Genomics Approaches for Better Understanding the Biological Basis of Fruit Ripening and Quality

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Abstract

Genomics tools are nowadays commonly used in many plant science labs and are rapidly spreading for studying transcriptome profiles throughout fruit development and discovering new genes involved in processes modulating quality traits. In fact, transcript profiling (TP) has the potential to reveal transcriptional hierarchy during development for thousands of genes, as well as providing expression data for many genes of unknown or putative function. By using both direct and indirect TP methods, a body of new information is now available concerning ripening regulation in both climacteric and non-climacteric fruits, and a number of genes differentially expressed during the transition from unripe to ripe fruit and related to the evolution of quality parameters have been identified. Concerning direct TP methods, isolated ESTs have been used for digital expression analysis throughout fruit development in several fruit species and for comparative genomics investigations. The comparative approach has allowed to identify genes putatively encoding transcription factors induced at ripening in both grapes and peaches indicating that some regulatory elements are in common in non-climacteric and climacteric fruits. Among direct TP analyses, cDNA-AFLP has been widely used in several fruit types including grape berry: using this technique, we have identified 92 and 82 genes differentially regulated in skins of grape berries during extended ripening off- (detachment) and on- (late harvest) plant, respectively. Some of these genes are in common but others are specifically induced or repressed by each treatment and may be responsible for some quality traits characterizing late-harvest or partially dehydrated grape berries. Considering the indirect analyses, the first peach microarray (μPEACH 1.0) containing oligo-probes corresponding to 4806 unigenes has been constructed and used for comparing transcriptome of pre-climacteric and climacteric peach fruit: 267 and 109 genes appear up- and down-regulated, respectively, during this transition. Among these, genes responsible for typical peach fruit traits (pulp pigmentation) and others, already associated to ripening in other species, but never studied in peach have been identified.

INTRODUCTION

The typical changes characterizing fleshy fruit ripening basically include modifications of pigmentation, texture, and flavour that, together with the absence of defects, disorders and/or diseases, define quality and the shelf-life of the commodity. All these changes are the results of a chain of events starting with the modulation of gene transcription, but including also translational and post-translational regulation. The application of genome-scale gene expression profiling tools is rapidly spreading for elucidating ripening mechanisms and understanding the biological basis of quality: in this context, tomato represents the model system due to the availability of extensive germplasm, ripening mutants, comprehensive genetic maps and markers, efficient transformation techniques and the development of genomic tools as BAC libraries, EST (Expressed Sequence Tag) collections and the advanced genome sequencing project (Fei et al., 2004; Giovannoni, 2004; Giovannoni and El-Rakshy, 2005). However, the ripening

process is currently studied using genomics approaches also in other fruit species where both direct and indirect analyses of transcriptome have been applied (Bonghi and Trainotti, 2006). Two important crops, grapes and peaches, have recently received particular attention and, due to the increased number of specific ESTs isolated and the availability of arrays, new information concerning specific and common mechanisms regulating ripening of these two fruit species is now available

EST REPERTOIRE AND DIGITAL EXPRESSION ANALYSES

An in silico expression analysis to 135,541 ESTs from 58 libraries representing different tissues from seven cultivars of *Vitis vinifera* has been performed by da Silva et al. (2005). A hierarchical cluster analysis has shown that expression profiles in fruit are different from those of other tissues. Marked changes in expression profiles are observed during the transition from pre-veraison to veraison and post-veraison berries. The hierarchical cluster analysis based on grape ESTs highlighted unknown genes that are co-regulated with already known GRIP (grape ripening induced) genes (Davies and Robinson, 2000), thus allowing to assign them a putative function during berry ripening.

Within the activity of the Italian Consortium for Genomics Studies in *Prunus* (ESTree Consortium, 2005) and working on EST collections from peach ripe mesocarp, we have pointed out that genes involved in ethylene biosynthesis and cell wall metabolism are highly represented at climacteric. Three transcription factors (TFs) homologous to *SEPALLATA3*, *atb2*, *bHLH61*, belonging to MADS, bZIP and bHLH families, respectively, have been identified (Ziliotto et al., 2005).

By performing a digital expression analysis on 83,675 sequences included in the TIGR Grape Gene Index, Fei et al. (2004) compared ESTs from green versus veraison grape berry and identified 95 ripening-induced genes. A similar analysis has been performed in our lab on 15,032 ESTs isolated from immature (S3 stage, 2417 ESTs) and mature (S4 stage, 12,615 ESTs) peach: a total of 164 ripening-induced and 113 ripening-repressed genes were annotated by BLAST against the GeneBank non redundant database (http://www.ncbi.nlm.nih.gov) and then classified into corresponding categories according to their putative functions. As reported in Fig. 1, marked differences between induced and repressed genes are present particularly in cell wall and hormone response categories.

The cDNA sequences of the 95 grape and 164 peach ripening-induced genes were compared at the translated amino acid level using the BLASTX algorithm (cutoff e-value $^{-10}$). 11 peach genes appeared to have homologues in the group of 95 grape genes: among these, genes encoding TFs, as *SEPALLATA 3* (MADS-box family), have been identified (Table 1). In grape berry, a putative role in regulation of ripening has been assigned to the *SEPALLATA3*-like transcription factor: expression analysis demonstrated that in both grapes (Boss et al., 2002) and peaches (Ziliotto et al., 2004) specific transcripts accumulate at ripening possibly indicating a common role of this TF in ripening regulation of both climacteric and non-climacteric fruit.

cDNA-AFLP

This technique has been used to study transcriptome profile changes occurring at ripening in several fruit species as raspberry (Jones et al., 2000), grape (Venter et al., 2001; Burger and Botha, 2004), strawberry (Martelli et al., 2003) and peach (Ziliotto et al., 2005). These works have identified, by comparing cDNA-AFLP profiles and sequences with those present in databases, candidate genes possibly involved in the regulation of the transition from immature to mature fruit. Considering *Vitis vinifera*, we have recently used this approach in the attempt of elucidating the physiological and metabolic changes occurring in epicarp during extended ripening of wine grape berry. Extended ripening of wine grape berries has recently attracted the interest of many wineries for the production of non-traditional and/or new style wines (e.g. late-harvest, “passiti”, reinforced wines). In order to produce these wine types, berries are allowed to over-ripe on-plant or, after harvest, undergo a more or less intense (according to the

enological purpose) dehydration process. In both cases, marked changes of berry composition occur due not only to metabolite concentration but also to physiological and metabolic events developmentally regulated (senescence-related) and/or specifically induced by water loss. These changes have profound effects on the quality traits, the organoleptic evolution and the ageing processes of the resulting wines. Research data on wine grape berry overripening and or/ postharvest dehydration is scant and is mostly related to compositional changes. Following the application of these techniques, in general sugars increase, glucose/fructose and malic/tartaric acid ratios decrease, pH and sugar/organic acid ratio increase and metabolic changes involving aromas and phenolic compounds also occur. With the aim of better understanding the processes occurring during wine grape extended ripening we have applied the cDNA-AFLP technique to compare freshly harvested, late-harvested (picked 7 days later) and off-plant dehydrated (7 days post-harvest, about 15% weight loss) berries. 92 and 82 genes differentially regulated in skins following off- (detachment) and on- (late harvest) plant extended ripening, respectively, have been identified. When compared to control samples (freshly harvested berries), 51 genes appeared repressed and 41 induced in detached berries, whereas 58 were repressed and 24 induced in on-plant over-ripe berries. Some (19 induced and 45 repressed) of these genes are in common but others are specifically up- or down-regulated by each treatment: as reported in Fig. 2, 22 and 6 genes are specifically induced and repressed, respectively, in detached berries, while only 5 and 13 appear to be positively and negatively modulated, respectively, in late-harvested berries. Following sequencing and comparison analysis with sequences present in the dbEST database (http://www.ncbi.nlm.nih.gov), differentially expressed genes showing significant similarity have been grouped into functional categories according to the putative function of the corresponding *Arabidopsis* gene. Even though the precise function of several of the isolated gene remains to be identified, signal transduction, transport and stress response are the three functional categories best represented (Table 2). Some genes appear strictly related to metabolic pathways affecting berry quality and composition: one example is represented by a putative isoflavone reductase, specifically induced by dehydration, that could be involved in the changes occurring in the flavonoid composition of the berry skins.

MICROARRAY

The development of nanotechnologies and their application for biological studies together with the increasing number of nucleotide sequences and cDNAs have led to the development of miniaturised hybridization-based approaches as the microarray, widely used also in plant science (Rensink and Buell, 2005). Following the pioneering application of this technology on strawberry, where a gene involved in aroma biosynthesis has been characterized (Aharoni et al., 2000), the microarray approach has been used to study transcriptome changes during development, ripening and/or postharvest in other fruits as tomato (Alba et al., 2004a and b), Citrus (Pons et al., 2005; Shimada et al., 2005), pear (Fonseca et al., 2005), grape (Terrier et al., 2005; Waters et al., 2005) and peach (ESTree Consortium, 2005; Trainotti et al., 2006). In this latter species, the use of the microarray μPEACH 1.0 containing about 4,800 oligonucleotide probes corresponding to genes expressed in peach fruit throughout development, has allowed to identify 267 and 109 genes that are up- and down- regulated, respectively, in the transition from pre-climacteric to climacteric. Besides genes encoding ethylene receptors and transcription factors, a number of genes involved in quality traits and showing differential expression have been observed: in particular, a new pectin-methyl esterase and two new expansins have been discovered. In addition, several genes encoding enzymes acting in the iso-prenoid biosynthetic pathway appear induced at ripening: a β-carotene hydroxylase, responsible for the formation of β-cryptoxanthin, the most abundant carotenoid of yellow peaches, has been identified.

Some of the previously mentioned microarrays have been cross-used for comparative genomics studies (e.g. within *Solanum* genus, Moore et al., 2005) or for

studying the effects of specific treatments. In order to evaluate the different behaviour of two strawberry cultivars stored under high CO_2 concentration, Ponce-Valadez et al. (2005) have performed specific hybridization using the tomato TOM1 microarray pointing out that specific genes might be involved in conferring different CO_2 sensitivity. A similar approach has been performed in our lab where the transcriptome changes of ripe and partially dehydrated (about 15% of weight loss) wine grape berry skins have been analysed using the µPEACH1.0 microarray. In Table 3, the list of induced and repressed genes is reported: several differentially expressed genes following grape berry post-harvest dehydration belong to stress/defence and signal transduction functional categories indicating the activation of specific metabolic pathways with possible consequences on the skin composition. Of particular interest appears the gene named Snakin1 (down-regulated in partially dehydrated grapes) whose encoded protein has been associated, in potato, with enhanced resistance to pathogens including *Botrytis* (Segura et al., 1999). If this behaviour will be confirmed also in grapes where *Botrytis* represents, in certain production areas, one of the most important spoilage causes, monitoring Snakin1 transcription could represent an useful indicator to assess the degree of resistance/ susceptibility and the different attitude of grape cultivars to extended ripening.

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Tables

Table 1. Common genes induced in both peach fruit and grape berry at ripening detected through in silico analysis. Peach ID, grape ID (as in TIGR Grape Gene Index at http://www.tigr.org/), putative function, e-value and putative functional category are reported.

peach ID	grape ID	putative function	e value	putative functional category
53	TC31726	S-adenosylmethionine synthetase 1	0.00	metabolism
68	TC32310	calcineurin B [P. sativum]	2.00E-66	signal transduction
75	TC31656	ubiquitin precursor - common sunflower	0.00	protein biosynthesis/degradation
250	TC25236	actin [S. rebaudiana]	0.00	cell structure/maintenance
332	TC31690	putative ripening-related protein [V. vinifera]	2.00E-77	unclassified
496	TC25267	expansin [V. labrusca x V. vinifera]	1.00E-124	cell wall
534	TC25663	bZIP transcription factor ATB2 [G. max]	6.00E-55	transcription factor
790	TC31821	calmodulin [A. thaliana]	1.00E-109	signal transduction
975	TC38413	MADS-box protein 4	7.60E-31	transcription factor
1282	TC31873	alcohol dehydrogenase 2 [V. vinifera]	1.00E-166	metabolism
2820	TC31552	putative metallothionein-like protein [V. vinifera]	5.00E-98	defense/stress response

Table 2. Induced (grey) and repressed (white) genes in epicarp of grape berry following off- (detachment) and on- (late harvest) plant extended ripening. Grape ID and putative function were assigned on the basis of BLAST results against dbEST databases using TBLASTX algorithm. Functional categories have been assigned according to those of the corresponding *Arabidopsis* gene.

Grape ID	Putative Function	e value	Functional categories
Vvi.10363	expressed protein [Oryza sativa]	1.00E-109	Energy metabolism
Vvi.2048	expressed protein [Arabidopsis thaliana]	0	
Vvi.1791	succinyl-CoA ligase [GDP-forming] beta-chain putative [Arabidopsis thaliana]	3.00E-138	
Vvi.8005	pyruvate decarboxylase, putative [Arabidopsis thaliana]	2.00E-21	
Vvi.836	UDP-GlcNAc:dolichol phosphate N-acetylglucosamine-1-phosphate transferase, putative [Arabidopsis thaliana]	3.00E-119	
Vvi.1484	pyruvate dehydrogenase E1 component beta subunit, chloroplast [Arabidopsis thaliana]	5.00E-58	
Vvi.7593	isoflavone reductase, putative [Arabidopsis thaliana]	7.00E-108	Secondary metabolism
Vvi.1745	cytochrome P450 family protein [Arabidopsis thaliana]	0	
Vvi.1745	cytochrome P450 family protein [Arabidopsis thaliana]	0	
DT030572	Phytochelatin synthetase-like protein [Arabidopsis thaliana]	6.00E-174	Stress responses
Vvi.7599	expressed protein [Arabidopsis thaliana]	0	
Vvi.7789	putative dehydration-responsive protein RD22 precursor [Oryza sativa]	5.00E-134	
CN914431	expressed protein [Malus x domestica]	3.00E-133	
AJ779498	Populus euphratica cDNA library	9.00E-13	
Vvi.1660	DNAJ heat shock protein, putative [Arabidopsis thaliana]	0	
Vvi.11954	expressed protein [Arabidopsis thaliana]	3.00E-30	
DT031088	Vitis vinifera cDNA library	3.00E-52	
AY954628	stress-inducible protein kinase [Glycine max]	4.00E-10	
Vvi.6889	putative nuclear RNA binding protein A [Oryza sativa]	1.00E-168	DNA binding
Vvi.639	putative RNA-binding protein [Oryza sativa]	6.00E-80	
Vvi.1591	putative ubiquitin-conjugating enzyme E2 [Oryza sativa]	1.00E-91	Protein metabolism
Vvi.7134	NAD-dependent epimerase/dehydratase family protein [Arabidopsis thaliana]	0	
Vvi.1514	ubiquitin carboxyl-terminal hydrolase family 1 protein [Arabidopsis thaliana]	1.00E-165	
Vvi.5412	expressed protein [Arabidopsis thaliana]	8.00E-164	
Vvi.6768	unknown protein, contains zinc finger domain [Oryza sativa]	1.00E-20	
Vvi.1026	eukaryotic translation initiation factor SUI1, putative [Arabidopsis thaliana]	2.00E-174	Transcription
Vvi.8865	protein phosphatase 2C, putative / PP2C, putative [Arabidopsis thaliana]	0	Signal transduction
Vvi.1591	calcium-binding EF hand family protein [Arabidopsis thaliana]	1.00E-97	
Vvi.7921	protein tyrosine phosphatase/kinase interaction sequence protein (PTPKIS1) [Arabidopsis thaliana]	0	
Vvi.6829	multi-copper oxidase type I family protein [Arabidopsis thaliana]	8.00E-133	
Vvi.878	serine/threonine protein kinase, putative [Arabidopsis thaliana]	0	
Vvi.8865	protein phosphatase 2C, putative / PP2C, putative [Arabidopsis thaliana]	0	
DV940295	Vitis vinifera cDNA library	2.00E-117	
Vvi.6760	protein phosphatase 2C, putative / PP2C, putative [Arabidopsis thaliana]	0	
Vvi.13678	serine/threonine protein phosphatase PP1 isozyme 4 (TOPP4) / phosphoprotein phosphatase 1 [Arabidopsis thaliana]	2.00E-34	
CN579622	Malus x domestica cDNA clone	3.00E-21	
Vvi.13151	AMP-dependent synthetase and ligase family protein [Arabidopsis thaliana]	1.00E-33	
CO494678	fbr-sw fibre secondary wall protein [Gossypium hirsutum]	2.00E-43	Transport
Vvi.13128	chloroplast ADP, ATP carrier protein (AATP1) [Arabidopsis thaliana]	9.00E-37	
Vvi.7007	putative H+-transporting ATP synthase [Oryza sativa]	2.00E-117	
Vvi.7007	putative H+-transporting ATP synthase [Oryza sativa]	2.00E-117	
DN496672	cDNA library Populus tremula	5.00E-46	
Vvi.3320	DC1 domain-containing protein [Arabidopsis thaliana]	0	
Vvi.7061	putative permease 1 [Oryza sativa]	5.00E-137	
DT006636	Vitis vinifera cDNA library	4.00E-43	
Vvi.2593	expressed protein [Arabidopsis thaliana]	3.00E-59	
Vvi.7824	Cwf15 / Cwc15 cell cycle control family protein [Arabidopsis thaliana]	5.00E-91	unknown
Vvi.4988	senescence-inducible chloroplast stay-green protein 1 [Lycopersicon esculentum]	3.00E-129	
Vvi.13187	expressed protein [Arabidopsis thaliana]	0	
DT012236	Vitis vinifera cDNA library	4.00E-34	

Table 3. Induced (●) and repressed (○) genes in epicarp of grape berry (cv. Raboso Piave) following post-harvest dehydration identified using μPEACH 1.0 array. Peach and grape IDs are those reported in Peach Genome Oligo Set Version 1.0 (http://omad.operon.com/download/index.php) and in TIGR Grape Gene Index (http://www.tigr.org/), respectively. Putative function and functional categories have been assigned as reported in Table 2.

	Peach ID	Grape ID	Putative function	e value	Functional Categories
●	3280	TC50088	Alkaline/neutral invertase	6,50E-52	Carbohydrate Metabolism
○	1695	TC38467	Phosphoglycerate kinase cytosolic	6,60E-102	
○	1956	TC45293	Alcohol dehydrogenase 2	1,00E-180	
○	5462	TC40801	Pyrophosphate fructose 6-P 1-phosphotransferase	5,40E-104	
○	112	TC40057	Pyruvate decarboxylase	2,50E-92	Cellular metabolism
○	299	TC40562	5-methyltetrahydropteroyltriglutamate homocysteine methyltransferase	0,00E+00	
○	1251	TC45173	Glutaredoxin	3,00E-40	
○	4882	TC48302	Acyl-CoA synthetase	3,10E-38	
●	143	TC50077	Cellulose synthase-like protein D4	6,30E-63	Cell wall metabolism
○	941	TC38812	Expansin	2,90E-97	
○	4993	TC46450	Caffeoyl-CoA-O-methyltrasferase	3,30E-100	
○	5294	TC38737	Cinnamyl alcohol dehydrogenase	2,70E-84	
●	2912	TC50143	Gluthatione reductase	1,10E-18	Defense/stress
●	3041	TC44289	Ankynin	1,10E-18	
●	3326	TC45246	Thioredoxin	4,20E-41	
●	3462	TC38300	Heat schock protein 70	3,30E-125	
○	373	TC44990	Dehydrin	3,80E-13	
○	1292	TC45126	Snakin-1	1,80E-31	
○	1315	TC38954	Dehydration-induced protein ERD15	6,70E-48	
●	2671	TC38867	RAD23-like protein	1,30E-34	DNA repair
●	889	TC38661	26S protease regolatory	1,20E-219	Protein metabolism
●	1730	TC45856	UIP2	5,70E-44	
○	271	TC45475	Adenosyhomocysteinase	1,90E-66	
●	3056	TC42182	Ser/Thr protein kinase isolog	6,20E-55	Transcription
●	5125	TC50135	Protein kinase	1,90E-36	
●	1295	TC51602	Similar to At4g30400	8,50E-15	Signal transduction
●	1310	TC38700	AtNAC2	2,50E-118	
●	2499	TC45046	Similar to At5g25190	4,10E-55	
●	2536	CF606025	WRKY trascription factor	1,20E-34	
○	1824	TC38692	Putative RING-H2 zinc finger protein	1,00E-180	
●	149	TC50077	SAC2	6,70E-23	Transport
●	4723	TC39829	Aquaporin	3,20E-11	

Figures

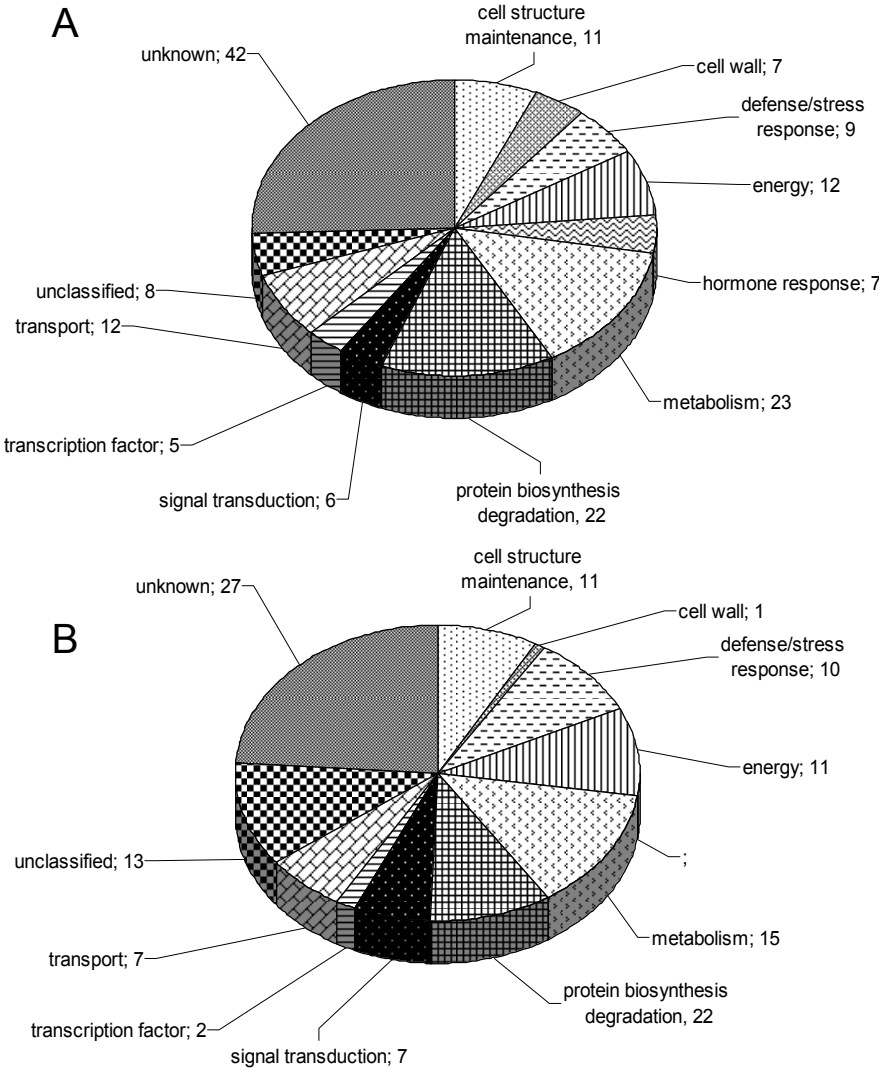

Fig. 1. Classification of the induced (A) and repressed (B) peach ESTs during the transition from immature to mature fruit based on their homologies to sequences available in public databases.

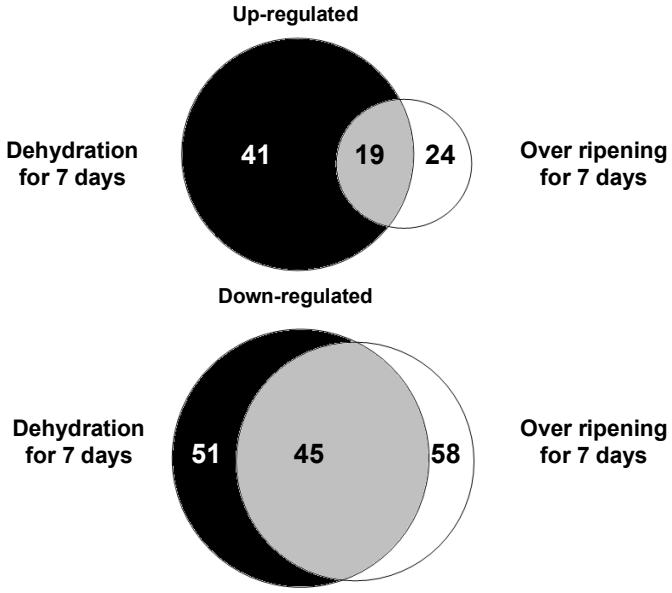

Fig. 2. Venn diagrams reporting the number of genes up- and down-regulated following postharvest dehydration (black) and over ripening (white) in epicarp of wine grape berries (cv. Raboso Piave). The number of genes induced or repressed in common by both treatments is reported in the grey area.