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RESEARCH PAPER

Strigolactones contribute to shoot elongation and to the formation of leaf margin serrations in *Medicago truncatula* R108

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Abstract

Strigolactones were recently identified as a new class of plant hormones involved in the control of shoot branching. The characterization of strigolactone mutants in several species has progressively revealed their contribution to several other aspects of development in roots and shoots. In this article, we characterize strigolactone-deficient and strigolactone-insensitive mutants of the model legume *Medicago truncatula* for aerial developmental traits. The most striking mutant phenotype observed was compact shoot architecture. In contrast with what was reported in other species, this could not be attributed to enhanced shoot branching, but was instead due to reduced shoot elongation. Another notable feature was the modified leaf shape in strigolactone mutants: serrations at the leaf margin were smaller in the mutants than in wild-type plants. This phenotype could be rescued in a dose-dependent manner by exogenous strigolactone treatments of strigolactone-deficient mutants, but not of strigolactone-insensitive mutants. Treatment with the auxin transport inhibitor *N*-1-naphthylphtalamic acid resulted in smooth leaf margins, opposite to the effect of strigolactone treatment. The contribution of strigolactones to the formation of leaf serrations in *M. truncatula* R108 line represents a novel function of these hormones, which has not been revealed by the analysis of strigolactone mutants in other species.

Key words: *Medicago truncatula*, elongation, leaf, mutant, phytohormone, shoot, strigolactone.

Introduction

Strigolactones (SL) are carotenoid-derived metabolites long known for their ability to trigger the germination of parasitic plant seeds (Cook *et al.*, 1966). They also play a role in plant–microbe symbiotic interactions, as stimulants of the growth and metabolism of arbuscular mycorrhizal fungi (Akiyama *et al.*, 2005; Besserer *et al.*, 2006, 2008). In addition to these effects in the rhizosphere, we and others have proposed that SL or related compounds also act *in planta* as phytohormones, contributing to the control of shoot branching (Gomez-Roldan *et al.*, 2008; Umehara *et al.*, 2008). This discovery stemmed both from the elucidation of the biosynthetic origin of SL (Matusova *et al.*, 2005) and from long-standing studies of the control of shoot architecture in several plant species (reviewed in Xie *et al.*, 2010; Ruyter-Spira *et al.*,

Abbreviations: SL, strigolactone; CCD, carotenoid cleavage dioxygenase; NPA, *N*-1-naphthylphtalamic acid.

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2013). Central to these pioneer studies are a series of mutants in *Arabidopsis thaliana* (*max—more axillary growth*), pea (*rms—ramosus*), rice (*d—dwarf*) and Petunia (*dad—decreased apical dominance*), isolated in forward genetic screens on the basis of their enhanced shoot branching phenotypes. The physiological characterization of these mutants including, in particular, a series of graft experiments, revealed that they lack or are insensitive to an unknown mobile signal able to suppress shoot branching. Cloning of the mutated genes in *Arabidopsis* showed that two carotenoid cleavage dioxygenase (CCD) isoforms, CCD7 and CCD8, are necessary for the synthesis of this signal (Sorefan *et al.*, 2003; Booker *et al.*, 2004). As carotenoid cleavage was proposed to be necessary for SL production (Matusova *et al.*, 2005), the hypothesis that the unknown signal could be SL was investigated. Indeed, pea and rice shoot branching mutants are either SL-deficient or SL-insensitive, and SL exhibits all the expected properties of the long sought-after signal (Gomez-Roldan *et al.*, 2008; Umehara *et al.*, 2008). The SL biosynthetic pathway has since been further elucidated with the discovery of additional enzymes and intermediates (Alder *et al.*, 2012). Studies of SL perception and signalling have identified two key actors, an α/β hydrolase probably acting as the SL receptor (Hamiaux *et al.*, 2012) and an F-box protein essential for signal transduction and integration (Stirnberg *et al.*, 2007).

Early analyses of SL-deficient and SL-insensitive mutants have mainly focused on shoot branching or tillering enhancement, although other phenotypes including dwarfism, altered root growth, reduced shoot diameter, delayed leaf senescence, decreased flower size, and modifications of leaf shape parameters have also been documented. In the last few years, more extensive characterization of the SL mutants in *Arabidopsis*, pea, and rice has established a role for SL in root architecture and root hair development (Kohlen *et al.*, 2011; Kapulnik *et al.*, 2011), mesocotyl elongation (Hu *et al.*, 2010), vascular secondary growth (Agusti *et al.*, 2011), adventitious rooting (Rasmussen *et al.*, 2012), seedling response to light (Tsuchiya *et al.*, 2010), and seed germination (Toh *et al.*, 2012). Furthermore, the characterization of SL-deficient mutants or transgenic lines in other plant species has revealed the contribution of SL to fruit development (Kohlen *et al.*, 2012), tuber differentiation (Roumeliotis *et al.*, 2012), and nodule formation (Foo and Davies, 2011). It therefore seems that SL, like other plant hormones, exert a wide range of effects in various physiological contexts. This is fully consistent with observations that SL signalling crosstalks with several other plant hormonal pathways (Vanstraelen and Benkova, 2012). It is likely that other effects of SL remain to be discovered, and the study of additional plant species should contribute to a more comprehensive assessment of SL functions.

The mechanisms by which SL affect these various aspects of plant development have not been fully elucidated. For example, two models have been put forward to explain the respective roles of auxin and SL in the control of shoot branching. One model proposes that SL dampens polar auxin transport in the main stem in a systemic manner (Bennett *et al.*, 2006). This would prevent auxin export from the buds that is necessary for bud growth (Crawford *et al.*, 2010). The other model favours a local action of SL in buds as second messengers of auxin (Dun *et al.*, 2013). These divergent views are not mutually exclusive and may reflect, to some extent, differences in the plant species and physiological contexts in these independent studies (Dun *et al.*, 2006). Among other known functions of SL, the modulation of primary root growth has been associated with modifications of the auxin gradient at the root tip (Ruyter-Spira *et al.*, 2011), whereas stimulation of vascular secondary growth seems to occur independently or downstream of auxin transport (Agusti *et al.*, 2011) and enhanced root hair elongation is at least partly independent from auxin (Kapulnik *et al.*, 2011). The emerging picture is that a universal mode of SL action is not to be discovered, and that SL can display opposite properties depending on the target cells and physiological context (de Saint-Germain *et al.*, 2013).

Among legumes, *Medicago truncatula* Gaertn. has emerged as an attractive model species for the study of aerial development. Many mutants with shoot or leaf phenotypes have been identified through forward genetics screens, and extensive reverse genetic resources have been generated. The aim of the present study was to investigate the role of SL in the regulation of aerial development in *M. truncatula*. We first identified transposon insertional mutants affected in SL biosynthesis or response genes. Both types of mutants displayed a bushy phenotype, which could be attributed to reduced internode elongation rather than to enhanced shoot branching. In addition, mutant phenotypic characterization in combination with SL treatments revealed a novel function for SL in leaf margin development.

Materials and methods

Identification of M. truncatula *SL-related genes*

A BLAST search was performed with the amino acid sequences of pea CCD7 and CCD8, and *Arabidopsis* D14, against the NCBI database. For phylogenetic analysis, sequences were aligned using the MUSCLE program (Edgar, 2004). Maximum-likelihood trees were built with MEGA6 (Tamura *et al.*, 2013), using Jones-Taylor-Thornton (JTT) as the amino acid substitution model and the nearest-neighbour-interchange (NNI) heuristic method. The partial deletion (95%) mode was used to treat gaps and missing data. Accession numbers of all genes used in this analysis are listed in Supplementary Table S1.

Plant material and growth conditions

All mutants were obtained from the Noble Foundation *Tnt1* insertion library (Tadege *et al.*, 2008) by PCR screening (Cheng *et al.*, 2014). Plants homozygous for the presence of a *Tnt1* insertion in the target gene were selected by PCR. The position of *Tnt1* insertions was confirmed by PCR and sequencing. Mutant lines *ccd7-1* and *ccd8-1* were backcrossed twice to the R108 wild type. In the F2 progeny of the second backcross, homozygous lines carrying a *Tnt1* insertion in the *CCD* gene were selected. Wild-type siblings identified in this progeny were used for comparison.

Plants were grown on an inert substrate (OilDri, Brenntag, France) and fertilized with a modified Long Ashton nutrient solution (Balzergue *et al.*, 2011). Plants were kept in a growth chamber under a 16 h photoperiod at 24 °C. For the analysis of shoot architecture, 40-day-old plants were photographed and shoot length was measured with a ruler.

Treatments

The synthetic strigolactone analogue GR24 was obtained from Chiralix (The Netherlands) and NPA was purchased from Sigma. Stock solutions were prepared in acetone for GR24, and in DMSO for NPA, and diluted 1000-fold in water supplemented with 0.05% Tween 20 to reach the indicated concentrations. Control treatments were performed with the solvent(s) alone, diluted 1000-fold in water supplemented with 0.05% Tween 20.

Plants were treated five times a week throughout the culture by application of 100 µl of solution directly to the primary shoot apex with a pipette.

Leaf shape analysis

After four to five weeks of growth, several fully expanded leaves were collected. The three leaflets of each leaf were carefully separated from the rachis and petiole, and leaflets were scanned at 600 dpi resolution using an Epson scanner. Images were analysed using ImageJ software to obtain solidity values.

Statistical analyses

All analyses were carried out using Statgraphics Centurion software (SigmaPlus). Two-sample comparisons were performed using the unequal variance t-test. Multiple comparisons were performed by one-way ANOVA followed by Fisher's LSD test, or using non-parametric tests when normality or homoscedasticity criteria were not satisfied. In that case datasets were analysed using the Kruskal-Wallis test followed by pairwise comparisons with the Mann-Whitney test. A Bonferroni correction was applied to take into account multiple testing, so that differences are reported at a 0.05 significance level.

Results and discussion

Identification of Tnt1*-insertion SL mutants*

Previous studies have demonstrated that SL biosynthesis involves the cleavage of a carotenoid substrate (Matusova *et al.*, 2005; Alder *et al.*, 2012). Enzymes able to carry out such a cleavage are classified into two main groups (Vallabhaneni *et al.*, 2010). While 9-*cis*-epoxycarotenoid dioxygenases (NCEDs) are specialized isoforms associated with the biosynthesis of abscisic acid, the carotenoid cleavage dioxygenases (CCDs) collectively use a wider range of substrates and contribute to varied physiological functions. CCDs can be further divided into four clades represented by the *Arabidopsis* isoforms CCD1, CCD4, CCD7, and CCD8 (Auldridge *et al.*, 2006). The involvement of CCD7 and CCD8 in SL synthesis is now firmly established in several species including pea (*Pisum sativum*), a close relative of *M. truncatula*. CCD7 and CCD8 act successively in the SL biosynthetic pathway (Alder *et al.*, 2012), so that a loss-of-function mutant of either of the corresponding genes is SL-deficient as demonstrated by biochemical analyses (e.g. Gomez-Roldan *et al.*, 2008 for pea; Umehara *et al.*, 2008 for rice).

We searched for *M. truncatula* orthologues of pea *CCD7* and *CCD8* genes. For *CCD7*, one strong homologous gene and four less closely related genes were identified. A search with the pea *CCD8* sequence yielded the same five genes, which therefore probably comprised the whole set of *M. truncatula CCD* genes. Phylogenetic analysis (Supplementary Fig. S1A) revealed that one of these genes (hereafter named *MtCCD7*) fell into the *CCD7* clade, and another into the *CCD8* clade (*MtCCD8*). The remaining three genes were more closely related to *CCD1* or *CCD4*. At the amino acid level, MtCCD7 and MtCCD8 respectively share 88% and 91% identity (93% and 95% similarity) with their pea orthologues.

Mutants of *MtCCD7* and *MtCCD8* were identified by PCR-based reverse screening from a collection of *M. truncatula Tnt1* retrotransposon insertion lines (Tadege *et al.*, 2008; Cheng *et al.*, 2014). *Tnt1* insertion lines were generated using the R108-1(c3) line, which is highly embryogenic and more amenable to genetic transformation than the widely used Jemalong A17 ecotype (Trinh *et al.*, 1998). Two mutant alleles (*ccd7-1* and *ccd7-2*) of *MtCCD7*, and one for *MtCCD8* (*ccd8-1*) were isolated. They all harbour a *Tnt1* insertion in the coding sequence (Supplementary Fig. S2) leading to a premature STOP codon, and can therefore be considered KO mutants.

The α/β hydrolase D14/DAD2 recently emerged as a strong candidate for the SL receptor (Hamiaux *et al.*, 2012; Nakamura *et al.*, 2013). The binding of SL to D14 triggers downstream responses and *d14* mutants in several species are insensitive to SL (Arite *et al.*, 2009; Hamiaux *et al.*, 2012; Waters *et al.*, 2012). D14 belongs to a multigene family in which close relatives called D14-like can be identified (Delaux *et al*., 2013). Only D14 seems to be essential for SL perception as indicated by binding capacities and phenotypes of mutants of *D14-like* genes (Waters *et al.*, 2012; Kagiyama *et al.*, 2013).

Five genes homologous to *Arabidopsis D14* could be found in the *M. truncatula* genome. Only one of these, hereafter called *MtD14*, falls into the *D14* clade (Supplementary Fig. S1B). The remaining four can be classified as *D14-like1* or *D14-like2*. A mutant harbouring a *Tnt1* insertion in the second exon of *MtD14* (*d14-1*, Supplementary Fig. S2) was identified by PCR-based reverse screening (Cheng *et al.*, 2014).

Aerial architecture of SL mutants

Shoot development in *M. truncatula* follows a complex but ordered pattern (Bucciarelli *et al.*, 2006; Moreau *et al.*, 2006). In brief, the main shoot axis produces growth units called metamers comprising an internode, a leaf, and an axillary bud. Under our growth conditions, internodes on the main axis remain very short in the early stages of growth. Axillary buds from metamers 1 to 4 (m1–m4) grow out to form axillary shoots, that elongate substantially and adopt a prostrate growth habit. The main axis then elongates vertically and produces additional metamers, from which new axillary shoots can later emerge. Overall, one axillary bud is present at each leaf axil and eventually grows out into a shoot of higher order.

After several weeks of growth, both *ccd7-1* and *ccd8-1* mutants appeared smaller and more compact than the wild type (Fig. 1A). While *M. truncatula* wild-type plants adopted a trailing growth habit as axillary shoots became longer and heavier, mutant plants did not. SL-insensitive *M. truncatula d14-1* mutants displayed an aerial phenotype very similar to that of *ccd7-1* and *ccd8-1* mutants (Fig. 1B), establishing a firm link between this phenotype and the SL pathway.

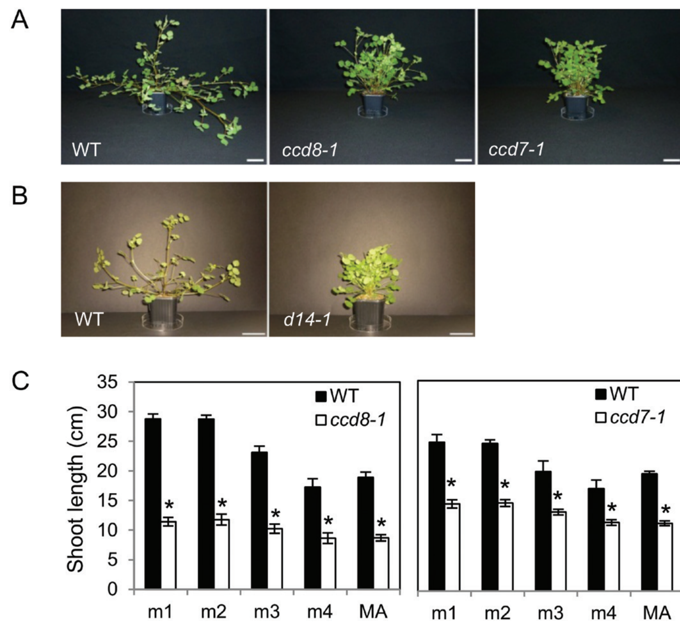

Fig. 1. Shoot architecture of strigolactone mutants. (A, B). Photographs of 40-day-old plants. Bar=5 cm. Mutants *ccd7-1* and *ccd8-1* (A) and *d14-1* (B) were examined in separate experiments. (C) Shoot lengths of the first axes (emerging from metamers m1 to m4) and of the main axis (MA) measured after 40 days of growth. Mutants were compared with their respective wild-type siblings. Values correspond to the mean±SEM of 5–6 plants per genotype. Asterisks indicate statistically significant differences between the mutant and wild type for each axis (unequal variance t-test, $P<0.05$). (This figure is available in colour at *JXB* online.)

The bushy appearance of SL-deficient *M. truncatula* mutants could not be attributed to enhanced shoot branching as indicated by the observation that in the wild type as well as the mutants, most axillary buds eventually developed into shoots. For example, 40-day-old wild-type and *ccd8-1* mutant plants, respectively, harboured an average of 8.38±0.6 and 8.38±0.26 first-order shoots, and 4±0.76 and 3.86±1.6 second-order shoots. Thus, the mutant and wild type could not be distinguished on the basis of bud outgrowth patterns. This situation is in contrast with other species studied for the role of SL in development: wild-type plants usually exhibit limited shoot branching and the number of outgrowing lateral buds increases in SL mutants. An exception is the legume *Lotus japonicus*, which exhibits profuse basal shoot branching at the cotyledonary node. Still, in this species, many buds in the aerial stem remain dormant, which allows for enhanced aerial branching in *LjCCD7* RNAi knock-down lines, in addition to an increased number of basal shoots (Liu *et al.*, 2013). To reveal a putative function for SL in the control of shoot branching in *M. truncatula*, it would be necessary to define growth conditions that limit the development of axillary shoots in the wild type. A starting point could be the observation that growing *M. truncatula* in the absence of a nitrogen source results in a complete inhibition of axillary shoot development, accompanied by a marked reduction of aerial organ growth (Bucciarelli *et al.*, 2006). One might be able to define an intermediate nitrogen supply level that does not impair plant growth too severely, while limiting axillary shoot development in the wild type, to investigate whether SL-deficient mutants exhibit enhanced shoot branching in such conditions.

The compact phenotype of the SL-deficient mutants could be attributed to reduced shoot elongation, as documented by the shoot length of the first four metamers and the main axis (Fig. 1C). Similar results were obtained with the *ccd7-2* mutant allele (Supplementary Fig. S3A). This phenotype persisted throughout the plants' life cycle, and therefore did not reflect a mere growth delay. Dwarfism has been reported in SL-deficient mutants of several species, and has been suggested to be an indirect consequence of enhanced branching, through reduced resource allocation to a larger number of shoots (Kohlen *et al.*, 2012) or tillers (Zou *et al.*, 2006). Our observations indicate that it is not the case in *M. truncatula*, as reduced shoot length in SL-deficient mutants is observed in the absence of any effect on shoot branching. This conclusion is consistent with the recent report of de Saint Germain *et al.* (2013), where it is proposed that in pea plants the effects of SL on shoot branching and internode elongation are independent. Exogenous application of the synthetic SL analogue GR24 (Zwanenburg *et al.*, 2009) at the primary shoot apex partly rescued the reduced shoot elongation observed in SL-deficient mutants of *M. truncatula* (Supplementary Fig. S3). This contrasts with the observations of de Saint Germain *et al.* (2013) in pea, where application of GR24 to the shoot tip did not stimulate shoot elongation. One hypothesis in that report was that SLs are unstable and therefore a single application might not be sufficient to obtain visible effects. In our case, GR24 was applied repeatedly over several weeks, which could account for the observed effect on shoot elongation. In any case, the positive effect of GR24 on shoot elongation of *M. truncatula* further supports our conclusion that SL deficiency is the cause of dwarfism in the *Mtccd7* and *Mtccd8* mutants.

Leaf shape alterations

When wild-type and SL mutant plants were grown side by side for the analysis of shoot architecture, we noticed differences in leaf shape. Apart from the first unifoliate leaf, *M. truncatula* bears compound leaves with three leaflets. Leaflets of R108 plants display an overall circular shape with serrations on the leaf margin. In both SL-deficient mutants serrations seemed shallower than in the wild type, as illustrated in Fig. 2A, B. To investigate whether this novel phenotype was due to SL deficiency, mutant plants were treated with the synthetic SL analogue GR24 at different concentrations applied directly at the primary shoot apex. These treatments clearly modified the leaf shape: GR24-treated leaflets displayed fewer, deeper, and wider serrations than mock-treated leaflets (Fig. 2A, B).

A morphometric analysis was undertaken to quantify these effects. We chose to focus on serration size as serration number is difficult to determine accurately, especially for the smaller ones. Among commonly used shape descriptors, solidity (Neal and Russ, 2012) was the most appropriate to quantify differences in serration size. Solidity corresponds to the ratio of leaflet area over convex area, i.e. the proportion of pixels in the convex area that are also in the leaflet

(Fig. 2C): the smaller the value, the larger the indentations. Solidity is affected only by irregularities at the surface perimeter, and not by overall form. Solidity was significantly higher in both SL-deficient mutants as compared with the wild type (Fig. 2D, E), confirming our initial visual observations that serrations were shallower in the SL-deficient mutants.

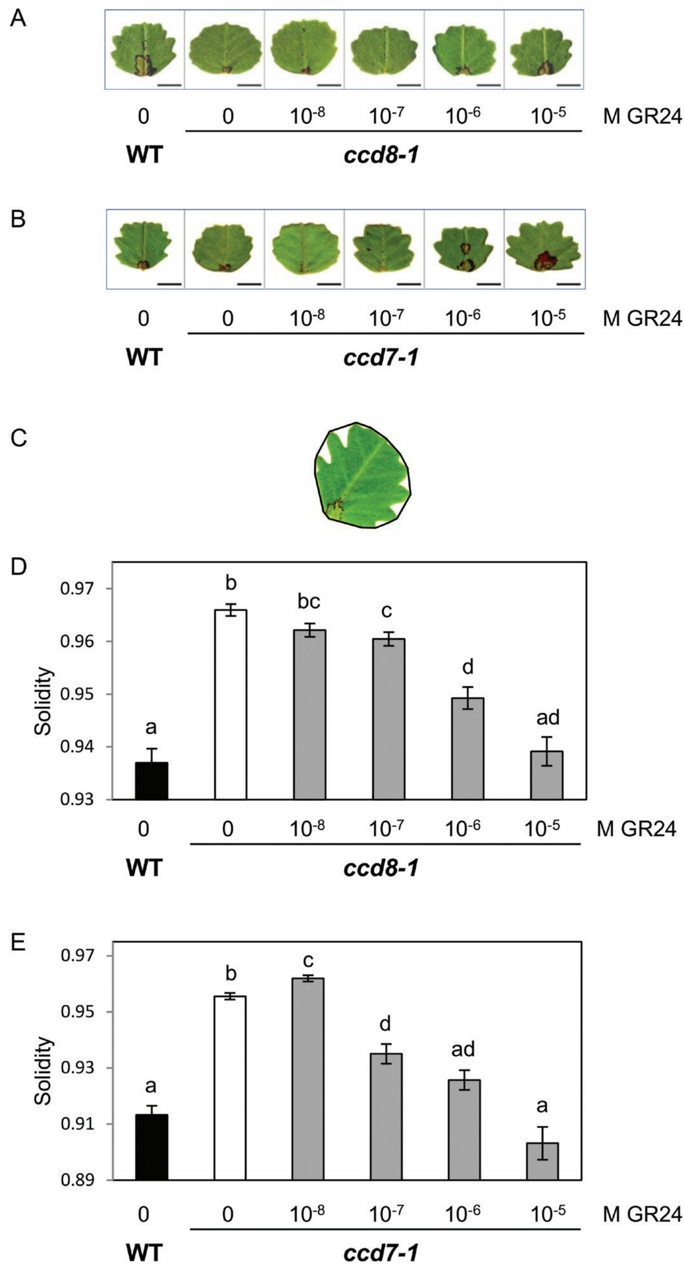

Fig. 2. Exogenous SL can rescue the leaflet serration phenotype of SL-deficient mutants. GR24 was applied to the shoot tip at the indicated concentrations. (A, B) Scanned images of one representative leaflet in each condition. Representative leaflets with a solidity value equal to the average solidity of all leaflets in this condition were selected. Bar=5 mm. (C) Illustration of solidity as a shape descriptor. The convex hull (black line) delimits the convex area of each leaflet. Solidity is calculated as the ratio of leaflet area/convex area. (D, E) Solidity values for leaflets of *ccd8-1* and *ccd7-1* mutants and their respective wild-type siblings, treated or not with GR24. Values correspond to the mean±SEM (*n*=49–85 leaflets for each condition). Different letters indicate statistically significant differences according to Mann-Whitney's test (*P*<0.05 after Bonferroni adjustment). (This figure is available in colour at *JXB* online.)

Furthermore, solidity decreased as the applied concentration of GR24 increased. Concentrations of 10^{-7} M and higher significantly affected solidity, and application of 10^{-6} –10^{-5} M GR24 could restore this shape descriptor back to wild-type levels (Fig. 2D, E). The analysis of leaf shape in the second *ccd7* mutant allele gave similar results (Supplementary Fig. S4). Finally, SL-insensitive *d14-1* mutants displayed the same leaf serration phenotype as the SL-deficient *ccd7* and *ccd8* mutants, but the shape of their leaflets was not modified by application of 10^{-5} M GR24 (Fig. 3). Together, these results demonstrate a close link between the canonical SL pathway and leaflet margin morphology in *M. truncatula* R108. It is noteworthy that the compound leaf structure does not seem to be disturbed in SL mutants. This is consistent with the hypothesis that partly distinct mechanisms account for the formation of compound leaves and leaf margin serrations in *M. truncatula* (Zhou *et al.*, 2011), and suggests that SL affects a process specific to leaf margins.

Genetic studies in *Arabidopsis* have shed light on the mechanisms that drive the formation of leaf serrations. In the model proposed by Bilsborough *et al.* (2011), the growth-repressing transcription factor CUC2 allows the formation of convergence points of polar auxin transport. This process, together with auxin's ability to stimulate its own polar transport, results in the accumulation of auxin at discrete points along the leaf margin. As auxin inhibits the expression of *CUC2*, a pattern of interspersed auxin and CUC2 maxima is created along the leaf margin, leading to differential growth and to the formation of serrations. In agreement with this model, impairment

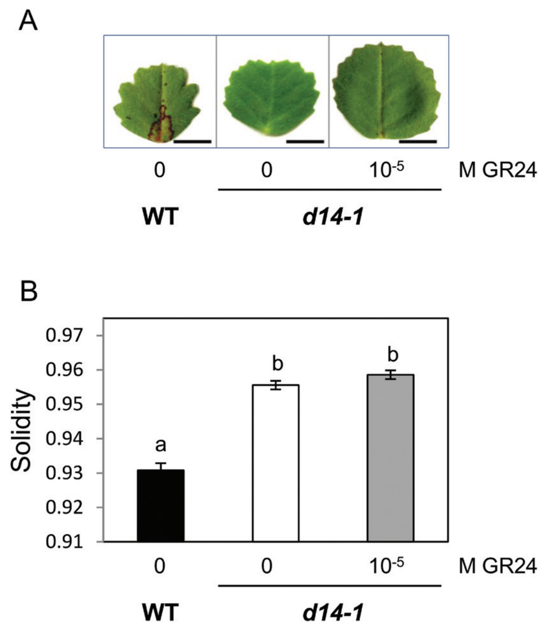

Fig. 3. Exogenous SL does not affect the leaflet shape phenotype of SL-insensitive mutants. Mutant plants were treated with 10^{-5} M GR24 applied to the shoot tip. (A) Scanned images of one representative leaflet under each condition. Bar=5 mm. (B) Leaflet solidity values for control WT plants and *d14-1* mutants treated or not with GR24. Values correspond to the mean±SEM (*n*=53–127 leaflets for each condition). Different letters indicate statistically significant differences according to Mann-Whitney's test (*P*<0.05 after Bonferroni adjustment). (This figure is available in colour at *JXB* online.)

of auxin transport in *Atpin1* mutants or following treatment with the auxin transport inhibitor *N*-1-naphthylphtalamic acid (NPA) results in the loss of leaf serrations (Hay *et al.*, 2006). The smooth leaf margin phenotype of *M. truncatula* R108 mutants of *MtPIN10* suggests that auxin transport is also required for the formation of leaf serrations in this species (Zhou *et al.*, 2011; Peng and Chen, 2011).

To investigate the relationship between SL, auxin transport, and serrations in *M. truncatula*, we compared the effects of SL and NPA on wild-type leaflets (Fig. 4). Consistent with the report of Zhou *et al.* (2011), NPA treatment resulted in smoother leaf margins with few and shallow serrations, or even no serrations at all except the terminal one (Fig. 4A). Similar results were obtained with *ccd7-1* mutants (Fig. 4B). Morphometric analysis confirmed the opposite effects of GR24 and NPA on the formation of serrations (Fig. 4C, D). These results do not lend support to an impact of SL on leaflet serrations through reduced auxin transport, as has been proposed for their effect on shoot branching (Crawford *et al.*, 2010). They do not rule out this mechanism either, as SL and NPA probably affect auxin transport in different ways, both qualitatively and quantitatively. Indeed, SL and NPA seem to target distinct auxin transporters (Shinohara *et al.*, 2013; Kim *et al.*, 2010), and they reduce auxin transport to different extents (Crawford *et al.*, 2010). It is also possible that their spatial or temporal range of action is different. A more detailed examination of the consequences of GR24 treatment on PIN turnover in leaf cells and on auxin distribution along the leaflet margin would be needed to determine whether SL control the formation of serrations by altering auxin transport. Alternatively, similar to some of their other functions in plant development, SL may act on leaf serrations either downstream of auxin or via a separate pathway.

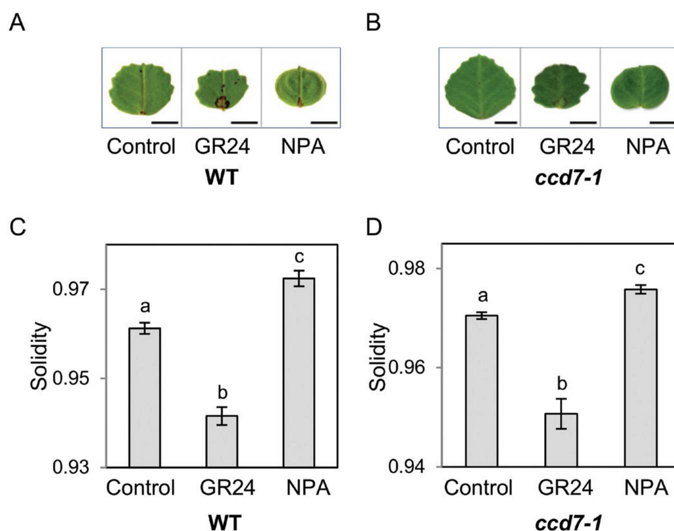

Fig. 4. GR24 and NPA effects on leaflet serrations. Wild-type (A, C) and *ccd7-1* mutant plants (B, D) were treated with 5×10^{-6} M GR24 or NPA, or the solvents alone (control). (A, B) Scanned images of one representative leaflet in each condition. Bar=5 mm. (C, D) Solidity values. Values correspond to the mean±SEM (*n*=37–72 leaflets for each condition). Different letters indicate statistically significant differences according to Mann-Whitney's test (*P*<0.05 after Bonferroni adjustment). (This figure is available in colour at *JXB* online.)

Other *M. truncatula* mutants with a leaf serration phenotype have been described, such as *lol1/ago7* (Zhou *et al.*, 2013) and *mtphan* (Ge *et al.*, 2014). In these cases, however, the serration shape was distinct from the SL mutants, and was accompanied by additional phenotypes that we did not observe. This suggests that different mechanisms account for the alteration of leaf serrations in these different mutant backgrounds. Moderate alterations of leaf shape have been reported previously for SL-deficient mutants in other species, notably a lower length/width ratio and other modifications related to lamina overall shape and position on the petiole (Beveridge *et al.*, 1997; Stirnberg *et al.*, 2002; Challis *et al.*, 2013). Nonetheless, the present article is to our knowledge the first report of a role for SL in the formation of leaf margin serrations. As leaf shape has been examined in great detail in *Arabidopsis* SL mutants (Hepworth, 2012), a serration phenotype is unlikely to have gone unnoticed in this species. The so far unreported effect of SL on leaf serrations may reflect particularities in leaf morphogenesis in *M. truncatula.* The inverted repeat-lacking clade (IRLC) of Fabaceae comprising *Medicago* spp is already known to use a different mechanism to control compound leaf development compared with the rest of the vascular plants (Champagne *et al.*, 2007). Other authors have also underlined the strong context-dependency of the mechanisms and pathways governing leaf shape (Zhou *et al.*, 2013). When primary shoot apices of the commonly used *M. truncatula* Jemalong A17 ecotype were treated with SL, no effect on the formation of leaf serrations could be observed (Supplementary Fig. S5), although Jemalong is able to respond to SL application to the root system by a decreased formation of lateral roots (De Cuyper *et al.*, 2014). This discrepancy indicates that the leaf SL response observed in R108 is not a general property in the *Medicago* genus. The R108 ecotype is morphologically different from other *M. truncatula* accessions. Its increased stem elongation (Schnurr *et al.*, 2007) and deeper leaf serrations may reflect an enhanced sensitivity to SL, at least in aerial organs. It will be interesting to determine whether these characteristics were already present in the original R108 ecotype, or appeared during the *in vitro* selection of the highly embryogenic R108-1(c3) line (Hoffmann *et al.*, 1997). In the latter case, a putative contribution of enhanced strigolactone sensitivity to embryogenic potential would be worth investigating.

In conclusion, we show here that both SL-deficient and SL-insensitive mutants of *M. truncatula* plants displayed a compact architecture that was attributed to reduced shoot elongation rather than enhanced shoot branching. In addition, the SL-deficient and SL-insensitive mutants displayed a modified leaf shape, with reduced serrations at the leaf margin. Exogenously applied SL could rescue the serration phenotype of the SL-deficient mutants in a dose-dependent manner, but had no effect on SL-insensitive mutants. These observations demonstrate the importance of SL in the formation of leaf serrations in *M. truncatula* R108. This novel function of SLs represents another example of the versatility of SL action depending on the target tissue and species context (de Saint-Germain *et al.*, 2013), and highlights the usefulness of investigating SL functions in a wide range of

plant species. It remains to be investigated whether the effect of SL on leaf margin serrations is widespread and could have contributed to the evolutionary diversification of leaf shape.

Supplementary data

Figure S1. Phylogeny of strigolactone biosynthesis and response genes.

Figure S2. Position of *Tnt1* insertions in the different mutant alleles.

Figure S3. Shoot elongation phenotype of the *ccd7-2* mutant allele; partial rescue of the shoot elongation phenotype by exogenous SL application.

Figure S4. Leaflet serration phenotype of *ccd7-2* mutants.

Figure S5. Effect of GR24 on leaflet serrations in *M. truncatula* ecotype A17.

Table S1. Sequence accession numbers.

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