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Profiling of sugar transporter genes in grapevine coping with water deficit

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ABSTRACT

The profiling of grapevine (*Vitis vinifera* L.) genes under water deficit was specifically targeted to sugar transporters. Leaf water status was characterized by physiological parameters and soluble sugars content. The expression analysis provided evidence that VvHT1 hexose transporter gene was strongly down-regulated by the increased sugar content under mild water-deficit. The genes of monosaccharide transporter VvHT5, sucrose carrier VvSUC11, vacuolar invertase VvGIN2 and grape ASR (ABA, stress, ripening) were up-regulated under severe water stress. Their regulation in a drought-ABA signalling network and possible roles in complex interdependence between sugar subcellular partitioning and cell influx/efflux under Grapevine acclimation to dehydration are discussed.

1. Introduction

Grapevine (*Vitis vinifera* L.) is the most widely cultivated and economically important fruit crop in the world (FAOSTAT data 2010; http://faostat.fao.org/). Even though it is considered as relatively drought tolerant [1], water deficit remains a major environmental cue, affecting grape and wine quality [2–4].

In plants, water availability appears as a determinant factor for cell and organ growth, for photosynthesis and carbohydrates redistribution between source and sink organs [5]. Carbohydrate allocation to long distance, at the level of the plant, as well as to short distance, in sugar partitioning at cellular level, requires sugar transporters. They are membrane proteins belonging to the major facilitator superfamily (MFS) whose subfamilies of sucrose transporters (SUTs) and monosaccharide transporters (MSTs) are the most studied [6–8]. The irreversible sucrose hydrolysis by invertases inside the different cell compartments (cell wall, cytoplasm, vacuole) gives rise to glucose and fructose, suggesting a fine-tuning of the sugar transport activity [9].

In this regard, some evidence has been provided that drought and other related abiotic stresses affect the expression of sugar transporter genes. In *Arabidopsis*, transcript accumulation of the tonoplast monosaccharide transporters TMT1 and TMT2 is increased in response to drought, salt and cold treatment [10], the expression of the putative sugar transporter ERD6 (early responsive to dehydration) is induced by dehydration [11], and the expression of ESL1, an ERD6-like transporter is enhanced by drought, salt and ABA [12]. Recently, *AtSUC2* and *AtSUC4* have been reported as induced by salt, osmotic, low temperature and ABA treatments [13]. In rice, the expression of the Golgi monosaccharide transporter OsGMST1 is positively correlated to salt treatment [14]. The sucrose transporter OsSUT2 was up-regulated during drought and salinity treatments in rice photosynthetic leaves [15].

The transcriptional regulation of grapevine sugar transporters has been studied in response to sugars [16] and to abscisic acid – ABA [17]. The expression analysis of *VvHT1* hexose transporter gene allowed us to identify the grape ASR (abscisic acid, stress and ripening), named VvMSA, at the cross-talk of sugar and ABA signalling [18]. A functional duality of ASRs, as hydrophyllins, required for the direct protection of cell macromolecules under stress conditions [19], and as expression regulators, at the example of VvMSA [18], has been already suggested [20]. This latter function seems closely related to the control of sugar metabolism and

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trafficking, as demonstrated by tomato *ASR1* overexpression and repression in transgenic potato and tobacco plants [21,22].

The present work was dedicated to the expression profiling of twenty-one sugar transporter genes, encoding the members of hexoses (VvHT), sucrose (VvSUC) and polyols (VvPMT) transporter subfamilies in grapevine leaves under water-deficit stress.

2. Materials and methods

2.1. Plant material

Vitis vinifera cv. Ugni blanc plants were in vitro propagated on Murashige and Skoog medium half strength macro elements (Duchefa, The Netherlands), supplemented with vitamins, 20 g l^{-1} sucrose and 0.5 g l^{-1} charcoal activated, under a 16 h photoperiod (light intensity of 40 μmol m^{-2} s^{-2}), at 24 °C, for 2 months. Then plants were transferred to greenhouse and grown on soil/vermiculite (2/1; v/v), at 23 °C/18 °C, 16 h/8 h day/night and 29% relative humidity, until they had 8–10 leaves (80 cm tall). For greenhouse acclimation, the regular watering was supplemented once a week with 100 ml of a nutrient mixture [23]. Water deficit was induced on a half of the plants, by arrest of watering for 16 days, and subsequent rewatering for 8 days. During this period, the control plants were watered daily. Three independent biological experiments were performed consisting each in 2 treatments (control and water deficit/rewatering), 8 time points (day 0, 1, 4, 8, 12, 16, 20, 24) and 3 plants per time point and treatment.

2.2. Physiological parameters

The 4th fully developed leaf was used for stem water potential measurement as described by Cramer et al. [24]. The petiole was cut and Ψ_{stem} was measured with a pressure chamber (PMS Instrument, USA). The same detached 4th leaves, were immediately weighted – fresh weight (FW), then immerged into water for 24 h in the dark to obtain turgid fresh weight (TW), and dried at 80 °C for 24 h to obtain the dry weight (DW). The Relative Water Content ($RWC = [(FW - DW)/(TW - DW)] \times 100\%$) and the Relative Growth Rate ($RGR = (\ln DW_{t2} - \ln DW_{t1})/(t2 - t1)$) [25] were calculated.

2.3. Sugar extraction and HPLC analysis

Powder of the pooled 5th fully developed leaves was freeze-dried for 96 h with a Free®Zone Freeze Dry System (Labconco, USA). After a triple extraction of soluble sugars from 100 mg of freeze-dried powder with 10 ml of methanol:chloroform:water (v/v/v; 12/5/3), samples were sonicated (30 s), and centrifuged (10 min, 1200×*g*). The supernatants were pooled and diluted with water (v/v; 5/3). Two ml of aqueous phase were vacuum evaporated, dissolved in 800 μl of deionized water, and soluble sugars were analysed by HPLC (series 200 Perkin Elmer, USA) with water as eluent (0.6 ml min^{-1}; 85 °C).

2.4. Macroarray analysis

Total RNA isolation from the 5th fully developed leaves, cloning of specific cDNA fragments for genes of interest and reference genes (*VvActin*, *VvEF1α*, *VvEF1γ* and *VvGAPDH*), macroarray spotting and hybridization were performed as described in Afoufa-Bastien et al. [26]. Specific primers are listed in Table S3. After quantification (Typhoon TRIO Imager, GE Healthcare, UK), signals for each gene of interest were normalized using the mean of the 4 reference genes.

2.5. Real-time RT-qPCR analysis

Reverse transcription was carried out on 2 μg of DNase-treated total RNA according to manufacturer protocol (Promega, USA). RT-qPCR was performed in reaction mixture (5 μl of 100-fold diluted cDNA, 125 nM of each primer and 10 μl of Power SYBR®green PCR Master Mix), with the program (2 min at 50 °C, 10 min at 95 °C, followed by 40 cycles with 15 s at 95 °C and 1 min at 60 °C) using a 7500 Fast Real-Time PCR System (Applied Biosystem, USA). The reference gene was *VvGAPDH*.

2.6. Promoter sequence analysis

Search for *cis*-regulatory elements in promoter sequences was performed using the PLAnt Cis acting regulatory DNA Elements database (PLACE) (http://www.dna.affrc.go.jp/PLACE/index.html).

2.7. Statistical analysis

GraphPad Prism 5 software (http://www.graphpad.com) was used for Student's *t* test and Pearson correlation, and Multi Experiment Viewer 4.8 software (http://www.tm4.org) for Heat-Map and Hierarchical Clustering of macroarray data.

3. Results

3.1. Water deficit effect on morphological and physiological parameters

A water deficit treatment was applied to greenhouse-grown grapevine plants by withholding water supply during 16 days, followed by 8 days of rewatering. The effect of water deficit was clearly visible by the wilting of shoot apexes and the pending of mature leaves after day 8 (Fig. 1A and B).

Stem water potential (Ψ_{stem}), relative water content (RWC), dry weight and relative growth rate (RGR) measurements were performed every four days on control plants and plants under water-deficit. In control plants, the Ψ_{stem} was constant at −0.32 MPa during the experiment. In plants under water deficit, Ψ_{stem} decreased slightly from −0.33 MPa to −0.45 MPa. After day 4, Ψ_{stem} decreased gradually and reached −2.38 MPa at day 16. During rewatering Ψ_{stem} quickly recovered to −0.70 MPa and −0.46 MPa at days 20 and 24, respectively (Fig. 1C).

The RWC was measured as a second indicator of grapevine water status, reflecting the metabolic activity in tissues [27]. In watered control plants, the RWC values remained stable around 95.2% during the 24 days of the experiment. In plants under water deficit, as observed for Ψ_{stem} values, RWC started to decrease after day 4 and reached 78.9% after 16 days of water deprivation. In a few days (16–24 day), the rewatering restored RWC levels similar to those of control plants (Fig. 1D).

The dry weight appeared as a valuable parameter of plant response to water-deficit stress, independent from the changing tissue capacity to restore turgid weight under severe water deficit. The measurement of the dry weight (Fig. 1E) showed a progressive increase during the experiment for well-watered control plants. Conversely, in plants submitted to water depletion the dry weight was maintained nearly to the initial level, and it only increased after rewatering. In parallel, the RGR (Fig. 1F) calculated for water-depleted plants was similar to control plants between day 4 and day 8, lower between 12 and 16 days and recovered near the control level after rewatering. These both physiological parameters confirmed the high sensitivity of the cell growth to water status.