RESEARCH PAPER

Kiwifruit SVP2 gene prevents premature budbreak during dormancy

Rongmei Wu1, Tianchi Wang1, Ben A.W. Warren1, Andrew C. Allan1,2, Richard C. Macknight3 and Erika Varkonyi-Gasic1,*

1 The New Zealand Institute for Plant & Food Research Limited (Plant & Food Research) Mt Albert, Private Bag 92169, Auckland Mail Centre, Auckland 1142, New Zealand
2 School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand
3 Department of Biochemistry, University of Otago, PO Box 56, Dunedin 9054, New Zealand

* Correspondence: erika.varkonyi-gasic@plantandfood.co.nz

Received 14 November 2016; Editorial decision 9 January 2017; Accepted 18 January 2017

Editor: Ruediger Simon, Heinrich Heine University

Abstract

MADS-box genes similar to Arabidopsis thaliana SHORT VEGETATIVE PHASE (SVP) have been implicated in regulation of flowering in annual species and winter dormancy in perennial species. However, the underlying regulatory mechanisms remain to be identified. In this study, the role of kiwifruit SVP2 was explored using ectopic transgenic expression in kiwifruit species with different chilling requirements and the model species tobacco, followed by transcriptomic analysis of transgenic kiwifruit plants. Ectopic expression of SVP2 affected the duration of dormancy in a high-chill kiwifruit Actinidia deliciosa. This effect could be overcome by sufficient winter chilling. SVP2 had a minimal effect on the duration of dormancy in a low-chill kiwifruit A. eriantha. Expression in a tobacco cultivar with photoperiodic regulation of flowering resulted in retarded vegetative growth but no impact on flowering. Transcriptomic analyses of the kiwifruit SVP2 transgenic and control lines identified 92 significantly differentially expressed genes potentially involved in SVP2-mediated growth repression during dormancy, suggesting a role complementary to abscisic acid (ABA). This study has demonstrated that kiwifruit SVP2 has an integrative role in suppression of meristem activity to prevent precocious budbreak before the fulfilment of winter chilling requirements.

Key words: ABA, Actinidia, budbreak, bud dormancy, dehydration, kiwifruit, SVP, transcriptome.

Introduction

In temperate horticultural woody perennials, winter dormancy is of particular importance both to avoid unfavourable winter conditions, and to synchronize budbreak and flowering in the following spring (Cooke et al., 2012; Yamane, 2014). Winter dormancy is a dynamic process, defined as a period between bud set in the autumn and budbreak in the spring, when no visible growth occurs. Dormancy has been divided into para-, endo-, and eco-dormancy phases (Lang et al., 1987). Para-dormancy is the suspension of growth caused by factors outside the meristem but within the plant, such as apical dominance. Endo-dormancy is the deepest state of dormancy, when budbreak is prevented by endogenous factors specific to the meristem, which stop the growth even under favourable external conditions. Eco-dormancy is when
the growth capacity is restored in the meristem, but remains suspended because of unfavourable external environmental factors and can be released when conditions become permissive. A certain amount of chilling in a bud is often required for the transition from endo-dormancy to eco-dormancy (Lang et al., 1987; Rohde et al., 2007).

Bud development can be dissected into bud formation, acclimation to dehydration and cold, and dormancy. Each of these steps is associated with specific sets of regulatory and marker genes and metabolites (Ruttink et al., 2007). Recent studies of metabolites and gene expression reconstruct the temporal sequence of events during bud development. At least three main regulatory programmes control the onset of dormancy, namely signal perception, hormone alteration, and transcription factors (Shim et al., 2014). Similarly, multiple functional categories of differentially expressed genes (DEGs) have been identified during dormancy release, including stress response, sugar metabolism, hormone response, cell cycle and DNA processing, energy generation, transcription factors, and signal transduction (Fabbroni, 2009). This has been further reinforced by a number of independent studies (Walton et al., 2009; Leida et al., 2012; Liu et al., 2012; Nishitani et al., 2012; Bai et al., 2013; da Silveira Falavigna et al., 2013; Ueno et al., 2013; Zhong et al., 2013; Howe et al., 2015). However, genetic regulation of dormancy remains largely unknown.

The first suggestion that MADS-box genes might be important regulators of dormancy came from a study of the peach (Prunus persica) evergreening (evt) mutant. Deletion of six tandem arrayed DORMANCY-ASSOCIATED MADS-BOX (DAM) genes in peach resulted in a complete lack of dormancy under cold or short-day (SD) induction, while the expression of a subset of these genes was elevated during endo-dormancy (Bielenberg et al., 2008; Li et al., 2009; Yamane et al., 2011). Similarly, a negative correlation of expression with endo-dormancy release was observed for six tandem arrayed DAM genes predicted to act as transcriptional repressors in Japanese apricot (Prunus mume) (Yamane et al., 2008; Sasaki et al., 2011). Ectopic expression of one of these genes in transgenic poplar resulted in premature growth cessation and terminal bud set, demonstrating a role in growth inhibition in the model woody perennial plant (Sasaki et al., 2011). Genes encoding homologs of DAM transcription factors are differentially regulated during dormancy in many horticultural woody perennials (Mazzitelli et al., 2007; Bielenberg et al., 2008; Diaz-Riquelme et al., 2009; Li et al., 2010; Ubi et al., 2010; Sasaki et al., 2011; Yamane et al., 2011; Liu et al., 2012; Bai et al., 2013; da Silveira Falavigna et al., 2013; Mimida et al., 2015; Porto et al., 2016), suggesting a conserved role in dormancy, and a major quantitative trait locus (QTL) for chilling requirement and bloom date overlapped the peach genomic regions where DAM genes are located (Zhebentyayeva et al., 2014). However, the underlying mechanism and mode of action remain poorly understood and the genetic evidence from diverse species is limited.

DAM proteins are closely related to Arabidopsis thaliana flowering time regulators SHORT VEGETATIVE PHASE (SVP) and AGAMOUS-LIKE 24 (AGL24). Arabidopsis SVP and AGL24 are central regulators in the flowering regulatory network, with high sequence similarity but opposite functions. Their mode of action includes interaction with other proteins, resulting in either repressing or activating complexes that regulate floral transition and maintain floral meristem identity (Michaels et al., 2003; Gregis et al., 2006; Lee et al., 2007, 2014; Liu et al., 2007, 2009; Li et al., 2008), or direct binding to the CarG motifs in floral activators, such as FLOWERING LOCUS T (FT) and SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1) (Lee et al., 2007; Posé et al., 2013). In a herbaceous perennial leafy spurge (Euphorbia esula), DAM-like and FT-like genes are reciprocally and differentially expressed during winter dormancy transition, implying a similar mechanism in regulation of dormancy (Horvath, 2009).

Woody perennials usually have multiple SVP homologues, resulting from lineage- and species-specific expansions within the SVP/AGL24 MADS-box subfamilies (Wells et al., 2015). Four SVP genes have been identified in the kiwifruit species Actinidia chinensis and A. delicosa, with differential ability to delay flowering in Arabidopsis. Expression of SVP1, SVP2, and SVP4 was elevated in kiwifruit buds over the winter dormancy period, and the relative transcript abundance was higher in colder regions, suggesting roles in bud dormancy and flowering (Wu et al., 2012). In contrast, SVP3 accumulation in buds did not demonstrate seasonal changes, but ectopic expression caused abnormal flower development, reduced petal pigmentation, and abnormal fruit and seed development, supporting a role in repression of reproductive development (Wu et al., 2014). To understand the mechanism of SVP-mediated regulation of kiwifruit bud dormancy, budbreak, and flowering, SVP2 was ectopically expressed in a high chilling requirement species A. delicosa, a low chilling requirement species A. eriantha, and in Nicotiana tabacum ‘Maryland Mammoth’. Detailed physiological and transcriptional analyses of 35S:SVP2 A. delicosa transgenic lines were performed.

Materials and methods

Plant transformation and growth conditions

SVP2 coding sequence under the control of the Cauliflower mosaic virus (CaMV) 35S promoter (Wu et al., 2012) was transformed into Agrobacterium tumefaciens strain EHA105 for transformation into kiwifruit A. delicosa ‘Hayward’ [A. delicosa (A. Chev.) C.F. Liang et A.R. Ferguson, also referred to as A. chinensis var. delicosa (A.Chev.) A. Chev.] and A. eriantha Benth. The same construct was transformed into A. tumefaciens strain GV3101 for transformation into tobacco (N. tabacum ‘Maryland Mammoth’). A reporter gene uidA (GUS) under the control of the CaMV 35S promoter (35S:GUS) in appropriate Agrobacterium strains was used to transform control plants. The transformation procedure for A. delicosa was previously described (Wang et al., 2006, 2007). Transformation of A. eriantha was according to a previously described protocol (Wang et al., 2006, 2007), with modifications to media composition. The regeneration medium contained half-strength Murashige and Skoog (1/2 MS) agar medium (Murashige and Skoog, 1962), 2 mg l⁻¹ 6-benzylaminopurine (BAP), 1 mg l⁻¹ zeatin, 0.2 mg l⁻¹ indole-3-butyric acid (IBA), 300 mg l⁻¹ timetin, and 150 mg l⁻¹ kanamycin. The shoot elongation medium contained 1/2 MS, 0.1 mg l⁻¹
zeatin, 0.5 mg l⁻¹ IBA, 300 mg l⁻¹ timentin, and 50 mg l⁻¹ kanamycin. Once their roots were established, transgenic plants were transferred to soil and grown in a containment glasshouse for 18 months at Plant & Food Research, Auckland, New Zealand. Budbreak time and flowering time for transgenic A. deliciosa and A. eriantha were assessed in the following spring season. Nicotiana tabacum transformation was carried out on young leaf discs excised from in vitro grown shoots (Horsch et al., 1985). Transgenic tobacco plants were grown in a containment glasshouse at 20 °C under SD conditions (8/16 h light/dark). The seeds from these transgenic plants were collected and germinated on 1/2 MS agar medium (Murashige and Skoog, 1962) supplemented with 50 μg ml⁻¹ kanamycin. Following the segregation tests, two homozygous lines were chosen and six T₂ generation plants of each line were used for detailed analysis.

For clonal propagation, A. deliciosa 35S:SVP2 Line 1 young shoots were collected and surface sterilized using 25% bleach (containing 1.25% sodium hypochlorite) for 20 min, followed by rinsing with sterile water five times. The nodes with axillary buds were excised and transplanted to MS medium. New shoots initiated from these axillary buds, and subsequently seven clonal plants were generated. Once roots were established, plants were transferred to ambient containment glasshouse conditions over 18 months. To initiate dormancy, plants were maintained in SD conditions for 6 weeks (18 °C, 14 h dark and 10 h light intensity at 300–600 μmol s⁻¹ m⁻²) and subsequently subjected to 4 weeks of fluctuating temperature conditions (14–20 °C during the day and 4–10 °C at night) with an average 9.5 h day length (maximum light intensity at 1000–2000 μmol s⁻¹ m⁻²). After 100% leaf drop, lateral buds were collected and the plants were subjected to chilling at 3–7 °C for up to 8 weeks.

Dormancy status was determined as described previously (Voogd et al., 2015). Briefly, stem cuttings with a single lateral bud were excised on a regular basis from each plant, the lower ends were immersed in water and maintained at budbreak forcing conditions (20 °C, 14 h photoperiod of white light and 70–80% humidity), and the number of days until visible budbreak was recorded. A minimum of three cuttings for each plant were used.

RNA extraction and expression studies

Total RNA was extracted from kiwifruit tissue as previously described (Chang et al., 1993). Total RNA was isolated from tobacco leaf using the Trizol reagent (Invitrogen). A 5 μg aliquot of total RNA was treated with DNase I (Ambion) and reverse transcribed at 37 °C using the BluePrint® Reagent kit for reverse transcription–PCR (RT–PCR) (TaKaRa) according to the manufacturer’s instructions. Amplification and quantification were carried out using the LightCycler® 480 System and SYBR Green I Master Mix (Roche Diagnostics). Reactions were performed in quadruplicate, and a non-template control was included in each run. Thermal cycling conditions were 95 °C for 5 min, followed by 50 cycles of 95 °C for 10 s, 60 °C for 10 s, and 72 °C for 20 s, followed by a melting temperature cycle, with constant fluorescence data acquisition from 65 °C to 95 °C. The data were analysed using the ratio of target to reference and calculated with the LightCycler®480 software 1.5 (Roche Diagnostics). The expression was normalized to previously characterized reference genes, kiwifruit Actin (Wu et al., 2012) and tobacco Nta-Tub1 (Pattanaik et al., 2010). Primer sequences used in this study are listed in Supplementary Table S1 at JXB online.

Analysis of differentially expressed genes

The DEGs between each sample set were detected with DESeq v2.10 (Anders and Huber, 2010). The cut off of Padj<0.05 value, followed by the absolute value of logFC (log2 fold change) of not less than 1.0 were considered as significantly differentially expressed genes. The gene expression unit was calculated using the RPKM method (reads per kilobase of transcript per million mapped reads). Annotations were obtained by BLAST of amino acid sequence to Arabidopsis amino acid sequences. Hierarchical clustering as well as heatmap analysis of DEGs were described in McAtee (2014). The best Arabidopsis (TAIR 10) hit was used for Gene Ontology (GO) term classification, and significance was established by singular enrichment analysis (SEA) coupled with available background data of Arabidopsis [false discovery rate (FDR) ≤0.05], using AgriGO Version1.2 (Du et al., 2010). The abiotic stress response and hormone response were established using the Arabidopsis eFP Browser (http://bbc.botany.utoronto.ca/efp/cgi-bin/efpWeb.cgi) (Winter et al., 2007).

Results

Overexpression of SVP2 delays budbreak in a high-chill kiwifruit A. deliciosa

To evaluate the role of SVP2 gene in kiwifruit, transgenic A. deliciosa lines with SVP2 cDNA driven by the CaMV 35S
promoter were generated, using the standard transformation protocol (Wang et al., 2012) and the CaMV 35S promoter-driven uidA (GUS) construct as control. *Actinidia deliciosa* has a high chilling requirement, long dormancy, and late spring budbreak. Normal adventitious shoot formation and growth were observed with the control construct, but the initiation of adventitious buds and shoot elongation were impaired when the *SVP2* construct was used. After multiple transformation experiments, four independent transgenic lines were obtained, with varying levels of *SVP2* transgene expression (Fig. 1A). No difference in autumn growth cessation, leaf drop, timing of bud-set, and bud formation could be detected between any of the *SVP2* transgenic and control lines over the period of 2 years, but a significant delay in the first visible budbreak during the spring season in both years was observed for *SVP2* lines. This delay correlated with levels of *SVP2* transgene expression and was most prominent in Line 1 (Fig. 1B, C). This line showed slow and weak growth and remained significantly smaller than the control lines over a period of 2 years (Fig. 1C, D).

**Cold treatment of SVP2 A. deliciosa transgenic lines**

In glasshouse conditions, *A. deliciosa* plants perceived insufficient chilling because of mild winters in Auckland, New Zealand. To address how chilling conditions related to the *SVP2*-mediated delay of budbreak, we selected *SVP2* Line 1 with the highest transgene expression for further analysis. Seven clonal plants were generated from Line 1, which all expressed the *SVP2* gene (Fig. 2A). The plants were allowed to grow in the containment glasshouse for a period of 18 months, before dormancy was induced by conditions mimicking autumn and early winter. Four control plants were subjected to the same treatment. Dormant plants were exposed to cold treatment for 8 weeks, and three single node cuttings were collected weekly to evaluate dormancy status (Fig. 2B). Average budbreak time demonstrated negative correlation with the duration of chilling (Fig. 2C). Without chilling, no budbreak was observed in cuttings taken from either control or *SVP2* plants. In *SVP2* plants subjected to cold, a significantly delayed budbreak compared with control plants was detected for up to 4 weeks of cold treatment. A delay of 23, 12, and 8 d was recorded for plants treated for 1, 2, and 3 weeks, respectively. After 4 weeks, average budbreak was still delayed by 8 d, but this delay decreased gradually after 5 weeks of cold treatment to no detectable difference after 8 weeks of cold treatment (Fig. 2C).

**Overexpression of SVP2 in a low-chill kiwifruit A. eriantha**

The restored budbreak timing of sufficiently chilled 35S:*SVP2* transgenic *A. deliciosa* prompted us to study the role of *SVP2* in a kiwifruit which has a low chilling requirement, *A. eriantha*. In this kiwifruit species, *SVP* gene sequences and expression are highly comparable and therefore likely to be

---

*Fig. 1.* Constitutive expression of *SVP2* delays budbreak in *Actinidia deliciosa*. (A) Relative expression of *SVP2* in four 35S:*SVP2* transgenic plants and two control plants. The expression was normalized to kiwifruit *Actin*. Error bars represent the SE of four replicate reactions. (B) Days to budbreak after 100% leaf drop in late autumn. The first visible leaf in spring was recorded as budbreak. (C, D) Transgenic *A. deliciosa* plants and control plants in the middle of spring.
functionally conserved as previously described in *A. chinensis* and *A. deliciosa* (Wu et al., 2012, 2014) (Fig. 3A). An additional advantage of *A. eriantha* is the fast reproductive maturity and prolific flowering in glasshouse conditions (Wang et al., 2006), facilitating the study of the role of *SVP2* in reproductive onset and development. Initial attempts at regeneration and transformation using standard protocols optimized for *A. eriantha* (Wang et al., 2006) were unsuccessful. Transformation with the control construct resulted in normal callus formation, initiation of adventitious buds, and subsequent growth, but browning and aborted shoot tip development were recorded with the *SVP2* construct (Supplementary Fig. S1). Similar results were obtained in an attempt to transform another kiwifruit species, *A. chinensis*, suggesting that overexpression of *SVP2* plays a detrimental role in regeneration and growth.

Overexpression of *SVP2* affects plant growth and seed germination, but not flowering time and petal colour in transgenic tobacco

Less efficient transformation in all tested kiwifruit species and delayed budbreak in *A. deliciosa* suggested that *SVP2* played a detrimental role in vegetative growth that could be overcome by changes in growth conditions (chilling and modified media composition), while not affecting flower development and petal colour, in contrast to reports for kiwifruit gene *SVP3* (Wu et al., 2014). To investigate whether kiwifruit *SVP2* had a conserved growth-restriction effect and further evaluate a role in flowering time and reproductive development, *SVP2* cDNA driven by the CaMV 35S promoter was transformed into an SD flowering tobacco variety ‘Maryland Mammoth’. The progeny of two independent transgenic tobacco lines were subjected to detailed analysis. Seed germination and root growth were delayed compared with those in controls (Fig. 4A–D; Table 2). Significant differences were observed in the height of transgenic *SVP2* plants, although the plant architecture and secondary growth were visually similar (Fig. 4E–H; Table 2). Flowering time and the number of flowers produced were similar, but more sterile flowers were found on *SVP2* plants than on controls (Table 2). Occasional homeotic conversion of stamen to petal was observed (Fig. 4I, J); however, the petal pigmentation and expression of the tobacco anthocyanin regulators, bHLH genes, *NiAn1a* and *NiAn1b*, and R2R3 MYB, *NtAN2* (Pattanaik et al., 2010; Bai et al., 2011) were comparable with those in control lines (Fig. 4K).
Overexpression of SVP2 in Actinidia eriantha leads to transcriptomic changes during winter dormancy

To understand the molecular mechanisms underlying SVP2-mediated growth repression in kiwifruit, a transcriptome analysis of SVP2 transgenic lines (t) and control lines (c) at different dormancy stages was performed. Sampling times corresponded to (i) the endo-dormant stage following 100% leaf drop; (ii) transition to eco-dormancy after exposure to winter temperature; and (iii) initiation of budbreak. The dormancy status of bud samples was confirmed by single node cutting assays. Budbreak was delayed in SVP2 plants at the first two time points, and SVP2 plants remained mostly

Table 1. Phenotypic analysis of 35S:SVP2 transgenic Actinidia eriantha

Days to budbreak were recorded as days from 100% leaf drop to the first visible budbreak. Number of breaking buds was recorded as the total number of developing shoots at the end of spring. Days to flowering were recorded as days from the first visible budbreak to the appearance of the first floral bud. Number of flowers was counted as total flowers per line.

| Transgenic lines | Days to budbreak | Number of breaking buds | Days to flowering | Number of flowers |
|------------------|------------------|-------------------------|------------------|------------------|
| Line 1           | 40               | 28                      | 18               | 11               |
| Line 2           | 43               | 21                      | 18               | 11               |
| Line 3           | 45               | 23                      | 14               | 3                |
| Line 4           | 40               | 25                      | 18               | 5                |
| Line 5           | 40               | 20                      | 25               | 5                |
| Line 6           | 45               | 23                      | 25               | 7                |
| Line 7           | 43               | 22                      | 25               | 35               |
| Control 1        | 40               | 32                      | nil              | 0                |
| Control 2        | 40               | 34                      | 32               | 1                |
| Control 3        | 40               | 34                      | 25               | 3                |
| Control 4        | 40               | 20                      | 18               | 22               |
Kiwifruit SVP2 delays budbreak

Endo-dormant at the first time point (Supplementary Fig. S2). No difference in budbreak time was detected between SVP2 and control lines at the third sampling point. For that reason, only SVP2 transgenic plant bud samples at this time point were analysed further. A total of 15 libraries were prepared and 60–70 million RNA-seq reads were generated for each library. PCA of these RNA-seq reads demonstrated clear separation between sampling dates, but less variation between SVP2 transgenic and control lines at corresponding sampling dates. The sample set at time point 3 was more variable, reflecting transcriptomic changes at an advanced developmental phase just before visible budbreak (Supplementary Fig. S2).

Comparison of SVP2 transgenic and control plant transcriptomes identified 253 genes significantly differentially expressed in the buds collected at the first time point (t1-c1) (Supplementary Table S3), and 226 in the buds collected at the second time point (t2-c2) (Supplementary Table S4), with 92 in the common set, 54 and 38 consistently up- and down-regulated, respectively, in SVP2 transgenic plants (Fig. 5A; Supplementary Table S5). Annotation of the closest Arabidopsis homologue found that 76 DEGs in t1-c1 and 41 DEGs in t2-c2 have been previously identified as direct Arabidopsis SVP targets (Tao et al., 2012; Gregis et al., 2013), while 69 DEGs in t1-c1 and 58 DEGs in t2-c2 have been associated with dormancy in poplar, leafy spurge, and kiwifruit (Horvath et al., 2008; Walton et al., 2009; Howe et al., 2015). Functional classification using GO enrichment analysis identified several categories of biological processes that were significantly affected in the SVP2 transgenic

---

**Table 2. Phenotypic analysis of 35S:SVP2 transgenic tobacco**

Data are presented as means and the SE of six individuals for each lines. Days for seed germination were recorded as days from sterilization to visible germination on MS plates. Total leaf number was counted when the first floral bud was visible. Plant height was expressed as centimetres when the first visible floral bud appeared. Total number of flowers and sterile flowers were counted on inflorescences.

| Transgenic Lines | Seed germination (d) | Total leaf number | Plant height (cm) | Total number of flowers | Number of sterile flowers |
|------------------|----------------------|-------------------|-------------------|-------------------------|--------------------------|
| Line 1           | 11 ± 2.3             | 18.7 ± 0.4        | 42.5 ± 3.3        | 26.1 ± 1.2              | 21.0 ± 2.5               |
| Line 2           | 13 ± 1.2             | 20.0 ± 1.2        | 31.1 ± 1.1        | 32.5 ± 1.5              | 27.7 ± 4.1               |
| Control 1        | 7 ± 0.0              | 19.3 ± 0.4        | 56.6 ± 3.6        | 28.0 ± 2.5              | 13.5 ± 1.6               |
| Control 2        | 7 ± 0.0              | 22.3 ± 0.4        | 49.0 ± 2.1        | 34.0 ± 2.5              | 14.5 ± 0.5               |

---

**Fig. 4.** Constitutive expression of SVP2 affects vegetative development in transgenic tobacco ‘Maryland Mammoth’. (A, B) Seed germination of 35S:SVP2 plants compared with control plants, 25 d after seeds stratification on MS plates. (C, D) Slow root formation in transgenic 35S:SVP2 plants compared with control plants. (E–H) Two lines of transgenic 35S:SVP2 plants compared with control plants under SD conditions. (I, J) Mutant transgenic SVP2 flower compared with control. The arrow indicates the petaloid stamen in the transgenic flower. (K) Relative expression of NtAn1a, NtAn1b, NtAN2, and the SVP2 transgene in petals of transgenic plants compared with control plants. Black and grey bars represent relative expression of two independent lines. The expression of each gene was normalized to tobacco Ntα-Tub1. Error bars represent the SEs for four replicate reactions.
plants, most notably stress response in the t1-c1 and t2-c2 sets. The molecular functions of catalytic and transferase activity were significantly enriched in the common gene set (Supplementary Table S6). Further interrogation of the closest Arabidopsis homologue expression data available through the eFP browser (Winter et al., 2007) revealed that a large proportion of identified genes responded to abiotic stress or plant hormone treatments, most commonly osmotic and cold stress and abscisic acid (ABA) treatment. Of the 92 common genes at both time points, 31 were homologous to Arabidopsis genes that are ABA or osmotic/drought responsive (Supplementary Table S5).

Analysis of SVP2 plant transcriptomes across different dormancy stages identified five types of expression patterns for the common set of 92 DEGs over the three time points (t1, t2, and t3); increasingly up- or down-regulated from endo-dormancy to budbreak, transiently up- or down-regulated during progression to eco-dormancy, and expressed to a similar level over the dormancy period (Fig. 5B). The accuracy and reproducibility of the transcriptome analysis results was confirmed by real-time RT–PCR analysis of a subset of candidate genes (Supplementary Fig. S3).

**Discussion**

**SVP2 delays shoot outgrowth but may not be sufficient for the onset of dormancy in kiwifruit**

In many woody perennials, SVP genes have been associated with winter dormancy. In particular, DAM genes from...
**Prunus persica** and *P. mume* have been advanced as key regulators of winter dormancy (Bielenberg et al., 2008; Jiménez et al., 2010; Sasaki et al., 2011; Yamane et al., 2011). As these *Prunus* spp. are recalcitrant to transformation, Sasaki et al. (2011) demonstrated a potential role by heterologous expression of *Prunus DAM6* in poplar. Overexpression of kiwifruit SVP2 in kiwifruit provides a system to ratify the growth inhibitory function of *SVP* genes by ectopic expression in the species of origin. The low regeneration efficiency suggested that overexpression of *SVP*-like genes strongly inhibited outgrowth of plants in tissue culture, potentially explaining the absence of reports on the role of these genes in the species from which they were isolated.

In natural conditions, onset of kiwifruit bud dormancy can be induced by autumn SD and cooler/fluuctuating temperature conditions (Brundell, 1976; Lionakis and Schwabe, 1984). The SVP2 kiwifruit lines exhibited no difference in the morphology of the shoot apex and axillary bud formation in comparison with control plants. In particular, premature growth termination and early bud set have not been observed in transgenic *A. deliciosa* or *A. eriantha* SVP2 lines grown in ambient conditions in summer (long days). The autumn SD and low temperature conditions did not visibly enhance the leaf senescence, leaf drop, and bud set in transgenic lines. Instead, transgenic plants showed a delay in axillary budbreak in the spring, suggesting that SVP2 was associated with maintenance of deep bud dormancy. This is in contrast to findings reported in transgenic poplar where expression of *Prunus DAM6* resulted in premature growth cessation followed by terminal bud set (Sasaki et al., 2011). A possible explanation is that the onsets of terminal and lateral bud dormancy rely on somewhat different mechanisms. In kiwifruit, the shoot tip aborts instead of forming a terminal bud; abortion is preceded by growth cessation and is initiated by tissue necrosis in the subapical zone (Foster et al., 2007). The timing of shoot tip abortion is negatively correlated with the shoot expansion rate and can occur at any time, resulting in short or long shoots. This high developmental plasticity makes visual observations of growth cessation in kiwifruit difficult; however, evidence from both kiwifruit and tobacco SVP2 lines confirms a role in growth inhibition. Delayed germination followed by slower root and shoot development all indicate that SVP2 can act as a growth repressor in tobacco. In addition, both SVP2 and *Prunus DAM6* performed a role in lateral bud endo-dormancy in kiwifruit and poplar, respectively (Sasaki et al., 2011), as demonstrated by delayed shoot outgrowth. Therefore, SVP2 in kiwifruit performs as a growth repressor once dormancy has been established, but may not be sufficient to suppress kiwifruit growth in permissive conditions. We therefore propose that SVP2 has a key role in suppressing meristem activity in dormant axillary buds.

**Winter chilling can over-ride SVP2-mediated growth inhibition in kiwifruit**

Plant dormancy has been divided into three well-defined phases, para-, endo-, and eco-dormancy (Lang et al., 1987). While growth can resume during para- and eco-dormancy, accumulation of chilling is required to release endo-dormancy, to allow budbreak and floral competency in the following spring (Linsley-Noakes and Allan, 1987; Walton et al., 2001; Snelgar et al., 2008). The normal chilling requirement for *A. deliciosa ‘Hayward’* is ~800 h (Linsley-Noakes and Allan, 1987), after which dormancy is fully alleviated. Insufficient chilling results in delayed budbreak followed by reduced flower and fruit development. Ectopic SVP2 therefore mimics the effects of insufficient chilling, further delaying budbreak, either by maintenance of deep dormancy or by reduction of shoot outgrowth rate. This effect is gradually reduced and becomes negligible after chilling for the period of ~800 h (5 weeks), suggesting that elevated SVP2 is not sufficient to suppress growth once adequate chilling requirements are met. This finding is consistent with our observations in the low-chill kiwifruit species *A. eriantha*, where elevated SVP2 had only a minor effect, strongly suggesting that kiwifruit SVP2 does not play a role in chilling-mediated dormancy release. Instead, it would appear that SVP2 prevents premature growth before full chilling is perceived.

Interestingly, elevated expression of SVP2 in shoot buds during dormancy and its decline prior to budbreak (Wu et al., 2012) suggests transcriptional regulation of SVP2 action. This is consistent with other reports of elevated DAM gene expression during dormancy and the suggestions that winter chilling repressed DAM gene expression, resulting in dormancy release (Horvath et al., 2008; Yamane et al., 2008; Li et al., 2009). However, the failure of ectopically expressed SVP2 to maintain dormancy after sufficient chilling indicated additional regulation at the post-transcriptional level. Possible mechanisms are unknown and may include post-transcriptional or post-translational modifications, differential protein stability, or alternative protein–protein interactions. Degradation of SVP protein and differential interactions with other MADS-box protein partners have been established as important during floral transition in Arabidopsis (Michaels et al., 2003; Gregis et al., 2006; Liu et al., 2007, 2009; Lee et al., 2014) and may be instrumental in regulation of dormancy and budbreak in other plant species, including kiwifruit.

**SVP2 affects vegetative growth but has no obvious effect on reproductive development and petal colour**

Previously, we reported that kiwifruit SVP2 and SVP3 had a differential ability to delay flowering in Arabidopsis and rescue the Arabidopsis *svp41* phenotype (Wu et al., 2012). Despite their high sequence similarity, only SVP3 was capable of delaying flowering and complementing the *svp41* mutant. Conversely, elevated SVP3 had no obvious effect on vegetative growth, dormancy, or flowering time in transgenic *Actinidia* or tobacco (Wu et al., 2014), consistent with the lack of increased expression in shoot buds during dormancy (Wu et al., 2012). Instead, elevated SVP3 delayed flower development and reduced petal pigmentation in transgenic *A. eriantha* and tobacco, through interference with transcription of the key anthocyanin pathway regulators (Wu et al., 2014). While SVP2 had no effect on the timing of flower development and
Transcriptomic analysis indicates that SVP genes may mimic the ABA effect

Transcriptomic changes in SVP2 transgenic lines revealed 92 putative SVP2 target genes, significantly up- and down-regulated over two stages of dormancy. Almost half of the genes were typically regulated in response to stress, most often osmotic and cold treatment, with a subset also identified as ABA-responsive genes. These results are consistent with previous findings of coinciding expression of DAM4–DAM6 and several ABA and drought stress response genes during dormancy in peach cultivars (Leida et al., 2012). ABA is an important growth inhibitor previously associated with dormancy; ABA was elevated during endo-dormancy and dropped following the transition to eco-dormancy in several species (Rinne et al., 1994; Le Bris et al., 1999; Rohde and Bhalaria, 2007; Horvath et al., 2008). Consequently, genes associated with response to ABA are often cold, drought, and stress regulated and preferentially expressed during endo- and eco-dormancy (Horvath et al., 2008). ABA affects dormancy progression through its action on dehydrins or membrane permeability (Campoy et al., 2011). Accordingly, kiwifruit genes identified as differentially expressed in SVP2 lines often show homology to well-described genes associated with the dehydration process. Responsive to dehydration 22 (RD22) is a molecular link between ABA signalling and abiotic stress, and its expression has been used as a reliable ABA early response marker in many plants (Shinozaki and Yamaguchi-Shinozaki, 2000; Matus et al., 2014). RD22 has been associated with grape bud dormancy (Mathiason et al., 2009) and Arabidopsis seed dormancy (Yamaguchi-Shinozaki and Shinozaki, 1993).

In this current study, two kiwifruit transcripts with homology to RD22 were highly up-regulated at the dormancy stage, but gradually declined prior to budbreak in transgenic SVP2 lines. The Early-Responsive to Dehydration Stress (ERD) genes have been collectively characterized in Arabidopsis as genes that are rapidly induced by dehydration stress. Three transcripts with similarity to an ERD-like gene together with a late embryogenesis abundant protein (ATECP31) were down-regulated in SVP2 overexpression lines, suggesting that different dehydration pathways existed between transgenic and control lines. Other osmotic- and ABA-responsive genes included ASPARAGINASE B1, GLYOXYLASE 17, and Vacuolar processing enzyme, which were all up regulated in SVP2 transgenic lines. Predicted SVP2 targets also include multiple protein kinases and phosphatases, potentially involved in ABA-induced signal transduction, and several transcription factors also associated with stress and ABA.

It is unclear at this stage if ABA metabolism itself is affected by overexpression of SVP2 in kiwifruit. One of the ABA biosynthesis pathway genes, NCED3, was elevated in the SVP2 transgenic lines in the early stage of dormancy (t1-c1), but not differentially expressed at t2-c2. Similarly, we found no evidence of major differences in ABA concentration between transgenic SVP2 and control lines in the samples corresponding to those collected for RNA-seq analysis during dormancy (data not shown). Therefore, it is possible that SVP2 mimics the ABA effect by targeting of genes and pathways associated with the dehydration process. In that case, SVP2 may be targeting the dehydration response only before sufficient chilling is perceived. After sufficient chilling, this pathway may be disrupted and the presence of SVP2 becomes insufficient to repress growth. Interestingly, a significant overlap between kiwifruit SVP2 and Arabidopsis SVP targets was revealed; in particular, in the t1-c1 set, 30% of top Arabidopsis hits have been reported to be directly regulated by SVP (Tao et al., 2012; Gregis et al., 2013), suggesting conservation of the mechanism of action between taxa. However, many of the well-defined Arabidopsis SVP target genes, such as homologues of FT and SOC, were not affected by elevated SVP2 expression, consistent with no demonstrated role for SVP2 in flowering and suggesting that SVP2 preferentially controls only specific aspects of dormancy.

In summary, this study has demonstrated a growth-inhibiting role for kiwifruit SVP2, mediated by ABA and dehydration response pathways, regulating the timing of meristem activity to avoid unfavourable winter conditions and prevent precocious budbreak.

Supplementary data

Supplementary data are available at JXB online.

Fig. S1. Transformation of Actinidia eriantha.

Fig. S2. Evaluation of A. delicosa transgenic and control buds at three stages for the RNA-seq experiment.

Fig. S3. qPCR validation of RNA-seq expression profiles.

Fig. S4. High resolution image of hierarchical clustering presented in Fig. 5.

Table S1. qPCR primer sets used in the RNA-seq validation.

Table S2. Summary of RNA-seq experiments.

Table S3. List of the differentially expressed genes (log2 >1, Padj<0.05) between transgenic SVP2 (t1) and control (c1) in bud samples collected on 28 June 2013.

Table S4. List of the differentially expressed genes (log2 >1, Padj<0.05) between transgenic SVP2 (t2) and control (c2) in bud samples collected on 14 August 2013.

Table S5. List of the common set of differentially expressed genes in buds collected at both time points (t1-c1 versus t2-c2).

Table S6. List of GO enrichment analysis results (FDR <0.05) for differentially expressed genes in t1-c1, t2-c2, and the common gene set differentially expressed at both time points.
Acknowledgements

The authors wish to thank Peter McAtee, Cecilia Deng, and Ross Crowhurst for assistance in bioinformatics analysis, Tim Holmes for photography, Sakuntala Karunaietnam and Andrew Gleave for cloning support, Monica Dragulescu and Wade Wadasinghe for maintenance of plants in the glasshouse, Roger Hellens and Jo Putterill for advise on the project design and progress, and Anne Gunson and Cath Kingston for critical reading of the manuscript. Special thanks to Eric Walton for inspiring our interest in regulation of kiwifruit dormancy and guidance during earlier stages of kiwifruit SVP gene research. This work was funded by the New Zealand Ministry of Business, Innovation & Employment, contract C10X0816 MeriNET. The authors declare no conflict of interest.

References

Anders S, Huber W. 2010. Differential expression analysis for sequence count data. Genome Biology 11, R106.

Bai S, Saito T, Sakamoto D, Ito A, Fuji H, Moriguchi T. 2013. Transcriptome analysis of Japanese pear (Pyrus pyrifolia Nakai) flower buds transitioning through endodormancy. Plant and Cell Physiology 54, 1132–1151.

Bai Y, Pattanaik S, Patra B, Werkman JR, Xie CH, Yuan L. 2011. Flavonoid-related basic helix-loop-helix regulators, NIA1a and NIA1b, of tobacco have originated from two ancestors and are functionally active. Planta 234, 363–375.

Bielenberg DG, Wang Y, Li ZG, Zhebentyayeva T, Fan SH, Reighard GL, Scorza R, Abbott AG. 2008. Sequencing and annotation of the evergrowing locus in peach [Prunus persica (L.) Batsch] reveals a cluster of six MADS-box transcription factors as candidate genes for regulation of terminal bud formation. Tree Genetics and Genomes 4, 495–507.

Brundell DJ. 1976. The effect of chilling on the termination of rest and flowering bud development of the Chinese gooseberry. Scientia Horticulturae 4, 175–182.

Campoy J, Ruiz D, Egea J. 2011. Dormancy in temperate fruit trees in a global warming context: a review. Scientia Horticulturae 130, 357–372.

Chang SJ, Puryear J, Cairney J. 1993. A simple and efficient method for isolating RNA from pine trees. Plant Molecular Biology Reporter 11, 113–116.

Cooke JE, Eriksson ME, Junttila O. 2012. The dynamic nature of bud dormancy in trees: environmental control and molecular mechanisms. Plant, Cell and Environment 35, 1707–1728.

Crowhurst RN, Gleave AP, MacRae EA, et al. 2008. Analysis of expressed sequence tags from Actinidia: applications of a cross species EST database for gene discovery in the areas of flavor, health, color and ripening. BMC Genomics 9, 351.

da Silveira Falavigna V, Porto DD, Buffon V, Margis-Pinheiro M, Pasquali G, Revers LF. 2013. Differential transcriptional profiles of dormancy-related genes in apple buds. Plant Molecular Biology Reporter 32, 796–813.

Díaz-Riquelme J, Ljajevtzy D, Martínez-Zapater JM, Carmona MJ. 2009. Genome-wide analysis of MikkCC-type MADS box genes in grapevine. Plant Physiology 149, 354–369.

Du Z, Zhou X, Ling Y, Zhang Z, Su Z. 2010. agrigO: A GO analysis toolkit for the agricultural community. Nucleic Acids Research 38, W64–W70.

Fabbroni C. 2009. Kiwifruit bud release from dormancy: effect of exogenous cytokinins. PhD thesis, The University of Bologna. http://amendottoro.unibo.it/1996/1/Fabbroni_Cristina.pdf.

Foster TM, Seleznynova AN, Barnett AM. 2007. Independent control of organogenesis and shoot tip ablation are key factors to developmental plasticity in kiwifruit (Actinidia). Annals of Botany 100, 471–481.

Gregis V, André F, Sessa A, et al. 2013. Identification of pathways directly regulated by SHORT VEGETATIVE PHASE during vegetative and reproductive development in Arabidopsis. Genome Biology 14, R56.

Gregis V, Sessa A, Colombo L, Kater MM. 2006. AGL24, SHORT VEGETATIVE PHASE, and APETALA1 redundantly control AGAMOUS during early stages of flower development in Arabidopsis. The Plant Cell 18, 1373–1382.

Han M, Gleave AP, Wang T. 2010. Efficient transformation of Actinidia arguta by reducing the strength of basal salts in the medium to alleviate callus browning. Plant Biotechnology Reports 4, 129–138.

Horsch R, Fry J, Hoffmann N, Eicholtz D, Rogers SG, Fraley R. 1985. A simple and general method for transferring genes into plants. Science 227, 1299–1321.

Horvath D. 2009. Common mechanisms regulate flowering and dormancy. Plant Science 177, 523–531.

Horvath DP, Chao WS, Suttle JC, Thimmapuram J, Anderson JV. 2008. Transcriptome analysis identifies novel responses and potential regulatory genes involved in seasonal dormancy transitions of leafy spurge (Euphorbia esula L.). BMC Genomics 9, 536.

Howe GT, Horvath DP, Dharmawardhana P, Priest HD, Mockler TC, Strauss SH. 2015. Extensive transcriptome changes during natural onset and release of vegetative bud dormancy in populus. Frontiers in Plant Science 6, 999.

Huang S, Ding J, Deng D, et al. 2013. Draft genome of the kiwifruit Actinidia chinensis. Nature Communications 4, 2640.

Jiménez S, Reighard GL, Bielenberg DG. 2010. Gene expression of DAM5 and DAM6 is suppressed by chilling temperatures and inversely correlated with bud break rate. Plant Molecular Biology 73, 157–167.

Lang GA, Early JD, Martin GC, Darnell RL. 1987. Endodormancy, paradoxydormancy, and ecodormancy—physiological terminology and classification for dormancy research. Hortscience 22, 371–377.

Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. Nature Methods 9, 357–359.

Le Bris M, Michaux-Ferrière N, Jacob Y, Poupet A, Barthe P, Guigonis J-M, Le Page-Degivry M-T. 1999. Regulation of bud dormancy by manipulation ofABA in isolated buds of Rosa hybrida cultured in vitro. Functional Plant Biology 26, 273–281.

Lee JH, Chung KS, Kim S-K, Ahn JH. 2014. Post-translational regulation of SHORT VEGETATIVE PHASE as a major mechanism for thermoregulation of flowering. Plant Signaling and Behavior 9, e28193.

Lee JH, Yoo SJ, Park SH, Hwang I, Lee JS, Ahn JH. 2007. Role of SVP in the control of flowering time by ambient temperature in Arabidopsis, Genes and Development 21, 397–402.

Leida C, Conesa A, Liácer G, Badenes ML, Ríos G. 2012. Histone modifications and expression of DAM6 gene in peach are modulated during bud dormancy release in a cultivar-dependent manner. New Phytologist 193, 67–80.

Li D, Liu C, Shen L, Wu Y, Chen H, Robertson M, Hellwell CA, Ito T, Meyerowitz E, Yu H. 2008. A repressor complex governs the integration of flowering signals in Arabidopsis. Developmental Cell 15, 110–120.

Li Z, Reighard GL, Abbott AG, Bielenberg DG. 2009. Dormancy-associated MADS genes from the EVG locus of peach [Prunus persica (L.) batsch] have distinct seasonal and photoperiodic expression patterns. Journal of Experimental Botany 60, 3821–3830.

Li ZM, Zhang JZ, Mei L, Deng XX, Hu CG, Yao JL. 2010. PISVP, an SVP homolog from trifoliate orange (Poncirus trifoliata L. Raf.), shows seasonal periodicity of meristem determination and affects flower development in transgenic Arabidopsis and tobacco plants. Plant Molecular Biology 74, 129–142.

Linsley-Noakes G, Allan P. 1987. Effects of winter temperatures on flower development in two clones of kiwifruit Actinidia delicosa (A. Chev.) CF Liang et AR Ferguson. Scientia Horticulturae 33, 249–260.

Lionakis SM, Schwabe W. 1984. Bud dormancy in the kiwi fruit, Actinidia chinensis planch. Annals of Botany 54, 467–484.

Liu C, Xi W, Shen L, Tan C, Yu H. 2009. Regulation of floral patterning by flowering time genes. Developmental Cell 16, 711–722.

Liu C, Zhou J, Bracha-Drori K, Kalovsky S, Ito T, Yu H. 2007. Specification of Arabidopsis floral meristem identity by repression of flowering time genes. Development 134, 1901–1910.

Liu G, Li W, Zheng P, Xu T, Chen L, Liu D, Hussain S, Teng Y. 2012. Transcriptomic analysis of “Sul” pear (Pyrus pyrifolia white pear group) buds during the dormancy by RNA-Seq. BMC Genomics 13, 700.

Mathisson K, He D, Grimplet J, Venkateswari J, Galbraith DW, Or E, Fennell A. 2009. Transcript profiling in Vitis riparia during chilling requirement fulfillment reveals coordination of gene expression patterns with optimized bud break. Functional and Integrative Genomics 9, 81–96.
Matsu T, Awa F, Espinoza C, et al. 2014. Inspection of the grapevine
BURP superfAMILY highlights an expansion of RD22 genes with distinctive
expression features during development and ABA-mediated stress
responses. PLoS One 9, e113072.

Mazzeliti L, Hancock RD, Haupts S, et al. 2007. Co-ordinated gene
expression during phases of dormancy release in raspberry (Rubus idaeus
L.) buds. Journal of Experimental Botany 58, 1035–1045.

McAtee PA. 2014. The transcriptional regulation of Actinidia chinensis
‘Hort16A’ fruit ripening. PhD thesis, The University of Auckland. https://
researchspace.auckland.ac.nz/handle/2292/23544.

Michaels SD, Ditt P, Gustafson-Brown C, Pelaz S, Yanofsky M, Amasino RM. 2003. AGL24 acts as a promoter of flowering in
Arabidopsis and is positively regulated by vernalization. The Plant Journal 33,
867–874.

Mimida N, Saito T, Moriguchi T, Suzuki A, Komori S, Wada M. 2015.
Expression of dormancy-associated mads-box (DAM)-like genes in apple.
Biologia Plantarum 59, 237–244.

Murashige T, Skoog F. 1962. A revised medium for rapid assays with
tobacco tissue cultures. Physiol. Plant 15, 473–479.

Nishitani C, Saito T, Ubi BE, Shimizu T, Itai A, Saito T, Yamamoto T,
Moriguchi T. 2012. Transcriptome analysis of Pyrus pyrifolia leaf
buds during transition from endodormancy to ecodormancy. Scientia
Horticulturae 147, 49–55.

Pattaika S, Kong Q, Zaitlin D, Werkman JR, Xie CH, Patria B, Yuan L. 2010. Isolation and functional characterization of a floral tissue-specific
R2R3 MYB regulator from tobacco. Planta 231, 1061–1076.

Porto DD, da Silveira Falavigna V, Arenhart RA, Perini P, Buffon F,
Anzanello R, dos Santos HP, Fialho FB, de Oliveira PRD, Revers LF. 2016. Structural genomics and transcriptional characterization of the
dormancy-associated MADS-box genes during bud dormancy progression
in apple. Tree Genetics and Genomes 12, 1–15.

Posé D, Verhage L, Ott F, Yant L, Mathieu J, Angenent GC, Immink RG, Schmid M. 2013. Temperature-dependent regulation of flowering by
antagonistic FLM variants. Nature 493, 414–417.

Rinne P, Tuominen H, Junttila O. 1994. Seasonal changes in bud
dormancy in relation to bud morphology, water and starch content, and
abscisic acid concentration in adult trees of Betula pubescens. Tree
Research 14, 549–561.

Rohde A, Bhalerao RP. 2007. Plant dormancy in the perennial context.
Trends in Plant Science 12, 217–223.

Rohde A, Ruttink T, Hostyn V, Sterck L, Van Driessche K, Boerjan W. 2007. Gene expression during the induction, maintenance, and release
of dormancy in apical buds of poplar. Journal of Experimental Botany 58,
4047–4060.

Ruttink T, Arend M, Morreel K, Storme V, Rombouts S, Fromm J,
Bhalerao RP, Boerjan W, Rohde A. 2007. A molecular timetable for
apical bud formation and dormancy induction in poplar. The Plant Cell 19,
2370–2390.

Sasaki R, Yamane H, Ooka T, Kotatsu H, Kitamura Y, Akagi T, Tao R. 2011. Functional and expression analyses of PrnDAM genes associated with
endodormancy in Japanese apricot. Plant Physiology 157, 485–497.

Shim D, Ko JH, Kim WC, Wang Q, Heatley DE, Han KH. 2014. A molecular framework for seasonal growth–dormancy regulation in
perennial plants. Horticulture Research 1, 14059.

Shinozaki K, Yamaguchi-Shinozaki K. 2000. Molecular responses to
dehydration and low temperature: differences and cross-talk between two
stress signaling pathways. Current Opinion in Plant Biology 3, 217–223.

Sniglar W, Hall A, McPherson H. 2008. Modelling flower formation of
kiwifruit (Actinidia delicosa) from winter chilling. New Zealand Journal of
Crop and Horticultural Science 36, 273–284.

Stacklies W, Redestig H, Scholz M, Walther D, Selbig J. 2007. pcaMethods—a bioconductor package providing PCA methods for
incomplete data. Bioinformatics 23, 1164–1167.

Tao Z, Shen L, Liu C, Liu L, Yan Y, Yu H. 2012. Genome-wide
identification of SOC1 and SVP targets during the floral transition in
Arabidopsis. The Plant Journal 70, 549–561.

Ubi BE, Sakamoto D, Ban Y, Shimada T, Ito A, Nakajima I,
Takemura Y, Tamura F, Saito T, Moriguchi T. 2010. Molecular
cloning of dormancy-associated MADS-box gene homologs and their
characterization during seasonal endodormancy transitional phases of
Japanese pear. Journal of the American Society for Horticultural Science
135, 174–182.

Jeno S, Klopp C, Leple JC, Deroy J, Noriot C, Léger V, Prince E,
Kremer A, Plomion C, Le Provost G. 2013. Transcriptional profiling
of bud dormancy induction and release in oak by next-generation
sequencing. BMC Genomics 14, 236.

Voogd C, Wang T, Varkonyi-Gasic E. 2015. Functional and expression analyses of kiwifruit SOC1-like genes suggest that they may not have a
role in the transition to flowering but may affect the duration of dormancy.
Journal of Experimental Botany 66, 4699–4710.

Walton EF, Podivinsky E, Wu RM. 2001. Bimodal patterns of floral gene
expression over the two seasons that kiwifruit flowers develop. Physiologia
Plantarum 111, 396–404.

Walton EF, Wu RM, Richardson AC, et al. 2009. A rapid transcriptional
activation is induced by the dormancy-breaking chemical cyanamide in
kiwifruit (Actinidia delicosa) buds. Journal of Experimental Botany 60,
3835–3848.

Wang T, Atkinson R, Janssen B. 2007. Choice of agrobacterium strain
for transformation of kiwifruit. Acta Horticulturae 929, 143–148.

Wang T, Karunametnam S, Wu R, Wang Y-Y, Gleave A. 2012. High
efficiency transformation platforms for kiwifruit (Actinidia spp.) functional
genomics. Acta Horticulturae 929, 143–148.

Wang T, Ran Y, Atkinson RG, Gleave AP, Cohen D. 2006.
Transformation of Actinidia eriantha: a potential species for functional
genomics studies in Actinidia. Plant Cell Reports 25, 425–431.

Wells CE, Vendramin E, Jimenez Tarado S, Verde I, Bielenberg DG.
2015. A genome-wide analysis of MADS-box genes in peach [Prunus
persica (L.) Batsch]. BMC Plant Biology 15, 41.

Winter D, Vinegar B, Nahal H, Ammar R, Wilson GV, Provart NJ.
2007. An ‘Electronic Fluorescent Pictograph’ browser for exploring and
analyzing large-scale biological data sets. PLoS One 2, e1718.

Wu R, Wang T, McGie T, Voogd C, Allan AC, Hellens RP, Varkonyi-
Gasic E. 2014. Overexpression of the kiwifruit SVP3 gene affects
reproductive development and suppresses anthocyanin biosynthesis in
petals, but has no effect on vegetative growth, dormancy, or flowering time.
Journal of Experimental Botany 65, 4985–4995.

Wu RM, Walton EF, Richardson AC, Wood M, Hellens RP, Varkonyi-
Gasic E. 2012. Conservation and divergence of four kiwifruit SVP-like
MADS-box genes suggest distinct roles in kiwifruit bud dormancy and
flowering. Journal of Experimental Botany 63, 797–807.

Yamaguchi-Shinozaki K, Shinozaki K. 1993. The plant hormone
abscisic acid mediates the drought-induced expression but not the seed-
specific expression of rd22, a gene responsive to dehydration stress in
Arabidopsis thaliana. Molecular and General Genetics 238, 17–25.

Yamane H. 2014. Regulation of bud dormancy and bud break in
Japanese apricot (Prunus mume Siebold & Zucc.) and peach [Prunus
persica (L.) Batsch]; a summary of recent studies. Journal of the Japanese
Society for Horticultural Science 83, 187–202.

Yamane H, Kashiwa Y, Ooka T, Tao R, Yonemori K. 2008. Suppression
subtractive hybridization and differential screening reveals endodormancy-
associated expression of an SVP/AGL24-type MADS-box gene in lateral
vegetative buds of Japanese apricot. Journal of the American Society for
Horticultural Science 133, 708–716.

Yamane H, Ooka T, Kotatsu H, Hosaka Y, Sasaki R, Tao R. 2011.
Expression regulation of PrpDM5 and PrpDM6, peach (Prunus
persica) dormancy-associated MADS-box genes, by low temperature and
dormancy-breaking reagent treatment. Journal of Experimental Botany 62,
3481–3488.

Zhententyayeva TN, Fan S, Chandra A, Bielenberg DG, Reighard
GL, Okie WR, Abbott AG. 2014. Dissection of chilling requirement and
bloom date QTLs in peach using a whole genome sequencing of sibling
trees from an F2 mapping population. Tree Genetics and Genomes 10,
35–51.

Zhong W, Gao Z, Zhuang W, Shi T, Zhang Z, Ni Z. 2013. Genome-
wide expression profiles of seasonal bud dormancy at four critical phases
in Japanese apricot. Plant Molecular Biology 83, 247–264.