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RESEARCH PAPER

Molecular analysis of post-harvest withering in grape by AFLP transcriptional 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

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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 fruitset 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 weighted; 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 BstT/C 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 $\gamma[^{33}P]$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.cribi.unipd.it). The tag sequences were used for BLASTN and BLASTX (Altschul *et al*., 1990) searches against the DFCI Grape Gene Index database (http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/gimain.pl?gudb=grape) and the non-redundant UNIPROT database (http://www.expasy.uniprot.org), respectively, using an *E*-value cut-off of 5×10^{-4}. Gene Ontology terms (http://www.geneontology.org) were assigned to each sequence using the BLASTN and BLASTX results.

Table 1. *Sampling time-points and corresponding physiological data*

Sampling time point	Days before or after ripening	Per cent weight	Brix degree
Post fruit-set; PFS	–92 d		
Pre-véraison; PRV	–65 d		
Véraison; V	–41 d		
Ripening; R	0	100%	22.10°
Off-plant withering I; WI	+22 d	83.20%	28.60°
Off-plant withering II; WII	+41 d	77.40%	30.00°
Off-plant withering III; WIII	+74 d	70.20%	32.20°
Off-plant withering IV; WIV	+99 d	67.30%	32.80°
On-plant withering I; WI	+22 d	101.10%	24.80°
On-plant withering II; WII	+41 d	98.20%	26.20°
On-plant withering III; WIII	+74 d	97.60%	26.10°

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 TC55334, 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 specifity allow analysing the expression profile of genes with high homology (Vuylsteke *et al*., 2007). The procedure of purification, amplification, and sequecing 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/tgi/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

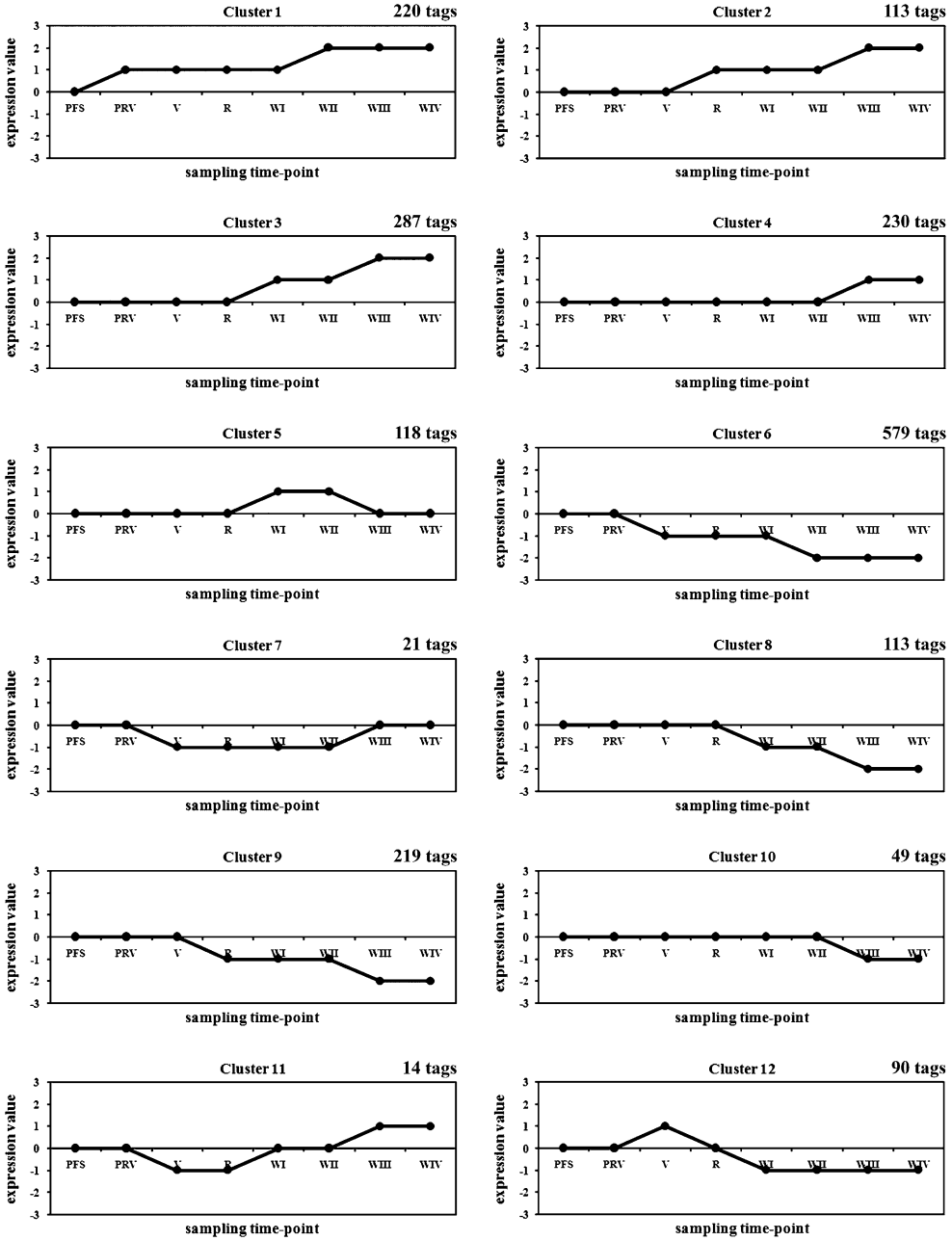

Fig. 1. Expression profiles of the 12 main clusters. The number of AFLP-TP tags belonging to each cluster is reported. For each cluster, the graph reports the mean expression values calculated using expression values of all tags in the cluster over the four development sampling time-points (PFS, PRV, V, and R) and the off-plant withering sampling time-points (WI, WII, and WIII).

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 developmental time points (PFS, PRV, V, R) and for the three time points common to both withering processes (WI, WII, 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

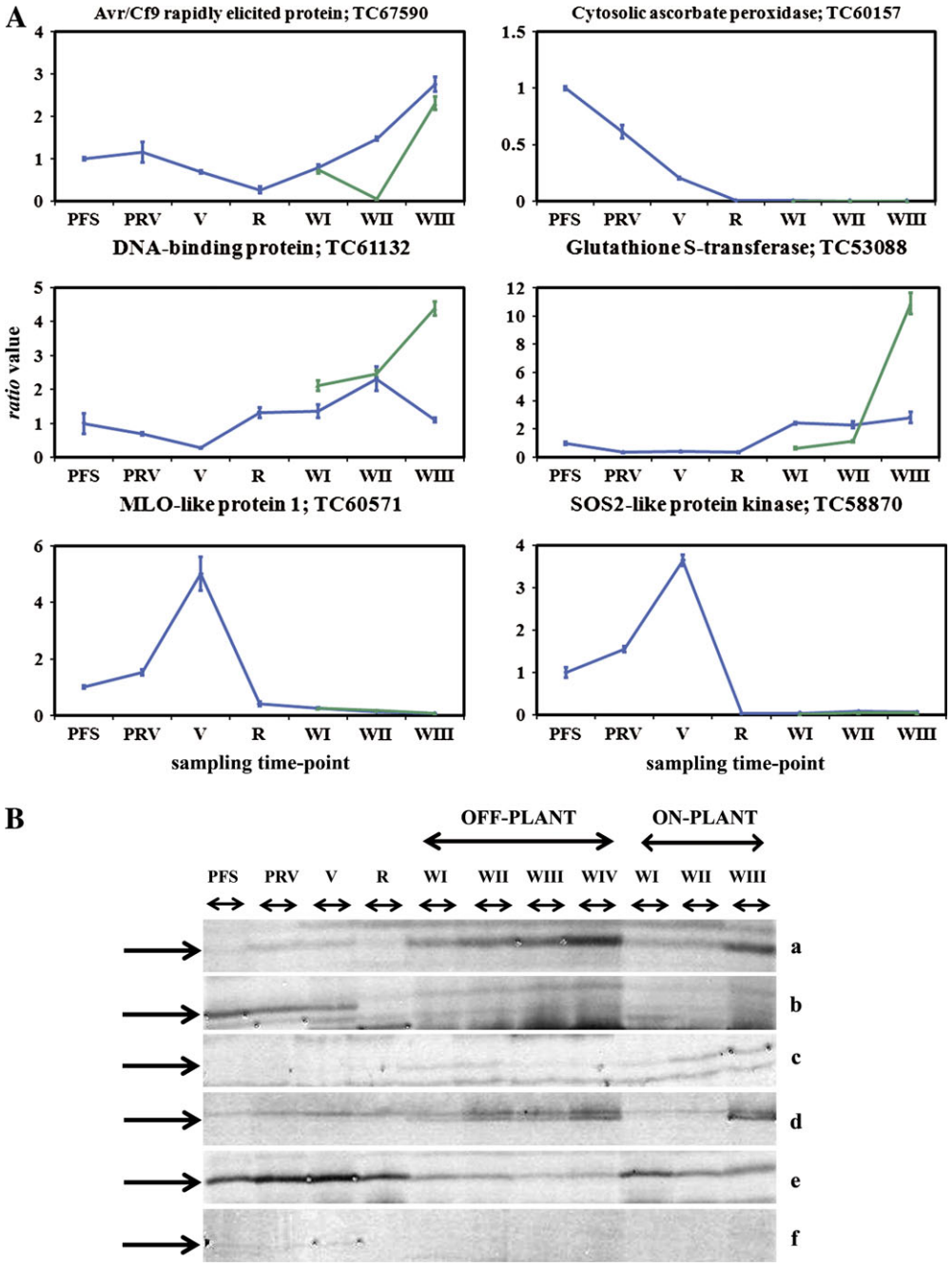

Fig. 2. (A) Real-time RT-PCR expression profiles of six AFLP-TP tags. Gene expression profiles expressed as a *ratio* value for each sampling time point relative to the post-fruit set (PFS) (±SE, n=3 technical replicates). Solid blue line: gene expression profile for development (PFS, PRV, V, and R) and for the off-plant withering sampling time points (WI, WII, WIII) (circles). Dotted green line: expression profile for the on-plant withering sampling time points (WI, WII, WIII) (triangles). (B) AFLP-TP expression profiles for the six tags analysed by real-time RT-PCR: (a) Avr9/Cf9 rapidly elicited protein, (b) cytosolic ascorbate peroxidase, (c) DNA-binding protein, (d) glutathione *S*-transferase, (e) MLO-like protein 1, (f) SOS2-like protein kinase. The expression profiles include the four development sampling time-points (PFS, PRV, V, R), the four off-plant sampling time-points (WI, WII, WIII, and WIV) and the three on-plant sampling time-points (WI, WII, and WIII). The off-plant WIV was not analysed by real-time RT-PCR.

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

Table 2. *Annotated cDNA-AFLP-TP tags from cluster 3*

Description	Accession[a]	*E*-value[b]
Secondary metabolic process: phenylpropanoid biosynthetic process		
4-Coumarate-CoA ligase-like	TC57438	6.16E-34
Phenylalanine ammonia-lyase	TC66528	3.13E-78
Phenylalanine ammonia-lyase	TC66528	1.83E-77
Secondary metabolic process: lignan metabolic process		
Polyphenol oxidase	TC58764	5.46E-68
Polyphenol oxidase	TC58764	8.80E-65
Secondary metabolic process: stilbene metabolic process		
Resveratrol synthase	TC52907	9.45E-52
Stilbene synthase	TC53668	7.85E-10
Stilbene synthase	TC59572	2.64E-97
Stilbene synthase	TC52790	2.22E-49
Secondary metabolic process: flavonoid metabolic process		
Chalcone-flavonone isomerase	TC55034	2.60E-06
Secondary metabolic process: terpenoid metabolic process		
Limonoid UDP-glucosyltransferase	TC65435	1.20E-06
Response to stimulus		
Cytosolic ascorbate peroxidase	TC51718	2.78E-102
Gag-pol polyprotein	TC69867	1.08E-35
Glutathione *S*-transferase GST24	TC53088	6.02E-64
MLO-like protein 6 (AtMlo6)	Q94KB7	2.82E-15
MutT domain protein-like	TC67034	1.05E-11
Reverse transcriptase	TC51865	4.30E-05
Non-LTR retroelement reverse transcriptase	CD007484	6.00E-11
Sorbitol related enzyme	TC58983	8.86E-28
SRE1a	TC61558	4.92E-07
Metabolic process: transcription		
AREB-like protein	TC52653	1.54E-32
bZIP transcription factor	TC54438	3.29E-19
DNA-binding protein	TC61132	2.72E-34
MYBR2	TC61058	7.06E-59
NAM-like protein	TC69267	1.77E-10
Transcription factor IIA	TC65001	1.15E-69
Metabolic process: translation		
26S proteosome regulatory subunit	Q6Z8F7	1.96E-04
4.5S. 5S. 16S, and 23S mRNA	TC70523	9.94E-37
60S acidic ribosomal protein	TC60834	8.79E-24
Hamamelis virginiana large subunit 26S ribosomal RNA gene	TC65768	1.89E-18
Ribosomal S29-like protein	TC65685	8.25E-06
RNA binding	TC69367	5.46E-26
Metabolic process: protein metabolic process		
COP9 signalosome complex subunit 3	Q8W575	3.51E-03
COP9 signalosome complex subunit 7	TC52949	1.70E-08
Mitogen-activated protein kinase	Q8GT86	5.73E-06
Phosphatase	TC60297	1.49E-58
Proteasome subunit beta type 7-A precursor	TC68818	8.50E-06
Ubiquitin	TC53245	3.66E-46
Ubiquitin	TC52385	3.25E-38
Cellular component organization and biogenesis		
Histone H2A.3	TC54193	2.40E-08
Myosin-like protein	TC57562	2.63E-24
Structural maintenance of chromosomes	Q6Q1P4	4.55E-04
Topoisomerase-like protein	Q8LDN5	6.00E-04
Transport		
ADP, ATP carrier	TC67277	7.19E-20
Cytochome b_5	TC52244	1.10E-08
Cytochrome B561-like. partial	TC58099	1.30E-65
Cytochrome oxidase	TC62100	2.00E-06
Cytochrome P450 mono-oxygenase CYP83C	TC61438	4.09E-51
Mitochondrial carrier protein	TC63333	4.21E-07
Potassium transport 7	Q9FY75	7.77E-23
Probable oxidoreductase At4g09670	TC63817	1.87E-08
Ras-related protein RAB8-5	TC60446	9.08E-31
Syntaxin 43	TC52593	4.69E-08
Metabolic process		
Acetyltransferase	Q9ASS8	5.44E-08
Acyl-coenzyme A oxidase, peroxisomal precursor	TC58112	2.63E-65
α-Glucan phosphorylase, H isozyme	TC53692	3.14E-14
4.5-DOPA dioxygenase extradiol-like protein. putative	Q6L3J4	7.41E-12
γ-Glutamylcysteine synthetase	TC56558	1.32E-15
Inositol 1.3.4-trisphosphate 56-kinase	Q1S3P6	2.33E-04
Invertase inhibitor-like protein	Q9LSN2	3.83E-05
Ketol-acid reductoisomerase, chloroplast precursor	TC68860	5.29E-61
Lysophospholipase-like protein	TC56357	1.29E-63
NADH ubiquinone oxidoreductase PSST subunit	TC64663	9.00E-81
Poly(ADP)-ribose polymerase	TC56033	1.70E-04
Plastid α-amylase	Q5BLY1	1.16E-16
S-adenosyl methionine synthase	TC67664	3.10E-05
Solanesyl diphosphate synthase	TC55340	5.20E-35
Biological process		
ATP binding	TC63053	2.73E-68
Cellular retinaldehyde-binding/triple function. C terminal	TC55679	4.28E-30
Cig3	Q8W417	2.01E-59
DNA-binding protein-like	CB009535	4.13E-26
Kelch repeat containing F-box protein family-like	TC57688	2.96E-18
KH domain-containing protein	TC63964	3.30E-07
Latency associated nuclear antigen	TC71005	2.18E-40
Legumin-like protein	TC52209	1.31E-64
Legumin-like protein	TC52209	1.26E-62
NADPH-ferrihaemoprotein reductase	CD007176	1.50E-06
RING finger-like protein	CB920519	3.18E-38
Ring finger family protein	TC56727	7.72E-60
Surfeit 1 homologue	TC70786	3.20E-05
Zinc finger protein	Q0KIL9	5.56E-16

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

molecular processes that take place after berry picking to be formulated.

Phenolic compounds

Among the AFLP tags induced by withering, there were three transcripts with homology to two different phenylalanine ammonia lyase (*PAL*) genes (TC69585; TC66528), and two tags encoding 4-coumarate-CoA ligase (4CL)-like proteins (TC57438) (Tables 2, 3). Therefore, berry dehydration appears to induce general phenylpropanoid metabolism, which generates precursors for many different categories of phenolic compounds. Eight tags corresponding to *STS* genes (TC52790, TC52907, TC53668, TC59572, TC60946, NP1227286) were induced by withering (Tables 2, 3) suggesting a strong stilbene production. Stilbenes are synthesized constitutively in

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	NP1227286	7.56E-52
Stilbene synthase	TC59572	6.24E-99
Stilbene synthase	TC60946	3.05E-04
Secondary metabolic process: terpenoid metabolic process		
Limonoid UDP-glucosyltransferase	TC65435	1.30E-09
Response to stimulus		
Avr9/Cf-9 rapidly elicited protein	CA813698	1.79E-46
Dehydrin 1a	TC61998	3.14E-32
Disease resistance response protein	Q9LID5	5.29E-35
Syringolide-induced protein	Q8S901	6.16E-08
Metabolic process: transcription		
Ethylene response factor	TC52148	6.60E-74
Ethylene-responsive element binding protein	TC62980	1.25E-04
Eukaryotic initiation factor 4B	Q9M7E8	6.90E-26
RING finger-like protein	CB920519	1.25E-17
SUPERMAN-like zinc finger protein	TC60860	8.85E-16
WRKY6	TC59548	4.10E-08
Metabolic process: translation		
26S ribosomal RNA	TC70629	2.03E-24
40S ribosomal protein S12	Q9XHS0	6.27E-09
60S ribosomal protein L3	O65076	4.64E-17
Hamamelis virginiana large subunit 26S ribosomal RNA gene	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		
22.0 kDa class IV heat shock protein precursor	P30236	3.09E-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		
Aspartate aminotransferase	TC55957	4.45E-51
Aspartate aminotransferase	CB006657	4.90E-08
Chloroplast outer membrane protein	Q56WJ7	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
Sucrose transporter-like protein	TC51830	3.18E-22
Metabolic process		
Dopamine β-mono-oxygenase N-terminal domain-containing protein	TC62500	8.00E-09
Fructose-bisphosphate aldolase	TC54602	3.55E-77
LEDI-5c protein	TC61395	9.25E-31
Lipoxygenase	Q8GSM3	2.03E-04
Phosphoglycerate kinase, cytosolic	TC52072	9.61E-102
Plastidic aldolase NPALDP1	TC59070	5.66E-22
Ribose-5-phosphate isomerase	TC59181	8.03E-91
Transaldolase	Q8H706	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*., 2004; Tornielli *et al*., 2005). 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*., 2007*a*, *b*; Grimplet *et al*., 2007). Preharvest water-deficit stress does not necessary 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 rhamnosyltransferase	TC70498	6.46E-37
Response to stimulus		
TMV response-related gene product	TC57457	1.00E-40
Thioredoxin domain-containing protein 9	TC56954	5.48E-76
Metabolic process: transcription		
HMG-I and HMG-Y, DNA-binding	Q1RZ01	4.61E-06
MYB-like DNA-binding domain protein	TC52565	1.89E-08
Putative VP1/ABI3 family regulatory protein	O04346	1.03E-11
Similarity to metallothionein-I gene transcription activator	Q9FLM8	7.38E-06
Metabolic process: translation		
30S ribosomal protein S16	TC53443	4.62E-06
60S ribosomal protein L12	TC52607	7.57E-37
Metabolic process: protein metabolic process		
Pepsin A	TC58741	1.15E-43
Probable prefoldin subunit 5	TC58696	5.13E-72
Putative tyrosine phosphatase	Q5ZEJ0	3.53E-22
S-locus receptor-like kinase RLK14	CB971388	7.50E-06
Transport		
ADP ribosylation factor 002	TC51848	1.18E-68
Putative cytochrome b_5	O22704	2.79E-25
Receptor-like protein kinase-like	TC54030	9.74E-71
Metabolic process		
Acyl-ACP thioesterase	TC60833	6.76E-17
α-Glucan water dikinase	TC54189	2.23E-45
ATP/GTP nucleotide-binding protein	Q9FII8	4.00E-06
β-Mannan endohydrolase	TC67062	2.80E-05
B-keto acyl 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
HMG-CoA synthase 2	TC68763	1.22E-22
Ketol-acid reductoisomerase, chloroplast precursor	TC68860	4.77E-61
Long-chain acyl-CoA synthetase	TC59981	9.30E-31
Molybdenum cofactor biosynthesis	TC70221	9.50E-43
Phosphoenolpyruvate carboxykinase	TC60028	5.57E-41
Photosystem I reaction center subunit N, chloroplast precursor	TC53444	2.58E-56
Pyrophosphate-dependent phosphofructo-1-kinase	TC70414	1.91E-100
Ribonuclease HII	Q53QG3	2.41E-06
Transaldolase ToTAL2	TC59186	1.24E-14
Biological process		
CaLB protein	P92940	1.00E-07
Cellular retinaldehyde-binding/triple function, C terminal	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 rRNA tRNA-Val rps12 rps7 ndhB	TC60315	6.60E-36
WD-40 repeat family protein-like	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
Thioredoxin-like protein	TC63581	1.84E-43
Metabolic process: transcription		
MADS-box transcripion factor	TC51812	2.76E-04
Metabolic process: protein metabolic process		
Serine/threonine protein phosphatase BSL2 homologue	Q2QM47	1.40E-12
Cellular component organization and biogenesis		
Actin-like	TC58881	3.14E-09
Cellulose synthase-like protein CslG	TC55634	1.56E-13
Transport		
ATP synthase γ chain	TC68806	3.78E-76
Metabolic process		
Acyl-CoA thioesterase	TC55739	1.29E-48
Carbonate dehydratase	O81875	8.36E-10
Phosphoribosylformylglycinamidine	Q84XV9	1.42E-20
Pyruvate kinase	TC60979	1.01E-55
S-adenosyl methionine synthase-like	TC62371	7.03E-20
Biological process		
Coenzyme Q biosynthesis protein	TC70287	1.71E-23
Cyclic nucleotide phosphodiesterase	CB918027	1.74E-14
Heterogeneous nuclear ribonucleoprotein A2/B1-like	TC62660	3.06E-33
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 (Grimplet *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*., 2007*a*, *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 *Vine*-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 lipoxygenase (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 lipoxygenase 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 H_2O_2 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 *S*-transferase (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 MYBR2 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[a]	Withering off-plant	Withering on-plant	*E*-value[b]	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
At3g11200/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 SUI1 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 HlyD	TC60298			9.43E-39	Transport
Aspartate aminotransferase, chloroplast precursor	TC55957			4.45E-51	Metabolic process
Lipoxygenase 2.2, chloroplast precursor	Q8GSM3			2.3E-04	Metabolic process
Plastidic aldolase NPALDP1	TC59070			5.66E-22	Metabolic process
Solanesyl diphosphate synthase	TC55340			5.2E-35	Metabolic process
Sorbitol related enzyme	TC58983			8.86E-28	Metabolic process
Ca^{2+}-binding EF hand protein	TC67340			1.41E-111	Biological process
Germin-like protein protein subfamily 3 member 2 precursor	TC52213			2.12E-06	Biological process
Histidine kinase	TC64607			4.36E-10	Biological process
Putative RNA-binding protein	Q9SFV5			8.95E-21	Biological process
pux2 (PLANT UBX DOMAIN-CONTAINING PROTEIN 2); nucleic acid binding	TC67882			4.98E-14	Biological process
Zinc finger protein	Q0KIL9			5.56E-16	Biological 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 BSL2 homologue	Q2QM47			1.4E-12	Metabolic process- protein metabolic process
Pectinesterase-like protein	Q9LZZ0			9.38E-24	Cellular component organization and biogenesis
Probable protein NAP1	TC52510			1.68E-18	Cellular component organization and biogenesis
Structural maintenance of chromosomes 1 protein	TC65126			3.92E-94	Cellular component organization and biogenesis
Acyl-CoA thioesterase	TC55739			1.29E-48	Metabolic process
Carbamoyl-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 δ-3	TC68914			2.33E-22	Biological process
GTP-binding protein LepA homologue	Q9FNM5			1.29E-10	Biological process
LIM domain protein PLIM1	TC51770			4.86E-44	Biological process
Nascent polypeptide associated complex α 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

Table 6. *Continued*

Description	Accession[a]	Withering off-plant	Withering on-plant	*E*-value[b]	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	Q9XHS0			6.27E-09	Metabolic process-translation
S15 ribosomal protein	Q8L4R2			5E-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			4.9E-08	Metabolic process
Chlorophyll *a/b* binding protein	TC56895			1.95E-79	Metabolic process
Chlorophyll *a/b* binding protein	TC65556			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

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

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 pectin-esterase-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 (Sterjiades *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

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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