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Shotgun proteomic analysis of photoperiod regulated dormancy induction in grapevine

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ABSTRACT

Certain grapevine genotypes become dormant in response to decreasing photoperiod and others require low temperature or both environmental cues to induce dormancy. This study used a proteomic approach to gain an understanding of the underlying molecular events involved in bud dormancy commitment. Two F_2 siblings (F2-110 and F2-040) with differences in photoperiod induced dormancy responsiveness were subjected to long day (LD, 15 h, paradormancy maintenance or dormancy inhibition) or short day (SD, 13 h, endodormancy commitment) treatment. Proteins were extracted at two time points (28 days and 42 days) of LD and SD photoperiod exposure, and label-free quantitative shotgun proteomic analysis was performed for three biological replicates of each treatment and time point. A total of 1577 non-redundant proteins were identified in the combined dataset of eight different conditions (2 genotypes, 2 photoperiods and 2 timepoints, available via ProteomeXchange with identifier PXD001627). Genotype specific patterns of budbreak and protein expression were detected in response to the differential photoperiod treatment at the two time points. Peroxidases, dehydrogenases and superoxide dismutases were more abundant at 42 SD than at 28 SD in the dormancy responsive F2-110, suggesting that oxidative stress response related proteins could be markers of endodormancy commitment in grapevine buds.

1. Introduction

Grapes are a prominent and economically important temperate fruit crop. Wine regions worldwide are spread across various climatic zones, contributing to the diversity of viticulture. Grapevines (*Vitis* spp.) grown in temperate continental regions are exposed to characteristic cold and dry winters which induce winter dormancy. Growth cessation and winter dormancy is a complex developmental process programmed in response to annual environmental cues, which is important in breeding new grapevine cultivars suitable for continental climates. Daylength, temperature and water availability are vital abiotic environmental signals for flowering, growth synchronization and dormancy induction in grapevine [1,2]. Daylength, also referred to as photoperiod, is the length of light exposure to plants. Daylength changes throughout the year according to geographic latitudes and seasons. It is one of the key environmental cues that grapevines employ to recognize seasonal changes. Studies of *V. riparia* and hybrid cultivar Seyval indicate that *V. riparia* responds to decreasing photoperiod by becoming dormant and increasing in freezing tolerance whereas Seyval requires low temperature and decreasing photoperiod to induce dormancy [1–8]. *V. riparia* is native to North America and is well adapted to northern temperate climates. It is phylloxera resistant and is extensively used as a commercial rootstock and in rootstock and scion breeding. Seyval, a hybrid wine cultivar, is derived from a complex hybridization of *Vitis vinifera* and *Vitis rupestris* and is also phylloxera resistant. Growth is maintained and buds are paradormant in long photoperiods in both genotypes [2,5]. *V. riparia* responds quickly to SD at warm temperatures, exhibiting growth cessation, shoot tip abscission and bud endodormancy induction within 28 days of short photoperiods in contrast to Seyval which remains paradormant under the same SD treatment and requires the addition of low temperature to induce endodormancy. Gene expression studies show specific molecular pathways are activated during the transition from paradormancy to endodormancy in different *Vitis* species [5,9–11]. Although the influence of photoperiod induced differential gene expression has been studied in different *Vitis* species by transcriptomic analysis the proteomics approach has been applied to shoots, but not to explore bud dormancy in grapevine [6]. A F2 genetic model system derived by selfing a single F1 (*V. riparia* x Seyval) that segregates for photoperiod responsiveness has been for identification of regulatory mechanisms involved in grape bud dormancy for breeding and mapping programs [3,11,12]. This mapping population provides the ability to explore dormancy induction in

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siblings as opposed to the more widely divergent species level differences. The availability of this grapevine genetic model system and mass spectrometry based proteomics provides a powerful tool for studying the underlying molecular networks and biological processes, to enhance our understanding of paradormancy and endodormancy regulation in grapevine. Gel based electrophoresis techniques have been predominantly used for proteomic studies in grapevine [13–18] with a few exceptions which involve shotgun proteomics techniques such as iTRAQ [19–22] and label-free quantitation techniques [23]. Proteomics analysis of grapevine buds has been limited to investigations using one and two-dimensional (2D) gel electrophoresis to study bud development process [2,13]. In addition, many of the published proteomic studies are limited by the fact that they did not have access to a complete grape genome sequence; rather, they used databases compiled from all available plant species [14,17,19,22], or *Vitis* expressed sequence tags (ESTs) [15,16] for protein identification. These approaches work reasonably well, but do not always present a complete picture. The availability of a grape genome assembly [24,25] enables high throughput mass spectra approaches with searches against sequence data thus providing improved protein coverage and identification. Sequence availability coupled with shotgun quantitative proteomics provides powerful tools for protein identification from complex mixtures. Shotgun label-free proteomics is renowned for its accuracy, reproducibility, high throughput [26,27] and is cheaper, less time-consuming, and less labour intensive when compared to 2-D gel based proteomic approaches. This facilitates the identification of the presence and absence of proteins and differences in protein abundance, between multiple samples. Quantitative shotgun proteomics has been used to study protein interactions in Cabernet Sauvignon grapevines exposed to water deficit conditions [23] and extreme hot and cold temperatures [26]; however, it has not been employed in grapevine bud dormancy analyses. Previous transcriptomic studies have shown strong differences in gene expression during a temporal cycle of grapevine bud paradormancy to endodormancy. We hypothesise that the changes in gene expression will provide distinct differences in protein expression related to the dormancy phases. Here we use the first shotgun label-free quantitative proteomic analysis of F2 siblings (F2-040 and F2-110) with differential dormancy induction characteristics to identify and quantify proteins specific to paradormancy and endodormancy induction.

F2-110 and F2-040 were exposed to two different photoperiods of long day (LD, 15 h, paradormancy maintenance or endodormancy inhibiting) and short day (SD, 13 h, endodormancy inducing) and proteins from age matched buds were analysed at two different time points of differential photoperiod treatment using Filter Aided Sample Preparation (FASP) [28,29] coupled with gas phase fractionation (GPF) [30,31]. Quantitation was based on spectral counting using Normalised Spectral Abundance Factors (NSAF) [32,33]. Examining the molecular processes which are triggered by different photoperiods will enhance our understanding of the signalling networks involved in dormancy induction and growth cessation. This will aid in identification of potential molecular markers for dormancy phase description and could facilitate breeding of grapevine cultivars with appropriate dormancy timing mechanisms for a changing climate.

2. Materials and methods

2.1. Growth of plant material and imposition of photoperiod treatments

A mapping population of 141 individuals of F_2 hybrids were developed by selfing single F_1 plants from a cross between a North American grapevine species *Vitis riparia* (USDA PI 588289) and a hybrid *Vitis* cultivar 'Seyval' (Seyve-Villard 5-276) as described in [1,3]. Two siblings from this population, F2-110 and F2-040, each representing the photoperiod response phenotype most like the grandparents (*V. riparia* and Seyval respectively) for dormancy induction were selected and propagated for this study. One hundred twenty plants each of two different genotypes; F2-110 ('*V. riparia* like') and F2-040 ('Seyval like') were generated. Potted, spur-pruned 2- to 6-year-old vines of F2-110 and F2-040 were removed from cold storage and grown in long photoperiod (LD, 15 h) at 25/20 ± 3 °C day/night temperatures with 600–1400 mol $m^{-2} s^{-1}$ photosynthetic photon flux in a climate-controlled unshaded glass greenhouse (En Tech Control Systems Inc., Montrose, Minn.) in Brookings, South Dakota (44.3 N). Vines were grown in 19 L pots at 1 pot per 0.4 m^2 with four shoots trained vertically. When the grapevines reached 12–15 nodes (30 days post bud break), they were randomized into two groups for photoperiod treatments: LD or SD (15 h, paradormancy maintenance or dormancy inhibition and 13 h, dormancy induction, respectively). Five days after randomization (35 days post bud break) the differential photoperiod was imposed. Plants continued with LD and the SD photoperiod treatment was imposed under the same temperature conditions. SD was imposed using an automated white-covered black-out system (Van Rijn Enterprises Ltd., Grassie, Ontario). Each experimental unit was composed of ten vines and there were three replicates/plots for each genotype and time point in each photoperiod treatment. Buds were harvested for each experimental unit replicate between 8:30 and 11:30 a.m. at 28 and 42 days of the LD or SD treatments. Buds were harvested from nodes 3 to 12 from the shoot base. Fresh weight and dry weight were determined for node 5 from each plant (ten buds per replicate) and bud percent water content (%WC) was determined (fresh weight – dry weight/ fresh weight). The number of nodes of periderm were counted from shoot base in each treatment. The buds for protein extraction were immediately frozen in liquid nitrogen and stored at −80 °C for future RNA and metabolite extraction. A total of 24 samples resulted (i.e. three biological replicates of F2-110 LD, F2-110 SD, F2-040 LD, F2-040 SD harvested at 28 d and 42 d respectively). Decapitated vines were returned to LD and monitored for bud break at 7 and 14 days. Bud break was indicated at modified Eichorn - Lorenz stage 4 as described in [34]. Percent bud break for all buds remaining on the vine were recorded at 7 and 14 days post harvest.

2.2. Protein extraction and protein assay

Approximately 1 g (fresh weight) of bud samples were ground in liquid nitrogen and proteins were extracted using the phenol-extraction protocol as described in [17]. Proteins were precipitated using methanol-chloroform [35]. Protein concentration was determined by the Pierce BCA protein assay (Thermo, San Jose, CA).

2.3. In-solution digestion and peptide extraction

Protein pellets were digested in-solution by a modified Filter Aided Sample Preparation (FASP) method as described in [28,31]. Protein extracts (250 µg) were dissolved in 200 µL 50% TFE, 0.1 M NH_4HCO_3, 50 mM DTT, heated (50 °C, 20 min) and concentrated to 20 µL in Amicon Ultra 0.5 mL 30 K ultrafiltration devices (Millipore). An aliquot of 100 µL 50% TFE, 0.1 M NH_4HCO_3, 50 mM iodoacetamide was added, incubated in the dark for 1 h at room temperature and centrifuged (14,000*g*, 45 min). Alkylated proteins were washed using 200 µL of 50% TFE, 0.1 M NH_4HCO_3 (four times), centrifuged (14,000*g*, 45 min), and the flow through was discarded. To the ~20 µL retentates in the ultrafiltration devices, 1 µL of 0.25 µg/µL Lys-C (Sigma) and 24 µL of 50% TFE, 0.1 M NH_4HCO_3 was added and incubated overnight at 30 °C. Trypsin digestion followed Lys-C digestion by addition of 2.5 µL of 1 µg/µL trypsin (Promega), 350 µL 20% acetonitrile (ACN), 50 mM NH_4HCO_3 and incubation at 37 °C for 8 h. The reaction was stopped with 10 µL 50% formic acid and resulting peptides were centrifuged into new ultrafiltration receptacles (14,000*g*, 45 min). This was followed by two rinses of the ultrafiltration devices using 100 µL 50% ACN, 2% formic acid and centrifugation (14,000 *g*, 45 min). Each extract was dried in a Speedvac to near dryness and reconstituted with 60 µL 2% TFE, 2% formic acid.