Recovery from water stress affects grape leaf petiole transcriptome.

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Title: Recovery from water stress affects grape leaf petiole transcriptome.

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Abstract

Fast and efficient recovery from water stress is a key determinant of plant adaptation to changing meteorological conditions modulating transpiration, i.e. air temperature and humidity. We analysed transcriptomic responses during rehydration after water stress in grapevine leaf petioles, where embolism formation and repair take commonly place, and where metabolic changes related to embolism recovery are expected to be particularly important.

We compared gene expression of recovering plants with irrigated controls, upon high and low transpiration conditions, using cDNA microarrays. In parallel, we assessed the daily dynamics of water relations, embolism formation and repair, and leaf abscisic acid concentration.

In recovering plants, the most affected gene categories were secondary metabolism, including genes linked to flavonoid biosynthesis; sugar metabolism and transport, including several aquaporin genes. The physiological dynamics of recovery were lower and the number of differentially expressed probes was much lower upon low transpiration than found in actively transpiring grapevines, suggesting the existence of a more intense metabolic reorganization upon high transpiration conditions and of a signal eliciting these responses. In plants recovering under high transpiration, abscisic acid concentrations significantly increased, and, in parallel, transcripts linked to abscisic acid metabolism and signalling (ABA-8'-hydroxylase, serine-threonine kinases, RD22 proteins) were upregulated; a trend that was not observed upon low transpiration.

Our results show that recovery from water stress elicits complex transcriptomic responses in grapevine. The increases observed in abscisic acid cellular levels could represent a signal triggering the activation of responses to rehydration after stress.

Keywords: Aquaporins, Abscisic acid, Drought, Embolism, Microarrays, Transpiration, *Vitis*.

Abbreviations: ABA (Abscisic acid), IRR (Irrigated), WS (Water stress), REC (Recovery), HCFM (Hydraulic Conductance Flow Meter), qRT-PCR (Quantitative Real - Time PCR).

Introduction

Drought is a common environmental stress for grapevines, which are traditionally grown in soils characterized by low water content even when irrigated. Indeed, a mild water stress is considered beneficial to improve the quality of grapes, especially of those that are further processed for winemaking (Chaves et al. 2007).

Rehydration induces the reversal of many effects of water stress, but the dynamics of these processes are diverse: increase of water potential may be rapid (hours or even minutes), while recovery of transpiration and photosynthesis may require days (Kirschbaum 1988; Lovisolo et al. 2008a; Flexas et al. 2009; Zufferey et al. 2011). Non-irrigated crops in temperate climates and irrigated crops under arid climates are subjected to continuous cycles of water stress and rewatering. It is thus crucial for plants to enhance the efficacy and speed of recovery from water stress, in order to maximise growth and production.

Rehydration from water stress has been studied at the molecular level in desiccation-tolerant plants (Vicre et al. 2004). In Arabidopsis, using a transcriptomic approach, a specific activation of genes was shown, implying that water stress recovery is not a simple reversal of stress (Oono et al. 2003). However, to our knowledge, no information is available about transcriptomic changes occurring upon rehydration in crop plants.

Among short-term plant adaptations to water stress, xylem cavitation is one of the most effective, in particular where negative pressures drive water across relatively long distances, as in water-stressed grapevines (Schultz and Matthews 1988; Choat et al. 2010). As for several other effects of water stress, xylem embolism decreases when droughted plants are rewatered. Embolism formation is controlled by biophysical (surface tension of the xylem sap) and anatomical (vessel diameter, pit membrane structure) factors. On the contrary, embolism recovery is not simply a biophysical reversal of embolism formation, but it is tightly controlled and regulated by plant metabolism, as shown by the fact that it can occur at negative water potentials (Holbrook and Zwieniecki 1999; Melcher et al. 2001; Brodersen et al. 2010) and that it can be impaired by treatments affecting metabolic activity (Bucci et al. 2003; Salleo et al. 2004; Lovisolo and Schubert 2006; Lovisolo et al. 2008b). Several studies have provided clues about possible metabolic signals and processes that could control xylem embolism recovery, following diverse

85 experimental approaches (Sakr et al. 2003; Salleo et al. 2004; Domec et al. 2006; Lovisolo and
86 Schubert 2006; Lovisolo et al. 2008b; Secchi and Zwieniecki 2010, 2011). However, scarce
87 molecular evidence is available to date about genes that could play a role in the embolism
88 recovery process.
89 The goal of this study was to profile gene expression during rewatering of water-stressed plants.
90 We used as experimental system the grapevine, which normally experiences important xylem
91 embolism under water stress (Schultz and Matthews 1988; Lovisolo and Schubert 2006). We
92 sampled leaf petioles, where the daily changes in embolism formation and repair have previously
93 been shown to follow a trend similar to what observed in shoots, and where metabolic events
94 related to embolism recovery are expected to be particularly important (Lovisolo et al. 2008a).
95 Indeed, since in grape tissues, physiological responses determined by water stress and embolism
96 recovery strictly occur in perixylematic parenchyma cells (Salleo et al. 2004), petioles, because of
97 their intrinsic anatomic characteristics, are well suited to study molecular changes triggering
98 cycles of embolism formation and repair.
99 The effects of rehydration on gene expression were assessed in plants grown under different
100 transpiration regimes, which are expected to affect the rate of recovery processes (Holbrook and
101 Zwieniecki 1999; Galle et al. 2009; Brodersen et al. 2010).
102 The study was performed on *Vitis vinifera* cv Grenache, a genotype particularly suited to
103 investigate responses to drought, since, being a typical isohydric variety, it well tolerates long-
104 lasting water stress conditions (Schultz 2003; Soar et al. 2004; Vandeleur et al. 2009).
105
106
107 **Materials and methods**
108
109 Plant material
110
111 Seventy-two, two-years-old plants of *Vitis vinifera* L. 'Grenache' grafted on *Vitis riparia* × *Vitis*
112 *berlandieri* 420A (Vivai Cooperativi Rauscedo – San Giorgio della Richinvelda (PN), Italy), were
113 grown in a partially-climate-controlled glasshouse under natural light and photoperiod conditions.
114 Each plant grew in a 10 l container filled with a substrate composed of a sandy-loam soil (pH 7.0;

115 available P 7.9 mg kg^{-1}; organic matter 1.37%; cation exchange capacity 4.58 meq 100 g^{-1})/expanded clay/peat mixture (2:1:1 by weight). From budbreak (10 February) to the beginning of
117 the experimental period, plants were irrigated twice every week to maintain water container
118 capacity (Lovisolo and Schubert 1998). Lateral shoots were removed and plants had 25 leaves at
119 the time of the first experiment, and 30 at the time of the second experiment.
120
121 Experimental set-up
122
123 Recovery from water stress was studied in two different experiments, where rehydration was
124 applied on water stressed plants under different levels of atmospheric water demand. In the first
125 experiment, water stress was applied on 36 plants in a period of low atmospheric evaporative
126 water demand (start June; Experiment A). A second experiment was carried out on a different set
127 of 36 plants under conditions of high atmospheric evaporative water demand (end July:
128 experiment B). Maximum temperature and minimum relative humidity in the days of
129 measurement and sampling were respectively 26°C and 60% in June, and 38°C and 24% in July.
130 In order to induce water stress, irrigation was withheld in each experiment on two thirds of the
131 plants until leaf water potential had reached levels lower than -1.20 MPa. At the beginning of the
132 first following day ("experimental day"), one half of the droughted plants was rehydrated (at 07.00
133 h) by watering pots to container capacity. Three treatments were thus obtained: irrigated (IRR),
134 water stress (WS), and recovery from water stress (REC), each applied to 12 plants.
135 Starting one hour after rewatering, we followed for 12 hours the dynamics of leaf water potential,
136 leaf gas exchange, embolism formation and repair, and ABA content in leaves. Measurements
137 were done in both experiments at 2, 5, 8, and 11 h after rewatering (HAR: corresponding to 9.00;
138 12.00; 15.00; 18.00 h), at each time point on a different set of three plants per treatment. Petioles
139 for molecular analyses were also sampled on the same time points.
140
141 Measurements of leaf gas exchange, leaf water potential, extent of xylem embolism, and ABA
142 analysis
143

144 Leaf transpiration rate, stomatal conductance and vapour pressure deficit (VPD) were recorded
145 using an infra-red gas analyser ADC-LCPro+ system (The Analytical Development Company Ltd)
146 on one leaf per plant inserted in the central region of the shoot (nodes 6 to 18 from the shoot
147 base). Leaf water potential (Ψ_{leaf}) was thereafter measured on two different transpiring leaves per
148 plant inserted in the central region of the shoot, using a Scholander-type pressure chamber (Soil
149 Moisture Equipment Corp.).
150 Xylem embolism was assessed immediately after and on the same plants where Ψ_{leaf} had been
151 measured, on leaf petioles, following the protocol described by Lovisolo et al. (2008a) and using a
152 Hydraulic Conductance Flow Meter (HCFM-XP of Dynamax Inc.) (Tyree et al. 1995). Briefly, one
153 further leaf per plant was chosen in the central region of the shoot and petioles were excised
154 underwater 1 cm from the nodes by bending the shoot and submerging the petiole into a water
155 container. Petioles were connected underwater to the HCFM, and a few seconds after starting the
156 measurement the leaf was cut 1 cm above the petiole insertion. The extent of xylem embolism was
157 determined by comparing the initial hydraulic conductivity (Khi) of petioles with their maximum
158 final hydraulic conductivity (Khf), recorded after removing air emboli by means of transient water
159 flushing, and was expressed as percent loss of conductivity PLC (%) = 100 × (Khf - Khi)/(Khf). The
160 extent of embolism recovery was estimated as difference between PLC of irrigated plants and of
161 recovering plants ($PLC_{REC-IRR}$). Conductivity measurements were performed within 1 h from start of
162 sampling.
163 Leaf ABA content was measured on the same leaf used for conductivity measurements, following
164 the method described in (Lovisolo et al. 2008a).
165
166 RNA extraction, microarray construction and hybridization
167
168 For REC and IRR treatments, RNA was extracted from petioles collected at the same time points
169 where physiological measurements were made, whereas in experiment B we extracted RNA only
170 from petioles collected at 8 HAR. Extraction of RNA from petioles of WS plants was done in both
171 experiments on samples collected at 8 HAR. Three petioles (about 2 g in total), one respectively
172 from the apical, central and basal parts of each plant, were detached, frozen in liquid nitrogen,
173 and stored at -80°C. After grinding petioles in liquid nitrogen, total RNA was extracted following

174 the protocol described by (Carra et al. 2007). RNA integrity and quantity were checked with a
175 2100 Bioanalyzer (Agilent), using the RNA LabChip® assay. Once known the exact RNA yields,
176 samples were treated with the proper concentration of DNase I, RNase-free (Fermentas: 50 U μL^{-1})
177 in according with the manufacturer's instructions, to successfully avoid any risk of DNA
178 contamination. Moreover, to further enhance purity of the DNAse-treated RNA samples, before
179 microarray analysis, a phenol : chloroform : isoamyl alcohol 25 : 24 : 1 (by vol.) purification step,
180 followed by precipitation in sodium acetate and absolute ethanol, was also performed, and RNA
181 quality and concentrations were checked again.
182 Microarray experiments were performed using the grape AROS V1.0 platform
183 (http://www.operon.com), which is based on a set of 14562 70-mer oligonucleotide probes, each
184 specific for one of the ESTs and EST contigs (named TCs, Tentative Consensuses) contained in the
185 *Vitis vinifera* Gene Index (VvGI, release 5) (http://compbio.dfci.harvard.edu/cgi-
186 bin/tgi/gimain.pl?gudb=grape). The glass chips were prepared at CRIBI (University of Padova,
187 Italy), as described by Trainotti et al. (2006). The probes, harbouring a 5' amino linker, were
188 spotted onto glass chips (MICROMAX Glass Slides Superchip™ I, Perkin Elmer) in 48 subgrids (4
189 columns x 12 rows) and each probe was spotted twice in the same chip. Twelve probes carrying
190 the role of positive controls were distributed in each subgrid. Reference spots, obtained from a mix
191 of all positive control oligos, were deposited at constant positions in each subgrid to have
192 information about the spatial variability of the hybridization signal. Twelve randomly generated
193 negative controls were included in each subgrid.
194 Fluorescently labelled cDNA was synthesized, purified, and used for hybridization of the
195 microarrays, as described in Supplemental Materials and methods.
196
197 Microarray experimental design and data analysis
198
199 The effects of water stress were analysed by comparing gene expression in WS and IRR leaf
200 petioles collected at 8 HAR: to this aim, WS and IRR tissue samples from both experiments were
201 respectively pooled before RNA extraction. The effects of rehydration were analysed by comparing
202 gene expression of REC and IRR leaf petioles collected at the same time after rewatering. In Exp.
203 A, a time course of transcriptional regulation under recovery was obtained by carrying out

comparisons at all sampling times (2; 5; 8 and 11 HAR), while in Exp. B expression changes were analysed only at 8 HAR. Each comparison was based on two biological replicates, where the green-fluorescent dye (Cy3) was coupled to either the WS or REC cDNAs and the red-fluorescent dye (Cy5) to the control (IRR) cDNA, and a technical replicate obtained by swapping the dye-labelling on the same cDNA samples. The total number of slides used was thus 12 for Exp. A (4 time points x 1 comparison x 3 replicates), 3 for Exp. B (1 time point x 1 comparison x 3 replicates) and 3 for WS versus IRR comparison.

Microarray slides were scanned as described by Trainotti et al. (2006), using a two-channel confocal microarray scanner (ScanArray1 Lite, Perkin-Elmer), setting the laser power emission between 77% and 82% and the Photomultiplier tube (PMT) between 73% and 75%. Microarray images were analyzed using TIGR Spotfinder v.2.2.4 (www.tm4.org/spotfinder.html), applying the Otsu algorithm, and visually rechecked. The resulting raw data were normalized by applying a Locally Weighted Linear Regression (LOWESS) using the Microarray Data Analysis (TIGR MIDAS, v.2.19, www.tm4.org/midas.html) software.

Normalized expression data were statistically analysed by one-class unpaired Significance Analysis of Microarray (SAM) (Tusher et al. 2001) using the Multi Experiment Viewer (TIGR MeV, v.4.0, www.tm4.org/mev) software, and applying a 66% cut-off filter prior to the analysis. Clones with significant changes in expression were identified at values giving a 10% false discovery rate (FDR). Only those probes resulting significant at the SAM analysis and that contemporarily reached the expression ratio threshold of $\log_2=1$ were retained for further analyses. Probe annotations were updated by query of the respective TC nucleotide sequence at the VvGI database (release 5) against the UniProt/TrEMBL protein database, using the blastx algorithm with a minimum significance value (e-value) of $1e^{-10}$, using the software Blast2GO v.1.7.2 (www.blasto2go.de). Probes were further annotated following the Gene Ontology (GO) functional classification (biological process) (Ashburner et al. 2000) (www.geneontology.org), using the GO mapping step of Blast2GO. GO terms of differentially expressed probes were thus grouped into functional classes using the AgBase GOslimViewer software with Plants filter (http://agbase.msstate.edu/cgi-bin/tools/goslimviewer_select.pl). Functional classes were arbitrarily pooled into higher-order functional categories (Suppl. Table S1). The homogeneity of frequencies of hits falling in the functional categories was finally tested by a χ^2 test.

234 Quantitative PCR analysis of gene expression

235

236 First-strand cDNA was synthesized in triplicate starting from 10 μg of total RNA, extracted as
237 described above, using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems)
238 and according to the manufacturers' instructions.
239 Gene-specific primers (reported in Suppl. Table S2) for quantitative Real Time-PCR (qRT-PCR)
240 assay were designed using the Primer Express® software (v3.0, Applied Biosystems) on the
241 complete *Vitis* cDNA sequences, retrieved from the Genoscope 12X database
242 (http://www.genoscope.cns.fr), corresponding to the TC sequences of the target probes. Only in
243 the case of aquaporin genes, qRT-PCR primers were designed on the heterogeneous 3'
244 untranslated region (UTR), in order to discriminate among the different isoforms of the target
245 aquaporin genes. Reactions were carried out in the StepOnePlus™ Real-Time PCR System
246 (Applied Biosystems) and the SYBR Green method (Power SYBR® Green PCR Master Mix, Applied
247 Biosystems) was chosen for quantifying the amplification results, as previously described
248 (Hugueney et al. 2009). Gene expression was quantified after normalization to the housekeeping
249 genes ubiquitin (*Vv*UBI, VvGI release 7 TC117219) and actin1 (*Vv*ACT1, VvGGI release 7
250 TC134791). Results were calculated as expression ratios (relative quantity, RQ) between either WS
251 or REC samples and IRR controls.

252

253

254 **Results**

255

256 Physiological responses to drought and rehydration

257

258 During both periods of water deprivation (ten days in Exp. A and seven days in Exp. B), leaf water
259 potential (Ψ_{leaf}) progressively decreased to about -1.4 MPa, with no significant differences between
260 the two experiments. Nevertheless, Ψ_{leaf} recovered at different rates in the two experiments: in
261 Exp. A, Ψ_{leaf} reached -0.4 MPa within five hours from rewatering, but it took two hours only to
262 reach the same level in Exp. B (Fig. 1).

263 Diurnal vapour pressure deficit (VPD) was about 12 mbar bar^{-1} with slight variations along the
264 experimental day in Exp. A, while in Exp. B VPD increased above 30 mbar bar^{-1} in the morning,
265 and remained higher than in Exp. A during the rest of the day (Fig. 2a).
266 Stomatal conductance (g_s) increased up to 15.00 h (8 HAR) in Exp. A to maximum values around
267 0.35 mol H_2O m^{-2} s^{-1}, while in Exp. B maximum g_s of irrigated plants was about one third than in
268 Exp. A. In WS plants, g_s values were very low (less than 0.05 mol H_2O m^{-2} s^{-1}) in both
269 experiments. In REC plants, g_s was similar to that of WS plants at the beginning of the day, and
270 then it increased reaching about 50% that of IRR controls (Fig. 2b).
271 Leaf transpiration (E) was high in Exp. A, but it was even higher in Exp. B, due to the high VPD
272 and notwithstanding the low stomatal conductance. In IRR plants, E increased up to 15.00 h (8
273 HAR) in Exp. A to about 4 mmol m^{-2} s^{-1}, while in Exp. B maximum E was about 6 mmol m^{-2} s^{-1}. In
274 WS plants, E was low and similar in both experimental periods. The transpiration rate (E) of REC
275 plants followed a pattern intermediate between IRR and WS plants, and was higher in Exp. B than
276 in Exp. A (Fig. 2c). Net photosynthesis (A), which was not affected by VPD, showed a pattern
277 similar as observed for stomatal conductance (Fig. 2d), resulting in lower values of instantaneous
278 water use efficiency (WUEi = A/E) in Exp. B than in Exp. A. Sub-stomatal CO_2 concentration was
279 correspondingly lower in REC plants than in IRR controls in Exp. A. On the contrary, it was
280 higher in REC than in IRR plants in Exp. B, suggesting a non-stomatal regulation of
281 photosynthesis (Fig. 2e).
282 Water stress induced extensive xylem embolism, with PLC values between 60 and 70% (Fig. 3).
283 Rehydration induced a steady decrease of PLC in both experiments, albeit with different
284 intensities: in the 11 h after rewatering, PLC decreased to 26% in Exp. A and to 60% in Exp. B.
285 The time course of petiole PLC confirmed that grapevines are submitted to diurnal cycles of
286 embolism formation and repair. These cycles were evident in IRR plants, and occurred (even if not
287 significantly) also in REC plants of Exp. B. On the contrary, almost no daily PLC variation was
288 observed in WS plants in both experiments (Fig. 3).
289 In Exp. A, under low transpiration rate, ABA concentration in leaves remained constant at a basal
290 level of about 2000 pmol g^{-1} DW in IRR controls. In REC plants, ABA concentration showed no
291 significant differences in comparison to IRR controls in the first and last parts of the day (3, 5 and

11 HAR), but it was lower than in IRR controls at 8 HAR. On the contrary, in Exp. B, ABA concentration significantly increased in REC plants as compared to IRR controls (Fig. 4).

Patterns of gene regulation under water stress and rehydration

In the microarray comparison between WS and IRR plants, the number of probes spotted on the chip that showed significant hybridization differences after SAM analysis (FDR 10%), and that also satisfied the expression ratio threshold chosen (log_2>1), was 358, where 207 probes were upregulated and 151 downregulated.

On the base of the same threshold parameters, in plants recovering after rehydration, in conditions of low transpiration (Exp. A), 144 probes showed significant expression differences in at least one of the four comparisons. In particular, 21 probes were upregulated and 123 downregulated. Most differences in gene expression were observed just after rewatering (2 HAR), where probes showing a significant difference were 118 at 2 HAR, 11 at 5 HAR, 25 at 8 HAR, and 30 at 11 HAR. In last, in plants recovering under high transpiration (Exp. B), 407 probes showed significant hybridization differences and, among them, 179 underwent transcriptional activation, whereas 228 were downregulated.

Functional grouping of GO hits assigned most differentially expressed genes to the categories response to stress, cell growth and morphogenesis, reproduction, cell cycle and homeostasis, and protein and aminoacid metabolism. Significant differences from equal distribution at the χ^2 test were observed, in particular in Exp B, where the incidence of hits falling in the photosynthesis and energy, carbohydrate metabolism, and transport was significantly higher, while hits in the cell wall and morphogenesis and reproduction, cell cycle and homeostasis were significantly less (Table 1).

The functional categories were also differently affected by up- and downregulation in the different treatments and experiments as indicated by the ratio between the percentage of upregulated probes and of downregulated probes (or vice-versa) for each category (referred in the text respectively as U/D and D/U). The comparison of expression levels in Exp. A (all data points pooled together) showed positive effects of the rehydration treatment in the cell growth and

morphogenesis (U/D = 4.3), lipid metabolism (4.3), and carbohydrate metabolism (2.1) categories, and a negative effect on the response to stress (D/U = 4.06), secondary metabolism (3.57) and nucleic acid metabolism categories (2.8). In Exp. B, positive effects of the rehydration treatment were observed on the secondary metabolism (U/D = 4.26) and carbohydrate metabolism (2.00), and negative effects on the photosynthesis and energy (D/U = 9.05) categories (Fig. 5).

Among the 144 probes differentially regulated in REC plants compared to IRR controls in Exp. A, 32 were also differentially affected by water stress (13 upregulated and 19 downregulated). Among the 407 probes differentially regulated in REC plants compared to IRR controls in Exp. B, 93 were also significantly affected by water stress (47 upregulated and 46 downregulated), while 22 were also differentially regulated by rehydration in Exp. A (2 upregulated and 17 downregulated) (Fig. 6).

Expression changes upon recovery

Within each functional category, differential regulation exclusively induced by rehydration (REC) often followed different patterns in the two experiments (Suppl. Tables S4 and S6).

Photosynthesis and energy

In conditions of low transpiration, at the first time point (2 HAR), three transcripts involved in respiration, photorespiration and fermentation (cytochrome c reductase: TC62094; glycolate oxidase: TC52919; alcohol dehydrogenase: TC63091) were downregulated. A RUBISCO binding protein, thought to act as chaperone for RUBISCO assembly (TC52052), was activated at 5 HAR, while other photosynthesis-related genes, such as a chlorophyll binding protein (TC56895), were downregulated at later time points. Nevertheless, comparing the two experiments, the repression of energy metabolism was more evident upon high transpiration conditions, where several genes, as chlorophyll-binding proteins (TC54828, TC56028, TC55189, TC57521, CB341233, TC65556), subunits of photosystems I and II (TC54542, TC52528, TC61693), and oxygen-evolving enhancer proteins associated to photosystem II (TC54765, TC53930), were significantly downregulated.

One transcript involved in fermentation (alcohol dehydrogenase, TC63091) was downregulated in both conditions.

Carbohydrate metabolism

Under low transpiration a pattern of general downregulation was observed for this category, at the beginning of the time-course (2 HAR), where a sucrose synthase (TC59920) and a glycosyltransferase (TC53455) were the most downregulated transcripts. Upon high transpiration, the expression of enzymes involved in galactose metabolism (e.g. GDP-mannose 3,5 epimerase: TC58885; TC55380), and of a fructokinase (TC57339) was reduced, while a gene encoding an alpha-amylase (TC67671) was significantly activated.

Nucleic acid metabolism, transcription and translation

In condition of low transpiration, this category was dominated by the downregulation of zinc-finger transcription factors (BM437359, TC62891), and by the activation of a DEAD-box RNA-binding protein (TC65154). On the contrary, upon high transpiration, we did not observe significant expression changes in transcription, with the only exception of a MYBA1 transcription factor (TC64719), a key regulator of flavonoid biosynthesis, which was upregulated in parallel with the many transcripts of this pathway (see below).

Secondary metabolism

In Exp. A, several genes involved in flavonoid biosynthesis (3-phosphoshikimate 1-carboxyvinyltransferase-EPSP synthase: TC69881; chalcone synthase: TC67855; phenylalanine-ammonia lyase: TC61248 and TC66528; flavanone-3-hydroxylase: TC67181; leucoanthocyanidin dioxygenase: TC69652,) were downregulated at 2 and at 5 HAR. Two GSTs (TC61062 and CB342131) were also downregulated at 2 HAR, in agreement with a possible function in flavonoid transport into vacuoles.

On the contrary, in Exp. B most of the genes involved in phenylpropanoid and flavonoid biosynthesis were coordinately activated by rehydration, including (in biosynthetic order): 4-coumarate-CoA ligase (TC57438), chalcone synthase (TC67409), chalcone isomerase (TC55034), dihydroflavonol 4-reductase (TC53437; TC51699), flavonol synthase (TC57653), UFGT (TC63201), and anthocyanidin reductase (TC55505).

Transport

Upon low transpiration conditions, this category was not particularly affected: only three transcripts were differentially expressed at 2 and 5 HAR. On the contrary, in Exp. B, genes linked to transport were strongly activated, in particular several members of the PIP-type aquaporin family, including three PIP1 (TC51776, TC60619, TC55780) and two PIP2 (TC58240, TC69190). Also one transporter, a TT12-like protein (TC62162, TC69833), possibly related to flavonoid accumulation was found among upregulated genes in this category.

Response to endogenous stimuli and signal transduction

In Exp. A, this functional class was overall downregulated. Reduced expression was observed for two Ca- and calmodulin-binding proteins (CB009010, CF208534), a RING-finger protein (TC53052), possibly involved in protein/protein interactions, a RLK-type S/T protein kinase (BM437868), member of a large family of plant genes that are involved in responses to biotic and abiotic stress (Lehti-Shiu et al. 2009), and a LEA protein (BE846412), reflecting a downscaling of osmotic adaptations. A downregulation of Ca^{2+} and calmodulin-binding proteins (TC53352; TC55454) was observed also in Exp. B; however an inactivation of protein kinases and receptor-like kinases (TC66054, TC63041, TC53071) was specifically observed only upon high transpiration conditions. In these conditions, ethylene and IAA pathways were also inactivated, through the downregulation of an ethylene receptor (TC52433), of ACC oxidase (TC56709), and of AUX/IAA proteins (TC51731, TC64438, TC60981). Always in Exp. B, we observed the activation of the major gene responsible for ABA catabolism (ABA 8'-hydroxylase, TC67543) (Nambara and Marion-Poll 2005).

Cell growth and morphogenesis

Upon low transpiration, in most gene categories early downregulation of genes activated by water stress was the rule, whereas a different pattern was observed for probes involved in cell growth, such as XET (TC63269), and BRU1, a brassinosteroid-induced protein showing extensive homology with XET (Zurek and Clouse 1994), which were upregulated later in the day. This was confirmed by analysis of the probes affected by both water stress and recovery, among whom those involved in this category (e.g. two XETs, CF212592 and CB346454) followed a consistent late reversal of the effects of water stress (Suppl. Table S6). On the contrary, upon high transpiration, this category was affected in an opposite way, as several probes involved in cell

411 enlargement, such as arabinogalactan proteins (TC65487; TC67718), a COBRA-like protein
412 involved in definition of cell polarity (TC68223), an expansin (TC68519) and two cellulose
413 synthases (TC56114; TC59569) were downregulated and only one probe, encoding phytosulfokine
414 (TC66424) and involved in cell proliferation, was upregulated.
415
416 Many of the results above reported were confirmed also through the analysis of those probes that
417 were contemporarily regulated by WS and by REC treatments. For instance, in Exp. A, the two
418 XET genes (CF212592 and CB346454) upregulated by REC were instead downregulated by WS
419 (Suppl. Table S5).
420 In Exp. B, several probes involved in photosynthesis (e.g. RUBISCO, CB809963 and TC70003)
421 were downregulated both by REC and by WS, whereas two probes involved in secondary
422 metabolism (4-coumarate-CoA ligase TC62308 and leucoanthocyanidin reductase TC61972) were
423 upregulated by both treatments (Suppl. Table S7).
424
425 Validation of microarray data by qRT-PCR
426
427 Expression changes of a group of transcripts, selected among those significantly differentially
428 expressed in microarrays, were quantified by Quantitative Real-Time PCR (qRT-PCR) in petiole
429 extracts used for the comparisons WS/IRR (11 genes) and REC/IRR of Exp. B sampled at 8 HAR
430 (14 genes). For the majority of target genes (8/11 genes for WS/IRR and 14/14 genes for
431 REC/IRR), the pattern of up- or downregulation, observed in microarray results, was confirmed by
432 qRT-PCR analysis, but in the case of three transcripts in the WS/IRR comparison (sucrose
433 transporter, potassium transporter, glucose-6-P translocator), the patterns diverged (Suppl. Table
434 S3). These variations probably depend on the fact that i) some regions of the Vitis genome are still
435 not definitely described (Zenoni et al. 2011), and ii) we amplified cDNA from the Grenache
436 cultivar, while the sequenced genome originates from the PN40024 clone, and cultivar-specific
437 polymorphisms are commonly observed in grapevine. For these reasons, the high specificity of
438 qRT-PCR in detecting target transcripts could have induced the formation of amplicons different
439 than expected.
440

441 **Discussion**

442

443 Physiological and molecular processes of recovery from water stress are affected by transpiration
444 intensity

445

446 As judged from water potential and gas exchange measurements, in both experiments, plants
447 reached the same levels of water stress, comparable to those normally observed in *Vitis vinifera* cv
448 'Grenache' (Lovisolo et al. 2010). However, recovery from water stress took place under different
449 temperature and evaporative conditions, resulting in higher VPDs and lower stomatal
450 conductances in Exp. B than in Exp A. Higher transpirations in Exp. B occurred thanks to the
451 higher VPDs, despite vines in that experiment experienced low levels of stomatal conductance
452 even in irrigated conditions, as predicted in near-isohydric grape cultivars downregulating
453 stomatal opening when VPD increases (Soar et al. 2006).
454 The dynamics of leaf water potential recovery were different in the two experiments, and under
455 low transpiration leaf water potential following rehydration increased more slowly than in Exp. B.
456 This observation implies that, upon high transpiration, water flew rapidly along the non-
457 embolized petiole xylem and parenchyma cells, notwithstanding the fact that at rewatering the
458 non-embolized conductivity was lower than upon low transpiration (18% vs 37% in Exp. A,
459 measured as 100% minus PLC). This induced a rapid increase of leaf water potential, while, upon
460 low transpiration, leaves drew less water, thus recovering water potential at a slower pace.
461 Embolism recovery also followed different dynamics in the two experiments. Following water
462 stress, plants showed a high level of petiole xylem embolism (PLC) in both experiments. In the 11
463 hours after rehydration, PLC decreased in the REC plants to a similar extent (about 20% more
464 than irrigated controls) in both conditions of transpiration, as evidenced by the REC-IRR line in
465 Fig. 3. However, recovery was faster under high transpiration, where it was completed by about
466 two thirds at 5 HAR; while the same level of recovery in Exp. A was reached after 8 HAR.
467 Are the different dynamics of water potential end embolism recovery causally connected? A first
468 hypothesis can be based on embolism recovery being essentially controlled by xylem water
469 tension, which we estimate to be equal to leaf water potential as soil water potential was kept
470 constant in the two experiments. In this hypothesis, upon rewatering water would flow along the

non embolized and the recovering vessels to the leaves, thus increasing leaf water potential and releasing tension, which would in turn allow physical re-absorption of the cavitations. This hypothesis is satisfied by the fact that embolism recovery was faster upon high transpiration, where stress recovery was faster.

However, recovery from water stress has been shown to consist not only in a passive hydraulic process, but also to be based on active mechanisms supported by metabolic processes (Holbrook and Zwieniecki 1999; Oono et al. 2003; Salleo et al. 2004). Thus a second, additional hypothesis to explain the different dynamics of recovery in the two experiments is that high transpiration may evoke a non-hydraulic signal able to induce regulatory and metabolic processes. The obvious candidate for such role is ABA, which is transported in the grapevine xylem (Downton et al. 1988), and whose delivery to the leaf could be modulated by leaf transpiration rate. In a previous paper (Lovisolo et al. 2008a), we reported a surge of ABA concentration in petioles recovering from water stress under high transpiration, with levels even higher than in petioles of water stressed plants, and we argued that this was due to fast transport of ABA to recovering leaves by the transpiration stream. In this study, we confirm that, under high transpiration, leaf ABA concentration increases in REC leaves compared with IRR leaves, but furthermore we show that, under low transpiration conditions (Exp. A), this ABA surge is absent and a slight increase of ABA concentration can be measured only in the last part of the day. The different patterns of ABA accumulation in recovering plants under different levels of transpiration were in agreement with corresponding changes of stomatal conductivity, confirming reports of stomatal closure under recovery from water stress at high transpiration (Lovisolo et al. 2008a; Flexas et al. 2009). These results open the hypothesis that, in addition to its well-known role in controlling stomatal conductance in grape (Soar et al. 2004), ABA could also trigger active processes of recovery from water stress in presence of high transpiration.

Transcriptome modifications induced by rehydration may underlie water potential and embolism recovery

Under both transpiration regimes, rewatering induced changes in gene expression profiles. In Exp A, where we profiled expression changes at four time-points along the day following rewatering,

501 the highest number of differentially expressed probes was observed at the first sampling point (2
502 HAR). At this stage many genes, involved in particular in carbohydrate, nucleic acid and protein
503 metabolism, secondary metabolism, responses to stress, and signal transduction underwent
504 negative regulation, suggesting a general inactivation of stress-induced metabolisms and, in
505 parallel, of the related signalling processes. Later on (11 HAR), genes involved in growth and
506 morphogenesis were activated. Thus, in grape petioles, the recovery process under low
507 transpiration conditions broadly follows the model described in Arabidopsis by Oono et al. (2003),
508 with an early reversal of stress followed by an activation of growth and development.
509 The physiological differences observed during recovery under different transpiration regimes were
510 mirrored by quantitative and qualitative differences of gene expression. Comparing samples
511 collected at the same time after rewatering (8 HAR), in Exp. A only 25 probes showed a significant
512 alteration in transcript concentration, whilst in Exp. B 407 probes were differentially expressed.
513 Even though the genes differentially expressed by water stress are subtracted from those
514 differentially regulated by rewatering in Exp. B (i.e. if expression changes of these genes are
515 supposed to be only a later effect of water stress) the results are respectively 12 and 314,
516 indicating that a much more intense gene expression reorganization takes place upon rewatering
517 upon high than low transpiration conditions.
518 A few trends of gene regulation, observed under recovery in conditions of low transpiration, were
519 confirmed under high transpiration. This was the case of the general downregulation of genes
520 involved in photosynthesis. This suggests that, during recovery from water stress, photosynthesis
521 is limited in petioles, in agreement with the reduction of the efficiency of the photosynthetic
522 system, which was described in grape and tobacco leaves recovering from water stress in
523 comparable conditions (Flexas et al. 2009; Galle et al. 2009).
524 However, under high transpiration new and unexpected patterns of gene regulation emerged. One
525 was the case of the general inactivation of genes involved in cell growth and morphogenesis (as
526 arabinogalactans and expansins). This observation, in agreement with the downregulation of
527 photosynthesis, suggests a general arrest of growth processes in leaf petioles recovering under
528 high transpiration. Downregulation of growth and photosynthesis could allow a redirection of
529 metabolism towards active processes of recovery, which could be at the base of the faster recovery
530 observed in these conditions.

Secondary metabolism was inhibited under recovery in Exp. A, while it was generally activated in Exp. B; in particular, genes representing most of the phenylpropanoid and flavonoid biosynthetic pathways were activated. In addition, a transcription factor (VvmybA2), which specifically upregulates anthocyanidin biosynthesis in grape (Kobayashi et al. 2002; Fournier-Level et al. 2009), and two glutathione-S-transferases (GSTs) were also activated. Besides other metabolic roles, evidences for GST involvement in vacuolar transport of flavonoids have already been reported in grape (Ageorges et al. 2006).

Moreover, since secondary metabolism also provides substrates for the polymerization of lignin and condensed tannins, which are present in the xylem of young grape stems (Jordao et al. 2001), the activation of this gene category could be necessary if new xylem vessels are developing during recovery from water stress, in order to sustain an important water demand from the transpiring leaves.

The Transport functional group was generally activated by rehydration, together with several amino acid permeases, which could be involved in a reorganization of protein biosynthesis, and a few MATE transporters. A role for this protein family in anthocyanin delivery into vacuoles has recently been demonstrated by Gomez et al. (2011). However, the main feature emerging from the expression data was the activation of plasma membrane aquaporins (PIPs). The members of this subfamily of proteins are normally expressed in different grape leaf organs, including petioles (Baiges et al. 2001), where they facilitate transcellular water transport. The role of petiole aquaporins in the recovery process may be double. On one side, aquaporins can contribute to accelerate axial cell-to-cell movement of water through the cortical parenchyma of leaf petioles, favouring recovery of water potential: this hypothesis, however, must take into account that, at the time of sampling (8 HAR), the water potential had completely recovered since 2 hours at least. Another possibility is that they contribute to refill embolized vessels by facilitating radial water flow from parenchyma cells to xylem vessels, in agreement with the fact that embolism recovery was still active at the moment of sampling. Several studies have proposed the involvement of aquaporins in embolism recovery, where they would facilitate the transfer of water from parenchyma cells to embolized xylem vessels (Martre et al. 2002; Sakr et al. 2003; Kaldenhoff et al. 2008; Secchi and Zwieniecki 2010). Although our observation cannot prove such a role, it is interesting to note that the activation of aquaporins was gene-specific: only the water-transporting

561 VvPIP2;1 was upregulated, together with three PIP1 genes, which do not transport water when
562 expressed in *Xenopus* oocytes (Vandeleur et al. 2009) and are though to have regulatory functions
563 (Kaldenhoff and Fischer 2006).
564 The accumulation of soluble sugars in the embolized xylem vessels has been proposed to serve as
565 an osmotic driving force for attracting water (through aquaporins) within the embolized vessels
566 that are at negative pressures (Salleo et al. 1996), or as a signal of vessel embolism (Secchi and
567 Zwieniecki 2011). In recovering shoots, we observed the activation of an alpha-amylase, which
568 could be involved in hydrolysing starch, as proposed in poplar by Secchi and Zwieniecki (2011).
569 On the contrary, no activation of sugar transporters was observed, indicating that either this
570 process is less important in grape than in poplar, or that these transporters are post-
571 transcriptionally regulated.
572
573 An ABA signal may induce molecular responses to rehydration in grapevine
574
575 Gene expression changes in recovering plants under conditions of low and high transpiration were
576 often contrasting, implying that different signals are originated in these two conditions. One of
577 these signals can be ABA, as we observed no differences in ABA concentration between water-
578 stressed and recovering plants under low transpiration, while, under high transpiration, we
579 measured a surge of ABA in recovering plants. In a previous paper (Lovisolo et al. 2008a), we
580 speculated that the ABA surge in leaves recovering upon high transpiration depends on passive
581 transport from the roots, due to the rise in water flux following rehydration, and accordingly, in
582 the present work, we do not see activation of ABA biosynthetic genes in recovering petioles, but
583 only of ABA-metabolizing genes, such as ABA-8'-hydroxylase. The ABA signal activates wide
584 transcriptional responses in Arabidopsis (Seki et al. 2002; Matsui et al. 2008). When applied to
585 grape berries, ABA activates expression of flavonoid biosynthetic genes and pathogen response
586 proteins (Jeong et al. 2004; Giribaldi et al. 2010). In recovering petioles under high transpiration,
587 the increase in ABA concentration was matched by well-known transcriptional effects of ABA,
588 such as the activation of RD22 proteins and of ABA-catabolyzing enzymes, such as ABA-8'-
589 hydroxylase. Other probes upregulated in this experiment represent genes known to be activated
590 by ABA, such as TC54154, a calmodulin-like protein (Delk et al. 2005), and TC56494, a serine-

591 threonine kinase (Kobayashi et al. 2004, 2005). An increase of endogenous ABA upon recovery
592 may represent an after-effect of water stress, controlling, besides stomatal closure, other
593 checkpoints of water transport throughout the plant during rehydration, thus affecting the speed
594 and dynamics of recovery from water stress. One such role of ABA could be in the control of
595 aquaporin expression. It has been shown that ABA can upregulate PIP-type aquaporins in roots of
596 Arabidopsis (Jang et al. 2007), maize (Parent et al. 2009) and tobacco (Mahdieh and Mostajeran
597 2009), and in leaves of bean (Montalvo-Hernandez et al. 2008). The results of other studies, where
598 high transpiration upregulates shoot aquaporins but where ABA was not measured, could also be
599 explained by such this mechanism. Transpiration demand triggers an increase in PIP2 expression
600 in rice roots at the cell surface around the xylem (Sakurai-Ishikawa et al. 2011); shade-grown
601 poplars experiencing a sudden increase in light, enhancing transpiration, exhibit increased
602 transcript abundance of 15 aquaporin genes (Almeida-Rodriguez et al. 2011). The transcriptional
603 effect of ABA on aquaporins, however, may be complex: an attenuation of transpiration is required
604 for the enhancement of membrane permeability and the upregulation of aquaporins in the plasma
605 membrane of maize protoplasts (Morillon and Chrispeels 2001). In Arabidopsis leaves, xylem-
606 borne ABA represses water mesophyll conductance, possibly through inactivation of aquaporins
607 (Shatil-Cohen et al. 2011). ABA could thus selectively act on specific members of the aquaporin
608 family, in order to alleviate the effects of water stress. Further research, involving e.g enhanced
609 xylem delivery of exogenous ABA to embolized xylem vessels, will be needed to test this
610 hypothesis.
611
612
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614
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References

Ageorges A, Fernandez L, Vialet S, Merdinoglou D, Terrier N, Romieu C (2006) Four specific isogenes of the anthocyanin metabolic pathway are systematically co-expressed with the red colour of grape berries. Plant Sci 170: 372-383

Almeida-Rodriguez AM, Hacke UG, Laur J (2011) Influence of evaporative demand on aquaporin expression and root hydraulics of hybrid poplar. Plant Cell Environ 34: 1318-1331

Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM, Sherlock G (2000) Gene Ontology: tool for the unification of biology. Nat Rev Genet 25: 25-29

Baiges I, Schaffner AR, Mas A (2001) Eight cDNA encoding putative aquaporins in *Vitis* hybrid Richter-110 and their differential expression. J Exp Bot 52: 1949-1951

Brodersen CR, McElrone AJ, Choat B, Matthews MA, Shackel KA (2010) The dynamics of embolism repair in xylem: *in vivo* visualizations using High-Resolution Computed Tomography. Plant Physiol 154: 1088-1095

Bucci SJ, Scholz FG, Goldstein G, Meinzer FC, Sternberg LDL (2003) Dynamic changes in hydraulic conductivity in petioles of two savanna tree species: factors and mechanisms contributing to the refilling of embolized vessels. Plant Cell Environ 26: 1633-1645

Carra A, Gambino G, Schubert A (2007) A cetyltrimethylammonium bromide-based method to extract low-molecular-weight RNA from polysaccharide-rich plant tissues. Anal Biochem 360: 318-320

Chaves MM, Santos TP, Souza CR, Ortuno MF, Rodrigues ML, Lopes CM, Maroco JP, Pereira JS (2007) Deficit irrigation in grapevine improves water-use efficiency while controlling vigour and production quality. Ann Appl Biol 150: 237-252

Choat B, Gambetta GA, Shackel KA, Matthews MA (2009) Vascular function in grape berries across development and its relevance to apparent hydraulic isolation. Plant Physiol 151: 1677-1687

Choat B, Drayton WM, Brodersen C, Matthews MA, Shackel KA, Wada H, McElrone AJ (2010) Measurement of vulnerability to water stress-induced cavitation in grapevine: a comparison of four techniques applied to a long-vesseled species. Plant Cell Environ 33: 1502-1512

Delk NA, Johnson KA, Chowdhury NI, Braam J (2005) CML24, regulated in expression by diverse stimuli, encodes a potential Ca^{2+} sensor that functions in responses to abscisic acid, daylength, and ion stress. Plant Physiol 139: 240-253

Domec JC, Scholz FG, Bucci SJ, Meinzer FC, Goldstein G, Villalobos-Vega R (2006) Diurnal and seasonal variation in root xylem embolism in neotropical savanna woody species: impact on stomatal control of plant water status. Plant Cell Environ 29: 26-35

Downton WJS, Loveys BR, Grant WJR (1988) Stomatal closure fully accounts for the inhibition of photosynthesis by abscisic-acid. New Phytol 108: 263-266

Flexas J, Baron M, Bota J, Ducruet JM, Galle A, Galmes J, Jimenez M, Pou A, Ribas-Carbo M, Sajnani C, Tomas M, Medrano H (2009) Photosynthesis limitations during water stress acclimation and recovery in the drought-adapted *Vitis* hybrid Richter-110 (*V-berlandieri*x*V-rupestris*). J Exp Bot 60: 2361-2377

Fournier-Level A, Le Cunff L, Gomez C, Doligez A, Ageorges A, Roux C, Bertrand Y, Souquet JM, Cheynier V, This P (2009) Quantitative genetic bases of anthocyanin variation in grape (*Vitis vinifera* L. ssp sativa) berry: a quantitative trait locus to quantitative trait nucleotide integrated study. Genetics 183: 1127-1139

Galle A, Florez-Sarasa I, Tomas M, Pou A, Medrano H, Ribas-Carbo M, Flexas J (2009) The role of mesophyll conductance during water stress and recovery in tobacco (*Nicotiana sylvestris*): acclimation or limitation? J Exp Bot 60: 2379-2390

669 Giribaldi M, Geny L, Delrot S, Schubert A (2010) Proteomic analysis of the effects of ABA
670 treatments on ripening *Vitis vinifera* berries. J Exp Bot 61: 2447-2458
671 Gomez C, Conejero G, Torregrosa L, Cheynier V, Terrier N, Ageorges A (2011) In vivo grapevine
672 anthocyanin transport involves vesiclemediated trafficking and the contribution of anthoMATE
673 transporters and GST. Plant J 67: 960-970
674 Holbrook NM, Zwieniecki MA (1999) Embolism repair and xylem tension: do we need a miracle?
675 Plant Physiol 120: 7-10
676 Hugueney P, Provenzano S, Verries C, Ferrandino A, Meudec E, Batelli G, Merdinoglu D,
677 Cheynier V, Schubert A, Ageorges A (2009) A novel cation-dependent O-methyltransferase
678 involved in anthocyanin methylation in grapevine. Plant Physiol 150: 2057-2070
679 Jang JY, Lee SH, Rhee JY, Chung GC, Ahn SJ, Kang HS (2007) Transgenic Arabidopsis and
680 tobacco plants overexpressing an aquaporin respond differently to various abiotic stresses. Plant
681 Mol Biol 64: 621-632
682 Jeong ST, Goto-Yamamoto N, Kobayashi S, Esaka A (2004) Effects of plant hormones and shading
683 on the accumulation of anthocyanins and the expression of anthocyanin biosynthetic genes in grape
684 berry skins. Plant Sci 167: 247-252
685 Jordao AM, Ricardo-da-Silva JM, Laureano O (2001) Evolution of proanthocyanidins in bunch
686 stems during berry development (*Vitis vinifera* L.). Vitis 40: 17-22
687 Kaldenhoff R, Fischer M (2006) Functional aquaporin diversity in plants. Biochim Biophys Acta -
688 Biomembranes 1758: 1134-1141
689 Kaldenhoff R, Ribas-Carbo M, Flexas J, Lovisolo C, Heckwolf M, Uehlein N (2008) Aquaporins
690 and plant water balance. Plant Cell Environ 31: 658-666
691 Kirschbaum MUF (1988) Recovery of photosynthesis from water-stress in *Eucaliptus pauciflora* - a
692 process in 2 stages. Plant Cell Environ 11: 685-694
693 Kobayashi S, Ishimaru M, Hiraoka K, Honda C (2002) Myb-related genes of the Kyoho grape (*Vitis*
694 *labruscana*) regulate anthocyanin biosynthesis. Planta 215: 924-933
695 Kobayashi Y, Murata M, Minami H, Yamamoto S, Kagaya Y, Hobo T, Yamamoto A, Hattori T
696 (2005) Abscisic acid-activated SNRK2 protein kinases function in the gene-regulation pathway of
697 ABA signal transduction by phosphorylating ABA response element-binding factors. Plant J 44:
698 939-949
699 Kobayashi Y, Yamamoto S, Minami H, Kagaya Y, Hattori T (2004) Differential activation of the
700 rice sucrose nonfermenting1-related protein kinase2 family by hyperosmotic stress and abscisic
701 acid. Plant Cell 16: 1163-1177
702 Lehti-Shiu MD, Zou C, Hanada K, Shiu SH (2009) Evolutionary history and stress regulation of
703 plant receptor-like kinase/pelle genes. Plant Physiol 150: 12-26
704 Lovisolo C, Perrone I, Carra A, Ferrandino A, Flexas J, Medrano H, Schubert A (2010) Drought-
705 induced changes in development and function of grapevine (*Vitis* spp.) organs and in their hydraulic
706 and non-hydraulic interactions at the whole-plant level: a physiological and molecular update. Funct
707 Plant Biol 37: 98-116
708 Lovisolo C, Perrone I, Hartung W, Schubert A (2008a) An abscisic acid-related reduced
709 transpiration promotes gradual embolism repair when grapevines are rehydrated after drought. New
710 Phytol 180: 642-651
711 Lovisolo C, Schubert A (1998) Effects of water stress on vessel size and xylem hydraulic
712 conductivity in *Vitis vinifera* L. J Exp Bot 49: 693-700
713 Lovisolo C, Schubert A (2006) Mercury hinders recovery of shoot hydraulic conductivity during
714 grapevine rehydration: evidence from a whole-plant approach. New Phytol 172: 469-478
715 Lovisolo C, Tramontini S, Flexas J, Schubert A (2008b) Mercurial inhibition of root hydraulic
716 conductance in *Vitis* spp. rootstocks under water stress. Environ Exp Bot 63: 178-182
717 Mahdieh M, Mostajeran A (2009) Abscisic acid regulates root hydraulic conductance via aquaporin
718 expression modulation in *Nicotiana tabacum*. J Plant Physiol 166: 1993-2003

719 Martre P, Morillon R, Barrieu F, North GB, Nobel PS, Chrispeels MJ (2002) Plasma membrane
720 aquaporins play a significant role during recovery from water deficit. Plant Physiol 130: 2101-2110
721 Matsui A, Ishida J, Morosawa T, Mochizuki Y, Kaminuma E, Endo TA, Okamoto M, Nambara E,
722 Nakajima M, Kawashima M, Satou M, Kim JM, Kobayashi N, Toyoda T, Shinozaki K, Seki M
723 (2008) Arabidopsis transcriptome analysis under drought, cold, high-salinity and ABA treatment
724 conditions using a tiling array. Plant Cell Physiol 49: 1135-1149
725 Melcher PJ, Goldstein G, Meinzer FC, Yount DE, Jones TJ, Holbrook NM, Huang CX (2001)
726 Water relations of coastal and estuarine *Rhizophora mangle*: xylem pressure potential and dynamics
727 of embolism formation and repair. Oecologia 126: 182-192
728 Montalvo-Hernandez L, Piedra-Ibarra E, Gomez-Silva L, Lira-Carmona R, Acosta-Gallegos JA,
729 Vazquez-Medrano J, Xoconostle-Cazares B, Ruiz-Medrano R (2008) Differential accumulation of
730 mRNAs in drought-tolerant and susceptible common bean cultivars in response to water deficit.
731 New Phytol 177: 102-113
732 Morillon R, Chrispeels MJ (2001) The role of ABA and the transpiration stream in the regulation of
733 the osmotic water permeability of leaf cells. Proc Natl Acad Sci U S A 98: 14138-14143
734 Nambara E, Marion-Poll A (2005) Abscisic acid biosynthesis and catabolism. Ann Rev Plant Biol
735 56: 165-185
736 Oono Y, Seki M, Nanjo T, Narusaka M, Fujita M, Satoh R, Satou M, Sakurai T, Ishida J, Akiyama
737 K, Iida K, Maruyama K, Satoh S, Yamaguchi-Shinozaki K, Shinozaki K (2003) Monitoring
738 expression profiles of Arabidopsis gene expression during rehydration process after dehydration
739 using ca. 7000 full-length cDNA microarray. Plant J 34: 868-887
740 Parent B, Hachez C, Redondo E, Simonneau T, Chaumont F, Tardieu F (2009) Drought and
741 abscisic acid effects on aquaporin content translate into changes in hydraulic conductivity and leaf
742 growth rate: a trans-scale approach. Plant Physiol 149: 2000-2012
743 Sakr S, Alves G, Morillon RL, Maurel K, Decourteix M, Guilliot A, Fleurat-Lessard P, Julien JL,
744 Chrispeels MJ (2003) Plasma membrane aquaporins are involved in winter embolism recovery in
745 walnut tree. Plant Physiol 133: 630-641
746 Sakurai-Ishikawa J, Murai-Hatano M, Hayashi H, Ahamed A, Fukushi K, Matsumoto T, Kitagawa
747 Y (2011) Transpiration from shoots triggers diurnal changes in root aquaporin expression. Plant
748 Cell Environ 34: 1150-1163
749 Salleo S, Lo Gullo MA, Trifilo P, Nardini A (2004) New evidence for a role of vessel-associated
750 cells and phloem in the rapid xylem refilling of cavitated stems of *Laurus nobilis* L. Plant Cell
751 Environ 27: 1065-1076
752 Salleo S, LoGullo MA, DePaoli D, Zippo M (1996) Xylem recovery from cavitation-induced
753 embolism in young plants of *Laurus nobilis*: a possible mechanism. New Phytol 132: 47-56
754 Schultz HR (2003) Differences in hydraulic architecture account for near-isohydric and anisohydric
755 behaviour of two field-grown *Vitis vinifera* L. cultivars during drought. Plant Cell Environ 26:
756 1393-1405
757 Schultz HR, Matthews MA (1988) Resistance to water transport in shoots of *Vitis vinifera* L. -
758 relation to growth at low water potential. Plant Physiol 88: 718-724
759 Secchi F, Zwieniecki MA (2010) Patterns of PIP gene expression in *Populus trichocarpa* during
760 recovery from xylem embolism suggest a major role for the PIP1 aquaporin subfamily as
761 moderators of refilling process. Plant Cell Environ 33: 1285-1297
762 Secchi F, Zwieniecki MA (2011) Sensing embolism in xylem vessels: the role of sucrose as a
763 trigger for refilling. Plant Cell Environ 34: 514-524
764 Seki M, Ishida J, Narusaka M, Fujita M, Nanjo T, Umezawa T, Kamiya A, Nakajima M, Enju A,
765 Sakurai T, Satou M, Akiyama K, Yamaguchi-Shinozaki K, Carninci P, Kawai J, Hayashizaki Y,
766 Shinozaki K (2002) Monitoring the expression pattern of around 7,000 Arabidopsis genes under
767 ABA treatments using a full-length cDNA microarray. Funct Integr Genomics 2: 282-291

768 Shatil-Cohen A, Attia Z, Moshelion M (2011) Bundle-sheath cell regulation of xylem-mesophyll
769 water transport via aquaporins under drought stress: a target of xylem-borne ABA? Plant J 67: 72-
770 80
771 Soar CJ, Speirs J, Maffei SM, Loveys BR (2004) Gradients in stomatal conductance, xylem sap
772 ABA and bulk leaf ABA along canes of *Vitis vinifera* cv. Shiraz: molecular and physiological
773 studies investigating their source. Funct Plant Biol 31: 659-669
774 Soar CJ, Speirs J, Maffei SM, Penrose AB, McCarthy MG, Loveys BR (2006) Grape vine varieties
775 Shiraz and Grenache differ in their stomatal response to VPD: apparent links with ABA physiology
776 and gene expression in leaf tissue. Aust J Grape Wine Res 12: 2-12
777 Trainotti L, Bonghi C, Ziliotto F, Zanin D, Rasori A, Casadoro G, Ramina A, Tonutti P (2006) The
778 use of microarray mu PEACH1.0 to investigate transcriptome changes during transition from pre-
779 climacteric to climacteric phase in peach fruit. Plant Sci 170: 606-613
780 Tusher VG, Tibshirani R, Chu G (2001) Significance analysis of microarrays applied to the ionizing
781 radiation response. Proc Natl Acad Sci U S A 98: 5116-5121
782 Tyree MT, Patino S, Bennink J, Alexander J (1995) Dynamic measurements of root hydraulic
783 conductance using a high-pressure flowmeter in the laboratory and field. J Exp Bot 46: 83-94
784 Vandeleur RK, Mayo G, Shelden MC, Gilliham M, Kaiser BN, Tyerman SD (2009) The role of
785 plasma membrane intrinsic protein aquaporins in water transport through roots: diurnal and drought
786 stress responses reveal different strategies between isohydric and anisohydric cultivars of grapevine.
787 Plant Physiol 149: 445-460
788 Vicre M, Farrant JM, Driouich A (2004) Insights into the cellular mechanisms of desiccation
789 tolerance among angiosperm resurrection plant species. Plant Cell Environ 27: 1329-1340
790 Zenoni S, Ferrarini A, Giacomelli E, Xumerle L, Fasoli M, Malerba G, Bellin D, Pezzotti M,
791 Delledonne M (2010) Characterizationof transcriptional complexity during berry development in
792 *Vitis vinifera* using RNA-seq. Plant Physiol 152: 1787-1795
793 Zufferey V, Cochard H, Ameglio T, Spring JL, Viret O (2011) Diurnal cycles of embolism
794 formation and repair in petioles of grapevine (*Vitis vinifera* cv. Chasselas) J Exp Bot 62: 3885-3894
795 Zurek DM, Clouse SD (1994) Molecular cloning and characterization of a brassinosteroid -
796 regulated gene from elongating soybean (*Glycine max* L.) epicotyls. Plant Physiol 104: 161-170
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800 **Captions to figures**

801

802 **Fig. 1** Leaf water potential (Ψ_{leaf}) measured on Grenache plants irrigated (IRR), subjected to water
803 stress (WS), or rewatered (REC) in the two experiments (A and B). Arrow shows time of
804 rehydration. Bars are standard errors of the means (*n*=6).

805

806 **Fig. 2** Vapour pressure deficit (VPD) (**a**), stomatal conductance (g_s) (**b**), leaf transpiration (E) (**c**),
807 net photosynthesis A (**d**), and substomatal CO_2 concentration (Ci) (**e**) measured on Grenache
808 plants irrigated (IRR), subjected to water stress (WS), or rewatered (REC) in the two experiments
809 (A and B). Arrow shows time of rehydration. Bars are standard errors of the means (*n*=3)

810

811 **Fig. 3** Percent loss of conductivity (PLC) changes measured on leaf petioles of Grenache plants
812 irrigated (IRR), subjected to water stress (WS), or rewatered (REC) in Exp. A (**a**) and in Exp. B (**b**).
813 Arrow shows time of rehydration. Bars are standard errors of the means (*n*=3). REC-IRR values
814 (circles) have been calculated by subtracting to PLC values recorded in recovering plants the
815 values measured under irrigated conditions in order to draw a virtual trend line showing the net
816 incidence of embolism formation (increase of PLC) or repair (decrease of PLC) during plant
817 recovery.

818

819 **Fig. 4** Time course of foliar ABA contents measured in leaves of Grenache plants irrigated (IRR) or
820 rewatered (REC) in Exp. A (**a**) and in Exp B (**b**). Arrow shows time of rehydration. Bars are
821 standard errors of the means (*n*=3).

822

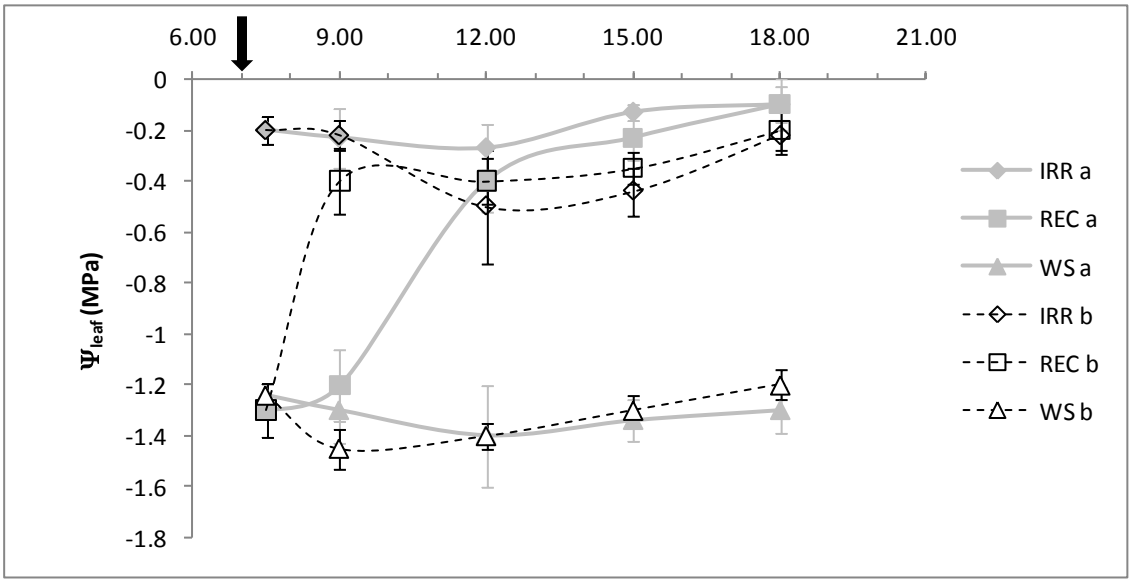
823 **Fig. 5** Percent incidence of GO hits of up- and downregulated probes showing significant
824 expression differences and reaching the $\log_2$>1 threshold of expression ratio in the REC plants
825 relative to IRR plants in all the time points of Exp. A (**a**), and in the REC plants relative to the IRR
826 of Exp. B (**b**).

827

828 **Fig. 6** Venn diagrams showing the number of probes whose expression was up- or downregulated
829 (reaching the $\log_2$>1 threshold of expression ratios) by water stress (WS) or rehydration (REC)

relative to IRR controls at all the time points in Exp. A (above left), and in Exp. B (above right), and by rehydration in the two experiments (REC A and REC B) (below).

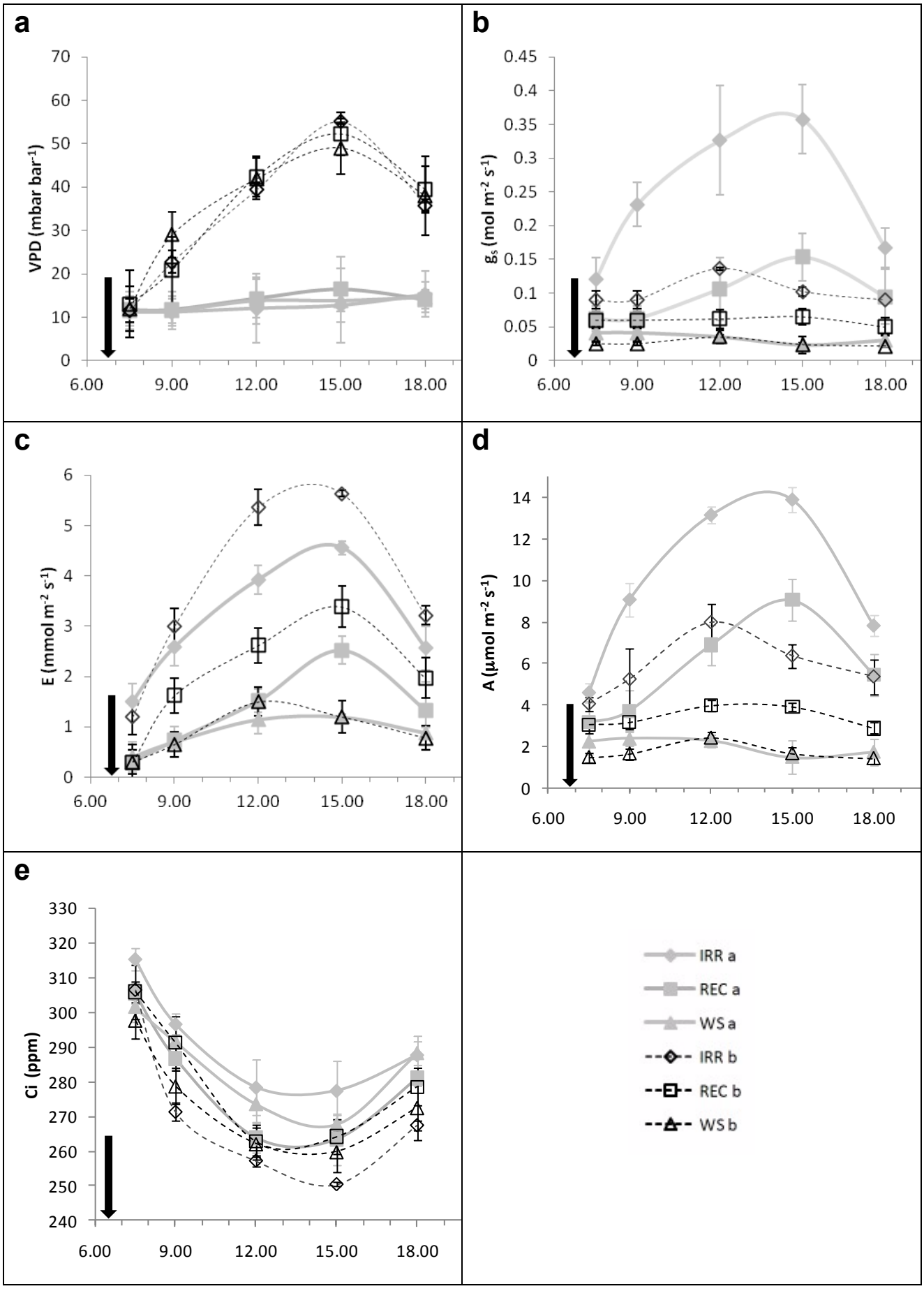
833 **Fig. 1**



834

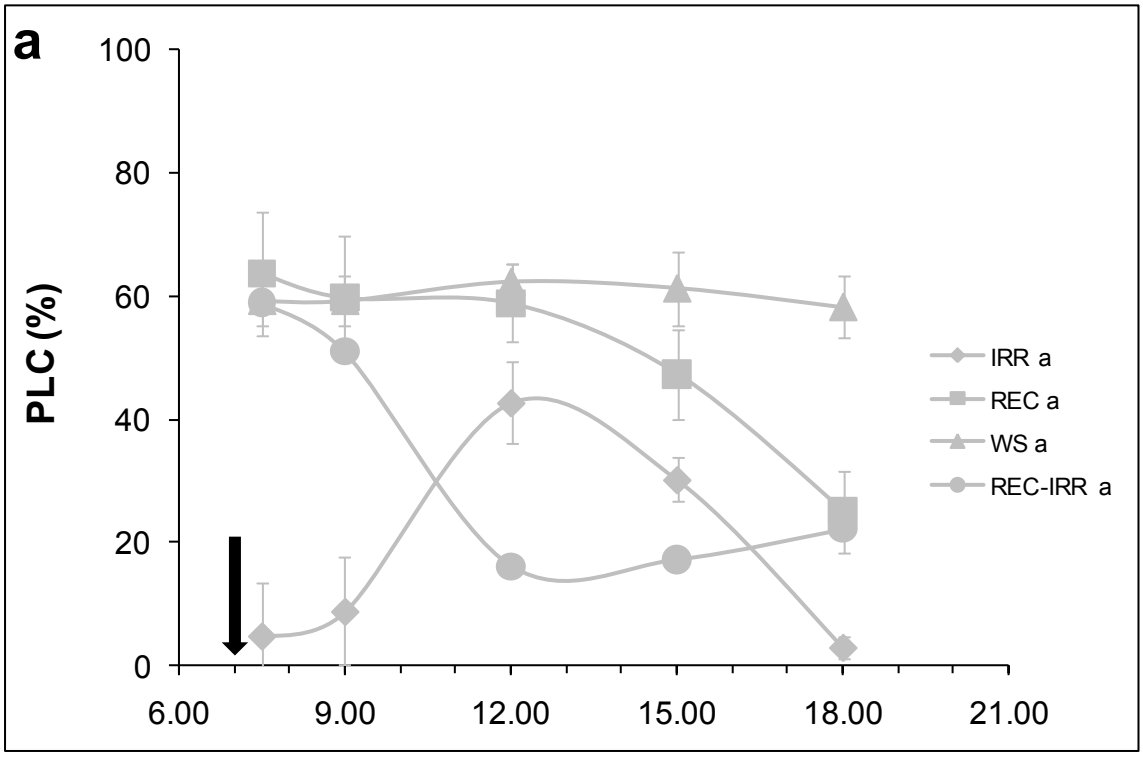
835

836 **Fig. 2**


a
VPD (mbar bar-1)
70
60
50
40
30
20
10
0
6.00
9.00
12.00
15.00
18.00
b
gs (mol m-2 s-1)
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
c
E (mmol m-2 s-1)
6
5
4
3
2
1
0
d
A (µmol m-2 s-1)
14
12
10
8
6
4
2
e
Ci (ppm)
330
320
310
300
290
280
270
260
250
240
IRR a
REC a
WS a
IRR b
REC b
WS b

837

838 **Fig. 3**



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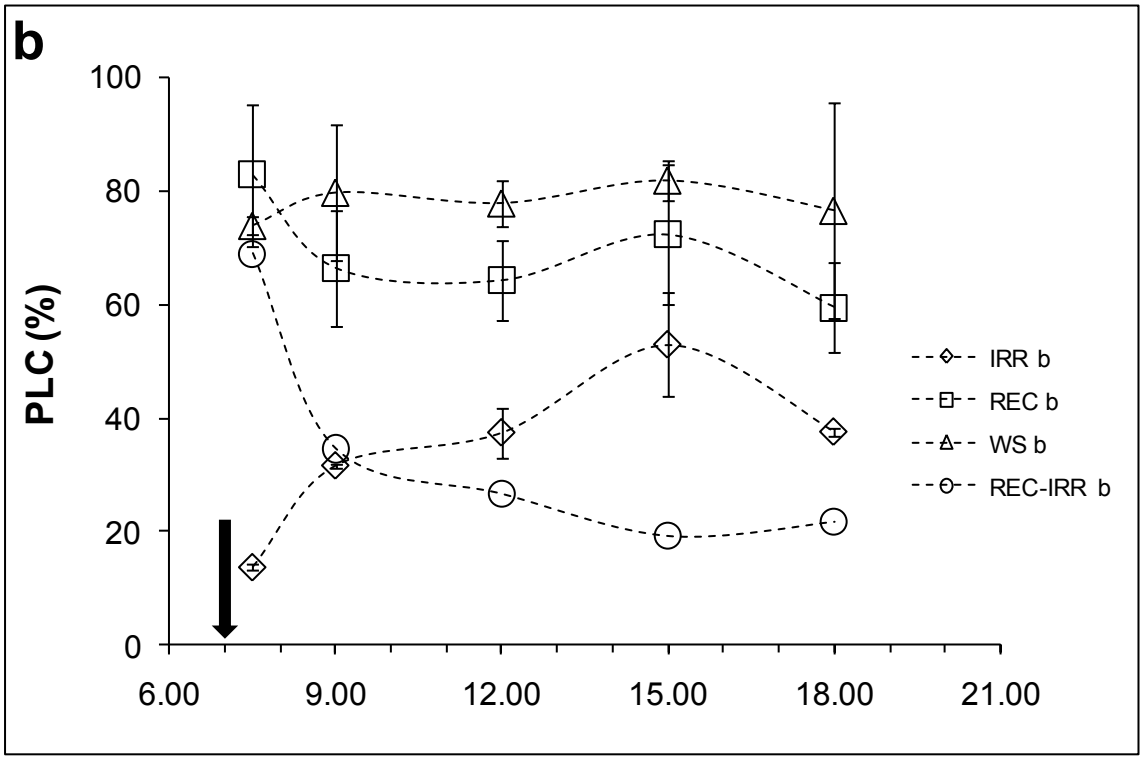
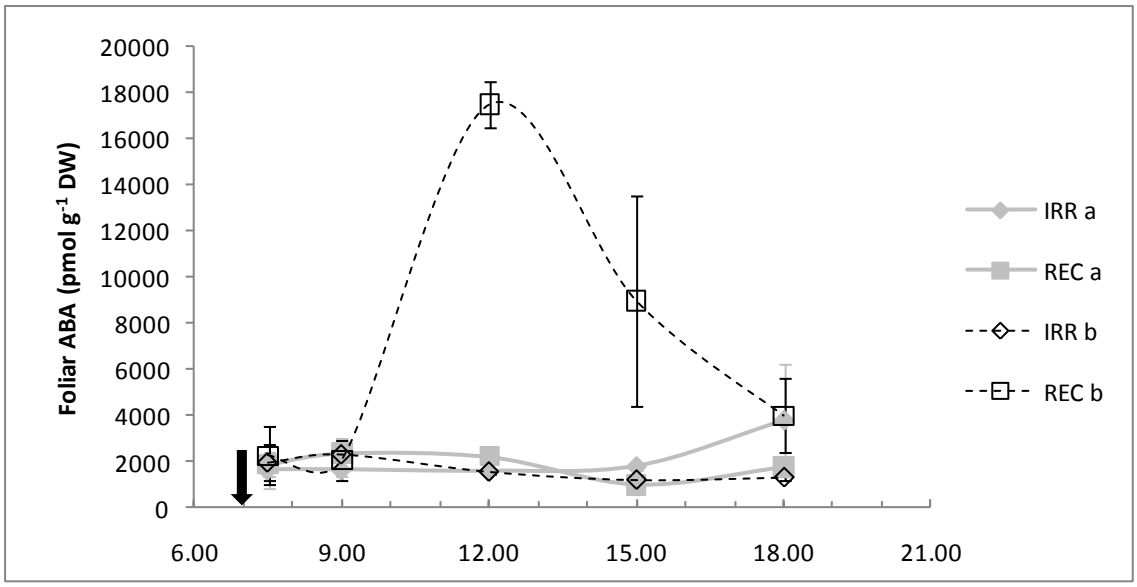


840

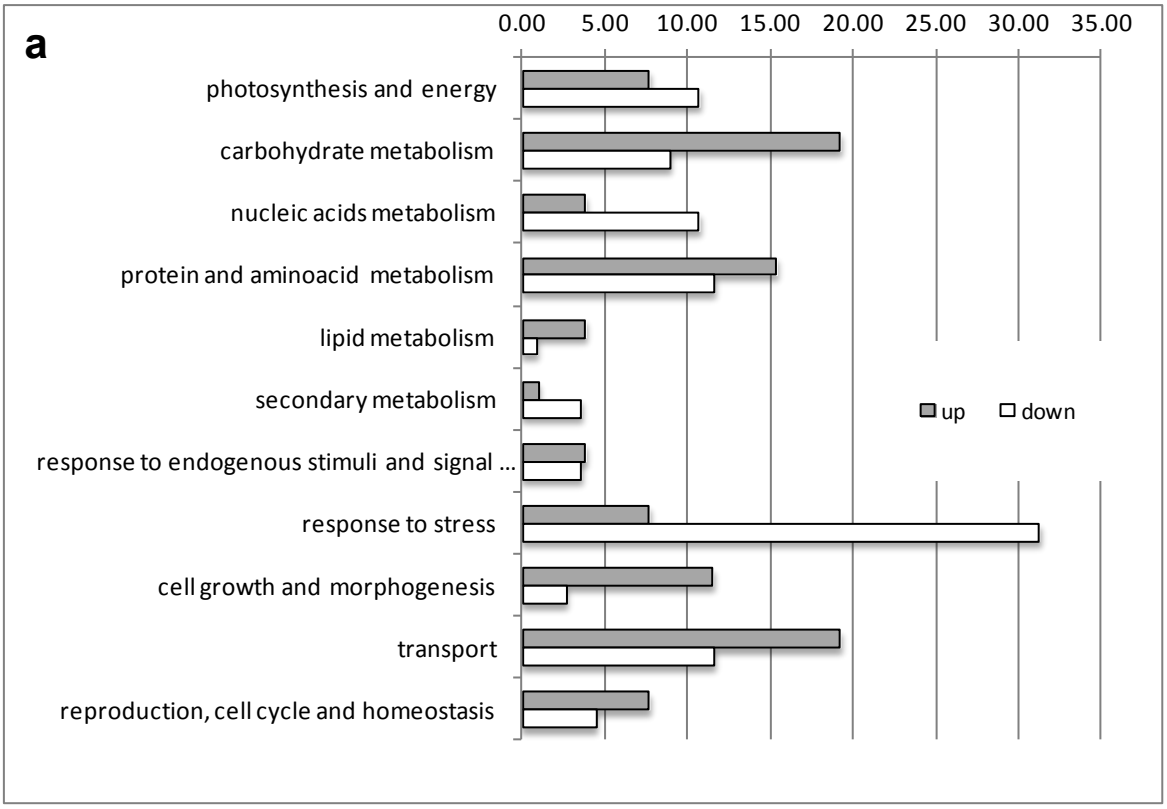
841

842 **Fig. 4**


Foliar ABA (pmol g-1 DW)
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
6.00
9.00
12.00
15.00
18.00
21.00
IRR a
REC a
IRR b
REC b

843

Fig. 5


a
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
photosynthesis and energy
carbohydrate metabolism
nucleic acids metabolism
protein and aminoacid metabolism
lipid metabolism
secondary metabolism
response to endogenous stimuli and signal ...
response to stress
cell growth and morphogenesis
transport
reproduction, cell cycle and homeostasis
up
down

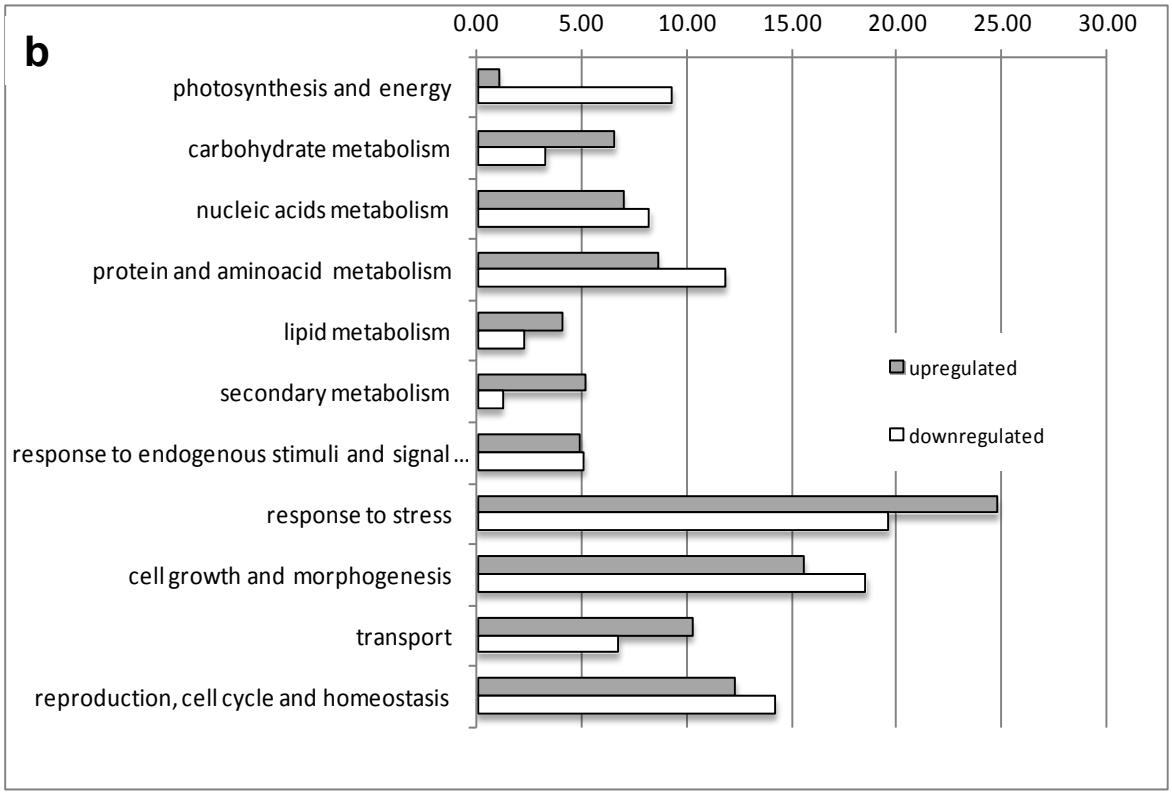
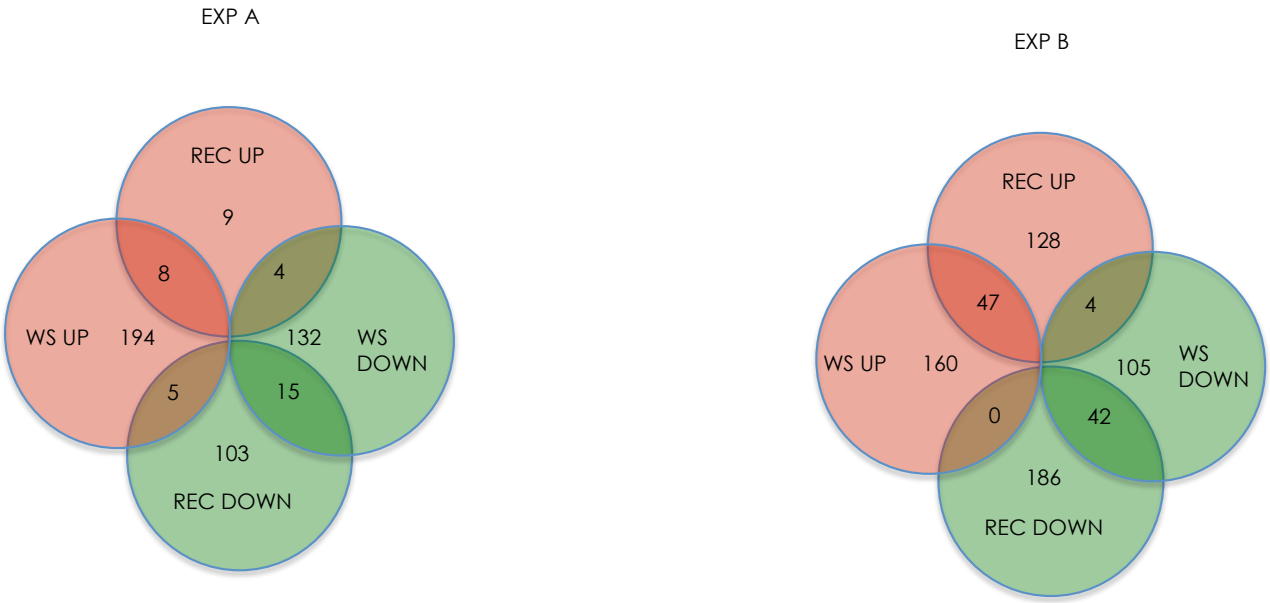

b
0.00
5.00
10.00
15.00
20.00
25.00
30.00
photosynthesis and energy
carbohydrate metabolism
nucleic acids metabolism
protein and aminoacid metabolism
lipid metabolism
secondary metabolism
response to endogenous stimuli and signal ...
response to stress
cell growth and morphogenesis
transport
reproduction, cell cycle and homeostasis
upregulated
downregulated

Fig. 6


EXP A
REC UP
9
8
4
WS UP
194
132
WS
DOWN
5
15
103
REC DOWN
EXP B
REC UP
128
47
4
WS UP
160
105
WS
DOWN
0
42
186
REC DOWN