RESEARCH PAPER

Strigolactone regulation of shoot branching in chrysanthemum (*Dendranthema grandiflorum*)

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Abstract

Previous studies of highly branched mutants in pea (*rms1–rms5*), Arabidopsis thaliana (*max1–max4*), petunia (*dad1–dad3*), and rice (*d3, d10, htd1/d17, d14, d27*) identified strigolactones or their derivates (SLs), as shoot branching inhibitors. This recent discovery offers the possibility of using SLs to regulate branching commercially, for example, in chrysanthemum, an important cut flower crop. To investigate this option, SL physiology and molecular biology were studied in chrysanthemum (*Dendranthema grandiflorum*), focusing on the *CCD8/MAX4/DAD1/RMS1/D10* gene. Our results suggest that, as has been proposed for Arabidopsis, the ability of SLs to inhibit bud activity depends on the presence of a competing auxin source. The chrysanthemum SL biosynthesis gene, *CCD8* was cloned, and found to be regulated in a similar, but not identical way to known *CCD8*s. Expression analyses revealed that *DgCCD8* is predominantly expressed in roots and stems, and is up-regulated by exogenous auxin. Exogenous SL can down-regulate *DgCCD8* expression, but this effect can be overridden by apical auxin application. This study provides evidence that SLs are promising candidates to alter the shoot branching habit of chrysanthemum.

Key words: Auxin, CCD8, chrysanthemum, shoot branching, strigolactone.

Introduction

Chrysanthemum (*Dendranthema grandiflorum*) accounts for 30% of world cut flower production. Conventionally, the lateral shoots are controlled by manual decapitation or removing the axillary buds, which is labour-intensive and accounts for about one-third of total cultivation costs. Therefore, chrysanthemum producers are in need of novel approaches to control shoot branching.

Shoot branching depends on both the formation of axillary buds in the axils of leaves and their subsequent outgrowth. Outgrowth is a more interesting target for breeders, since it allows greater flexibility in branching. Axillary shoot apical meristems are established in the axils of the leaves produced by the primary shoot apical meristem and after forming only a few leaves, they can enter a dormant state. These dormant buds may be reactivated by endogenous or environmental signals, contributing to the enormous diversity of plant architectures observed in nature (Dun *et al.*, 2006; Leyser, 2009).

Hormones play a central role in shoot branching control. Auxin inhibits bud outgrowth (Thimann and Skoog, 1933; Skoog and Thimann, 1934), whereas cytokinin promotes it (Sachs and Thimann, 1967). These hormones can efficiently regulate shoot branching, but their application also affects flowering, leaf development, and height, reducing their utility for the horticultural industry.

A series of branching mutants with fewer pleiotropic phenotypes has been identified. These include the *more axillary branching* (*max*) mutants of Arabidopsis, the *ramosus* (*rms*) mutants of pea (*Pisum sativum*), the *decreased apical dominance* (*dad*) mutants of petunia (*Petunia hybrida*), and the *dwarf* (*d*) or *high tillering dwarf* (*htd*) mutants of rice (*Oryza sativa*) (reviewed in Dun *et al.*, 2006; Leyser, 2009). Reciprocal grafting and double mutant studies demonstrate that these genes are involved in the production and signalling of a graft-transmissible, upwardly moving branch inhibitor, which was recently shown to be...
strigolactone or a derivative (SL) (Beveridge et al., 1996, 2000; Napoli, 1996; Foo et al., 2001; Morris et al., 2001; Turnbull et al., 2002; Sorefan et al., 2003; Booker et al., 2004, 2005; Snowden et al., 2005; Simons et al., 2007; Gomez-Roldan et al., 2008; Umehara et al., 2008).

To date, four loci involved in SL biosynthesis and one locus involved in SL signalling have been molecularly identified (Stirnberg et al., 2002; Sorefan et al., 2003; Booker et al., 2004, 2005; Lin et al., 2009). A sixth gene in the pathway has been described, represented by rice D14, which acts either in a biosynthetic step downstream of SL or in SL signalling (Arite et al., 2009). Of the biosynthetic genes, two encode divergent plastidic carotenoid cleavage dioxygenases. MAX4/RMS1/DAD1/D10 encodes CAROTENOID CLEAVAGE DIOXYGENASE (CCD8) (Sorefan et al., 2003; Snowden et al., 2005; Arite et al., 2007), whereas MAX3/RMS3/HTD1/D17/SICCD7 encodes CCD7 (Booker et al., 2004; Johnson et al., 2006; Zou et al., 2006; Vogel et al., 2009). CCD8 and CCD7 sequentially cleave a variety of carotenoid substrates (Booker et al., 2004; Schwartz et al., 2004; Auldridge et al., 2006; Alder et al., 2008). D27 encodes a plastidic iron-containing protein, which possibly acts at an early point in the pathway (Lin et al., 2009). MAX1 encodes a cytP450 predicted to act downstream of CCD7 and CCD8 (Booker et al., 2005). MAX2/RMS4/D3 encodes a nuclear-localized F-box protein (Stirnberg et al., 2002; Ishikawa et al., 2005; Johnson et al., 2006), involved in SL signalling (Beveridge et al., 1996; Booker et al., 2005; Stirnberg et al., 2007).

The mode of action of SLs and their relationship with auxin is a matter of debate. One hypothesis is that SLs inhibit branching by reducing auxin transport canalization, preventing the establishment of polar auxin transport out of axillary buds into the main stem, and thus preventing their activation. According to this model, SLs act systemically to modulate auxin transport, and their ability to inhibit buds depends on the presence of competing auxin sources, such as the primary shoot apex or other active buds, for common auxin transport pathways in the main stem (Prusinkiewicz et al., 2009). However, it has also been proposed that SLs act directly in axillary buds to inhibit them by some unknown, but auxin-transport-independent mechanism (Brewer et al., 2009).

Another point of debate is the extent of negative feedback in the SL pathway and the role of auxin in the process. In most species, auxin positively regulates the expression of both CCD8 and CCD7 (Sorefan et al., 2003; Bainbridge et al., 2005; Foo et al., 2005; Johnson et al., 2006; Arite et al., 2007; Hayward et al., 2009). However in rice, CCD7/HTD1 is not stably up-regulated by auxin (Zou et al., 2006; Arite et al., 2007). There is also good evidence for widespread feedback regulation in the SL pathway (Foo et al., 2005; Snowden et al., 2005; Arite et al., 2007; Hayward et al., 2009). CCD7 and CCD8 transcripts generally accumulate above wild-type levels in SL mutants. This effect is much stronger in pea than in the other species. In rice, significant up-regulation was observed for CCD8/D10 but not for CCD7/HTD1 in the dβ mutant background (Arite et al., 2007). The relationship between auxin and feedback regulation of SL biosynthesis is unclear. SL mutants transport increased amounts of applied auxin compared to wild-type (Beveridge et al., 2000; Bennett et al., 2006; Lazar and Goodman, 2006; Lin et al., 2009), and in Arabidopsis this correlates with the over-accumulation of auxin in the polar transport streams of their stems (Prusinkiewicz et al., 2009). Furthermore, double mutants defective in both auxin signalling and SL signalling, feedback up-regulation of CCD7/MAX3 or CCD8/MAX4 is mostly abolished. This suggests that feedback signalling is substantially mediated by auxin (Hayward et al., 2009). Consistent with this idea, as described above, in rice there is a correlation between auxin-inducibility and feedback regulation (Arite et al., 2007). However, the situation in pea is different since auxin treatments cannot achieve the particularly high level of CCD8/RMS1 and CCD7/RMS5 up-regulation observed in rms mutants, suggesting an auxin-independent feedback (Foo et al., 2005; Johnson et al., 2006). It is therefore clear that comparative approaches in diverse species have contributed to our understanding of SL function, but also raised some interesting questions about the degree of conservation of the system between species.

Thus the analysis of the SL pathway in additional species has the potential to contribute to both the development of applications for SLs in agriculture and horticulture, and to resolving questions about the mechanism of action of SLs. To these ends, work aimed at the evaluation of SL physiology in chrysanthemum, as well as the identification of chrysanthemum genes required for SL synthesis, is described in this paper.

Materials and methods

Plant materials and growth condition

Chrysanthemum (Dendranthema grandiflorum) cv. Jinba plantlets were propagated under sterile conditions in jars containing MS agar medium (Murashige and Skoog, 1962) and grown in a tissue culture room at 21 °C, with 16/8 h light/dark photoperiods and a light intensity of 100–120 μmol m−2 s−2. Arabidopsis thaliana Columbia (WT) and max4-1 were grown in 4 cm square compartment containing F2 compost (Levington Horticulture, Ipswich, UK) and transferred to a greenhouse at 21 °C, with 16/8 h light/dark photoperiods.

Hormone stocks

Napthalene acetic acid (NAA) stock solution was dissolved in 70% ethanol, and GR24 (LeagGen Labs, Orange CT USA) was dissolved in acetone were injected into the apical or basal blocks, as required, to give a final concentration of 5 μM. Solvent treatments were used as controls.

Split plate assay

The hormone responses of buds on isolated stem segments were assessed using the method described by Chattfield et al. (2000).

Isolation of the DgCCD8 genes

Total RNA was extracted from the stem and roots of chrysanthemum plantlets using Trizol (Mylab, China). cDNA was
synthesized using Superscript II reverse transcriptase (Invitrogen). Degenerate primers were designed from the amino acid sequence of the Arabidopsis MAX4, petunia DAD1, and pea RSM1 gene and used to amplify a fragment of the DgCCD8 gene from cDNA, with forward primer (5'-GTTGATGACATGGTGACAAG-3') and reverse primer (5'-GACCTCACATCTGAGGTTGAATCATC-3'). The amplification products were used to design the primers for Rapid Amplification of cDNA Ends (RACE), which was used to obtain full-length DgCCD8 transcripts. For 3' RACE, cDNA was synthesized with the adaptor oligo (dt) primer (5'-CTGACCTAGGATCCGGATCT-3') and a specific primer 3P (5'-GGTCTAGATCGAGGTTCAAAAAC-3'). The amplification was performed with primer AP5 (5'-GGCAGGCTCGACTAGTATGACGGGIGGGIIGGGIIG-3') and the specific primer 5P. This PCR product was used as the template in an additional nested PCR using specific primer 5PI (5'-TGGCATCTCGGGCCAGACACTAC-3') and primer AU5P (5'-GGOCCACGCCGTGAGATGACGGG-3). Amplified fragments were cloned into the pMD18-T vector and sequenced. According to the sequences, a forward primer from the 5'UTR region (5'-TACACCATTTACTACCATGTTG-3') and a reverse primer from the 3'UTR (5'-CCATCATCCAGAGGTTGAATCATC-3') were designed to isolate the complete DgCCD8 from both cDNA and genomic DNA. Genomic DNA was isolated from young leaves using the DNeasy Plant Mini Kit (Qiagen). The amplification products were used to identify the sequence of the chrysanthemum cDNAs and genomic clones, and the positions of introns in the genes.

Phylogenetic analysis

Orthologues of the known CCD8 genes were identified using InParanoid (Remm et al., 2001) to perform pairwise comparisons between each of the published or draft complete genome sequences: Arabidopsis thaliana (Rhee et al., 2003), Brachypodium distachyon (http://www.brachypodium.org/), Lotus japonicus (Sato et al., 2008), Medicago truncatula (http://www.medicago.org/), Oryza sativa (Ouyang et al., 2007), Physcomitrella patens (Rensing et al., 2008), Populus trichocarpa (Tuskan et al., 2006), Selaginella moellendorffii (http://genome.jgi-psf.org/Selmo1/Selmo1.home.html), Sorghum bicolor (Paterson et al., 2009), Vitis vinifera (Jaillon et al., 2007), and Zea mays (Schnable et al., 2009). The orthology of candidate genes was confirmed during phylogenetic reconstruction. Sequences were aligned using MUSCLE v3.6 (Edgar, 2004) using default settings. The maximum likelihood phylogeny was reconstructed under the Dayhoff substitution matrix using RAxML (Stamatakis, 2006). Support for the maximum likelihood phylogenies was estimated from 100 rapid bootstrap resamplings (Stamatakis et al., 2008).

Generation of transgenic plants

For complementation experiments, the ORFs of DgCCD8a and DgCCD8b were cloned into pART27 fusing them with the 35S promoter, and then these fusions were transferred into the binary vector pART27 (Gleave, 1992). The constructs were transformed into Arabidopsis thaliana mutant max4-1 plants via Agrobacterium tumefaciens strain GV3101 using the floral dip method (Clough and Bent, 1998). The independent transformants were screened on Arabidopsis thaliana salts (ATS) agar medium containing 50 mg 1-1 kanamycin. Independent, single insertion site, homozygous T3 lines were used to analyse the branching phenotype.

Decapitation assay

To quantify branching, a decapitation assay was used (Bainbridge et al., 2005). Seeds of max4-1, WT, [35S::DgCCD8a] max4-1 and [35S::DgCCD8b] max4-1 lines were sown in F2 compost and grown in a growth cabinet at 21 °C, 8/16 h light/dark photoperiods. After 28 d, plants were shifted to 16/8 h light/dark photoperiods to induce flowering. Primary bolts were decapitated once they reached 10–15 cm in height. Rosette branches were counted 10 d after decapitation.

Hormone treatments

For analysis of DgCCD8 expression after decapitation, auxin, or GR24 treatment, in vitro-grown chrysanthemum plantlet cuttings, 15 cm in height, were transferred into jars containing MS-agar medium with 5 μM GR24 or an equal volume of acetone. They were left intact or were decapitated, and Eppendorf tubes containing MS-agar medium with 5 μM NAA or an equal volume of ethanol were placed over the stumps. After 6 h treatment, the stem spanning the basal three nodes of six plantlets per treatment was excised and the tissue pooled to extract total mRNA, which was used to examine the accumulation of total DgCCD8 transcripts.

Semi-quantitative RT-PCR

Total RNA was extracted using an RNase Plant Mini Kit (Qiagen) and reverse transcribed using Superscript II (Invitrogen) according to the manufacturer’s manual. To investigate the total expression of DgCCD8 in chrysanthemum, the common primers RT1 (5'-GTGGAGAAGCAGACGGAACGT-3') and RT2 (5'-GTTGACCCACAAAAGCGTAAGC3') were used. Specific primers RT1 (5'-TTATACCGTGGGCTCTTCCCTC-3') and RT3 (5'-GCGACAATCATACAATGTCAA-3') were used to analyse the expression of DgCCD8b. Amplification of ribosomal RNA (Yang et al., 2005) was performed using 25 cycles as a normalization control. To analyse the transcription from the transgenes in [35S::DgCCD8a] and [35S::DgCCD8b] max4 lines, RT1 (5'-GTGGTAGAAGCAGACGGAACGT-3') and RT3 (5'-GTTGACTCGACTCGATGTCG-3') were used. UBQ was used as a normalization control with primers UF (5'-AACCCTTTGAGTTGGAATCATC-3') and UR (5'-GTCCTTCCTTCTGGAACGT-3')

Results

The inhibition of chrysanthemum buds by SL and auxin

To assess whether SLs can inhibit chrysanthemum bud outgrowth, the hormone responses of buds on one-node stem segments held between two agar blocks in Petri dishes were tested (Chatfield et al., 2000). The segments were treated with basally supplied GR24, a synthetic strigolactone, apically supplied auxin (NAA) or both. Although NAA was sufficient to inhibit bud outgrowth, GR24 alone was surprisingly ineffective (Fig. 1). However, the combined application of GR24 and NAA completely inhibited bud outgrowth and was more effective than NAA applied alone.

These results suggest that GR24 is only effective at inhibiting buds in the presence of an auxin source. To test this further, the activity of SLs supplied to two-node stem segments was determined, with the rationale that the second bud would provide a natural auxin source. In this configuration, the two buds respond in different ways to the treatments. The apical bud behaves similarly to buds in the one-node segment experiment (compare Fig. 1 with Fig. 2A). There is no effect of basally supplied GR24, but apical auxin has a strong inhibitory effect. By contrast, the
its known hexaploidy (Nazeer and Khoshoo, 1983), so the amino acids. Southern blot analysis showed that at least 1671 bp encoding a predicted protein of 556 amino acids.

DgCCD8a comprises 1740 bp containing an open reading frame of 1740 bp encoding a predicted protein of 556 amino acids. A 750 bp fragment with strong sequence similarity to the MAX4 family was successfully amplified. Amino acid sequence comparisons between these three genes and their orthologues from Arabidopsis, pea, petunia, and rice show that the predicted DgCCD8s are 60–61% identical to AtMAX4, 72–73% identical to PsRMS1, 78% identical to PhDAD1, and 58% identical to OsD10 (Fig. 3A).

To investigate further the relationship between the predicted CCD8 proteins, a phylogenetic tree of CCD8 proteins was constructed from a taxonomically diverse set of species (Fig. 3B). In line with taxonomy, DgCCD8s are placed in a well-supported clade with petunia.

Genomic fragments corresponding to DgCCD8 genes were isolated by PCR using a forward primer from the 5′-UTR regions and a reverse primer from the 3′-UTR regions of DgCCD8. Sequencing of clones led to the identification of five different CCD8-related genomic sequences. Comparison of the DgCCD8 cDNAs to the genomic sequence revealed that two genomic fragments correspond to the DgCCD8a and DgCCD8c cDNA, respectively. However, none corresponds to DgCCD8b. Thus, in total, six different DgCCD8 sequences, that are likely to represent two alleles at each of three DgCCD8 loci have been identified.

To determine whether the DgCCD8 genes are functionally conserved, the DgCCD8a and DgCCD8b ORFs from the Cauliflower Mosaic Virus 35S promoter (35S::DgCCD8a, 35S::DgCCD8b) were expressed in the Arabidopsis max4-1 mutant background. For each construct, multiple independent transgenic lines were generated, and those showing segregation patterns in the T2 consistent with a single insertion site (data not shown) were taken to homozygosity for detailed analysis. Branching was assessed using a decapitation assay previously shown to provide sensitive discrimination between wild-type and max4 mutant plants (Bainbridge et al., 2005). The mean number of rosette branches with a length of at least 2 cm was scored 10 d after decapitation to quantify the degree of rescue of the max4 mutant phenotype by the DgCCD8 genes. Figure 4B represents the three most strongly rescuing lines for each construct out of 11 35S::DgCCD8b and 15 35S::DgCCD8a max4 characterized lines. Constitutive expression of DgCCD8b reduced the mean number of branches from 10.8 in max4 to 5–6, resulting in plants indistinguishable from wild-type (WT), which had a mean of 5.25 branches. However, constitutive expression of DgCCD8a was less effective, reducing the number of branches to 7.05–7.35
This difference was not due to different levels of expression of the transgenes, because when RT-PCR was used to determine transcript abundance, the \textit{DgCCD8} transgenes were found to accumulate to similar levels between the \textit{35S::DgCCD8b} and \textit{35S::DgCCD8a} lines (Fig. 4C). Therefore, the increased effectiveness of \textit{DgCCD8b} in rescuing the \textit{max4} phenotype compared to \textit{DgCCD8a} is probably a post-transcriptional effect.

\textbf{Tissue specificity of \textit{DgCCD8} expression}

To gain an overall picture of the tissue specificity of \textit{DgCCD8} expression in chrysanthemum, the combined levels of all \textit{DgCCD8} transcripts in mRNA extracted from different tissues were examined by RT-PCR, using primers that do not discriminate between the different copies/alleles. \textit{DgCCD8} mRNA was detected in all tissues examined, except for the leaf. Expression in stems was higher than in roots and shoot apices (Fig. 5).

To gain insight into whether there is any sub-functionalization within the family, the expression pattern of \textit{DgCCD8b} was analysed further using specific primers. \textit{DgCCD8b} was not detected in shoot apices, but only in stem and root tissues, with strongest expression in the stem (Fig. 5).

\textbf{Induction of \textit{DgCCD8} expression by auxin}

To investigate auxin regulation of \textit{DgCCD8}, RT-PCR analysis was performed on basal stem segments following decapitation, with or without apical application of auxin (Fig. 6). Decapitation resulted in a drastic reduction in total \textit{DgCCD8} transcript abundance after 6 h but this reduction was successfully compensated by apical NAA application. These data indicate that auxin regulates \textit{DgCCD8} gene expression in a similar manner to that described in other species.

\textbf{Feedback control of \textit{DgCCD8} expression}

To investigate feedback control by SL on \textit{DgCCD8} expression, total \textit{DgCCD8} transcript abundance after 6 h treatment was compared with basal GR24 on intact and decapitated plants (Fig. 6). It was observed that, in intact plants, \textit{DgCCD8} expression is down-regulated by GR24 compared with untreated controls. However, this down-regulation is modest and is less than that observed upon decapitation. Furthermore, down-regulation by GR24 was not observed in decapitated plants, and \textit{DgCCD8} expression in decapitated plants treated with NAA was indistinguishable from the decapitated plants treated with both apical NAA and basal GR24.
The mechanisms of bud inhibition by SL and auxin

Inhibition of bud outgrowth by an actively growing shoot apex is mediated by apically-derived auxin transported down the main stem (Thimann and Skoog, 1933), which acts indirectly without entering the bud (Morris, 1977; Booker et al., 2003). SLs have been proposed to act as second messengers for auxin, relaying the inhibitory signal from the main stem into the buds (Brewer et al., 2009). An alternative model is that apically-derived auxin moving in the main stem inhibits bud activity by reducing the sink.

Discussion

The mechanisms of bud inhibition by SL and auxin

Inhibition of bud outgrowth by an actively growing shoot apex is mediated by apically-derived auxin transported down the main stem (Thimann and Skoog, 1933), which acts indirectly without entering the bud (Morris, 1977; Booker et al., 2003). SLs have been proposed to act as second messengers for auxin, relaying the inhibitory signal from the main stem into the buds (Brewer et al., 2009). An alternative model is that apically-derived auxin moving in the main stem inhibits bud activity by reducing the sink.
The effect of SL on the activity of solitary buds, in the absence of a competing auxin source has not previously been described, and has the potential to distinguish between these alternative models. When a solitary bud was treated with SL, it was activated with similar kinetics to an untreated bud. This argues against the idea that SLs act in a straightforward way by entering buds and inhibiting their activity. However, the result is entirely consistent with the idea that SLs act by dampening auxin transport canalization. Such a mechanism would only be effective in inhibiting bud activation in the presence of a competing auxin source, reducing the sink strength of the main stem for auxin. Accordingly, SL was effective at inhibiting solitary buds when apical auxin was simultaneously applied. Furthermore, SL was able to inhibit growth of the basal bud on an explant with two buds, while the active bud was unaffected. The active apical bud presumably exports auxin into the stem, as has been demonstrated for Arabidopsis buds, providing a competing auxin source similar to apical auxin application (Prusinkiewicz et al., 2009).

**Isolation of the chrysanthemum CCD8 genes**

To investigate further the operation of the SL pathway in chrysanthemum, the chrysanthemum orthologues of CCD8 were isolated, and their orthology was confirmed by phylogenetic analysis and functional complementation of the Arabidopsis ccd8/max4-1 mutant. Our results suggest that there are at least three loci encoding CCD8 in chrysanthemum, consistent with its known hexaploidy. Furthermore, some evidence was found for sub-functionalization within the family. The two cDNAs tested were apparently not equally effective in rescuing max4-1, despite differing by only three non-conserved amino acids, and the more effective rescuing cDNA is expressed in only a sub-set of the tissues where the CCD8 transcripts were detected.

Despite the high functional conservation of the CCD8 genes between species, there are interesting differences in expression pattern. In Arabidopsis (Auldridge et al., 2006), petunia (Snowden et al., 2005), and pea (Foo et al., 2005), root expression of CCD8 is at least 10 times higher than shoot expression. By contrast, in rice (Arite et al., 2007) and...
chrysanthemum, shoot expression exceeds root expression. These differences may reflect different contributions of the root and shoot in the SL-regulation of shoot branching in the different species.

Feedback regulation of DgCCD8 expression

Previously negative feedback regulation has been observed for CCD8s in all species examined (Foo et al., 2005; Snowden et al., 2005; Arite et al., 2007; Hayward et al., 2009). However, the molecular basis for this feedback is unresolved. Three non-exclusive mechanisms have been proposed (Hayward et al., 2009). SL biosynthesis genes may be directly negatively regulated by SLs; feedback could be mediated indirectly by auxin since SL signalling results in a decreased auxin export from buds, and auxin is required for optimal CCD8 gene expression; or indirect feedback may be mediated by an unknown signal, which in pea is suggested to depend on the RMS2 gene (Foo et al., 2005; Dun et al., 2006).

In our studies, decapitation and exogenous application of auxin result in down- and up-regulation of DgCCD8 expression, respectively, suggesting positive regulation by auxin similar to other species. Furthermore, GR24 can down-regulate the expression of the DgCCD8 gene, demonstrating feedback control. However, application of GR24 to decapitated plants did not further repress DgCCD8 expression. Moreover, GR24 could not down-regulate DgCCD8 expression in the presence of exogenous apically applied auxin. In both these situations, GR24 would be expected to have only a modest effect on the amount of auxin in the stem, and thus these results are consistent with the hypothesis that GR24 acts mainly via auxin to mediate feedback regulation on DgCCD8.

Conclusion

It has been demonstrated that chrysanthemum buds can be inhibited by GR24 and thus there is potential to use SLs for the regulation of branching in chrysanthemum horticultural production. However, large-scale exogenous treatment techniques need to be developed and the cost of GR24 or analogues needs to be reduced for this to be practicable. In addition, chrysanthemum CCD8 genes have been isolated, providing the opportunity to use genetic modification to improve the chrysanthemum growth habit. Our results also contribute data from an additional species to ongoing debates about the mode of action of SLs in the inhibition of shoot branching, and its relationship with auxin. The effects of GR24 on bud activity and DgCCD8 expression support the idea that SLs inhibit bud activation by modulating auxin transport canalization, and that auxin is a major mediator of feedback by SL on SL synthesis.

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References

Alder A, Holdermann I, Beyer P, Al-Babili S. 2008. Carotenoid oxygenases involved in plant branching catalyse a highly specific conserved apocarotenoid cleavage reaction. Biochemical Journal 416, 289–296.

Arite T, Iwata H, Ohshima K, Maekawa M, Nakajima M, Kojima M, Sakakibara H, Kyozuka J. 2007. DWARF10, an RMS1/MAX4/DAD1 ortholog, controls lateral bud outgrowth in rice. The Plant Journal 51, 1019–1029.
Arite T, Umehara M, Ishikawa S, Hanada A, Maekawa M, Yamaguchi S, Kyozuka J. 2009. d14, a strigolactone-insensitive mutant of rice, shows an accelerated outgrowth of tillers. Plant and Cell Physiology 50, 1416–1424.

Auldridge ME, Block A, Vogel JT, Dabney-Smith C, Mila I, Bouzayen M, Magallanes-Lundback M, DellaPenna D, McCarty DR, Klee HJ. 2006. Characterization of three members of the Arabidopsis carotenoid cleavage dioxygenase family demonstrates the divergent roles of this multifunctional enzyme family. The Plant Journal 45, 982–993.

Bainbridge K, Sorefan K, Ward S, Leyser O. 2005. Hormonally controlled expression of the Arabidopsis MAX4 shoot branching regulatory gene. The Plant Journal 44, 569–580.

Bennett T, Sieberer T, Willett B, Booker J, Luschnig C, Leyser O. 2006. The Arabidopsis MAX pathway controls shoot branching by regulating auxin transport. Current Biology 16, 553–563.

Beveridge CA, Ross JJ, Murfet IC. 1996. Branching in pea. Action of genes Rms3 and Rms4. Plant Physiology 110, 859–865.

Beveridge CA, Symons GM, Turnbull CGN. 2000. Auxin inhibition of decapitation-induced branching is dependent on graft-transmissible signals regulated by genes Rms1 and Rms2. Plant Physiology 123, 689–697.

Booker J, Auldridge M, Wills S, McCarty D, Klee H, Leyser O. 2004. MAX3/CCD7 is a carotenoid cleavage dioxygenase required for the synthesis of a novel plant signalling molecule. Current Biology 14, 1232–1238.

Booker J, Chatfield S, Leyser O. 2003. Auxin acts in xylem-associated or medullary cells to mediate apical dominance. The Plant Cell 15, 495–507.

Booker J, Sieberer T, Wright W, Williamson L, Willett B, Stirnberg P, Turnbull C, Srinivasan M, Goddard P, Leyser O. 2005. MAX1 encodes a cytochrome P450 family member that acts downstream of MAX3/4 to produce a carotenoid-derived branch-inhibiting hormone. Developmental Cell 8, 443–449.

Brewer PB, Dun EA, Ferguson BJ, Rameau C, Beveridge CA. 2009. Strigolactone acts downstream of auxin to regulate bud outgrowth in pea and Arabidopsis. Plant Physiology 150, 482–493.

Chatfield SP, Stirnberg P, Forde BG, Leyser O. 2000. The hormonal regulation of axillary bud growth in Arabidopsis. The Plant Journal 24, 159–169.

Clough SJ, Bent AF. 1998. Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. The Plant Journal 16, 735–743.

Dun EA, Ferguson BJ, Beveridge CA. 2006. Apical dominance and shoot branching. Divergent opinions or divergent mechanisms? Plant Physiology 142, 812–819.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32, 1792–1797.

Foo E, Buller E, Goussot M, Foucher F, Rameau C, Beveridge CA. 2005. The branching gene RAMOSUS1 mediates interactions among two novel signals and auxin in pea. The Plant Cell 17, 464–474.

Foo E, Turnbull CG, Beveridge CA. 2001. Long-distance signalling and the control of branching in the rms1 mutant of pea. Plant Physiology 126, 203–209.

Gleave AP. 1992. A versatile binary vector system with a T-DNA organisational structure conducive to efficient integration of cloned DNA into the plant genome. Plant Molecular Biology 20, 1203–1207.

Gomez-Roldan V, Fermas S, Brewer PB, et al. 2008. Strigolactone inhibition of shoot branching. Nature 455, 189–194.

Hayward A, Stirnberg P, Beveridge C, Leyser O. 2009. Interactions between auxin and strigolactone in shoot branching control. Plant Physiology 151, 400–412.

Ishikawa S, Maekawa M, Arite T, Onishi K, Takamure I, Kyozuka J. 2005. Suppression of tiller bud activity in tillering dwarf mutants of rice. Plant Cell Physiology 46, 79–86.

Jailion O, Aury JM, Noel B, et al. 2007. The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. Nature 449, 463–467.

Johnson X, Bricich T, Dun EA, Goussot M, Haurogne K, Beveridge CA, Rameau C. 2006. Branching genes are conserved across species. Genes controlling a novel signal in pea are coregulated by other long-distance signals. Plant Physiology 142, 1014–1026.

Lazar G, Goodman HM. 2006. MAX1, a regulator of the flavonoid pathway, controls vegetative axillary bud outgrowth in Arabidopsis. Proceedings of the National Academy of Sciences, USA 103, 472–476.

Leyser O. 2009. The control of shoot branching: an example of plant information processing. Plant, Cell and Environment 32, 694–703.

Lin H, Wang R, Qian Q, et al. 2009. DWARF27, an iron-containing protein required for the biosynthesis of strigolactones, regulates rice tiller bud outgrowth. The Plant Cell 21, 1512–1525.

Morris DA. 1977. Transport of exogenous auxin in two-branched dwarf pea seedlings (Pisum sativum L.). Planta 166, 91–96.

Morris SE, Turnbull CGN, Murfet IC, Beveridge CA. 2001. Mutational analysis of branching in pea. Evidence that rms1 and rms5 regulate the same novel signal. Plant Physiology 126, 1205–1213.

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15, 473–497.

Napoli C. 1996. Highly branched phenotype of the petunia dad1-1 mutant is reversed by grafting. Plant Physiology 111, 27–37.

Nazeer MA, Khoshoo TN. 1983. Variation in the chromosome complement of Chrysanthemum morifolium complex. Nucleus 26, 22–29.

Ouyang S, Zhu W, Hamilton J, et al. 2007. The TIGR Rice Genome Annotation Resource: improvements and new features. Nucleic Acids Research 35, D883–D887.

Paterson AH, Bowers JE, Bruggmann R, et al. 2009. The Sorghum bicolor genome and the diversification of grasses. Nature 457, 551–556.

Prusinkiewicz P, Crawford S, Smith RS, Ljung K, Bennett T, Ongaro V, Leyser O. 2009. Control of bud activation by an auxin transport switch. Proceedings of the National Academy of Sciences, USA 106, 17431–17436.
Remm M, Storm CE, Sonnhammer EL. 2001. Automatic clustering of orthologs and in-paralogs from pairwise species comparisons. Journal of Molecular Biology 314, 1041–1052.

Rensing SA, Lang D, Zimmer AD, et al. 2008. The Physcomitrella genome reveals evolutionary insights into the conquest of land by plants. Science 319, 64–69.

Rhee SY, Beavis W, Berardini TZ, et al. 2003. The Arabidopsis Information Resource (TAIR): a model organism database providing a centralized, curated gateway to Arabidopsis biology, research materials and community. Nucleic Acids Research 31, 224–228.

Sachs T, Thimann K. 1967. The role of auxins and cytokinins in the release of buds from dominance. American Journal of Botany 54, 136–144.

Sato S, Nakamura Y, Kaneko T, et al. 2008. Genome structure of the legume, Lotus japonicus. DNA Research 15, 224–228.

Schwartz SH, Qin X, Loewen MC. 2004. The biochemical characterization of two carotenoid cleavage enzymes from Arabidopsis indicates that a carotenoid-derived compound inhibits lateral branching. Journal of Biological Chemistry 279, 46940–46945.

Simons JL, Napoli CA, Janssen BJ, Plummer KM, Snowden KC. 2007. Analysis of the DECREASED APICAL DOMINANCE genes of petunia in the control of axillary branching. Plant Physiology 143, 697–706.

Skoog F, Thimann KV. 1934. Further experiments on the inhibition of the development of lateral buds by growth hormone. Proceedings of the National Academy of Sciences, USA 20, 480–485.

Snowden KC, Simkin AJ, Janssen BJ, Templeton KR, Loucas HM, Simons JL, Karunairetnam S, Gleave AP, Clark DG, Klee HJ. 2005. The Decreased apical dominance1/Petunia hybrida CAROTENOID CLEAVAGE DIOXYGENASE8 gene affects branch production and plays a role in leaf senescence, root growth, and flower development. The Plant Cell 17, 746–750.

Sorefan K, Booker J, Haurogne K, et al. 2003. MAX4 and RMS1 are orthologous dioxygenase-like genes that regulate shoot branching in Arabidopsis and pea. Genes and Development 17, 1469–1474.

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688–2690.

Stamatakis A, Hoover P, Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML Web servers. Systematic Biology 57, 758–771.

Stirnberg P, Furner IJ, Ottoline Leyser HM. 2007. MAX2 participates in an SCF complex which acts locally at the node to suppress shoot branching. The Plant Journal 50, 80–94.

Stirnberg P, van De Sande K, Leyser HM. 2002. MAX1 and MAX2 control shoot lateral branching in Arabidopsis. Development 129, 1131–1141.

Thimann KV, Skoog F. 1933. Studies on the growth hormone of plants. III. The inhibiting action of the growth substance on bud development. Proceedings of the National Academy of Sciences, USA 19, 714–716.

Turnbull CGN, Booker JP, Leyser HMO. 2002. Micrografting techniques for testing long-distance signalling in Arabidopsis. The Plant Journal 32, 255–262.

Tuskan GA, Difazio S, Jansson S, et al. 2006. The genome of black cottonwood, Populus trichocarpa (Torr. & Gray). Science 313, 1596–1604.

Umehara M, Hanada A, Yoshida S, et al. 2008. Inhibition of shoot branching by new terpenoid plant hormones. Nature 455, 195–200.

Vogel JT, Walter MH, Giavalisco P, et al. 2009. SICCD7 controls strigolactone biosynthesis, shoot branching and mycorrhiza-induced apocarotenoid formation in tomato. The Plant Journal 61, 300–311.

Yang DH, Yun PY, Park SY, et al. 2005. Cloning, characterization and expression of a Lateral suppressor-like gene from chrysanthemum (Dendranthema grandiflorum Kitamura). Plant Physiology and Biochemistry 43, 1044–1051.

Zou J, Zhang S, Zhang W, Li G, Chen Z, Zhai W, Zhao X, Pan X, Xie Q, Zhu L. 2006. The rice HIGH-TILLERING DWARF1 encoding an ortholog of Arabidopsis MAX3 is required for negative regulation of the outgrowth of axillary buds. The Plant Journal 48, 687–696.