Plant Physiology and Biochemistry 96 (2015) 241–253

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Roles of *DgD14* in regulation of shoot branching in chrysanthemum (*Dendranthema grandiflorum* 'Jinba')

Chao Wen, Lin Xi, Bin Gao, Keyong Wang, Suhui Lv, Yaping Kou, Nan Ma, Liangjun Zhao*

Beijing Key Laboratory of Development and Quality Control of Ornamental Crops, Department of Ornamental Horticulture and Landscape Architecture, China Agricultural University, No.2 Yuanmingyuan West Road, Haidian District, Beijing 100193, China

ARTICLE INFO

Article history:
Received 11 May 2015
Received in revised form
27 July 2015
Accepted 27 July 2015
Available online 29 July 2015

Keywords:
Chrysanthemum
DgD14
Strigolactone
Auxin
Phosphorus deficiency
Low nitrogen

ABSTRACT

Shoot branching plays an important role in determining plant architecture. Strigolactones (SLs) negatively regulate shoot branching, and can respond to conditions of low or absent phosphate or nitrogen. The *D14* gene is a probable candidate as an SL receptor in rice, petunia, and *Arabidopsis*. To investigate the roles of *D14* in shoot branching of chrysanthemum, we isolated the *D14* homolog *DgD14*. Functional analysis showed that DgD14 was a nuclear-localized protein, and restored the phenotype of *Arabidopsis d14-1*. Exogenous SL (GR24) could down-regulate *DgD14* expression, but this effect could be overridden by apical auxin application. Decapitation could down-regulate *DgD14* expression, but this effect could be restored by exogenous auxin. In addition, *DgD14* transcripts produced rapid responses in shoot and root under conditions of phosphate absence, but only a mild variation in bud and stem with low nitrogen treatment. Indistinct reductions of P levels in shoot were observed in plants grown under low nitrogen conditions. The absence of phosphate and low levels of nitrogen negatively affected plant growth. These results demonstrate that P levels in shoot had a close relationship with phosphate, whereas nitrogen did not directly regulate *DgD14* expression in shoot. Taken together, these results demonstrated that *DgD14* was the functional strigolactone signaling component in chrysanthemum.

1. Introduction

Shoot branching is a ubiquitous phenomenon in plant growth, and is also one of the basic characteristics of plants that plays an important role in determining plant architecture. Branching is a highly plastic determinant of plant shape to allow plants to respond to environmental stresses (Evers et al., 2011). The axillary buds grow into shoots regulated by genetic, hormonal, developmental, and environmental factors (Schmitz and Theres, 1999; Beveridge et al., 2003; McSteen and Leyser, 2005; Ongaro et al., 2008). Plant hormones, such as auxins and strigolactones (SLs), inhibit bud outgrowth, while cytokinins (CKs) promote branching, and thus these hormones interact to regulate bud breaking and branching (Beveridge, 2006; Leyser, 2009).

SLs were recognized relatively recently as a new class of carotenoid-derived signal that can directly regulate shoot branching (Beveridge, 2006; Gomez-Roldan et al., 2008; Umehara et al., 2008). A series of increased branching mutants in diverse plant species have been identified, including the more axillary growth (*max*) mutants of *Arabidopsis thaliana*, the dwarf (*d*) and high tillering dwarf (*htd*) mutants of *Oryza sativa*, the decreased apical dominance (*dad*) mutants of *Petunia hybrida*, and the ramosus (*rms*) mutants of *Pisum sativum* (Domagalska and Leyser, 2011). They have been bred to allow investigation of the molecular mechanisms of SL biosynthesis, transport, and signaling. Through analysis of these mutants, seven genes have been identified. *D27* encodes a novel chloroplast-located iron-containing protein, which is the first enzyme in the SL pathway (Lin et al., 2009; Alder et al., 2012; Waters et al., 2012a); *CCD7* and *CCD8* encode divergent plastidic carotenoid cleavage dioxygenases that function in the chloroplast (Sorefan et al., 2003; Booker et al., 2004; Snowden et al., 2005; Ledger et al., 2010; Pasare et al., 2013); while *MAX1* encodes a cytochrome P450 monooxygenase and catalyzed carlactone to carlactonoic acid in *Arabidopsis*, it works downstream of *CCD7* and *CCD8*, and is a particularly strong candidate for contributing to diversification of inputs upstream of *MAX2* (Booker et al., 2005; Lazar and Goodman, 2006; Challis et al., 2013; Abe et al., 2014). In addition, *MAX2* encodes an F-box protein, function in signaling pathways downstream of SLs and is required for response to SLs.

* Corresponding author.
E-mail address: zhaolj5073@sina.com (L. Zhao).

http://dx.doi.org/10.1016/j.plaphy.2015.07.030
0981-9428/

D14 encodes an α/β-fold hydrolase, which is proposed to act in signaling or in the hydrolysis of SLs to an active compound and provides specificity to signaling via *MAX2* mediates both SLs and karrikins signaling (Stirnberg et al., 2002; Umehara et al., 2008; Hamiaux et al., 2012; Waters et al., 2012b; Challis et al., 2013). Finally, *D53* encodes a protein that shares predicted features with the class I Clp ATPase proteins, and acts as a repressor of SL signaling (Jiang et al., 2013; Zhou et al., 2013).

Classic decapitation studies, grafting, application, and one/two-node assays (Chatfield et al., 2000; Brewer et al., 2013; Chen et al., 2013) have shown that auxins, CKs, and SLs play a major role in regulating bud outgrowth. Auxins act indirectly on entering the bud through two mechanisms, the auxin transport canalization model and the second messenger model. The auxin transport canalization model works as follows: if it is assumed that active growth, buds establish their own polar auxin transport stream (PATS) into the main stem, then high auxin concentrations in the main stem can prevent bud activation by reducing the sink strength of the main stem for auxin, thereby preventing the canalization of auxin transport out of the bud. In the second messenger model, auxins in the main stem regulate the synthesis of CKs and SLs that act as auxin second messengers within the bud and regulate branching (Booker, 2003; Ward et al., 2013). Auxins moving down the plant can increase the expression of SL biosynthesis genes and can also negatively regulate CK content (Tanaka et al., 2006). CKs and SLs are directly transported into axillary buds to regulate outgrowth (Kohlen et al., 2011). SLs regulate xylem sap CK (X–CK) levels through a feedback signal (Beveridge et al., 2000; Morris et al., 2001; Foo et al., 2005), but the CK levels do not account for the more branching phenotypes in the *rms* or *d10* mutants (Zhang et al., 2010). SLs and CKs can also act antagonistically on pea bud growth (Dun et al., 2012). Auxins, CKs, and SLs interact in multiple feedback loops, and provide a robust balance to regulate shoot branching (Ferguson and Beveridge, 2009; Domagalska and Leyser, 2011; Ward et al., 2013).

The biosynthesis of SLs can respond to conditions of low or absent phosphate (P) and/or nitrogen (N) to regulate plant architecture. P or N limitations in plants cause reduction in shoot:root ratio, increased SL levels in the roots, regulation of lateral root formation, and stimulation of arbuscular mycorrhizal symbiosis (Umehara et al., 2010; Kapulnik et al., 2011; Ruyter-Spira et al., 2011; Bonneau et al., 2013). The different responses of SL expression to P or N deficiency are related to the nutrient acquisition strategies of plants (Yoneyama et al., 2012). In red clover, tomato, and alfalfa, P deficiency enhances SL exudation (Yoneyama et al., 2007; Lopez-Raez et al., 2008; Yoneyama et al., 2012), while in sorghum, rice, Chinese milk vetch, lettuce, and marigold, deficiency of either N or P promotes SL exudation (Yoneyama et al., 2007, 2012; Jamil et al., 2011).

D14 encodes a protein of the α/β-fold hydrolase superfamily in rice, which has homologs in petunia (*DAD2* gene) and *Arabidopsis* (*AtD14* gene) (Arite et al., 2009; Hamiaux et al., 2012; Waters et al., 2012b). In petunia, SL-mediated interaction of DAD2 with PhMAX2A may trigger the SCF E3 ligase to target an unknown substrate for ubiquitination and degradation (Hamiaux et al., 2012). In *Arabidopsis*, the D14 protein family confers distinct responses to either SLs (D14) or karrikins (Kai2) (Waters et al., 2012b). In rice, *OsMADS57* interacts with *OsTB1* and control the outgrowth of axillary buds through *OsD14* (Guo et al., 2013). SL promotes D14-SCF^{D3}-mediated degradation of *D53* to regulate shoot branching (Jiang et al., 2013; Zhou et al., 2013).

Chrysanthemum (*Dendranthema grandiflorum*) is one of the most important commercial cut flowers, but requires manual decapitation or removal of lateral branches to maintain its architecture in commercial production. In chrysanthemum, the ability of SLs to inhibit bud activity depends on the presence of a competing auxin source, as auxins locally down-regulate biosynthesis of CKs in nodes. SLs also down-regulate the biosynthesis of CKs (Chen et al., 2013). The expression of *DgCCD8*, the SL biosynthesis gene, can be down-regulated by exogenous SL, but can be overridden by apical auxin application (Liang et al., 2010). *DgMAX2*, a key regulatory gene in SL signal transduction, is able to restore *max2-1* mutant branching to wild-type (WT) *Arabidopsis* (Dong et al., 2013). *DgBRC1* complemented the multiple branches phenotype of *Arabidopsis brc1-1*, its transcripts could respond to apical auxin supply and polar auxin transport (Chen et al., 2013).

In this study, we identified *DgD14*, a *D14* ortholog gene of the α/β-fold hydrolase superfamily, in chrysanthemum (*D. grandiflorum* 'Jinba'), and found that the expression of *DgD14* was inhibited by decapitation and induced by auxins. Furthermore, *DgD14* could produce a rapid response to absent phosphate or low N (LN) treatments. These findings provide new insights into the dynamics of the putative SL signaling component *DgD14* in chrysanthemum.

2. Materials and methods

2.1. Plant materials and growth conditions

Chrysanthemum plantlets were propagated under sterile conditions in jars containing MS agar medium (Murashige and Skoog, 1962), and then grown in a tissue culture room at 22–25 °C with a photoperiod of 16/8 h light/dark and a light intensity of 100–120 μmol m^{-2} s^{-1}.

For the decapitation assay and the sprayed hormone assay, the chrysanthemum cuttings (7 cm in height) were initially grown in vermiculite for 10 days, then plants were transferred into pots containing peat soil and vermiculite (1:1) in a greenhouse at 25/23 °C with a 16/8 h light/dark photoperiod.

2.2. Hormone stocks

Naphthaleneacetic acid (NAA; Sigma–Aldrich, Shanghai, China) stock solution was dissolved in 70% ethanol, and GR24 (LeadGen Labs, Orange CT USA) was dissolved in acetone. 6-benzylaminopurine (6-BA; Sigma–Aldrich, Shanghai, China) was dissolved in NaOH, while indole-3-acetic acid (IAA; Sigma–Aldrich, Shanghai, China) and gibberellin acid (GA; Sigma–Aldrich, Shanghai, China) were dissolved in ethanol.

2.3. Isolation of the full-length coding sequence for DgD14

Total RNA was extracted from stems with TRIzol Reagent (15596-026; Life Technologies/Invitrogen) and cDNA synthesis was performed using RevertAid First Strand cDNA Synthesis Kit (#K1621; Thermo Scientific). Primers were designed for *DgD14* cloning based on the sequence regions of *D14* genes that are conserved among *Arabidopsis AtD14*, *Petunia DAD2*, and rice *D14* genes. After obtaining a conserved domain fragment using forward primer P_for and reverse primer P_rev, the 3′ fragment of *DgD14* was amplified by the rapid amplification of cDNA Ends (RACE) method, using the 3′ RACE primers 3′-race1 and 3′-race2, and the 5′ fragment of *DgD14* amplified in the same way using the 5′ RACE primers 5′-race1 and 5′-race2. The products, amplified using PrimeStarHS DNA Polymerase (TaKaRa, Dalian, China), were cloned into pMD18-T vector (TaKaRa) and verified by sequencing (Zhongke Xilin Biotechnology, Beijing, China). Sequence alignment and phylogenic analysis were performed using the ClustalW and ESPript programs (http://www.genome.jp/tools/clustalw/) and the MEGA 5.0 program (http://www.megasoftware.net/) respectively. Phylogenetic trees were generated with MEGA 5.0 using the Neighbor-