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Auxin and cytokinin related gene expression during active shoot growth and latent bud paradormancy in *Vitis riparia* grapevine

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ABSTRACT

Cultural practices for canopy management in grapevines rely on intensive manipulation of shoot architecture to maintain canopy light levels. In contrast to common model plant systems used to study regulation of branch outgrowth, the grapevine has a more complex architecture. The node contains first, second and third order axillary meristems. The prompt bud (N + 1) develops into a summer lateral and a latent compound bud develops in the basal node of the summer lateral (N + 2, N + $3_{1,2}$). The outgrowth potential of latent buds was determined using common canopy management treatments (shoot tip decapitation and removal of summer laterals and leaves) and monitoring the rate of latent bud outgrowth. Two shoot node regions (apical and basal) with differential outgrowth potential were characterized and it was noted that the shoot tip, summer laterals and leaves in addition to node position contributed to the inhibition of latent bud outgrowth. To advance the understanding of the molecular regulation of bud outgrowth and paradormancy in the complex shoot architecture of grapevines, the expression of auxin and cytokinin genes involved in branching (amidase (*VrAMI1*), PINFORMED-3 (*VrPIN3*) and isopentenyl transferase (*VrIPT*)) were monitored in shoot tips and differentially aged buds of *Vitis riparia* grapevine shoots. In addition, Histone 3 (*VrH3*) and a hexose transporter (*VrHT1*) expression were monitored as a measure of tissue activity. The expression of *VrAMI1* and *VrPIN3* remained constant in actively growing shoot tips and decreased significantly with increasing bud maturation in paradormant buds. *VrHT1* expression was greater in buds than in any other plant tissue tested. *VrHT1* may have the potential to be used as an indicator of paradormancy status in grapevines. These characterizations in the complex architecture of the grapevine provide an excellent model system for molecular analysis of bud outgrowth and shoot architecture development.

Introduction

A developing shoot exerts control or apical dominance over its developing buds, inhibiting their outgrowth (Blazkova et al., 1999; Brown et al., 1967; Crabbe, 1984; Suzuki and Kitano, 1989). In woody plants this phase of bud development, termed paradormancy, is a transitional phase and an important component in the regulation of growth cycling and shoot architecture. During the growing season, axillary buds have the potential to grow if the shoot tip is damaged, the shoot angle is changed, or the shoot growth exceeds the apical dominance effect, allowing outgrowth of more distal buds. In temperate perennial plants paradormancy, or the inhibition of axillary bud outgrowth, is necessary to maintain the bud structure and promote its continued development for the next season's growth and flowering. At the end of the growing season, the paradormant buds transition into endodormancy, allowing buds to survive winter temperatures (Morrison, 1991).

Several studies in trees have investigated the relationship between paradormancy and bud outgrowth as influenced by the shoot tip and bud location (Crabbe, 1984; Suzuki et al., 1990; Suzuki and Kitano, 1989). However, these studies focused on simple architectural shoot systems where the phytomers consist of a node with an overwintering or latent bud in the leaf axil. In grapevines, the shoot architecture is more complex in that the phytomers consist of a node containing a prompt bud on which the latent or overwintering bud develops. The grapevine prompt bud is an axillary bud or first order meristem (N + 1), that is morphologically homologous to the meristem in a typical axillary bud. However, the prompt bud does not contain bud scales and usually grows out in the same growing season as it develops. As the prompt bud grows, reiterating the pattern of the main shoot, it is called a summer lateral (Fig. 1).

Abbreviations: ABA, abscisic acid; AMI1, amidase 1; HT1, hexose transporter; H3, Histone 3; IAA, indolyl-acetic acid; IPT, isopentenyl transferase; PIN3, PINFORMED-3.

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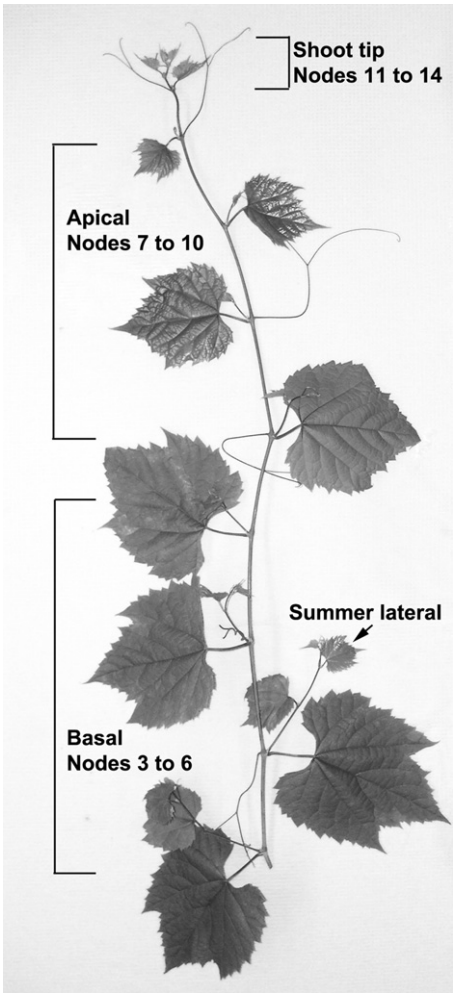

Fig. 1. Shoot morphology and node positions. Apical buds (nodes 7–10); basal buds (nodes 3–6).

The overwintering bud is the result of a latent bud developing in the axil of the basal leaf of the prompt lateral (Gerrath and Posluszny, 2007; Mullins et al., 1992). This bud is a second order axillary bud (N + 2) and two additional axillary buds form in its basal leaves and are third order axillary buds ($N + 3_{1,2}$), (May, 2000; Pratt, 1974). Thus the overwintering bud is a compound bud made up of meristems derived from morphologically different orders on the shoot. In the following spring a new shoot grows from the latent bud, and the prompt buds develop rapidly in the leaf axils. As the shoot continues to increase in node number, the prompt buds grow into lateral shoots and the latent buds develop at the basal node of the summer laterals.

In contrast to most trees, grapevines are pruned extensively, annually removing the majority of the previous year's growth; therefore the canopy structure is dependent upon the annual shoot architectural development. In grapevines this shoot architecture is strongly influenced by environment and genotype, and shoot pruning, leaf removal and summer lateral thinning used to manage the grapevine canopy (Keller et al., 2010; Lebon et al., 2004; Pallas et al., 2008). These cultural practices also influence the potential outgrowth of paradormant buds that are needed for the next growing season's shoot and crop production.

The greatest advances in understanding auxin and cytokinin interactions relative to bud outgrowth and shoot architecture have been addressed in herbaceous plants systems (Muller and Leyser, 2011). Auxins have been shown to play a role in budbreak induced by shoot bending and application of cytokinins and cytokinin-like compounds can induce bud break in woody plants (Abo-Hamed et al., 1981; Jackson, 2003; Or, 2009; Williams, 2000). However, there has been little investigation of the auxin and cytokinin regulation of the grapevine's complex architecture in relation to latent bud outgrowth versus maintenance of latent bud paradormancy. This study tests the tissue specific and temporal response of candidate genes known to influence axillary bud outgrowth potential in a grapevine species used in breeding programs for disease and pest resistance (Hemstad and Luby, 2000).

Table 1
Tissue removal treatments to determine bud outgrowth potential.

Treatment	Tissue removed	Node position from base of shoot (Fig. 1)
D	Four node shoot tip	11–14 plus apical tip
A	Summer laterals	7–10
B	Summer laterals	3–6
DA	D + A	11–14 plus apical tip + 7–10
DB	D + B	11–14 plus apical tip + 3–6
DAB	D + A + B	Apical tip + 3–14
DLAB	D + A + B	Apical tip + leaves and summer laterals at 3–14

Materials and methods

Plant material

Five to six-year-old-spur-pruned *Vitis riparia* Michx. ecodormant grapevines were root pruned and potted in 201 pots containing soil/peat/perlite (at 1:2:2 by volume) medium in climate controlled growth chambers (PGW36, Conviron, Winnepeg, Canada) with a 15 h photoperiod (450–500 μmol/s PPF) and 25/20 °C day/night temperatures. After budbreak, emerging shoots were thinned to two per vine. Plants were watered every other day and fertilized weekly with a complete nutrient solution (Prolific 20–20–20).

Bud outgrowth potential

After shoots reached a height of 14 nodes, grapevines were randomly assigned to control or one of seven tissue removal treatments (Table 1, Fig. 1). Rate of latent bud outgrowth was monitored using the bud stage scale of Coombe (1995), which ranges from stage 00 to stage 08. Three bud stages were monitored to determine bud break timing: stage 00 (no visible activity), stage 03 (wool emerging from the bud) and stage 05 (leaf tips emerging from the bud). After treatments were imposed, timing of latent bud outgrowth (budbreak) at nodes three to ten was monitored daily for 21 d in four replicates of each treatment.

Effects of treatment and bud position on the probability of bud break were evaluated by a logistic model. Analysis of shoot development data was conducted using general linear models. All linear regressions were performed by SAS software (http://www.sas.com/).

Temporal and spatial gene expression analysis

Grapevines used for the gene expression studies were grown in climate controlled growth chambers as described in bud outgrowth study. It should be noted that the initial length of the shoot was 14 nodes; however, the shoot continued to grow for 21 d. The node position was therefore standardized from the base of the shoot. The shoot tip is always the apex and four nodes indicated in Fig. 1, and