mesocotyl elongation in dark-grown rice seedlings (Hu et al., 2010). This was also achieved by regulating cell division but not cell size in this tissue located between the coleoptile node and the cotyledon (Hu et al., 2010). Consequently, SLs appear to regulate cell division but with varying effects according to tissue. Whereas SLs would repress cell division in axillary meristems via the transcription factor PsBRC1 and repress cell division in the mesocotyl of dark-grown rice plants (Hu et al., 2010), SLs would promote cell division in the stem to stimulate longitudinal growth and secondary growth of the stem. Whether the stimulatory effect of SLs on internode elongation and their effect on secondary growth are independent will need further investigation, in particular by identifying the transcription factor(s) involved in these responses. Candidates might be found among the TCP family of transcription factors, comprising 24 members in Arabidopsis including BRC1. Several members of this plant-specific protein family have been shown to activate or repress the transcription of cell cycle genes, very likely in coordination with other proteins (Berckmans and De Veylder, 2009; Martín-Trillo and Cubas, 2010).

Applying GR24 to the shoot tip of SL-deficient *rms1* plants had no effect on main stem internode elongation (Fig. 4), suggesting that SL function in stem elongation is unlikely to be performed at the shoot tip. Nevertheless, in dicotyledons, stem tissues result from cell divisions occurring in a region called the rib meristem/rib zone, located at the base of the shoot apical meristem and consequently not directly at the surface of the shoot apex (Kerstetter and Hake, 1997). The strong difference between the GR24 and GA_1 effect when applied to the shoot tip is intriguing. It is possible that GR24 treatment could not reach the rib zone, where cells divide and elongate rapidly. One important characteristic of SL molecules is their low stability, which is essential for their role in the rhizosphere to signal the presence of a host root (Parniske, 2008). Moreover, SL reception seems to involve the hydrolysis of SLs into inactive compounds by the D14/DAD2 α/β-fold hydrolase (Hamiaux et al., 2012). Therefore, the continuous supply of SL may be essential (Smith and Waters, 2012) to observe an effect on internode elongation, which is a prolonged process, whereas axillary bud outgrowth is more a "switch on/off" process. This would explain why we observed an effect of GR24 on shoot elongation using hydroponics with continuous feeding and not by a single GR24 application to the shoot tip. In contrast, axillary bud outgrowth can be inhibited by a single GR24 application directly onto the bud.

An indirect growth effect due to the smaller root system of *Zmccd8* has been suggested in maize to explain the dwarf phenotype of the mutant, which displays fewer nodal roots and shorter primary root than the wild type (Guan et al., 2012). In Arabidopsis, SLs have been shown to control different aspects of root growth (main and lateral roots, root hair; Koltai, 2011; Ruyter-Spira et al., 2011), and it is possible that root supply of GR24 influences root growth and, indirectly, shoot growth. Nevertheless, in pea, and in normal nutritive conditions, the root system is not much affected in the SL-deficient and SL-response *rms* mutants (Beveridge et al., 1997a; Foo et al., 2013; data not shown). By contrast, in another legume, *L. japonicus*, while transgenic *LjCCD7*-silenced SL-deficient plants have strongly reduced internode lengths, they have roots with higher biomass and a longer primary root than wild-type plants (Liu et al., 2013). Consequently, an indirect effect of the root system on internode length is unlikely to play a major role in the SL-related mutants based purely on effects on root growth. Of course, it remains possible that SL signaling in the roots regulates the level of another mobile signal that affects elongation.

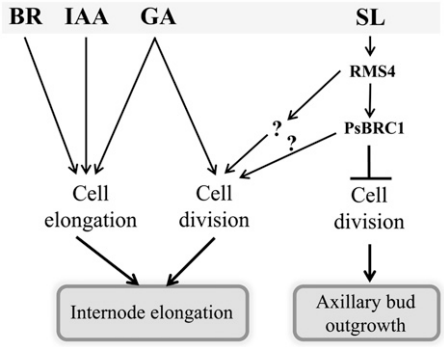

Figure 11. Model integrating the role of auxin (IAA), BR, GA, and SL on internode elongation in pea. Arrows indicate activation, and bars indicate repression. IAA and BR promote internode elongation by activating cell elongation; GA promotes internode elongation by activating both cell elongation and cell division; SL promotes internode elongation by cell division only independently from GA, via RMS4 and a transcription factor that, very likely, is not PsBRC1. In axillary buds, SL inhibits cell division via RMS4 and PsBRC1.

SL and GA Stimulate Stem Growth Independently

It is tempting to speculate that SLs in the roots or stem affect the levels of other signals that control stem elongation by affecting cell division, such as GA. Here, different approaches (genetic, physiological, molecular) were used to test whether SLs affect GA levels or signaling to control internode elongation. GA_1 levels were not correlated with dwarfism, and no effect of GR24 application on the transcript level of GA metabolism genes was found. We showed that SL-deficient and SL-response mutants were able to respond to GA treatment and that functional DELLA proteins were not

necessary for the SL regulation of internode elongation. Combined, our data strongly indicate that SLs do not act via GA to stimulate internode elongation in pea. Some results remain puzzling and will need further study, such as the reduced GA_1 levels observed in the SL-response *rms4* mutant. Nevertheless, it does not change our conclusion that SLs, independently of GA, stimulate cell division for the control of internode elongation in an RMS4-dependent pathway and possibly in a PsBRC1-independent pathway (Fig. 11). Another candidate for a SL-mediated signal in roots might be xylem-translocated cytokinins (CKs), which are considered to be anticorrelated in content with shoot SL signaling (Dun et al., 2009). CKs are known to play an important role in cell division, and xylem-CK content is known to be suppressed by reduced SL signaling. Unlike *rms* mutants, *Psbrc1* mutants do not contain depleted xylem sap CK levels, consistent with their taller stature. CKs are well known to affect cell proliferation in the shoot apical meristem, and reducing CK levels by overexpression of a catabolic *CYTOKININ OXIDASE* gene gives plants with shoot apical meristem containing fewer cells and slower leaf formation. Nevertheless, the role of CKs in the control of internode elongation still needs further investigation, as opposite results have been obtained in different species. Overexpression of a cytokinin oxidase to reduce endogenous levels of CK resulted in severe dwarf plants in tobacco due to both slower development and shorter internodes (Werner et al., 2001), but longer internodes were observed in transgenic poplar (*Populus tremula* × *tremuloides*) trees (Nieminen et al., 2008).

CONCLUSION

SLs were proposed to be central modulators of shoot architecture by modulating and coordinating shoot branching and secondary growth (Agusti and Greb, 2013). This study emphasizes the role of SLs in repressing or inducing the activities of different types of meristems to adjust shoot architecture to environmental conditions. We have shown by exogenous SL applications that control of branching and internode elongation can be independent. Whether, in natural conditions, these multiple actions of SLs are coordinated or not needs further investigation (Agusti and Greb, 2013).

MATERIALS AND METHODS

Plant Material

The dwarf branching pea (*Pisum sativum*) mutants *rms1-10* (M3T-884), *rms1-11* (M3T-988), and *rms4-3* (M3T-946) were obtained in the dwarf (*le*) cv Térèse and are described by Rameau et al. (1997). The *rms1-1* mutant (WL5237) obtained in the tall line Parvus and the *rms4-1* mutant (K164) obtained in the tall line cv Torsdag are described by Arumingtyas et al. (1992). The *rms1-2T* mutant line was obtained by backcrossing the *rms1-2* allele (derived from the cv Weitor background; Beveridge et al., 1997b) into the wild-type line Torsdag three times. The *Psbrc1* used in this study corresponds to BC3 (*Psbrc1* × Térèse). The mutant *Psbrc1*, identified by a TILLING (targeting-induced local lesions in genomes) approach from cv Caméor, is described by Braun et al. (2012). In the four *LE/le*, *RMS1/rms1* lines, *le RMS1* corresponds to cv Térèse and *le rms1* corresponds to the *rms1-10* branching mutant. The *LE RMS1* line was obtained by backcrossing nine times the *LE* allele from Torsdag in cv Térèse. The *LE rms1* line was obtained by crossing *LE RMS1* with the branching *rms1-10* mutant.

For Figure 8, the line HL178 *la cry-s* double mutant was crossed with the *rms1-10* line, and several slender F2 individuals (*la cry-s*) were selected phenotypically. Different F3 families, fixed for *la cry-s*, were followed that were either fixed for *RMS1* or *rms1* or segregating for *RMS1*. The branching phenotype of *rms1* plants was generally easy to identify; nevertheless, plants were genotyped for *RMS1* in the segregating F3 families. Because the *rms1* mutant and the double *la cry-s* mutant are not available in the same genetic background, comparisons of internode length were done for *rms1* F3 individuals (*rms1 rms1*) and nonbranching F3 individuals (*rms1 RMS1* and *RMS1 RMS1*) within the same segregating families (families 6–8 in Fig. 8). Other fixed *rms1* (families 9 and 10 in Fig. 8) and *RMS1* F3 (families 1–5 in Fig. 8) families were also analyzed for comparison.

The Arabidopsis (*Arabidopsis thaliana*) transgenic line expressing the GFP-RGA protein under the control of the *RGA* promoter is described by Silverstone et al. (2001).

Growing Conditions and Phenotype Measurements

Pea plants were grown in 16-h-photoperiod glasshouse conditions as described (Braun et al., 2012). For Figures 2 and 4, pea plants were grown in an 18-h photoperiod as described (Dun et al., 2013). Nodes were numbered acropetally from the first scale leaf as node 1. Internodes were numbered acropetally, with internode 1 extending between node 1 and node 2. The indicated stage (leaves expanded) corresponds to the number of nodes with fully expanded leaves. Bud and branch lengths were measured with digital calipers. For longer culture, one or two plants were cultivated per 2-L pot. To study the effect of branching on internode elongation, axillary buds were removed every day.

Hormonal Treatments

For Figure 2, solutions applied directly to the axillary buds contained 50% ethanol, 2% polyethylene glycol 1450, 0.1% acetone, and 0 or 1 μM (3 ng) GR24.

For shoot apex treatments (Fig. 4), 2 μL of solution containing 0 or 10 μM (6 ng) GR24 or 1.4 mM GA_3 (0.1 μg; Sigma) in 0.2% Tween 20 with 1% acetone and 5% ethanol was applied with a pipette to the unexpanded stipules of the shoot tip.

For hydroponics treatments, culture was as described by Braun et al. (2012), except that 33-L polyvinyl chloride opaque pots were used, one for each genotype. Acetone or GR24 (dissolved in acetone) was added to the hydroponic culture solution to give a final concentration of 0 or 3 μM GR24 and 0.01% acetone. The hydroponic culture solution was continuously aerated by an aquarium pump and was replaced weekly. One day after the beginning of the treatment, expanding stem tissue (internode 5) and epicotyls were harvested for gene expression analyses.

For GA treatment, 2 μL of solution containing GA_3 (Sigma) at the concentrations specified dissolved in 95% ethanol was applied to each of the two stipules at node 3 of the main stem on 8-d-old plants.

Gene Expression Analyses

Elongating internodes were harvested, total RNA was isolated and quantified, complementary DNA was synthesized, and real-time PCR gene expression analyses were performed as described (Braun et al., 2012). The analysis was done in two independent experiments. Primer sequences were as follows for GA synthesis genes and GA deactivation genes: *PsGA20ox1*, 5′-CATTCCATTAGGCCAAATTTCAAT-3′ and 5′-CTGCCCTATGTAAACAACTCTTGTATCT-3′; *PsGA3ox1*, 5′-TTCGAGAACTCTGGCCTCAAG-3′ and 5′-ATGTTCCTGCTAACTTTTTCATGGTT-3′; *PsGA2ox1*, 5′-CACAACCAATCAAGAACACAATTTC-3′ and 5′-CCCTTCTGCATCAAATCAAG-3′; *PsGA2ox2*, 5′-CCCTCCTGACCCCAGTGAAT-3′ and 5′-CTCACACTCACAAATCTTCCATTTG-3′. Transcript levels for the different genes were expressed relative to the expression of the *EF1α* gene (Johnson et al., 2006).

Segregation Studies

The cleaved-amplified polymorphic sequence marker for genotyping *RMS1* was based on amplification of the PCR fragment using primers RMS1-118F (5′-TTGGTTGGACTTCACTTTGAGG-3′) and RMS1-984R (5′-CACAACAAT-CAGCAATGACAGC-3′) and digestion with the *Cfr*13I enzyme (Fermentas).

Hormone Quantification

Harvested material (expanding internodes of 15-d-old plants) was extracted in cold 80% methanol and prepared for analysis by ultra-performance liquid chromatography-mass spectrometry as described previously (Tivendale et al., 2012). The internal standard was $[^2H_2]GA_1$, provided by Prof. Lewis Mander (Australian National University). The system consisted of a Waters Acquity H-series ultra-performance liquid chromatograph coupled to a Waters Xevo triple quadrupole mass spectrometer. A Waters Acquity UPLC BEH C18 column (2.1 × 100 mm × 1.7 μm particles) was used, with mobile phases A = 1% acetic acid (v/v) in water and B = acetonitrile. The program ran from 100% A to 85% A/15% B over 2 min, then to 77.5% A/22.5% B at 7 min. GA_1 eluted at approximately 6 min. The flow rate was 0.35 mL min^{-1}, and the column temperature was 35°C. Mass spectrometry was conducted in the electrospray and multiple reaction monitoring modes, monitoring negative ions. The ion source temperature was 130°C, the desolvation gas was nitrogen at 950 L h^{-1}, the cone gas was nitrogen at 50 L h^{-1}, the desolvation temperature was 450°C, the capillary voltage was 2.7 kV, and the cone voltage was 46 V. The transitions monitored, with collision energies in parentheses, were mass-to-charge ratio (*m/z*) 347.3 to 229.1 (28 V) and *m/z* 347.3 to 273.1 (22 V) for endogenous GA_1 and *m/z* 3 49.3 to 231.1 (28 V) and *m/z* 349.3 to 275.1 (22 V) for deuterated GA_1. The dwell time was 120 ms per channel.

Cell Measurements Using Scanning Electronic Microscopy

Freshly sampled tissues were cooled to −33°C by a Peltier cooling stage (Deben) and observed with a Hirox SH-1500 bench-top scanning electronic microscope. Internode 4 of 16-d-old plants was used, and its length was measured (eight plants per genotype). The epidermis cells of this internode were directly observed with a scanning electron microscope (MEB Hirox SH-1500). About 100 cells per plant were analyzed with ImageJ software (http://rsbweb.nih.gov/ij/), representing a total of 770 to 1,000 cells per genotype. The cell number in each internode 4 was estimated by the ratio of internode 4 length to mean cell length.

Confocal Laser Microscopy

Detection of GFP fluorescence was performed with a Zeiss LSM 710 confocal microscope. The excitation wavelength was 488 and 561 nm, and emission was collected at 565 to 720 nm for root tip imaging. For the experiments, root tips from 4-d-old transgenic plants expressing *pRGA::GFP-RGA* were mounted on standard microscope slides in the presence of water (5‰ acetone), 100 μM GA_3 (5‰ acetone), or 10 μM GR24 (5‰ acetone).

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession numbers DQ403160 (RMS5), X91658 and U58830 (PsGA20ox1), AF001219 and U85045 (PsGA3ox1), AF100955 (PsGA2ox1), and AF100954 (PsGA2ox2).

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Stem and stipule diameters of intact plants and plants with buds removed.

Supplemental Figure S2. Cell width of epidermal internode.

Supplemental Figure S3. Transcript levels of the SL biosynthesis gene *RMS5* after GR24 supply.

Supplemental Figure S4. Transcript levels of GA biosynthesis genes after GR24 supply.

Supplemental Figure S5. Transcript levels of GA catabolism genes after GR24 supply.

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