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Digital expression profiling of a grape-bud EST collection leads to new insight into molecular events during grape-bud dormancy release

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Abstract

The application of genomic approaches may serve as an initial step in broadening our understanding of the complex network of biochemical and cellular processes responsible for the regulation and execution of grape-bud dormancy release. However, bud tissue in general, and the dormant bud in particular, are under-represented in the public *Vitis* genomic resources. Here we describe a large-scale grape-bud EST collection representing a wide range of bud developmental stages. A collection of 5516 consensus sequences is presented, of which 59% were not included within the *Vitis* TIGR collection at the time of current analysis. About 22% of these transcripts bear no resemblance to any known plant transcript and thus corroborate the need for this targeted EST collection. The added value of the presented EST collection lies in the conferred ability to compare EST frequencies between the different cDNA libraries. Such comparison was implemented and allowed us to identify several genes/functions whose expression is altered in response to the dormancy-release treatment. Based on this analysis, it is suggested that oxidative stress, calcium signaling, intracellular vesicle trafficking and anaerobic mode of carbohydrate metabolism play a role in the regulation and execution of grape-bud dormancy release.

Keywords: Vitis; Bud dormancy; Oxidative stress; Vesicle trafficking; Carbohydrate metabolism; Expressed sequence tag (EST)

1. Introduction

The buds of temperate woody plants, including grapevine, undergo a dormancy cycle during the winter, which is induced by decreasing photoperiod and/or temperatures [1–7], and ends when plants have received adequate chilling temperatures and are exposed to favorable growing conditions [8–11].

In warm-winter regions, prolonged dormancy is a major obstacle for commercial production of temperate fruits, including grapevine, which is widely distributed in subtropical regions [10,12–14]. At present, the control of dormancy release by the use of artificial dormancy-breaking compounds is needed to compensate for lack of natural chilling and is indispensable for maintaining economic production of table grape in these regions [14–16]. However, the currently available effective compounds are both costly and entail a risk of bud damage due to their phytotoxicity [14,16,17].

An understanding of the biological mechanisms involved in bud-dormancy release is crucial for the manipulation of bud-break timing. Although extensive studies have been performed on various physiological aspects of dormancy [10,11,18–24], a characterization of the complex network of biochemical and cellular processes responsible for the regulation and execution of bud-dormancy release has not yet been achieved [25].

Application of hydrogen cyanamide (HC), which is the most useful dormancy-breaking agent for grape [12,15–17,26], provides a controlled system for the analysis of dormancy release in grape buds, with a relatively uniform response of the

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bud population. The controlled induction timing and the uniform response make this system an optimal tool for accurately defining the actual timing of induction of dormancy release, thereby enabling the detection of early changes following this induction [17,27–29].

The mechanism by which HC exerts its dormancy-breaking effect is not clear, but it has been shown that both catalase expression and activity are inhibited in grape buds shortly after HC application, leading to the accumulation of hydrogen peroxide [28,30–31]. We recently described GDBRPK, a transcript for a sucrose non-fermenting (SNF)-like protein kinase that is up-regulated following chemical induction of dormancy release by HC. We also described the simultaneous and remarkable transient induction of transcripts encoding pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) shortly after HC application. Based on these findings, we speculated that HC application leads to transient respiratory stress, which likely results in a temporary increase in the AMP-to-ATP ratio [28]. Since AMP is known as a stress signal that is sensed by SNF-like kinases, we suggested that the SNF-like GDBRPK could serve as the sensor of this signal [28,29]. However, the identification of such changes in expression patterns of single genes provides only partial clues to the possible involvement of specific metabolic pathways in the dormancy process.

To gain a broader perspective on the complex biochemical network responsible for the regulation and execution of the dormancy process, much more detailed insight is needed into the coordinated induction (or repression) of cassettes of metabolic pathways that act together during dormancy release in grape buds. An initial step in that direction is the application of genomic approaches to the characterization of alterations in gene expression following controlled endodormancy release. The large *Vitis* EST collection in the TIGR database and the *Vitis vinifera* Affymetrix microarray are important tools for grape genomics. Nevertheless, bud tissue in general, and the dormant bud in particular, are under-represented within these resources. Therefore, we generated an EST collection from grape buds that consists of randomly selected clones from four different cDNA libraries originated from young and mature buds at various developmental stages. In this work, we present a collection of 5516 consensus sequences, of which 59% were not included within the *Vitis* TIGR collection at the time of analysis. About 22% of these transcripts bear no resemblance to any known plant transcript, corroborating the need for this targeted EST collection.

An added benefit of the current collection lies in the conferred ability to compare EST frequencies between the different cDNA libraries. Such comparison was implemented and exposed genes/functions whose expression is induced/reduced in response to the dormancy-release treatment.

2. Materials and methods

2.1. Plant material

The experiments were conducted with mature, cordon-trained grapevines (*Vitis vinifera* cv. Perlette and cv. Superior) from commercial vineyards. All plants were subjected to the cultural practices commonly used in such vineyards. Mature buds were collected from canes originated from a cv. Perlette vineyard in the central Jordan Valley. Young buds were collected from green shoots of cv. Superior vines from a commercial vineyard located in the plain of Judea.

2.2. Collection of mature buds

2.2.1. Natural dormancy cycle

From November to January, vines at a cv. Perlette vineyard in the Jordan Valley were pruned at 2-week intervals to three-node spurs and the detached canes, each containing nine buds (in positions 4–12) were transferred to the lab. Canes were cut into single-node cuttings that were randomly mixed. Ten groups of 10 single-node cuttings were prepared on each collection date, placed in water and then forced for 21 days at 23 °C under a 12/12 h photoperiod. The bud-break percentages after 21 days were used to express the dormancy depth of the vines on the collection date as previously described [28]. Bud break was defined as the stage at which green tissue is visible beneath the bud scales. Buds were harvested from the detached canes from each collection date and were immediately transferred to liquid nitrogen and stored at −80 °C.

2.2.2. Chemical induction of dormancy release

Dormancy-breaking treatments were applied in mid-December. Vines were pruned as already described and the detached canes were divided into two groups. Canes of one group were sprayed with 5% Dormex, a commercial formulation containing 49% HC, and those of the other group were sprayed with water and served as controls. Both Dormex and water were sprayed with the addition of a 0.02% (v/v) Triton X-100 wetting agent. Sprayed canes were cut into single-node cuttings that were randomly mixed and placed in water in a growth chamber at 23 °C under neutral 12/12 h light/dark photoperiod. Buds were harvested from both HC-treated and control cuttings at selected time points after HC application (3, 6, 12 and 24 h, 2 day and 4 day), and were immediately transferred to liquid nitrogen and stored at −80 °C.

To assess the effect of treatment on dormancy release, 10 groups of 10 single-node cuttings were prepared from control and HC-treated canes and then forced, under the conditions described above. Bud-break percentages were calculated for 21 days after HC application.

2.2.3. Collection of young buds

Green buds were harvested from the axiles of the leaves at position 3–8 on young shoots of *Vitis vinifera* cv. Superior vines. Collection was started in March, 4 weeks after bud burst, and continued at weekly intervals throughout bud development until veraison, in mid-June.

2.3. Extraction of RNA and purification of poly(A)$^+$ RNA

RNA was extracted from frozen bud samples using a modified CTAB-LiCl protocol [32]. PolyA-mRNA was purified