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Research article

Comparison of distinct transcriptional expression patterns of flavonoid biosynthesis in Cabernet Sauvignon grapes from east and west China

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ABSTRACT

Flavonoids make a very important contribution to the organoleptic qualities of grapes and wines. In this work these were analyzed in Cabernet Sauvignon grown in Changli, Hebei Province in east China and Gaotai, Gansu Province in west China. These regions have distinctly different climates contributing to their different 'terroir'. RNA sequencing was performed to trace transcriptome changes in Cabernet Sauvignon berries at pea size, veraison and ripening, corresponding to E-L 31, 35 and 38. The accumulation of flavonols, flavan-3-ols and anthocyanins together with the expression of relevant genes were analyzed and compared between the two regions. The biosynthesis patterns were similar between two regions, but more flavonols, anthocyanins, and tri-hydroxylated flavonoids accumulated in grapes from Gaotai before berry harvest, possibly due to the higher transcript levels of the genes that encode biosynthetic enzymes and their potential candidate transcription factors. The lower levels of flavan-3-ols, mainly (−)-epigallocatechin, in the pre-veraison grapes from Changli, might be due to limited flow of carbon to the F3′5′H branch pathway, as the ratio of *F3′5′H* to *F3′H* was lower in these berries from Changli. It is suggested that the combination of climatic factors profoundly affect the flavonoid pathway in grapes from China, providing regionally specific metabolism patterns.

1. Introduction

Flavonoids play an important role in the interaction between the plants and their environment, protecting them against various biotic and abiotic stresses (Bohm, 1998). In addition, these compounds in wine grapes have attracted much attention for their important influence on the sensory quality of wine, as well as their potential contribution to human health through their antioxidant, antimicrobial, antiviral, and anticarcinogenic characteristics (Dixon et al., 2005).

Flavonoids are biosynthesized in plants through the widely-distributed secondary metabolic phenylpropanoid and flavonoid pathways. The major classes of flavonoid compounds found in grape berries include flavonols, flavan-3-ols (proanthocyanidins) and anthocyanins, which share a common upstream pathway involving some key enzymes, such as phenylalanine ammonia-lyase (PAL; EC:4.3.1.24), cinnamic acid 4-hydroxylase (C4H; EC:1.14.13.11), 4-coumarmate: CoA ligase (4CL; EC:6.2.1.12), chalcone synthase (CHS; EC:2.3.1.74) and chalcone isomerase (CHI;

Abbreviation: ANR, anthocyanidin reductase; C, (+)-catechin; C4H, cinnamic acid 4-hydroxylase; 4CL, 4-coumarmate: CoA ligase; CHS, chalcone synthase; CHI, chalcone isomerase; DEGs, Differentially expressed genes; DFR, dihydroflavonol 4-reductase; EC, (−)-epicatechin; ECG, (−)-epicatechin-3-O-gallate; EGC, (−)-epigallocatechin; FDR, False Discovery Rate; F3H, flavanone 3-hydroxylase; F3′H, flavonoid 3′-hydroxylase; F3′5′H, flavonoid 3′, 5′-hydroxylase; FLS, flavonol synthase; GDD, growingdegree-days; GST, glutathione-S-transferase; LAR1, leucoanthocyanidin reductase 1; LDOX, leucoanthocyanidin dioxygenase; PA, proanthocyanidin; PAL, phenylalanine ammonia-lyase; RAD, radiation dose of sunshine; STS, stilbene synthase; UFGT, UDP glucose-flavonoid 3-O-glucosyltransferase; RNA-Seq, RNA sequencing; WAF, weeks after flowering.

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EC:5.5.1.6). Flavonols are thought to contribute greatly to the wine color as cofactors for the anthocyanin copigmentation (Boulton, 2001). Flavan-3-ols are the second most abundant natural phenolic compounds after lignin. They can exist as polymeric proanthocyanidins (PAs) and are compounds of great importance to wine quality due to their astringent, bitter properties, and their contribution to long-term color stability (Vidal et al., 2003). In *Vitis vinifera* grapes, the principal individual anthocyanins are acylated and non-acylated 3-*O*-monoglucosides of delphinidin, cyanidin, petunidin, peonidin and malvidin. Both the amount and the proportions in their different structural forms are responsible for the color of red grapes and the resultant wines.

The influence of grape variety on composition and content of flavonoids has been studied widely (Monagas et al., 2003). However, even grapes of the same variety can have distinctive flavonoid profiles as a result of different environmental factors (Downey et al., 2006). Unlike some countries, China has a very scattered distribution of wine-growing regions, with a distance of over 2000 km from east to west as well as from south to north and there are altitude differences of over 2000 m. Thus, the climatic conditions vary greatly among the wine-making regions of China. Changli County, which is located in Hebei province (East China), is characterized by a wetter and warm climate, while Gaotai County is located in Gansu province some 1600 km to the west (West China) and has a cooler, drier climate.

In our previous work, we compared phenolic compounds in Cabernet Sauvignon wines from five different wine-growing regions in China, and found that wines from northwestern regions presented great differences in phenol profiles when compared to the wines from East regions (Li et al., 2011). To a large extent, the regional differences between the wines were caused by the complex process of grape berry development, which was controlled by the transcriptional regulation of mRNA. Therefore, it is useful to understand the molecular basis that controls berry development and consequently determines grape quality. It will provide a clearer understanding of grape metabolic mechanisms, and especially the effects of the environment on the quality of wine grapes. For this purpose many researchers have been encouraged to investigate the transcriptome and metabolome composition in developing berries through modern molecular biotechnology. In recent years, as the availability of the grapevine genome (Jaillon et al., 2007) and the development of novel high-throughput sequencing technologies such as RNA sequencing technology (RNA-Seq) (Mortazavi et al., 2008) has emerged as the first sequencing-based method to permit the entire transcriptome to be surveyed in a rapid and quantitative manner.

In the present study, the transcriptional profiles of the flavonoid biosynthesis pathway and the accumulation pattern of flavonoid compounds in Cabernet Sauvignon berries from the 2010 vintage, grown in Hebei Changli and Gansu Gaotai, were analyzed by RNA-Seq and high performance liquid chromatography–electrospray tandem mass spectrometry (HPLC-ESI-MS/MS) technology, respectively. Comparative analysis of the transcript and metabolite profiles in Cabernet Sauvignon berries between the two regions revealed region-specific patterns of flavonoid biosynthesis. These results provided a valuable insight into the regional effects on the quality of wine grapes in China and will contribute to the further research into flavonoid biosynthesis in wine grapes.

2. Material and methods

2.1. Plant material

This study was carried out in 2010 in nonirrigated vineyards located in Changli county (119°20′E; 39°48′N, clay sandy soil), Hebei province, and Gaotai county (99°41′E; 39°18′N, sandy soil), Gansu province of China. The vineyard in Changli is located on the plain with an altitude of about 212 m, and it is located on the desert edge zones at the altitude of about 1377 m in Gaotai. Cabernet Sauvignon (*V. vinifera* L.) vines (own-rooted) in both of the vineyards were planted in 2001, trained to a vertical shoot-positioned, arranged in north-south rows spaced 2.5 m × 1 m (inter and intrarow), and spur pruned cordon trellis with a bud load of ~12 nodes per meter of row length. The cordon was trained 75 cm aboveground with four pair of surmounting catch wires for a canopy wall extending ~1.2 m above the cordon. Berries were collected from 3 WAF (weeks after flowering) to commercial harvest (16 WAF or 17 WAF) according to the method described as follows. For each sample, three biological replicates were performed. As one biological replicate, 300-berry samples were selected randomly from at least one hundred clusters at similar positions from fifty whole vine selections. The samples were divided in three parts and 100 berries were processed immediately in order to monitor berry ripening, another 100 berries (without seeds) were stored at −80 °C for subsequent RNA extraction, and the rest had their skins removed by hand. The latter were then ground in liquid N_2 using a mortar, and were freeze dried using a LGJ-10 freeze drier at −50 °C. These skin powders were stored frozen at −40 °C in preparation for HPLC analysis of the flavonoids.

2.2. Determination of physiological parameters

Total soluble solids (°Brix) were assayed with a digital pocket refractometer (Atago, Tokyo) from juice crushed from every berry sample. Titratable acidity (g/L) of the grape juice was measured by titration to an endpoint of pH 8.4 with a strong base (0.5 M NaOH). All analyses were performed in triplicate.

2.3. RNA extraction and Illumina sequencing

Total RNA of whole berries (wihtout seeds) that had been finely ground in liquid nitrogen, were extracted using a Plant Total RNA Extraction Kit (Sigma Corporation, Sigma–Aldrich, Bornem, Belgium). RNA content and quality were assessed with an Agilent 2100 Bioanalyzer, and RNA integrity was confirmed by electrophoresis on 1% agarose gels containing formaldehyde. Total RNA from three biological replicates were pooled to generate one lane for each cDNA library construction. After extracting the total RNA from the samples, mRNA from six total extracts (~20 μg RNA) were pooled and enriched using oligo (dT) magnetic beads. After adding the fragmentation buffer, the mRNA was cleaved into short fragments of about 200 bp. The first strand cDNA was synthesized by random hexamer-primer using the mRNA fragments as templates. Then the buffer, dNTPs, RNase H and DNA polymerase I were added to synthesize the second strand. The double strand cDNA was purified with QiaQuick PCR extraction kit and washed with EB buffer for end repair and single nucleotide A (adenine) addition. Finally, sequencing adaptors were ligated to the fragments. The required fragments were purified by agrose gel electrophoresis and enriched by PCR amplification. The library products were ready for sequencing analysis via Illumina HiSeq™ 2000, which was performed by "HuaDa Gene" (http://www.genomics.org.cn/). Clean reads were mapped to reference sequences using SOAPaligner/soap2 (Li et al., 2009). A maximum of 2 mismatched bases were allowed in the alignment. The gene expression level was calculated by using RPKM (reads per exon kilo base per million mapped sequence reads) (Mortazavi et al., 2008). If there was more than one transcript for a gene, the longest one was used to calculate its expression level and coverage.