Available online at www.sciencedirect.com
SCIENCE DIRECT
Plant Science 170 (2006) 1111–1117
PLANT SCIENCE
www.elsevier.com/locate/plantsci

Cytokinin-induced changes in *CLAVATA1* and *WUSCHEL* expression temporally coincide with altered floral development in *Arabidopsis*

Donna L. Lindsay, Vipen K. Sawhney, Peta C. Bonham-Smith *

University of Saskatchewan, Biology Department, 112 Science Place, Saskatoon, Sask., Canada S7N 5E2

Received 14 July 2005; received in revised form 13 January 2006; accepted 28 January 2006
Available online 24 February 2006

Abstract

Application of the cytokinin N^6-benzylaminopurine (BAP) to the shoot apical meristem of *Arabidopsis thaliana* Landsberg *erecta* (L.) Heynh. induced aberrant flower development, including an increase in floral organ number resembling the *clavata* (*clv*) mutant phenotype. Transcriptome analysis of BAP-treated populations indicated that expression of *CLV1*, a gene encoding a receptor-like kinase involved in organ differentiation and stem-cell maintenance in shoot and floral apical meristems, was significantly reduced by the exogenous cytokinin. Time course analysis by RT-PCR of transcript levels in BAP-treated plants during flower development showed a decline and subsequent recovery of *CLV1* and a concurrent increase in *WUSCHEL* (*WUS*), consistent with the known suppression of *WUS* by CLV. *WUS* encodes a homeodomain protein linked to shoot meristem proliferation. The temporal coincidence of aberrant floral phenotype with changes in transcript levels of *CLV1* and *WUS* suggests that cytokinins regulate flower development through genes controlling shoot apical meristem activity.

Keywords: *WUSCHEL*; *CLAVATA*; Cytokinins; Shoot apical meristem; Flower development; *Arabidopsis*

1. Introduction

Cytokinins, a group of hormones named for their ability to induce cytokinesis [1] and primarily produced in root tips and actively growing tissues, are integral to plant development, including stimulating cell division, shoot initiation and mediating the flowering process (reviewed in [2]). Growth of the aerial portion of the plant is achieved by the activity of the shoot apical meristem, a highly specialized region of cells initiating primordia at its periphery while maintaining a central region of pluripotent cells [3]. Regulation of meristem function and maintenance is thought to be under the control of hormones; specifically, elevated endogenous cytokinin levels have been correlated to vegetative and floral organogenesis [4].

Previous studies on the regulatory role of cytokinins in plant development have involved exogenous treatments followed by documentation of altered phenotypes which resemble known mutants [5,6], analyses of mutants and transgenics with altered cytokinin levels [7–10], and microarray data on cytokinin-induced changes in gene expression [11,12]. Cytokinin induction of shoot proliferation supposes a connection with stimulation of meristematic activity and this hormone has been linked with the meristematic genes *STM* and *KNAT1* [9]; however, much remains to be resolved about the regulation of shoot meristem activity.

In the flowering process, numerous genes are responsible for floral meristem and organ identity, while genes associated with meristematic function can affect the number of organs initiated in each whorl; for example, *clavata1* (*clv1*), *clv2* and *clv3* mutants share a phenotype of increased floral organ number. One of the best-characterized pathways in shoot meristem function features *CLV1*, a leucine-rich repeat (LRR) receptor-like kinase integral to the balance between primordia differentiation and meristem proliferation. To that end, a functional CLV complex restricts the expression domain of *WUSCHEL* (*WUS*), which encodes a homeodomain protein that promotes meristematic cell identity [13,14].

In an earlier study it was shown that exogenous cytokinin N^6-benzylaminopurine (BAP) applied to wild type *Arabidopsis* induced floral phenotypes that resemble *clv1* [5]. Here, using microarrays and RT-PCR analyses, we show that cytokinin-induced floral phenotypes temporally coincide with changes in expression levels of *CLV1* and *WUS*, genes integral to the maintenance and function of the shoot apical meristem.

* Corresponding author. Tel.: +1 306 966 4457; fax: +1 306 966 4461.
E-mail address: bonhamp@duke.usask.ca (P.C. Bonham-Smith).

0168-9452/$ – see front matter
doi:10.1016/j.plantsci.2006.01.015

2. Methods

2.1. Plant growth

Arabidopsis thaliana Landsberg *erecta* (L*er*) and *clv1-1* (ABRC, Columbus, OH), were grown at 16 h light 23 °C ± 1°/8 h dark 19 °C ± 1°. N^6-benzylaminopurine (BAP; Sigma–Aldrich Co., St. Louis, MO) in 50 μl 1 M NaOH was warmed until dissolved, then diluted in 50 ml sterile distilled water (final pH 6.6) to 10^{-3} M; an optimum concentration for induction of aberrant floral phenotypes in *Arabidopsis* [5,15]. Three microliters of 10^{-3} M BAP in 0.05% $Tween_{20}$ (Sigma–Aldrich Co., St. Louis, MO) was applied with a Hamilton syringe to the surface of the shoot apex of plants when four to five rosette leaves were ≥1 mm (as the shoot apices were in transition from a vegetative to an inflorescence function). The phenotypes of the proximal 20 flower positions in the racemes of control and BAP-treated plants were recorded and photographed. Contrast and brightness of photos were adjusted using Adobe Photoshop.

2.2. Microarray samples

The main axes of 150 plants, including the shoot and root apical meristems, with leaves removed, were harvested and flash-frozen in liquid nitrogen 48 h after BAP treatment, for three separate biological replicates. Non-harvested plants from each BAP-treated and control population were allowed to mature and phenotypes recorded. RNA was extracted from the plant axes following the protocol of the RNeasy Plant Mini Kit (Qiagen Valencia, CA), then processed and hybridized to 22K Affymetrix *Arabidopsis* GeneChips® at the McGill University and Genome Québec Innovation Centre (http://genomequebec.mcgill.ca). Kensington Discovery Edition v1.8.2 (KDE) (InforSense, Boston, USA; London, UK; www.inforsense.com) software performed interpretation of raw spot emission, conversion to numerical output and normalization, involving a model-based background correction, quantile normalization at the probe level, and expression values were scaled so that all arrays had the same mean. The percentage of spots with P(resent) hybridization for each array: control 62.9%; BAP-treated replicates 63.9%, 65.5%, 66.6%.

2.3. RT-PCR

The main axes of 50 plants, including the shoot and root apical meristems, with leaves removed, were harvested and RNA isolated at 4, 24, 48, 96, and 192 h after BAP treatment at the four to five leaf stage. Time courses of three separate biological replicates of both BAP-treated and controls were collected; in each case, plants were retained for phenotype documentation. RT-PCR on total RNA was carried out using the OneStep RT-PCR Kit (Qiagen Valencia, CA). *18S* rRNA was the internal control. Gene specific primers: *CLV1* (5′) CTGCTTCTGA GTGTATGTCTTC, (3′) CGGATTTAGGAGGGTTAGTGAG; *WUS* (5′) TTCAACG-GAACAAACATGAC, (3′) GTGCATAG- GGAAGAGAGGAA; *18S* (5′) GGAGCGATT- TGTCTGGTT, (3′) TGATGACTCGC-GCTTACT; *18S* competitive primers (competimers) were the same sequences as *18S*-5′ and *18S*-3′ but with a dideoxythymidine at the 3′ end (*18S* sequences supplied by Kaplan and Guy, U of F, Gainesville, FL, USA). Optimization for *18S* primer:competimer ratio reduced the *18S* signal to better correlate with the gene specific signal [16]. The optimized protocol was 64 ng RNA, 1:6 ratio of 18S primers:competimers, 25 PCR cycles for amplification of *CLV1* and 37 PCR cycles for amplification of *WUS*, at 95 °C (30 s denaturing), 52°C/48 °C (*CLV1*/*WUS* 30 s annealing) and 72 °C (30 s extension). Transcript amplified by RT-PCR was analyzed with ethidium–bromide stained gels using Gel Doc 2000 Quantity One software. Ratios of the genes of interest to the *18S* internal controls were calculated and graphed. Statistical analysis was carried out with SAS version 8.2 for Windows (SAS Institute Inc., Cary, NC, USA). Differences were considered significant at $p \leq 0.05$.

2.4. Microarray data from Genevestigator

Tools within the microarray database Genevestigator (http://www.genevestigator.ethz.ch.) [17], Gene-Chronologer was used as an indicator of control levels of expression of specific genes during development and Meta-Analyzer was used to examine expression profiles of genes responding to various plant hormones (based on work from Yoshida's Laboratory, Saitama, Japan) and stress inducers.

3. Results

3.1. Exogenous BAP induces phenotypes resembling clv1-1

Flower development in *Arabidopsis* follows a conserved pattern of four whorls: a calyx of four sepals, a corolla of four

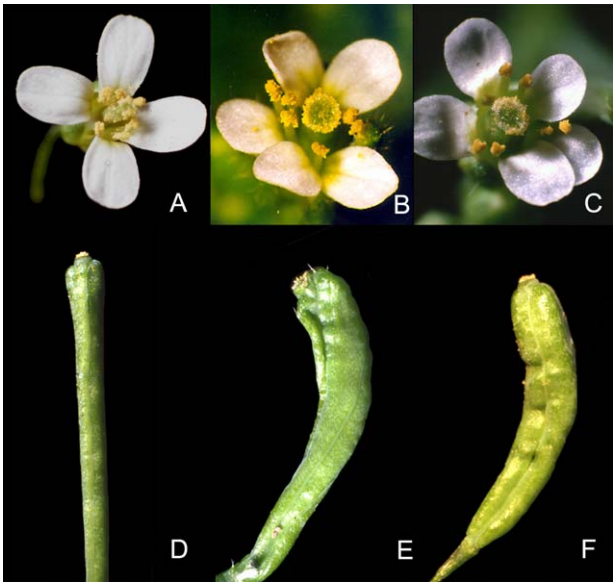

Fig. 1. (A) Wild type *Arabidopsis* flower. (B) Flower of BAP-treated wild type with increased floral organ number phenotype, similar to (C). (C) *clv1-1* flower with increased floral organ number. All petals; white colour tinge is an artefact of photography. (D) Wild type *Arabidopsis* L*er* silique. (E) Silique of BAP-treated wild type with increased carpel number, similar to (F). (F) *clv1-1* silique with increased carpel number.