RESEARCH PAPER

Molecular analysis of post-harvest withering in grape by AFLP transcripational profiling

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Abstract

Post-harvest withering of grape berries is used in the production of dessert and fortified wines to alter must quality characteristics and increase the concentration of simple sugars. The molecular processes that occur during withering are poorly understood, so a detailed transcriptomic analysis of post-harvest grape berries was carried out by AFLP-transcriptional profiling analysis. This will help to elucidate the molecular mechanisms of berry withering and will provide an opportunity to select markers that can be used to follow the drying process and evaluate different drying techniques. AFLP-TP identified 699 withering-specific genes, 167 and 86 of which were unique to off-plant and on-plant withering, respectively. Although similar molecular events were revealed in both withering processes, it was apparent that off-plant withering induced a stronger dehydration stress response resulting in the high level expression of genes involved in stress protection mechanisms, such as dehydrin and osmolite accumulation. Genes involved in hexose metabolism and transport, cell wall composition, and secondary metabolism (particularly the phenolic and terpene compound pathways) were similarly regulated in both processes. This work provides the first comprehensive analysis of the molecular events underpinning post-harvest withering and could help to define markers for different withering processes.

Key words: AFLP-TP, gene expression, grape berry withering, on- and off-plant withering processes.

Introduction

The study of grape development and post-harvest maturation is of great interest to plant biologists, providing particular insight into the genetic and environmental factors controlling berry ripening and the organoleptic properties of wine (Conde et al., 2007; Deluc et al., 2007; Grimplet et al., 2007; Pilati et al., 2007). Berries for sweet dessert wines (e.g. Recioto, Vin Santo) and dry fortified wines (e.g. Amarone) undergo a phase of post-harvest dehydration which can last up to 3 months, where metabolism is modified significantly and the sugar content increases (Kays, 1997). In post-harvest berries, the rate of water loss induces cell wall enzyme activity, increases respiration and ethylene production, and causes the loss of volatiles and changes in polyphenol levels (Hsiao, 1973; Bellincontro et al., 2004; Costantini et al., 2006). Air drying and its impact on turgor pressure also leads to major changes in fruit structure and texture, such as softening, a change in superficial cell architecture, the reduction of intercellular space, and cell squeezing (Ramos et al., 2004).

Studies of metabolic changes in Malvasia, Trebbiano, and Sangiovese grapes during post-harvest drying revealed that berry cells undergo an initial water stress response at 10–12% weight loss, characterized by the accumulation of abscisic acid (ABA), proline, and lipoxygenase. A second dramatic change in metabolism occurs at >19% weight loss, characterized by the accumulation of proline and an increase in alcohol dehydrogenase (ADH) activity. This two-step metabolism leads initially to the formation of C6 compounds, ethanol and acetaldehyde, which subsequently decrease due to the
formation of ethyl acetate (volatile acidity) (Costantini et al., 2006).

At the molecular level, very little is known about the post-harvest phase of fruit ripening, and the only previous studies in grape relate to the modulation of stilbene synthase and phenylalanine ammonia lyase genes (Versari et al., 2001; Tonutti et al., 2004). The aim of this study was to determine whether the known enzymatic and hormonal activities in withering grape berries reflect changes at the mRNA level. Gene expression profiles characterizing the on- and off-plant withering process in Vitis vinifera cv. Corvina were studied by amplified fragment length polymorphism-transcriptional profiling (AFLP-TP).

Materials and methods

Plant material and total RNA extraction
Clusters of Vitis vinifera cv. Corvina (clone 48) were harvested over the course of the 2003 growing season from an experimental vineyard in the Verona Province (San Floriano, Verona, Italy). Berries were sampled at eight time points from early fruit-set until the completion of withering (Table 1). The post-harvest ripening phase was analysed by sampling clusters directly from plants (on-plant withering) or by collecting clusters picked from the plant on the same date (off-plant withering) and stored in a special, naturally-ventilated room or ‘fruttaio’ lacking a controlled environment (Table 1).

Eight clusters were collected for each sampling time-point (about 1 kg). Five hundred berries were sampled from different positions of the eight clusters, discarding rotten or small undeveloped berries. Skin and flesh of 100 berries were separated, discarding seeds, and immediately frozen. The 400 remaining berries were weighed; weight percentages of on- and off-plant withering samples were calculated in comparison to the weight of the ripening sample (Table 1). The sugar content of the juice obtained from ripening and on- and off-plant withering berries was measured using a bench refractometer PR-32 (Atago Co., Ltd, Tokyo, Japan). Total RNA was extracted from skin and flesh samples according to Rezaian and Krake (1987).

AFLP-TP analysis

AFLP-based transcript profiling (AFLP-TP) (Breyne et al., 2003) was carried out starting from 10 μg of total RNA (half from the skin and half from the flesh) and using restriction enzymes BstYI and MseI (New England Biolabs, Beverly, MA, USA). For pre-amplification, a MseI primer without a selective nucleotide was combined with a BstYI primer containing a T or a C as a selective nucleotide at the 3' end. The pre-amplified samples were diluted 600-fold and 5 μl were used for the final selective amplifications with a BstTC primer with one more selective nucleotide (BstT0: 5'-GAC TGC GTA GTG ATC T-3' and BstC0: 5'-GAC TGC GTA GTG ATC C-3') and an MseI primer (Mse0: 5'-GAT GAG TCC TGA GTA A-3') with two selective nucleotides. All 128 possible primer combinations were used. Selective γ[^3P]ATP-labelled amplification products, were separated on a 6% polyacrylamide gel using the Sequigel system (Bio-Rad, Hercules, CA). Dried gels were exposed to Biomax films (Kodak, Rochester, NY). The mean number of fragments amplified with one primer combination was 75.

Differentially-expressed transcripts were identified by visual inspection of autoradiographic films and their profiles were visually scored (on a scale from −2 to 2; see Supplementary Table S2 at JXB online). Hierarchical clustering was carried out using a complete linkage algorithm and the Pearson correlation as a distance measure (Michael Eisen, Stanford University) (http://rana.lbl.gov/EisenSoftware.htm). Bands corresponding to differentially-expressed transcripts were excised from the gels and eluted in 100 μl distilled water. DNA was re-amplified under the conditions described above and purified on MultiScreen plates (Millipore, Billerica, MA, USA) prior to sequencing (BMR Genomics) (http://bmr.criibi.unipd.it). The tag sequences were used for BLASTN and BLASTX (Altschul et al., 1990) searches against the DFCI Genome database (http://compbio.dfci.harvard.edu/cgi-bin/tgi/tgi/index.pl;?gudb=grape) and the non-redundant UNIPROT database (http://www.expasy.uniprot.org), respectively, using an E-value cut-off of 5×10^-5. Gene Ontology terms (http://www.geneontology.org) were assigned to each sequence using the BLASTN and BLASTX results.

Real-time RT-PCR analysis

The transcriptional profiles of six AFLP-TP tags were analysed by real-time RT-PCR experiments using the SYBR® Green PCR master mix (Applied Biosystems, Foster City, CA, USA) and the Mx3000P® Real-Time PCR system (Stratagene, La Jolla, CA, USA). Gene-specific primers were designed for the six tags using the sequence information of the same tags and of the corresponding TC. A primer pair was also designed for TCS5334, encoding an actin protein. Primer sequences are listed in Supplementary Table S1 at JXB online. The real-time RT-PCR analysis was performed in a 25 μl reaction volume using a final primer concentration of 300 nM and cDNA synthesized from 40 ng of total RNA, in three replicates for each reaction. The PCR began with a 50 °C hold for 2 min and a 95 °C hold for 10 min followed by 40 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 20 s. Non-specific PCR products were identified by the dissociation curves. The amplification efficiency was calculated from raw data using LingRegPCR software (Ramakers et al., 2003). The relative expression ratio value was calculated for development time points and withering time points relative to the first sampling time point (post-fruit-set; PFS) according to the Pfaffl equation (Pfaffl, 2001). SE values were calculated according to Pfaffl et al. (2002).

Results and discussion

AFLP-TP analysis

AFLP-TP, a gel-based transcript profile method, is a genome-wide transcriptional analysis with some
advantageous features over microarrays. No prior sequence information is required for AFLP-TP analysis, the low start-up cost and its high specificity allow analysing the expression profile of genes with high homology (Vuylsteke et al., 2007). The procedure of purification, amplification, and sequencing of tags required by AFLP-TP analysis is time-consuming, labour-intensive and cannot be automated. However, the gene discovery possibility of AFLP-TP is still an important advantage which can complement the recently obtained genomic informations (French-Italian Public Consortium for Grapevine Genome Characterization, 2007; Velasco et al., 2007). For these reasons, an AFLP-TP analysis was used to obtain a large-scale description of the transcriptional changes of grapevine berries during withering, a process uncharacterized up to now. Other aspects of grape berry development have been investigated by microarray analysis, such berry ripening under normal and water stress conditions (Terrier et al., 2005; Waters et al., 2005; Deluc et al., 2007; Grimplet et al., 2007; Pilati et al., 2007; Lund et al., 2008).

Eight sampling times were chosen during the 2003 Vitis vinifera cv. Corvina growing season, four covering the entire period of berry development (Table 1) and up to four covering the subsequent 99 d post-ripening period (Table 1). In the latter case, two different withering processes, one on-plant and one off-plant, were considered. For the on-plant withering process, only the first three sampling points were used, due to the poor quality of the berries at the final stage (Table 1).

The kinetics of the withering processes was monitored by evaluating weight loss and the sugar content of berry juice (Table 1). For on-plant withering, a negligible weight loss was recorded (Table 1) because grape clusters connected to the shoot are not subjected to intense dehydration. The observed increase in sugar concentration is mainly due to the over-ripening process (Table 1).

During the 2003 growing season, temperature values higher than the seasonal average values and lower rainfall were recorded in the sampling area. These climatic conditions influenced berry development and resulted in the anticipated ripening. Similar conditions, recorded for the autumn season, could have affected withering and, in particular, dehydration, which characterizes the off-plant withering process.

AFLP-TP analysis was performed mixing an equal amount of total RNA extracted from skin and flesh tissues for each sampling time-point, to overcome problems related to RNA extraction efficiency. RNA yields from skin and flesh tissues could be negatively affected by polyphenol and sugar contents which, moreover, change during the berry development and withering processes. Because RNA extracted from whole berries derives from unknown quantities of skin and flesh RNAs and because these can be differently affected by the extraction procedure during the analysis, it was decided to mix equal amounts of skin and flesh RNAs and to maintain the same total RNA quantity over the whole experiment. Although this procedure can introduce some bias, it is believed that these are preferable to the analysis of an unknown and varying RNA content of samples.

The expression of approximately 9600 transcripts, representing almost one-third of the protein-coding genes predicted in the grapevine genome (French-Italian Public Consortium for Grapevine Genome Characterization, 2007), was analysed using 128 different BstYI+1/MseI+2 primer combinations for selective amplification. Among these transcripts, 2093 were found to be differentially expressed during berry development and/or withering. The differentially expressed tags were excised from the gels, and 1829 were successfully re-amplified by PCR using the appropriate selective AFLP-TP primers (data not shown). The PCR products yielded 1267 good-quality sequences which were used for BLASTN and BLASTX searches against the DFCI Grape Gene Index database (http://compbio.dfci.harvard.edu/cgi-bin/tgi/gimain.pl?gudb=grape) and the UNIPROT database (http://www.expasy.uniprot.org), respectively (see Supplementary Table S2 at JXB online). Gene Ontology terms were assigned to the sequences and were used to organize them into major functional categories (see Supplementary Fig. S1 at JXB online). No matches were found for 225 sequences.

Cluster analysis
The expression profiles of the 2093 differentially-expressed transcripts were visually scored relative to the first sampling time point which was arbitrarily attributed a zero value. Hierarchical clustering analysis was performed using a Pearson correlation (uncentred) distance and complete linkage clustering based on the scores from the four developmental and four post-harvest (off-plant withering) sampling points. Twelve main clusters were identified and their mean expression profiles are shown in Fig. 1.

Clusters 1 (10.51%) and 2 (6.36%) represent genes induced in early and late development, respectively, whereas clusters 3 (13.71%) and 4 (10.99%) represent genes specifically induced during early and late withering, respectively. Cluster 5 (5.64%) represents genes that are expressed transiently during withering. Clusters 6 (27.66%) and 9 (10.46%) represent genes that are repressed during early and late development, whereas clusters 8 (6.36%) and 10 (2.34%) represent genes that are specifically repressed during early and late withering, respectively. Cluster 7 (1.00%) represents genes that are transiently repressed during ripening and the first stage of withering. Cluster 11 (0.67%) represents genes that are repressed during late berry development but induced at the onset of withering. Finally, cluster 12 (4.3%) is the...
The reciprocal of cluster 11, i.e. genes up-regulated in late development but repressed during withering.

**Real-time RT-PCR experiments**

The expression profiles of six randomly-selected differentially-expressed genes were confirmed by real-time RT-PCR experiments using the same RNA samples. The analysis was carried out for the four development time points (PFS, PRV, V, and R) and for the three time points common to both withering processes (WI, WII, and WIII) (Fig. 2). The six tags represented an avr9/cf-9 rapidly-elicited protein, a cytosolic ascorbate peroxidase, a DNA-binding protein, a glutathione S-transferase, a MLO-like protein, and an SOS2-like protein kinase. The real-time RT-PCR expression profiles were similar to the profiles obtained by AFLP-TP (Fig. 2).

**Changes in gene expression during off-plant withering**

AFLP-TP analysis of grape samples allowed us to identify a number of transcripts specifically modulated during the
post-harvest withering process, i.e. those in clusters 3 and 4 (induced during early and late withering, respectively) and clusters 8 and 10 (repressed during early and late withering, respectively). These genes accounted for 33.4% of all differentially expressed transcripts, with an approximate 3:1 ratio of up-regulated to down-regulated genes. For each cluster, a list of tags with homology to sequences with known functions was prepared (Tables 2, 3, 4, 5).

Analysis of the AFLP-TP transcripts specifically modulated during berry dehydration allowed a model for the...
molecular compounds. These pathways involve a variety of enzymes, many of which are involved in the metabolism of phenolic compounds. These enzymes include cytochrome P450 mono-oxygenase, cytochrome oxidase, and glutathione-S-transferase, which are involved in the metabolism of various phenolic compounds.

The Phenolic compounds section is highly detailed, providing a comprehensive overview of the metabolic pathways involved in the production of various phenolic compounds in grapes. The section details the metabolic pathways involved in the production of stilbenes, such as resveratrol, and the metabolism of phenylpropanoids, such as flavonoids and lignans. The section also discusses the role of various enzymes, such as cytochrome P450 mono-oxygenase and cytochrome oxidase, in the metabolism of these compounds.
Table 3. Annotated cDNA-AFLP-TP tags from cluster 4

| Description | Accession\(^a\) | E value\(^b\) |
|-------------|----------------|-------------|
| Secondary metabolic process: phenylpropanoid biosynthetic process | | |
| 4-Coumarate-CoA ligase-like protein | TC57438 | 3.40E-30 |
| Phenylalanine ammonia-lyase | TC69585 | 9.91E-32 |
| Secondary metabolic process: lignan metabolic process | | |
| Dirigent-like protein pDIR14 | TC62196 | 2.02E-55 |
| Secretory laccase | TC54354 | 1.54E-19 |
| Secondary metabolic process: stilbene metabolic process | | |
| Resveratrol synthase | TC52907 | 7.14E-47 |
| Stilbene synthase | NPI227286 | 7.56E-52 |
| Stilbene synthase | TC59572 | 6.24E-09 |
| Stilbene synthase | TC60946 | 3.05E-04 |
| Secondary metabolic process: terpenoid metabolic process | | |
| Ligninoid UDP-glucosyltransferase | TC65435 | 1.30E-09 |

Response to stimulus

| Description | Accession\(^a\) | E value\(^b\) |
|-------------|----------------|-------------|
| Avr\(^9\)/Cl-9 rapidly elicited protein | CA813698 | 1.79E-46 |
| Dehydrin 1a | TC61998 | 3.14E-32 |
| Disease resistance response protein | Q9LID5 | 5.29E-35 |
| Syringoid induced protein | Q8S901 | 6.16E-08 |
| Metabolic process: translation | | |
| Ethylene response factor | TC52148 | 6.60E-74 |
| Ethylene-responsive element binding protein | TC629802 | 1.25E-04 |
| Eukaryotic initiation factor 4B | Q9MT7E8 | 6.90E-26 |
| RING-finger-like protein | CB920519 | 1.25E-17 |
| SUPERMAN-like zinc finger protein | TC60860 | 8.85E-16 |
| WRKY6 | TC59548 | 4.10E-10 |
| Metabolic process: translation | | |
| 26S ribosomal RNA | TC70629 | 2.03E-24 |
| 40S ribosomal protein S12 | Q9XHS0 | 6.27E-09 |
| 60S ribosomal protein L3 | Q65076 | 4.64E-17 |
| Hamamelis virginiana large subunit | TC65768 | 6.70E-12 |
| Protein synthesis initiation factor 4G | TC67911 | 1.23E-80 |
| Ribosomal protein L3 | Q1RYN6 | 4.39E-17 |
| S15 ribosomal protein | Q8L4R2 | 5.00E-04 |

Metabolic process: protein metabolic process

| Description | Accession\(^a\) | E value\(^b\) |
|-------------|----------------|-------------|
| 22.0 kDa class IV heat shock protein precursor | P30236 | 3.00E-04 |
| PLANT UBX DOMAIN-CONTAINING PROTEIN 2 | TC67882 | 5.00E-14 |
| SKP1 | TC57098 | 2.92E-33 |
| Ubiquitin-protein ligase | TC64169 | 3.03E-69 |
| Cellular component organization and biogenesis | | |
| H4 NEUCR Histone H4 | TC52370 | 8.27E-29 |

Transport

| Description | Accession\(^a\) | E value\(^b\) |
|-------------|----------------|-------------|
| Aspartate aminotransferase | TC55957 | 4.45E-51 |
| Aspartate aminotransferase | CB006657 | 4.90E-08 |
| Chloroplast outer membrane protein | Q56W37 | 3.00E-10 |
| Copper-transporting P-type ATPase | TC64839 | 4.10E-11 |
| Hexose transporter | Q3L7K6 | 9.00E-12 |
| Major facilitator superfamily MFS 1 | TC61509 | 8.98E-27 |
| Secretion protein HlyD | TC60298 | 9.43E-39 |
| Secretory carrier-associated membrane protein 1 | TC52744 | 5.01E-05 |
| Succinate transporter-like protein | TC51830 | 3.18E-22 |

Metabolic process

| Description | Accession\(^a\) | E value\(^b\) |
|-------------|----------------|-------------|
| Dopamine \(\beta\)-mono-oxygenase | TC62500 | 8.00E-09 |
| N-terminal domain-containing protein | | |
| Fructose-bisphosphate aldolase | TC54602 | 3.55E-77 |
| LED1-5c protein | TC61395 | 9.25E-31 |
| Lipoxygenase | QWSGS53 | 2.03E-04 |
| Phosphoglycerate kinase, cytosolic | TC52072 | 9.61E-102 |

Table 3. Continued

| Description | Accession\(^a\) | E value\(^b\) |
|-------------|----------------|-------------|
| Plasticid aldolase | TC59070 | 5.66E-22 |
| Ribose-5-phosphate isomerase | TC59181 | 8.03E-91 |
| Transaldolase | Q6H706 | 3.39E-16 |
| Trehalose-phosphate phosphatase | TC67690 | 1.50E-06 |
| Biological regulation | | |
| Response regulator 6 (TypeA response regulator 9) | TC62852 | 5.97E-41 |
| Biological process | | |
| Calcium-binding allergen | TC63220 | 4.05E-31 |
| Germin-like protein | TC52213 | 2.10E-06 |
| L. esculentum protein with leucine zipper | TC54217 | 2.00E-36 |

\(^a\) Accession number (DFCI Grape Gene Index, UNIPROT ID).
\(^b\) E-value from BLASTN and BLASTX searches.

seeds and are also produced in berry skin during development, and in response to biotic or abiotic stresses (Soleas et al., 1997). Significant resveratrol accumulation occurs during the post-harvest drying of berries of many grape cultivars, and this has already been linked to the high-level expression of stilbene synthase (STS) (Celotti et al., 1998; Tornielli, 1998; Versari et al., 2001). Given that STS is also induced during on-plant withering (see Supplementary Table S2 at JXB online), these results indicate that the induction of the expression of many STS genes is a characteristic of the berry post-ripening phase.

Among the up-regulated withering-specific transcripts, one chalcone isomerase (CHI) gene (TC55034) and two tags homologous to polyphenol oxidase (TC52784) (Table 2) were identified (Table 2). The transcriptional profile of the first gene suggests an activation of the flavonoid pathway during the withering process, while the transcriptional profile of the second one indicates a probable oxidation/polymerization of phenolic compounds.

Few previous studies have considered the production of phenolics in grape skin during the post-harvest drying process, and there is some conflict about the abundance of such compounds, with some reports citing a general reduction (Di Stefano et al., 1997; Borsa and Di Stefano, 2000) and others a general increase (Bellincontro et al., 2001). Given that STS is also induced during on-plant withering (see Supplementary Table S2 at JXB online), these results taken together, these results suggest that, in addition to the stilbene synthesis, some classes of flavonoids may also be produced during the withering process.

Small- and large-scale gene expression studies have already been performed on grapes under preharvest water-deficit stress (Castellarin et al., 2007a; b; Grimplet et al., 2007). Preharvest water-deficit stress does not necessarily cause a cell osmotic stress in berry tissues which is likely to occur during the post-harvest dehydration process analysed in this work. Although physiological events associated with pre- and post-harvest developmental stages are different, a similar positive modulation of genes involved in the phenylpropanoid pathway in lignin and...
Table 4. Annotated cDNA-AFLP-TP tags from cluster 8

| Description | Accession\(^a\) | E-value\(^b\) |
|-------------|----------------|--------------|
| **Secondary metabolic process:** flavonoid metabolic process | | |
| Anthocyanidin-3-glucoside | TC70498 | 6.46E-37 |
| shammossyltransferase | | |
| **Response to stimulus** | | |
| TMV response-related gene product | TC57457 | 1.00E-40 |
| Thiorodoxin domain-containing protein 9 | TC56954 | 5.48E-76 |
| **Metabolic process: transcription** | | |
| HMG-I and HMG-Y, DNA-binding protein | Q1RZ01 | 4.61E-06 |
| MYB-like DNA-binding domain protein | TC52565 | 1.89E-08 |
| Putative VP1/ABI3 family regulatory protein | O04546 | 1.03E-11 |
| Similarity to metallothionein-I gene transcription activator | Q2FLM8 | 7.38E-06 |
| **Metabolic process: translation** | | |
| 30S ribosomal protein S16 | TC53445 | 4.62E-06 |
| 60S ribosomal protein L12 | TC52607 | 7.57E-37 |
| **Metabolic process: protein metabolic process** | | |
| Pepino A | TC57841 | 1.15E-43 |
| Probable prefoldin subunit 5 | TC58696 | 5.13E-72 |
| Putative tyrosine phosphatase | Q5ZDE0 | 3.53E-22 |
| S-locus receptor-like kinase RLK14 | CB971388 | 7.00E-06 |
| **Transport** | | |
| ADP ribosylation factor 002 | TC51848 | 1.18E-68 |
| Putative cytchrome b\(\_\_\_\_\) | Q27064 | 2.79E-25 |
| Receptor-like protein kinase-like | TC54030 | 9.74E-71 |
| **Metabolic process** | | |
| Acyl-ACP thioesterase | TC60833 | 6.76E-17 |
| \(\alpha\)-Glucan water dikinase | TC54189 | 2.23E-45 |
| ATP/GTP nucleotide-binding protein | Q9FII8 | 4.00E-06 |
| \(\beta\)-Mannan endohydrolase | TC56962 | 2.80E-05 |
| \(\beta\)-ketol reductase | TC53435 | 9.70E-10 |
| C-type cytochrome biogenesis protein | TC68921 | 8.01E-08 |
| CDP-diacylglycerol-glycerol-3-phosphate-3-phosphatidyltransferase | TC64058 | 4.87E-06 |
| Diaminopimelate decarboxylase | TC68200 | 2.71E-64 |
| tHMG-CoA synthase 2 | TC68763 | 1.22E-22 |
| Ketal-acid reductoisomerase, | TC68860 | 4.77E-61 |
| chloroplast precursor | | |
| Long-chain acyl-CoA synthetase | TC59981 | 9.30E-31 |
| Molybdenum cofactor biosynthesis | TC70221 | 9.50E-43 |
| Phospholipid reductase | TC69028 | 5.77E-41 |
| Photosystem I reaction center subunit | TC53444 | 2.58E-56 |
| N. chloroplast precursor | | |
| Pyrophosphate-dependent | | |
| phosphofructo-1-kinase | TC70414 | 9.19E-100 |
| Ribonuclease HII | Q53QG3 | 2.41E-06 |
| Transaldolase ToTL2 | TC59186 | 1.24E-14 |
| **Biological process** | | |
| CaLB protein | P92940 | 1.00E-07 |
| Cellular retinaldehyde-binding/triple function, C terminus | TC55679 | 3.20E-14 |
| Cyclin dependent kinase inhibitor | TC52886 | 1.27E-50 |
| DREPP4 | TC70411 | 3.58E-37 |
| Fasciclin-like AGP-12 | TC51953 | 1.50E-30 |
| Nucleotide binding | TC67441 | 7.32E-08 |
| RNA binding | TC62986 | 7.15E-68 |
| tRNA-Ala tRNA-Ile 16S tRNA | TC60315 | 6.60E-36 |
| tRNA-Val rps12 rps7 ndhB | TC52339 | 2.41E-32 |

\(^a\) Accession number (DFCI Grape Gene Index, UNIPROT ID).
\(^b\) E-value from BLASTN and BLASTX searches.

Table 5. Annotated cDNA-AFLP-TP tags from cluster 10

| Description | Accession\(^a\) | E-value\(^b\) |
|-------------|----------------|--------------|
| **Response to stimulus** | | |
| Putative metallophosphatase | Q8VXF6 | 5.33E-25 |
| Thiorodoxin-like protein | TC65381 | 1.84E-43 |
| **Metabolic process: transcription** | | |
| MADS-box transcription factor | TC51812 | 2.76E-04 |
| **Metabolic process: protein metabolic process** | | |
| Serine/threonine protein phosphatase | Q2QM47 | 1.40E-12 |
| **Cellular component organization and biogenesis** | | |
| Actin-like | TC58881 | 3.14E-09 |
| Cellulose synthase-like protein | TC55634 | 1.56E-13 |
| **Transport** | | |
| ATP synthase γ chain | TC68806 | 3.78E-76 |
| **Biological process** | | |
| Acetyl-CoA thioesterase | TC55739 | 1.29E-48 |
| Carbonate dehydratase | Q8X4X9 | 1.42E-20 |
| Phosphoribosylformylglycinamidine | TC69079 | 1.01E-55 |
| Pyruvate kinase | TC62731 | 7.03E-20 |
| 3-adenosyl methionine synthase-like | TC70287 | 1.71E-23 |
| Cyclic nucleotide phosphodiesterase | CB918027 | 1.74E-14 |
| Heterogeneous nuclear ribonucleoprotein | TC62660 | 3.06E-33 |
| A2/B1-like | | |
| Neurofilament-H related protein | CD801715 | 6.50E-07 |

\(^a\) Accession number (DFCI Grape Gene Index, UNIPROT ID).
\(^b\) E-value from BLASTN and BLASTX searches.

stilbene biosynthesis was observed in skin tissues of ripening berries in response to water-deficit stress (Grimp-let et al., 2007), and during berry post-harvest withering. On the other hand, preharvest water stress accelerated ripening and induced the expression of flavonoid structural genes during berry development (Castellarin et al., 2007a, b), while the water stress caused by dehydration characterizing the off-plant withering had a minor influence on the flavonoid pathway.

**Terpenoid compounds**

Terpenoids contribute to the aroma of grapes and their products including wine (Lund and Bohlmann, 2006). AFLP-TP showed that two transcripts with homology to a limonoid UDP-glucosyltransferase (TC65435) were induced during the post-harvest drying (Tables 2, 3). In citrus fruits, limonoid UDP-glucosyl transferase catalyses the conversion of bitter tasting limonin to limonoid glucoside (Kita et al., 2000). There is no evidence for the presence of limonin in grape berries, but it is possible that this gene is involved in the modification of other terpenes or in the production of secondary metabolites and hormones (Kita et al., 2000).

A tag representing hydroxymethylglutaryl-CoA synthase (TC68763) was shown to be repressed during withering (Table 4). This enzyme is involved in the synthesis of hydroxymethylglutaryl-CoA (HMG-CoA),
which can be converted into isoprenoids via the mevalonate pathway (Sirinupong et al., 2005). These data suggest that the late terpene biosynthetic pathway is up-regulated whereas the production of terpene precursors is inhibited. A repression at ripening of a transcript encoding a key enzyme of the non-mevalonate IPP biosynthetic pathway, the 1-deoxy-d-xylulose 5-phosphate synthase was reported in grape berries under water-deficit stress (Grimplet et al., 2007).

**Cell wall metabolism**

Previous reports have described the expression patterns of cell wall-modifying enzymes during berry development and ripening, as well as concomitant changes in cell wall composition (Nunan et al., 1998, 2001; Vidal et al., 2001; Doco et al., 2003; Grimplet et al., 2007). There is no direct evidence for modification of the berry cell wall structure and composition during off-plant drying, but the increase in polyphenolic compounds reported in some studies (Tornielli et al., 2005; Pinelo et al., 2006) might depend on cell wall degradation. AFLP-TP analysis revealed the down-regulation of only two withering-specific tags putatively involved in cell wall metabolism, encoding a cellulose synthase (TC55634) and a β-mannan endohydrolase (TC67062) (Tables 4, 5).

**Response to stress**

It has recently been shown that berry ripening results in the accumulation of transcripts related to biotic and abiotic stress responses (Deluc et al., 2007; Pilati et al., 2007). Among the withering-specific AFLP-TP tags, there were transcripts encoding a gag-pol polyprotein (TC69867), a non-LTR reverse transcriptase (CD007484), and a reverse transcriptase (TC51865) (Table 2). These data suggest that an increase in transposable element activity is one component of the stress response to berry withering. Many transposable elements have been identified in the grapevine genome (Verriès et al., 2000; Pelsy et al., 2002; Pereira et al., 2005; French-Italian Public Consortium for Grapevine Genome Characterization, 2007; Velasco et al., 2007) and cis-acting sequences in the LTR of elements Tnt1, Tto1, and Vne-1 could be involved in the activation of defence genes in response to stress conditions (Grandbastien, 1998; Verriès et al., 2000).

Dehydration is likely to be the major stress factor affecting grape berries after harvest, since they lose over 30% of their weight through evaporation during off-plant ripening (Table 1). The up-regulation of DHN1a, encoding dehydrin 1a (TC61998), and of a trehalose-phosphate phosphatase mRNA (TC67690) (Table 3), supports this theory, since plant dehydrins counteract the water stress that occurs in cold, frost, drought, and saline conditions (Sanchez-Ballesta et al., 2004; Rorat, 2006). In *Vitis riparia* and in *V. vinifera*, DHN1a is induced in response to cold, drought, and ABA treatment (Xiao and Nassuth, 2006). This gene could protect the berry during the late withering stages, together with the increased production of trehalose by trehalose-phosphate phosphatase (Table 3) since increased trehalose levels protect *Escherichia coli* from stress including drought (Garg et al., 2002). The up-regulation of a sorbitol related enzyme (TC58983) (Table 2) could positively affect the synthesis of this sugar with a protective role against water stress in plant (Tao et al., 1995).

One transcript encoding a lipoygenase (Q8GSM3), an enzyme involved in the synthesis of C6 volatile compounds and signalling molecules that respond to stress (Croft et al., 1993), was isolated among the tags specifically induced in late withering (Table 3). During Malvasia grape berry drying, an increase in lipoygenase activity and the concomitant production of C6 compounds such as hexen-1-ol, hexanal, and (E)-hex-2-enal was reported (Costantini et al., 2006).

It has been suggested that grape ripening, unlike tomato and strawberry, is not accompanied by the induction of oxidative stress response genes (Terrier et al., 2005). However, an oxidative burst characterized by H₂O₂ accumulation duration *véraison* and by the modulation enzymes that scavenge reactive oxygen species (ROS) was recently described during berry development (Pilati et al., 2007). The AFLP-TP analysis identified two tags, encoding a cytosolic ascorbate peroxidase (TC51718) and a glutathione cytosolic reductase (TC53088), which were up-regulated during post-harvest drying (Table 2). This suggests that the post-ripening phase is characterized by oxidative stress and the corresponding response. Such response may not require the involvement of two thioredoxin-like proteins given that the corresponding transcripts (TC56954; TC63581) were down-regulated during withering (Tables 4, 5).

Despite the absence of pests and diseases, several genes involved in biotic stress responses were also induced during withering, including the *STS* genes discussed above. Other early-induced genes identified by AFLP-TP analysis included transcripts homologous to *Arabidopsis thaliana* MLO-like protein 6 (Q94KB7) and potato systemic acquired resistance-related protein SRE1a (TC61558) (Table 2). The involvement of MLO proteins in resistance to powdery mildew was reported in barley (Peterhänsel and Lahaye, 2005). Delayed induction was observed for other defence gene tags including those related to *A. thaliana* Avr9/Cf-9 rapidly elicited protein (CA813698) (Durrant et al., 2000), soybean syringolide-induced protein (Q8S901) which is induced in soybean cells treated with *Pseudomonas syringae* elicitors (Hagihara et al., 2004) and an *A. thaliana* disease resistance response protein (Q9LID5) (Table 3). A TMV response-related gene product (TC57457) was shown to be repressed during withering (Table 4).

Genes related to the general stress response, such as a sorbitol related enzyme, an Avr9/Cf-9 rapidly elicited
protein, and a disease resistance gene were also induced in ripening berries of grape plants in water-deficit conditions (Grimplet et al., 2007).

**Carbohydrate transport and metabolism**

Our AFLP-TP experiment showed that VvHT5 (Q3L7K6), which encodes a hexose transporter (HT) located in the plasma membrane (Hayes et al., 2007), is up-regulated late in the withering process (Table 3). This indicates that hexose transport, reported to be strongly active during ripening (Hayes et al., 2007), is probably also active during withering. Such activity may be responsible for the transport of sugars in different subcellular compartments.

The solute concentration in ripening berries increases in part due to water loss (Costantini et al., 2006; Di Stefano et al., 1997), but reactions related to hexose aerobic/anaerobic respiration, hexose conversion to malate, gluconeogenesis, and malate respiration might also increase during post-harvest drying (Zironi and Ferrarini, 1987; Bellincontro et al., 2006; Chkaiban et al., 2007).

The analysis showed that transcripts encoding glycolytic enzymes like aldolase (TC54602; TC59070) and phosphoglycerate kinase (TC52072) were up-regulated (Table 3), whereas a pyruvate kinase (TC60979) was repressed (Table 5) along with phosphoenolpyruvate carboxykinase (TC60028), which is involved in gluconeogenesis (Table 4). Taken together these results suggest that hexoses could be metabolized via the pyruvate pathway or conversion into malate, even if no transcripts directly involved in the latter pathway were identified, while de novo synthesis of such compounds seems to be inhibited.

**Ethylene metabolism**

Berry development is characterized by a weak spike in ethylene production around véraison with a concomitant increase in the activity of 1-aminocyclopropane-1-carboxylic acid oxidase, the enzyme responsible for the last step of ethylene biosynthesis (Chervin et al., 2004). Exogenous ethylene application affects the production of phenols and anthocyanins, and influences the aromatic quality of Aleatico berries, so ethylene is likely to be involved in the post-harvest withering process (Bellincontro et al., 2006). AFLP-TP analysis revealed the up-regulation of S-adenosyl methionine synthase (TC67664) (Table 2), which supports such a role.

Grimplet et al. (2007) also provides evidence of the induction of genes involved in ethylene biosynthesis and signalling in grape berry development and ripening under water-deficit stress conditions.

**Transcription factors**

Several transcription factor genes matched to the withering-specific AFLP-TP tags (Tables 2, 3, 4, 5). These included an up-regulated transcript related to a tobacco bZIP transcription factor (TC54438) (Table 2) that binds in vitro to G-box elements in the promoters of phenylpropanoid biosynthetic genes (Heinekamp et al., 2002). The putative grapevine homologue could potentially bind similar elements upstream of grapevine genes, such as those identified in the Vst1 and DFR promoters (Schubert et al., 1997; Gollop et al., 2002). Another induced transcript was homologous to the apple MYB2 factor (TC61058) (Table 2). In plants, MYB proteins regulate different cellular and developmental processes including secondary metabolism, cellular morphogenesis, and the response to growth regulators (Martin and Paz-Ares, 1997). In grapevine, the role of MYB proteins in the regulation of phenylpropanoid synthesis has been considered (Deluc et al., 2006, 2008; Bogs et al., 2007; Walker et al., 2007). The up-regulation of a transcript displaying homology to the Nicotiana attenuata WRKY6 factor (TC59548) was also observed (Table 3). This could be linked to the activation stress response genes, as observed in numerous plant species in the case of wounding, pathogen infection or abiotic stress (Ulker and Somssich, 2004).

Among the withering-specific genes, the transcript for grapevine MADS1 (TC51812) was repressed (Table 5). This MADS-box transcription factor may play a role in flower development before fertilization and in berry development after fertilization (Boss et al., 2001).

**On-plant and off-plant withering processes**

Transcriptional modulation during grape berry post-harvest ripening was also studied in bunches that were left attached to the plant in the vineyard. AFLP-TP analysis was carried out on overripe berries and the results were compared with those obtained from the off-plant withering in order to highlight major differences caused by attachment to the shoot.

Off-plant withered berries were characterized by significant water loss and increased sugar concentration, whereas there was negligible water loss and little sugar accumulation in the on-plant berries (Table 1). A comparative analysis of AFLP-TP expression profiles from the three shared sampling time points identified 167 transcripts that were modulated only during off-plant withering, while another 86 transcripts were modulated only during the on-plant process. Thus, only 253 tags with different transcription profile were detected on the whole. This comparative analysis suggests that common transcriptional changes characterize the two kinds of withering processes. This seems surprising for a non-climacteric fruit such as grape berry, in which the occurrence of different processes on-plant and off-plant could be hypothesized. Differences in gene expression seem to be due mainly to dehydration stress, occurring in the off-plant withering process. A list of tags homologous to sequences with a known function is provided in Table 6.

One notable difference between the two processes was the higher level of VvDHN1a in off-plant withered berries,
### Table 6. Annotated AFLP-TP tags specific for on-plant and off-plant withering

| Description | Accession | Wiltering off-plant | Wiltering on-plant | E-value | Ontology |
|-------------|-----------|---------------------|-------------------|---------|----------|
| **Modulated-off plant only (up-regulated)** | | | | | |
| Dehydrin 1a | TC61998 | | | 3.14E-32 | Response to stimulus |
| Myb like protein | TC62992 | | | 1.3E-76 | Metabolic process- transcription |
| At3gl1200/F11B9.12 | TC53420 | | | 1.21E-38 | Metabolic process- transcription |
| Eukaryotic initiation factor 4B | Q9M7E8 | | | 6.9E-26 | Metabolic process-translation |
| Protein synthesis initiation factor 4G | TC67911 | | | 1.23E-80 | Metabolic process-translation |
| Protein translation factor SU11 homologue | TC59193 | | | 6.96E-13 | Metabolic process-translation |
| 26S ribosomal RNA | TC70629 | | | 2.03E-24 | Metabolic process-translation |
| SKP1 | TC57098 | | | 2.92E-33 | Metabolic process-protein metabolic process |
| Putative chloroplast outer membrane protein | Q56WJ7 | | | 3E-10 | Transport |
| Ras-related protein RAB8-5 | TC60446 | | | 9.08E-31 | Transport |
| Secretion protein HylD | TC60298 | | | 9.43E-39 | Transport |
| Aspartate aminotransferase, chloroplast precursor | TC55957 | | | 4.45E-51 | Metabolic process |
| Lipoygenase 2.2, chloroplast precursor | Q8GSM3 | | | 2.3E-04 | Metabolic process |
| Eukaryotic initiation factor 4B | TC53420 | | | 1.21E-38 | Metabolic process- transcription |
| Protein synthesis initiation factor 4G | TC67911 | | | 1.23E-80 | Metabolic process-translation |
| Protein translation factor SU11 homologue | TC59193 | | | 6.96E-13 | Metabolic process-translation |
| 26S ribosomal RNA | TC70629 | | | 2.03E-24 | Metabolic process-translation |
| SKP1 | TC57098 | | | 2.92E-33 | Metabolic process-protein metabolic process |
| **Modulated-off plant only (down-regulated)** | | | | | |
| Laccase | TC68636 | | | 3.59E-13 | Secondary metabolic process-lignan metabolic process |
| TMV response-related gene product | TC57457 | | | 1.01E-40 | Response to stimulus |
| Class III HD-Zip protein 1 | TC57687 | | | 6.97E-21 | Metabolic process- transcription |
| Catalytic/protein phosphatase type 2C | TC66121 | | | 4.46E-58 | Metabolic process-protein metabolic process |
| F-box containing protein TIR1 | TC62557 | | | 1.04E-38 | Metabolic process-protein metabolic process |
| Protein-like kinase protein | TC69785 | | | 5.44E-32 | Metabolic process-protein metabolic process |
| Serine/threonine-protein phosphatase | Q2QM47 | | | 1.4E-12 | Metabolic process-protein metabolic process |
| BSL2 homologue | Q9LZ20 | | | 9.38E-24 | Cellular component organization and biogenesis |
| Pectinesterase-like protein | TC67882 | | | 4.98E-14 | Biological process |
| Zinc finger protein | Q0KIL9 | | | 5.56E-16 | Biological process |
| **Structural maintenance of chromosomes 1 protein** | TC56126 | | | 3.92E-94 | Cellular component organization and biogenesis |
| Acer-CoA thioesterase | TC55739 | | | 1.29E-48 | Metabolic process |
| Carbohydrate-phosphate synthase, large subunit | TC58441 | | | 1.097E-27 | Metabolic process |
| 4-Diphosphocytidyl-2-C-methyl-D-erythritol kinase | TC69609 | | | 1.4E-16 | Metabolic process |
| Photosystem I reaction centre subunit N chloroplast precursor | TC53444 | | | 5.36E-13 | Metabolic process |
| Replication factor C | TC58522 | | | 5.54E-49 | Metabolic process |
| Cyclase | TC53458 | | | 2.41E-36 | Biological process |
| Cyclin D-3 | TC68914 | | | 2.33E-22 | Biological process |
| GTP-binding protein LepA homologue | Q9FN5M | | | 1.29E-10 | Biological process |
| LIM domain protein PLIM1 | TC51770 | | | 4.86E-44 | Biological process |
| Nascent polypeptide associated complex z chain | TC51933 | | | 2.5E-07 | Biological process |
| Nucleic acid binding | TC61629 | | | 3.2E-29 | Biological process |
| Phospholipase-like protein | TC67981 | | | 5.49E-19 | Biological process |
which almost certainly reflects off-plant water loss and the role of VvDH1a in dehydration stress. A similar profile was observed for a transcript with homology to a tomato enzyme involved in sorbitol biosynthesis (Ohta et al., 2005). There were no major differences in genes involved in cell wall metabolism. However, tags encoding a pectinesterase-like protein (Q9LZZ0) and a laccase (TC68636) were down-regulated specifically in off-plant withered berries (Table 6).

Pectinesterase is involved in the process of fruit softening during ripening (Prasanna et al., 2007), and this would appear less important in off-plant withered berries as would the polymerization of monolignols by laccase (Stergiades et al., 1992). Possible down-regulation of cell wall lignification during the off-plant process is also supported by the repression of a tag homologous to a poplar Class III HD-Zip protein 1 (TC57687) (Table 6) which plays a role in wood formation (Ko et al., 2006). A putative glycine-rich protein was up-regulated in the on-plant withered berries, and such proteins also play a role in cell wall structure (Mousavi and Hotta, 2005).

In off-plant withered berries, a tag with homology to the A. thaliana NAP1 (TC52510) protein was repressed (Table 6). NAP1 helps to regulate the activity of the ARP3/3 complex, which controls actin polymerization, suggesting that on-plant withering may require the preservation of actin polymers (Brembu et al., 2004).

With respect to energy metabolism, transcripts involved in photosynthesis were down-regulated in off-plant withered berries, for example, the photosystem I reaction centre subunit N chloroplast precursor (TC53444). However, a tag matching solanesyl diphosphate synthase (TC55340) was up-regulated (Table 6). In A. thaliana, this enzyme is involved in the synthesis of the isoprenoid component of plastoquinone and ubiquinone (Jun et al., 2004), which take part in photosynthetic electron transfer in the chloroplast and

| Description | Accession<sup>a</sup> | Withering off-plant | Withering on-plant | E-value<sup>b</sup> | Ontology |
|-------------|------------------------|---------------------|--------------------|----------------------|----------|
| Putative preselin | Q6AUZ8 |  |  | 1.37E-07 | Biological process |
| Rieske iron-sulphur protein Tic55 precursor | TC58384 |  |  | 4.81E-24 | Biological process |
| WD-40 repeat family protein-like | TC52339 |  |  | 2.18E-18 | Biological process |
| **Modulated-on plant only (up-regulated)** | | | | |
| 22.0 kDa class IV heat shock protein precursor | P30236 |  |  | 3.09E-04 | Response to stimulus |
| BZIP transcription factor | TC61986 |  |  | 1.65E-76 | Metabolic process- transcription |
| Protein translation factor SUI1 homologue 1 | TC68660 |  |  | 8.89E-25 | Metabolic process-translation |
| 40S ribosomal protein S12 | Q9XH50 |  |  | 6.27E-09 | Metabolic process-translation |
| S15 ribosomal protein | Q8L4R2 |  |  | 5.0E-04 | Metabolic process-translation |
| 60S ribosomal protein L3 | O65076 |  |  | 4.64E-17 | Metabolic process-translation |
| H4 NEUCR Histone H4 | TC52370 |  |  | 8.27E-29 | Cellular component organization and biogenesis |
| Ferritin-3 chloroplast precursor | TC54876 |  |  | 3.59E-43 | Transport |
| Aspartate aminotransferase | CB006657 |  |  | 4.98E-08 | Metabolic process |
| Chlorophyll a/b binding protein | TC53695 |  |  | 1.95E-79 | Metabolic process |
| Chlorophyll a/b binding protein | TC55556 |  |  | 4.56E-17 | Metabolic process |
| Transaldolase | Q8H706 |  |  | 3.39E-16 | Metabolic process |
| Cellular retinaldehyde-binding/triple function C-terminal | TC55679 |  |  | 1.27E-25 | Biological process |
| Putative glycine-rich protein | TC57883 |  |  | 1.41E-25 | Biological process |
| Putative WD-40 repeat-protein | Q9M2Z2 |  |  | 1.18E-20 | Biological process |

| Modulated-on plant only (down-regulated) | | | |
| Eukaryotic translation initiation factor 5 | TC68348 |  |  | 1.01E-33 | Metabolic process-translation |
| Cysteine protease | TC66158 |  |  | 5.17E-145 | Metabolic process- protein metabolic process |
| Polyubiquitin | TC70093 |  |  | 3.67E-18 | Metabolic process- protein metabolic process |
| Ubiquitin | TC57081 |  |  | 7.82E-10 | Metabolic process- protein metabolic process |
| Kininogen-1 precursor | TC64953 |  |  | 9.4E-30 | Biological process |
| Nucleic acid binding | TC53554 |  |  | 2.21E-41 | Biological process |

<sup>a</sup> Accession number (DFCI Grape Gene Index, UNIPROT ID).

<sup>b</sup> E-value from BLASTN and BLASTX searches.
respiratory electron transfer in the mitochondrion (Jun et al., 2004). Chlorophyll a/b binding proteins (TC56895; TC65556) were up-regulated in on-plant withered berries (Table 6).

There were some differences between the two processes in terms of protein synthesis, with both induction and repression noted for tags corresponding to various ribosomal proteins and translation factors (Table 6). However, on-plant withering appeared to repress genes involved in protein recycling, such as polyubiquitin (TC70093) and ubiquitin (TC57081) (Table 6).

In terms of secondary metabolism, only genes involved in terpenoid biosynthesis showed any major differences between the post-harvest drying processes with the repression of a tag encoding a 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase (TC69609), an enzyme belonging to the mevalonate-independent pathway, in off-plant withered grapes (Table 6).

**Conclusion**

AFLP-TP analysis allowed genes to be identified whose steady-state mRNA levels were modulated during post-harvest withering, painting a broad picture of the transcriptional events underpinning post-harvest berry withering in the Corvina variety. The results must be evaluated considering the 2003 growing season as particularly hot and dry. Dehydration, the main stress that occurs during off-plant withering, triggers a number of different responses including the activation of canonical stress-response genes, the accumulation of osmolytes and the mobilization of transposable elements. The berry withering process could also be characterized in terms of the synthesis of phenolic and terpene compounds, ethylene biosynthesis, and hexose catabolism via the pyruvate pathway. Genes were also identified whose expression differed according to the type of withering process (on or off the vine), indicating that off-plant withering induced a deeper form of dehydration stress and induced the high level expression of stress response genes such as those encoding dehydrins and osmolyte biosynthetic enzymes. This experiment has made a significant contribution to understanding the molecular basis of grape berry withering and may help to identify useful markers for different withering processes.

**Supplementary data**

Supplementary data can be found at JXB online.

**Fig. S1.** Major functional categories of the differentially-expressed AFLP-TP tags.

**Table S1.** Sequences of real-time RT-PCR primers.

**Table S2.** Complete list of the AFLP-TP transcripts modulated during berry development, off-plant and on-plant withering.

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**References**

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215, 403–410.

Bellicentro A, De Santis D, Botondi R, Villa I, Mencarelli F. 2004. Different post-harvest dehydration rates affect quality characteristics and volatile compounds of Malvasia, Trebbiano and Sangiovese grape for wine production. *Journal of the Science of Food and Agriculture* 84, 1791–1800.

Bellacenna A, Fardelli A, De Santis D, Rotondi R, Mencarelli E. 2006. Post-harvest ethylene and 1-MCP treatments both affect phenols, anthocyanins, and aromatic quality of Aleatico grapes and wine. *Australian Journal of Grape and Wine Research* 12, 141–149.

Bogs J, Jaffé FW, Takos AM, Walker AR, Robinson SP. 2007. The grapevine transcription factor VvMYBPA1 regulates proanthocyanidin synthesis during fruit development. *Plant Physiology* 143, 1347–1361.

Borsa D, Di Stefano R. 2000. Evoluzione dei polifenoli durante l’appassimento di uve a frutto colorato. *Rivista Vitivinicola* 4, 25–35.

Boss PK, Vivier M, Matsumoto S, Dry IB, Thomas MR. 2001. A cDNA from grapevine (*Vitis vinifera* L.), which shows homology to AGAMOUS and SHATTERPROOF, is not only expressed in flowers but also throughout berry development. *Plant Molecular Biology* 45, 541–553.

Bremhu T, Winge P, Seem M, Bones AM. 2004. NAPP and PIRP encode subunits of a putative wave regulatory protein complex involved in plant cell morphogenesis. *The Plant Cell* 16, 2335–2349.

Breyne P, Dreesen R, Cannoot B, Rombaut D, Vandepoele K, Rombouts S, Vanderhaeghen R, Inzé D, Zabeau M. 2003. Quantitative cDNA-AFLP analysis for genome-wide expression studies. *Molecular Genetics and Genomics: MGG* 269, 173–179.

Castellarin SD, Matthews MA, Di Gaspero G, Gambetta GA. 2007b. Water deficits accelerate ripening and induce changes in gene expression regulation flavonoid biosynthesis in grape berries. *Planta* 227, 101–112.

Castellarin SD, Pfeiffer A, Sivilotti P, Degani M, Peterlunger E, Di Gaspero G. 2007a. Transcriptional regulation of anthocyanin biosynthesis in ripening fruit of grapevine under seasonal water deficit. *Plant, Cell and Environment* 30, 1381–1399.

Celotti E, Ferrarinì R, Conte LS, Giulivo C, Zironi R. 1998. Modifiche del contenuto di resveratrolo in uve di vitigni della Valpolicella nel corso della maturazione e dell’appassimento. *Vigne et Vins* 58, 83–92.

Chervin C, El-Kereamy A, Roustan JP, Latché A, Lamon J, Bouzyen M. 2004. Ethylene seems required for the berry development and ripening in grape, a non-climacteric fruit. *Plant Science* 167, 1301–1305.

Chkaiban L, Botondi R, A, de Santis D, Kefalas P. 2007. Influence of post-harvest water stress on lipoxygenase and alcohol dehydrogenase activities, and on the composition of some
volatile compounds of Gewürztraminer grapes dehydrated under controlled and uncontrolled thermohygroscopic conditions. *Australian Journal of Grape and Wine Research* **13**, 142–149.

Conde C, Silva P, Fontes N, Dias ACP, Tavares RM, Sousa MJ, Agasse A, Delrot S, Geros H. 2007. Biochemical changes throughout grape berry development and fruit and wine quality. *Food L*., 1–22.

Costantini V, Bellincontro A, De Santis D, Botondi R, Mencarelli F. 2006. Metabolic changes of Malvasia grapes for wine production during post-harvest drying. *Journal of Agricultural and Food Chemistry* **54**, 3334–3340.

Croft K, Juttner F, Shusarenko AJ. 1993. Volatile products of the lipoxypgenase pathway evolved from Phaseolus vulgaris (L.) leaves inoculated with *Pseudomonas syringae pv. phaseolicola*. *Plant Physiology* **101**, 13–24.

Deluc L, Barrieu F, Marchive C, Lauvergeat V, Decendit A, Richard T, Carde JP, Merillon JM, Hamdi S. 2006. Characterization of a grapevine R2R3-MYB transcription factor that regulates the phenylpropanoid pathway. *Plant Physiology* **140**, 499–511.

Deluc L, Bogs J, Walker AR, Ferrier T, Decendit A, Merillon JM, Robinson SP, Barrien F. 2008. The transcription factor VvMYBSb contributes to the regulation of anthocyanin and proanthocyanidin biosynthesis in developing grape berries. *Plant Physiology* **10.1104/pp.10.118919**.

Deluc LG, Grimplet J, Wheatley MD, Tillett RL, Quilici DR, Osborne B, Schooley DA, Schlauch KA, Cushman JC, Cramer GR. 2007. Transcriptomic and metabolite analyses of Cabernet Sauvignon grape berry development. *BMC Genomics* **8**, 429.

Di Stefano R, Borsa D, Gentilini N, Corino L, Trenti S. 1997. Evoluzione degli zuccheri, degli acidi fissi e dei composti fenolici dell’uva durante l’appassimento in fruttaio. [Evolution of sugars, acids and phenolic compounds of grape during drying in fruttaio]. *Rivista Viticoltura Enologia* **1**, 33–41.

Doco T, Williams P, Pauly M, O’Neill MA, Pellerin P. 2003. Polysaccharides from grape berry cell wall. II. Structural characterization of the xyloglucan polysaccharides. *Carbohydrate Polymers* **53**, 253–261.

Durrant WE, Rowland O, Piedras P, Hammond-Kosack KE, Jones JD. 2000. cDNA-AFLP reveals a striking overlap in race-specific resistance and wound response gene expression profiles. *The Plant Cell* **12**, 963–977.

French-Italian Public Consortium for Grapevine Genome Characterization. 2007. The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature* **449**, 463–467.

Garg AK, Kim JK, Owens TG, Ranwala AP, Choi YD, Ko JH, Prassinos C, Han KH. 2006. Developmental and seasonal expression of PtaHB1, a *Populus* gene encoding a class III HD-Zip protein, is closely associated with secondary growth and inversely correlated with the level of microRNA (miR166). *The New Phytologist* **169**, 469–478.

Lund ST, Bohlmann J. 2006. The molecular basis for wine grape quality: a volatile subject. *Science* **311**, 804–805.

Lund ST, Peng FY, Nayar T, Reid KE, Schlosser J. 2008. Gene expression analyses in individual grape (*Vitis vinifera*) berries during ripening initiation reveal that pigmentation intensity is a valid indicator of developmental staging within the cluster. *Plant Molecular Biology* **10.1007/s11103–008–9371-z**.

Martin C, Paz-Ares J. 1997. MYB transcription factors in plants. *Trends in Genetics* **13**, 67–73.

Mousavi A, Hotta Y. 2005. Glycine-rich proteins: a class of novel proteins. *Applied Biochemistry and Biotechnology* **120**, 169–174.

Nunan KJ, Davies C, Robinson SP, Fincher GB. 2001. Expression patterns of cell wall modifying enzymes during grape berry. *Planta* **214**, 257–264.

Nunan KJ, Sims IM, Baec A, Robinson SP, Fincher GB. 1998. Changes in cell wall composition during ripening of grape berries. *Plant Physiology* **118**, 783–792.

Ohta K, Moriguchi R, Kanahama K, Yamaki S, Kanayama Y. 2005. Molecular evidence of sorbitol dehydrogenase in tomato, a non-Rosaceae plant. *Phytochemistry* **66**, 2822–2828.

Pelsy F, Merdiningtya D. 2002. Complete sequence of Tvvi1, a family of Ty 1 copia-like retrotransposons of *Vitis vinifera L.*, reconstituted by chromosome walking. *Theoretical and Applied Genetics* **105**, 615–621.

Pereira HS, Barão A, Delgado M, Morais-Cecílio L, Viegas W. 2005. Genomic analysis of Grapevine Retrotransposon 1 (Gret 1) in *Vitis vinifera*. *Theoretical and Applied Genetics* **111**, 871–878.

Peterhansel C, Lahaye T. 2005. Be fruitful and multiply: gene amplification inducing pathogen resistance. *Trends in Plant Science* **10**, 257–260.

Pfaffl MW. 2001. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research* **29**, e45.

Pfaffl MW, Horgan GW, Dempfle L. 2002. Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic Acids Research* **30**, e36.
