

*Short-term response of wild grapevines
(Vitis vinifera L. ssp. sylvestris) to
NaCl salinity exposure: changes of some
physiological and molecular characteristics*

**Hend Askri, Samia Daldoul, Anis Ben
Ammar, Saloua Rejeb, Rahma Jardak,
Mohamed Nejib Rejeb, Ahmed Mliki &
Abdelwahed Ghorbel**

Acta Physiologiae Plantarum

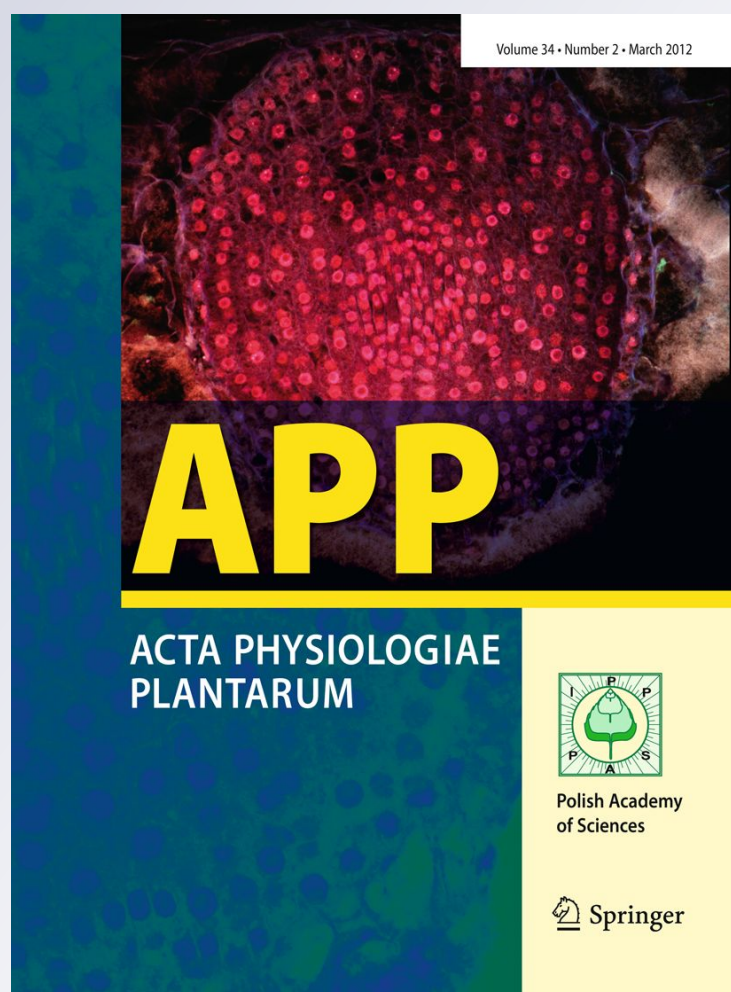
ISSN 0137-5881

Volume 34

Number 3

Acta Physiol Plant (2012) 34:957-968

DOI 10.1007/s11738-011-0892-8



Your article is protected by copyright and all rights are held exclusively by Franciszek Górski Institute of Plant Physiology, Polish Academy of Sciences, Kraków. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your work, please use the accepted author's version for posting to your own website or your institution's repository. You may further deposit the accepted author's version on a funder's repository at a funder's request, provided it is not made publicly available until 12 months after publication.

Short-term response of wild grapevines (*Vitis vinifera* L. ssp. *sylvestris*) to NaCl salinity exposure: changes of some physiological and molecular characteristics

Hend Askri · Samia Daldoul · Anis Ben Ammar ·
Saloua Rejeb · Rahma Jardak · Mohamed Nejib Rejeb ·
Ahmed Mliki · Abdelwahed Ghorbel

Received: 15 April 2011 / Revised: 12 October 2011 / Accepted: 31 October 2011 / Published online: 16 November 2011
© Franciszek Górski Institute of Plant Physiology, Polish Academy of Sciences, Kraków 2011

Abstract The physiological and molecular response to salt stress was studied in two wild grapevine (*Vitis vinifera* L. ssp. *sylvestris* or *Vitis sylvestris*) accessions “Khédhayria” and “Houamdia”, previously identified as salt-tolerant and salt-sensitive pair wise. Plants from both accessions were subjected to a progressive salt stress by the use of a nutritional solution containing up to 150 mM NaCl for 2 weeks. Salt stress adversely affected growth and water potential since the first day of exposure to 150 mM NaCl. However, chlorophyll fluorescence parameters were unchanged until 14 days of salt exposure. At that time point the predawn water potential (Ψ_{PD}), the non-photochemical quenching of fluorescence (NPQ) and the coefficient of photochemical quenching (q_p) were significantly less altered in the tolerant accession. At the molecular level semi-quantitative RT-PCR assays revealed a differential expression of (*Vs* α -gal/SIP and *Vs* DHN) genes within these contrasting accessions after exposure to 24 h and 14 days of salt. Comparably, the *Vs* RD22 gene had increased slightly after only 14 days of treatment in both accessions. These results were the first pieces of information reported on the early and late regulation of salt response genes in wild grapevines. Furthermore, genotype-dependent parameters such as NPQ, q_p , mRNA

levels of *Vs* α -gal/SIP and *Vs* DHN could be used to screen salt-tolerant wild grapevine genotypes.

Keywords Wild grapevines · Salt stress · Chlorophyll fluorescence · Salt-responsive genes · RT-PCR

Abbreviations

RT-PCR Reverse transcription-polymerase chain reaction
SD Standard deviation
IF Induction factor

Introduction

In Tunisia, grapevines are widely cultivated and are of economic importance. However, the growing problem of soil salinity greatly affects grapevine vegetative growth, yield and fruit quality (Prior et al. 1992; Stevens et al. 1999). Soils affected by salinity are progressing in Tunisia and represent more than 25% of the arable land (DG/ACTA 2005). Salinity induces complex effects on grapevines as a result of ionic (Fisarakis et al. 2001), osmotic (Downton et al. 1990) and nutritional (Garcia and Charbaji 1993) interactions. The initial and primary effect of salinity is osmotic (Munns 2002). However, the exact physiological mechanism of salt stress remains unknown. Several studies revealed that salt-tolerance mechanisms in grapevine involve several factors such as photosynthesis alteration (Hatami et al. 2010), ion accumulation (Shani and Al 2005), restriction of ions into roots (Storey et al. 2003) and shoots (Walker et al. 2004) and compatible solute

Communicated by J. V. Jorrin-Novo.

H. Askri (✉) · S. Rejeb · M. N. Rejeb
Institut National de Recherches en Génie Rural, Eaux et Forêts,
Laboratoire de Gestion des Risques Environnementaux
en Agriculture Irriguée, BP.10, 2080 Ariana, Tunisia
e-mail: askri.hend@yahoo.fr

S. Daldoul · A. B. Ammar · R. Jardak · A. Mliki · A. Ghorbel
Centre de Biotechnologie de Borj Cédria, Laboratoire
de Physiologie Moléculaire des Plantes, BP. 901,
2050 Hammam-lif, Tunisia

accumulation (Downton and Loveys 1981). Those features are frequently reported as discriminators between salt-tolerant and salt-sensitive cultivars (Daldoul et al. 2010).

One of the strategies adopted in overcoming salinity is the use of tolerant genotypes through the characterization of local genetic resources and the selection of potential tolerant genotypes (Daldoul et al. 2010; Agaoglu et al. 2004). In this respect, several research teams are focusing on the quantification of salt tolerance in various grapevine cultivars using transcriptomic (Daldoul et al. 2010; Cramer et al. 2006; Tattersall et al. 2007; Jellouli et al. 2010) and proteomic tools (Grimplet et al. 2009; Jellouli et al. 2008). So far a wide variability of the tolerance of grapevine varieties to salt stress has been reported (Cavagnaro et al. 2006) and *Vitis vinifera* cultivars revealed to be less tolerant than other *Vitis* species, especially those frequently used as rootstocks in saline environments (Alexander and Groot Obbink 1971; Antcliff et al. 1983; Walker et al. 2002). Currently most botanists regard the wild ancestral grape *V. sylvestris* as the primitive form of the cultivated grape because of the close morphological resemblance and free gene flow between them (Heywood and Zohary 1991) and consequently have reduced its taxonomic status to subspecies level within the *V. vinifera* crop complex (Levadoux 1956).

In Tunisia there are quite important wild grapevine populations (Zoghalmi et al. 2003) presenting a high genetic variability as well as a clear distinction from cultivated varieties (Snoussi et al. 2004). Such genetic differences have also been revealed at the transcriptional between cultivated and wild grapevines (Raymond et al. 2007). Nevertheless, wild grapevines remain less investigated regarding their genetic tolerance to salinity (Raymond et al. 2008), although they were proved to be resistant to many virus diseases and have a high adaptation potential to different soil types and climates (Ocete et al. 1995; Arnold et al. 1998).

There is no information to understand the response of wild vines to salinity. The aim of this study was to assess salt tolerance in this species in Tunisia. The analysis focused on wild vine response to salt stress with a particular emphasis on the physiological (growth and photosynthetic processes) and molecular levels.

Experiments were carried out in potting media at two time points (1 day and 14 days) with two wild accessions, sensitive and tolerant, cultivated in the presence of 150 mM NaCl to investigate some physiological changes and analyse the expression of selected salt-responsive genes (Daldoul et al. 2010). Based on this approach, we attempt to better understand the wild grapevine short-term response to salt stress at the vegetative stage and select pertinent parameters, useful for screening for salt tolerance, in this species.

Materials and methods

Plant material

The experiment was initially set up with six ecotypes of *Vitis sylvestris* collected from different localities in the north of Tunisia. The ecotypes were identified among prospection by Dr Zoghalmi (Zoghalmi et al. 2003). They are heterogeneous liana that exhibit high levels of morphological and genetic variations (Zoghalmi et al. 2009). Preliminary assays on the six accessions displayed varying tolerance to NaCl treatment (Askri et al. unpublished results). Two ecotypes have been selected for further investigation according to their growth responses to salinity, i.e., the tolerant ecotype (Khédhayria) displays less impaired growth under salt conditions than does its sensitive counterpart (Houamdia).

Growth conditions

Woody cuttings of *Vitis sylvestris*, accessions Khédhayria and Houamdia were obtained from two localities in the northwest of Tunisia and cultivated in peat for two months. Single two month-old year plants (a load of 12 to 14 buds per plants) of Khédhayria and Houamdia were transferred into 10-l pots of sandy soil and grown for two additional months in a greenhouse (16 h light period, PAR of $300 \mu\text{mol m}^{-2} \text{s}^{-1}$, minimum and maximum temperatures ranging between 19.9 and 31.5°C, respectively, and an average humidity of 74.2%). The pots were filled with an inert sandy soil (pH 7.4 and electrical conductivity (EC) 0.46 dS/m) and covered with a 5-cm layer of perlite to limit soil evaporation. Plants were spaced 0.5 m apart and 1.0 m between rows. Plants were trained vertically by one wire, the highest of which was located 1.5 m above the soil surface. Shoots were then trained along a horizontal trellis wire, 40 cm from the pot surface, to develop the cordon. The pots were irrigated with a long Ashton nutrient solution, giving 700 ml to each plant twice or three times a week. The soil water content was maintained above 75% of pot capacity and the amount of drainage was approximately 100 ml. Soil water content was directly measured twice a week with the gravimetric method. The plants were grown with the nutrient solution for 60 days. Salinization started in selected homogenous plants at the vegetative stage. Shoot lengths of Khédhayria and Houamdia were 77 and 60 cm, respectively.

Salt-stress treatment

Plants were irrigated with a Long Ashton solution to which NaCl was progressively added (3.5 mM $(\text{CaNO}_3)_2$, 3 mM KNO_3 , 2 mM NH_4NO_3 , 0.6 mM K_2HPO_4 , 1.5 mM

MgSO₄, 1.6 mM KH₂PO₄ and the micronutrients, 90 µM Fe-EDTA, 9.1 µM MnCl₂, 0.76 µM ZnSO₄, 0.7 µM CuSO₄, 46.3 µM H₃BO₃ and 0.21 µM (NH₄)₆MO₇O₂₄ with pH 6.0, EC 2 dS/m, Plenchette et al. 1982). To avoid osmotic shocks, salt concentrations were increased by 25 mM every two irrigations, until a final concentration of 150 mM (EC 18 dS/m at 25°C) was reached. No salt was added to the nutrient solution in control plants.

Generally investigations on the short-term effects of NaCl on physiological responses of cultivated grapevines are obtained among experiments undertaken with a maximum concentration of 100 mM NaCl (Downton and Loveys 1981; Shani and Al 2005; Fisarakis et al. 2001; Hamrouni 2009; Daldoul et al. 2010). Our growth data from previous experiments on grapevines revealed that cultivated grapevines (*Vitis vinifera*), classified as moderately sensitive to salt stress (Maas and Hoffman 1977), are generally more sensitive than wild species (Daldoul et al. 2010; Hamrouni 2009) and that the results obtained with 150 mM were more efficient in discriminating the wild accessions (Askri et al. unpublished results). In terms of tolerance to NaCl, the 150-mM concentration was adopted in the present study. For salt-treated plants NaCl soil solution level reached 100 and 150 mM, respectively, 21 and 28 days after the beginning of salt treatment. For molecular analysis plant material was harvested 24 h and 14 days after exposure to 150 mM NaCl. At the second time point, 14 days, the sensitive accession started to be necrotic. Thus continuing the experiment for longer would result in the death of this sensitive line.

Growth measurements

The growth characteristics measured were shoot length (cm), stems, leaves and root dry weights (g) on ten control and salt-stressed plants. Shoot length was measured weekly and the other parameters recorded before the beginning of salt stress (t_i : initial harvest) and 14 days after exposure to 150 mM NaCl (t_f : final harvest).

Salt tolerance of the ecotypes was determined by both, relative growth rate (RGR, day⁻¹) and shoot growth rate (SGR, cm day⁻¹). These two parameters were derived from the above measurements. RGR is the increase in plant material per unit of material per unit of time. It was calculated for stems (RGR_S), leaves (RGR_L), roots (RGR_R) and the entire plant (RGR_P) using the equation: $RGR = (\ln w_f - \ln w_i) / (t_f - t_i)$ (Hunt 1990). Where w_i and w_f were the dry weights determined at t_i and t_f (42 days after salinisation) respectively. SGR was calculated from the increase in shoot length measured every week from the beginning to the end of salt period based on the equation: $SGR = (L_2 - L_1) / (t_2 - t_1)$, where $L_2 - L_1$ were the lengths determined at time t_2 and t_1 , respectively (Fisarakis et al. 2001).

Leaf water potential and osmotic potential

Predawn leaf water potential (Ψ_{PD}) was measured weekly on three fully expanded mature leaves per treatment using a Scholander pressure chamber (Model 1000; PMS Instrument Co; Corvallis, OR, USA) at predawn (4:00 h) within 1 day and 14 days of exposure to 150 mM NaCl. Care was taken to minimise water loss during transfer of the leaf to the chamber by enclosing it in plastic bag immediately after excision. Leaf osmotic potential (Ψ_P) was determined using an osmometer (Herman Roebling, Type 13/13 DR, Berlin, Germany), using the leaves of the Ψ_{PD} measurements; after freezing the leaf blades with N₂ liquid cell sap was pressed on by a syringe (Moutinho-Pereira et al. 2001). After centrifugation (12,000×g, 3 min, 4°C), 100 µl of the cell sap were used for measurements in the osmometer (mOsmol/Kg H₂O).

Ψ_P (MPa) was calculated according to Van't Hoff equation, which applies strictly to ideal dilute solutions: $\Psi_P = -nRT$, where $n = \text{mOsmol (g H}_2\text{O)}^{-1}$, $R = 8.314 \times 10^{-6} \text{ MPa mol}^{-1} \text{ K}^{-1}$ and $T = 298.2 \text{ K}$.

Soil solution osmotic potential

Concentrations of total ions (Na⁺, K⁺, Ca²⁺, Mg²⁺, HCO₃⁻, Cl⁻ and SO₄²⁻) were determined in the soil extract from the saturated soil past (Askri et al. 2010). Soil solution osmotic potential (Ψ_s) was calculated according to the Van't Hoff equation, where $n = \text{mmol ml}^{-1}$ solution of the solute.

Leaf water content determination

At the end of the experiment leaf fresh weights and leaf dry weights of leaves, similar to those used for Ψ_{PD} and Ψ_P , were determined. Leaf water content (WC, %) was calculated as $(\text{fresh weight} - \text{dry weight}) \times 100 / \text{fresh weight}$.

Chlorophyll fluorescence measurements

Chlorophyll fluorescence emission from the upper leaf surface of intact plants was measured with a modulated fluorimeter (MiniPAM Photosynthesis Yield analyser, Walz, Effeltrich, Germany). The minimal (F_0) and maximal fluorescence (F_m) emissions were assessed in leaves at predawn and the maximum quantum efficiency of PSII photochemistry was calculated as $F_v/F_m = (F_m - F_0)/F_m$. The leaves were then continuously illuminated with a white actinic light to measure F_s and F'_m (steady-state and maximal fluorescence in light-adapted leaves, respectively). The parameter F'_0 (minimal fluorescence level in light adapted leaves was estimated following Baker and

Rosenqvist (2004) as $F'_0 = F_0/(F_v/F_m + F_0/F'_m)$. Non-photochemical quenching of fluorescence (NPQ) was calculated as $NPQ = (F_m - F'_m)/F'_m$ (Björkman and Demmig-Adams 1994). The coefficient of photochemical quenching (q_p) was calculated as $q_p = (F'_m - F_s)/(F'_m - F'_0)$ (Schreiber et al. 1986). The intrinsic efficiency of open PSII (Φ_{exc}) was calculated as $F'_v/F'_m = (F'_m - F'_0)/F'_m$ (Genty et al. 1989). The quantum yield of PSII electron transport (Φ_{PSII}) was calculated as $\Phi_{PSII} = [(F'_m - F_s)/F'_m]$ (Schreiber et al. 1995).

RNA purification

Pooled leaves sampled on three plants per treatment were ground in liquid nitrogen. Total RNA was isolated according to Daldoul et al. (2009). Removal of contaminating gDNA was achieved by a combination of RNase-free DNaseI treatment followed by a final purification with RNeasy MinElute Cleanup Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Quantity and quality of RNA samples were examined by spectrophotometry and gel electrophoresis.

Reverse transcription-polymerase chain reaction

A semi-quantitative RT-PCR two-step method was used to measure gene expression. First-strand cDNA synthesis was performed using SuperScript III Reverse Transcriptase (Invitrogen) on 2 µg total RNA and oligo dT primers (Invitrogen). Three replicates of RNA samples derived from leaves of control and salt-stressed *V. sylvestris*, namely accessions Khédhayria and Houamdia were used for gene expression analysis. RNA samples used were derived from leaves. The relative amount of gene expression for different candidate genes was determined within linear amplification ranges. One to two microliters cDNA template (equivalent to 50 and 100 ng RNA) was used for each PCR. Subsequent amplification of the cDNA

fragments was performed with Phusion™ High-Fidelity DNA Polymerase (New England Biolabs, Frankfurt). Number of cycles used and primer sequences designed for *Vs RD22* (accession number: [HS102336](#)), *Vs DHN* (accession number: [HS102334](#)) and *Vs α-gal/SIP* (accession number: [HS102333](#)) and *Vs EF1γ* (accession number: [HS102335](#)) control were optimized for each gene (Table 1). Signal intensities of each of the RT-PCR amplicons have been quantified using ImageJ 1.43 quantification software. Normalized signal intensities have been calculated by dividing the intensity for each candidate gene by the intensity of its respective *EF1γ* control signal. Induction factors (IF) were calculated for reasons of comparison from the normalized signal intensities of the stressed conditions divided by the average of the normalized signal intensities of the control conditions. Three repetitions (mean expression intensity) were performed.

Cloning and sequencing

PCR products were cloned into pGEM-T easy vector (Promega, USA) with T4 DNA ligase according to the manufacturer's instructions. The thermo competent *Dh5α* cells (New England Biolabs) were subjected to heat-shock transformation and screening of transformed colonies by culture on LB medium supplemented with antibiotic (100 mg/l Ampicillin). Plasmid DNA extraction was performed using Wizard® Plus SV Miniprep DNA Purification System (Promega, USA). Recombinant DNA plasmids were sequenced in the technological platform of the Center of Biotechnology (CBBC). Sequencing was achieved in both directions with T7 and SP6 primers using ABI-PRISM 3130 Genetic Analyser Sequencer (Applied Biosystems, USA). Analysis of DNA sequences were conducted with the BLAST tools provided by the National Center for Biotechnology Information (NCBI, <http://www.ncbi.nlm.nih.gov>, Altschul et al. 1997). Sequences were subsequently submitted to Gene Bank (NCBI).

Table 1 Primer sequences used for PCR amplifications

	Primer sequences	Cycling number	Annealing temperature (°C)	PCR product size (bp)
<i>Vs α-gal/SIP</i>	FW cgggtcggcgcttactcatctca	25 cycles	65	500
	RV ctcccaaaccacccaacacag	25 cycles	65	
<i>Vs DHN</i>	FW cggggcagggcagcaac	30 cycles	65	250
	RV gcagaaagctgatgcgaggctgc	30 cycles	65	
<i>Vs RD22</i>	FW TAGGGATGCAAACTAGCCACCT	25 cycles	55	300
	RV TGTAATTGTGTACTGCTGCTCGC	25 cycles	55	
<i>Vs EF1γ</i>	FW gcgggcaagagatacctcaa	25/30 cycles	57	258
	RV tcaatctgtctaggaaaggaag	25/30 cycles	57	

Statistical analysis

The experiment was arranged in a randomized complete block design (RB) with 5 to 10 replicates for physiological parameters and triplicate for transcript analysis. Data were expressed as mean $\pm$ SD. Means were compared by using the one-way and multivariate analysis of variance (ANOVA) followed by Fisher's LSD tests. Differences between individual means were deemed to be significant at $P < 0.05$. All analysis was performed using the "Statistica v 5.1" software.

Results

Growth response

At the end of the treatment salinity significantly reduced vine shoot length, shoot growth rate (SGR) and relative growth rate (RGR) of whole plant, leaves, stems and roots in both ecotypes (Table 2). However, Khédhayria showed a higher growth in whole plant than Houamdia due to higher shoot growth. In fact at the end of salt stress, a decrease of about 30% in RGR of shoots was noted in Khédhayria compared with up to 50% in Houamdia. The reduction in root growth was almost the same in both ecotypes and about 36 and 39%, respectively, in Khédhayria and Houamdia.

The development of the SGR in relation to salinity is shown in Fig. 1. The kinetic of this parameter could indicate how early salt stress effects are significant and how tolerant ecotypes are toward salt. Differences with the control treatment appear 21 days following the application of salinity stress from exposure to 150 mM NaCl in both accessions Khédhayria and Houamdia (Fig. 1a, b). The same trend was registered over salt treatment and statistical analysis indicated high significant differences

($P < 0.01$) after 1 day and 14 days' exposure to 150 mM NaCl. While comparing the behaviour of the two accessions toward stress conditions significant differences from early phase treatment (7 days of salt initialization) until the end of the experiment were observed. Over salt treatment, Khédhayria recorded higher SGR values than Houamdia (Fig. 1c).

Effects of salinity and accession on leaf water potential and leaf osmotic potential

Predawn leaf water potential (Ψ_{PD}) and leaf osmotic potential (Ψ_P) decreased in both Khédhayria and Houamdia accessions after 24 h of exposure to 150 mM NaCl (Table 3); however, no significant differences were observed between the two accessions. Depressive salt effects on Ψ_{PD} and Ψ_P were also maintained by the 14th day of treatment. The decrease of Ψ_{PD} was significantly ($P < 0.05$) more pronounced in the sensitive accession (Houamdia), while for Ψ_P statistical analysis did not show significant differences between the two accessions.

Effects of salinity and accession on chlorophyll fluorescence

There were no significant changes in the minimal Chl *a* fluorescence, (F_0). The maximal Chl *a* fluorescence, (F_m) and the maximum quantum efficiency of PSII photochemistry (F_v/F_m) occurred after 24-h exposure to 150 mM NaCl (Table 4). Similar results were observed in both accessions with parameters measured in light-adapted leaves (Φ_{exc} , Φ_{PSII} , q_p and NPQ). In contrast, after 14 days' exposure to 150 mM NaCl, Table 3 showed a significant decrease ($P < 0.05$) in Φ_{exc} , Φ_{PSII} and a high significant increase ($P < 0.01$) in NPQ. At this time point q_p was unchanged in the tolerant accession Khédhayria and slightly decreased in the sensitive Houamdia.

Table 2 Shoot length (L, cm), shoot growth rate (SGR, cm day⁻¹), relative growth rate in day⁻¹ of the whole plant (RGR_{pl}), leaves (RGR_L), stems (RGR_S) and roots (RGR_R) in stressed and control plants of Khédhayria and Houamdia accessions after 14 days' exposure to 150 mM NaCl

	<i>L</i>	SGR	RGR _{pl}	RGR _L	RGR _S	RGR _R
<i>Acc. Khédhayria</i>						
0 mM NaCl	285.0	4.74	0.052	0.049	0.058 ^a	0.049
150 mM NaCl	205.0	2.52	0.036	0.033	0.041 ^b	0.031
Decrease (% control)	28.1	46.9	31.8	33.4	28.4	35.7
<i>Acc. Houamdia</i>						
0 mM NaCl	231.6	4.26	0.043	0.038	0.056 ^a	0.031
150 mM NaCl	140.6	1.83	0.021	0.018	0.029 ^c	0.019
Decrease (% control)	39.2	57.0	50.0	53.4	47.4	39.5
<i>F values</i>						
Accession (A)	19.4**	6.4*	52.6**	68.2**	8.0**	28.8**
Salinity (S)	41.0**	103.4**	138.1**	139.5**	80.5**	29.7**
Interaction (S $\times$ T)	0.16 ^{ns}	0.21 ^{ns}	2.2 ^{ns}	1.65 ^{ns}	4.5*	0.82 ^{ns}

Values are mean ($\pm$ SD) of at least ten replications. Data labelled with different letters are significantly different at * $P < 0.05$; ** $P < 0.01$

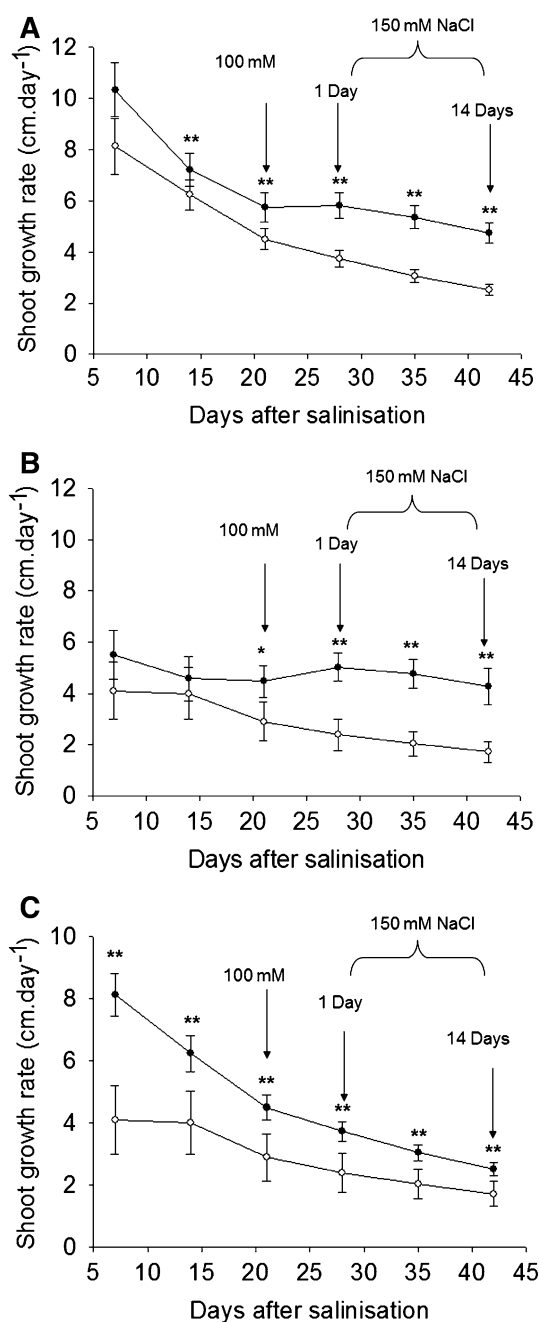


Fig. 1 Changes in shoot growth rate (SGR) of stressed (white circle) and control (black circle) in Khédhayria (a) and Houamdia accessions (b) over time. c reflects segregation of stressed plants of Khédhayria (black circle) and Houamdia accessions (white circle). Values are mean ($\pm$ SD) of at least ten replications. Data labelled with different letters are significantly different at * $P < 0.05$; ** $P < 0.01$

Differential expression of salt-responsive cDNAs under salt-stress condition

RT-PCRs carried out with primers designed for *Vv α -gal/SIP*, *Vv RD22* and *Vv DHN* genes which were identified in

Vitis vinifera yielded 500, 300 and 250 bp sequences, respectively. Quantification of these amplified fragments revealed a significant increase of mRNA corresponding to *Vs α -gal/SIP* gene after 24 h of salt stress in both Khédhayria and Houamdia accessions. However, after 14 days of salt stress, this transcript level was increased only in Houamdia with an induction factor of 2.7. Most of this increase happened during the first 24 h (Fig. 2a).

Vs RD22 gene showed no significant difference in gene expression after 24 h of salt stress in both accessions. After 14 days, this gene expression was slightly higher in the sensitive accession Houamdia (1.65-fold, Fig. 2b).

However, as for the expression of *Vs DHN* gene, there was an increase of the corresponding transcript in both stressed accessions with a higher level in the sensitive accession Houamdia. In this latter accession IF was 3.8 within the first 24 h and reached 5.57, 14 days after addition of salt (Fig. 2c).

Discussion

In our experiment the screening method used to select for salt tolerance in wild grapevines was based on rate of growth at the vegetative stage. Munns (2002) and Arzani (2008) discussed in reviews various screening methods based on physiological indicators and reported that salinity decreases both net photosynthesis and growth in higher plants at the vegetative stage. Growth parameters are usually adopted to investigate the short-term effect of salinity on the performance of grapevines in potting media (Fisarakis et al. 2001; Cramer et al. 2006). The two parameters considered in this study were relative growth rate (RGR) and shoot growth rate (SGR) at the vegetative stage. RGR which represents the efficiency of the plant as a producer of new material under control and stress conditions was reported the most useful single comparator of innate growth potential because it is independent of scale of organism (Hunt 1990). Also, SGR was used in grapes for screening for salt-tolerant varieties (Fisarakis et al. 2001). It was proved to be a very sensitive measure of growth in grapevine under water-deficit and salinity (Cramer et al. 2006).

Our results from RGR of the whole plant (RGR_{PI}), shoots (RGR_S), roots (RGR_R) and from SGR demonstrated the inhibition of growth induced by salt in both tolerant and sensitive wild grapevine accessions as indicated by Table 2. Furthermore, at the end of salt stress decrease in values of SGR are as consistent as those reported with RGR_S and RGR_{PI} . We can conclude that in grapevines under salt stress the SGR is as a good indicator of growth as RGR_S and/or RGR_{PI} . It also seems to be a genotype-dependent parameter. Indeed, the tolerant accession is significantly less affected especially at the

Table 3 Leaf predawn water potential Ψ_{PD} , leaf osmotic potential Ψ_P , soil solution osmotic potential Ψ_S and leaf water content WC in stressed and control plants of Khédhayria and Houamdia accessions after 1- and 14-day exposure to 150 mM NaCl

	Ψ_{PD} (MPa)	Ψ_P (MPa)	Ψ_S (MPa)	WC (%)
1 day after 150 mM NaCl				
<i>Acc. Khédhayria</i>				
0 mM NaCl	-0.20 ± 0.05^a	-0.79 ± 0.03^a		
150 mM NaCl	-0.73 ± 0.03^b	-1.41 ± 0.06^c	–	
<i>Acc. Houamdia</i>				
0 mM NaCl	-0.25 ± 0.05^a	-0.88 ± 0.03^b	–	
150 mM NaCl	-0.65 ± 0.13^b	-1.41 ± 0.03^c	–	
<i>F</i> values				
Accession (A)	0.14 ^{ns}	3.94 ^{ns}	–	
Salinity (S)	112**	765.98**	–	
Interaction (S $\times$ T)	2.29 ^{ns}	5.49*	–	
14 days after 150 mM NaCl				
<i>Acc. Khédhayria</i>				
0 mM NaCl	-0.20 ± 0.00^a	-1.03 ± 0.17^a	-0.17 ± 0.01^a	79.0 ± 1.6^a
150 mM NaCl	-0.72 ± 0.08^b	-1.39 ± 0.15^b	-0.85 ± 0.01^b	76.4 ± 1.0^b
<i>Acc. Houamdia</i>				
0 mM NaCl	-0.23 ± 0.03^a	-1.09 ± 0.18^a	-0.16 ± 0.01^a	79.6 ± 3.1^a
150 mM NaCl	-0.93 ± 0.08^c	-1.54 ± 0.26^b	-0.92 ± 0.01^b	75.1 ± 1.1^b
<i>F</i> values				
Accession (A)	15*	1.72 ^{ns}		0.3 ^{ns}
Salinity (S)	355.3**	20.91**		33.4**
Interaction (S $\times$ T)	8.07*	0.33 ^{ns}		2.4 ^{ns}

Values are mean ($\pm$ SD) of at least three replications. Data labelled with different letters are significantly different at * $P < 0.05$; ** $P < 0.01$

early phase (Fig. 1c). Thus, for grapes, the kinetics of SGR could be more advantageous than RGR because without destroying plant material we could exactly determine when salt effect and differences in ecotypes became statistically significant.

Concomitant decline in predawn leaf potential (Ψ_{PD}) and leaf water content (WC) was observed at the end of the experiment. Average WC of leaves in controlled and stressed plants was 79.3 and 75.8% of fresh weight, respectively. Calculated decrease of WC was of 5.6 and 3.3% in sensitive and tolerant accessions, respectively (Table 3). Furthermore, the osmotic potential (Ψ_P) in leaves was significantly lower than that registered for soil solution osmotic potential (Ψ_S). This situation allows plants to absorb water and solutes from soil solution. According to these results we are able to assume the lowering of osmotic leaf potential to an active osmotic potential. Munns (2002) and Shani and Al (2005) reported that the decrease in soil solution osmotic potential under salt stress begins almost as soon as salinity resulting in reduction of transpiration and biomass production of grapevines. Decrease in Ψ_P values was of 47.5 and 31.4% in the tolerant accession Khédhayria and sensitive Houamdia, respectively, suggesting that the tolerant ecotype has a better potential of osmotic adjustment.

Investigations on the effect of salt stress on chlorophyll fluorescence revealed differences in the response of the

tested grapevine accessions. The maximum quantum efficiency of PSII photochemistry (F_v/F_m) was constant. Average values of both accessions were 0.81 ± 0.01 and 0.80 ± 0.01 , after 1- and 14-day exposure to 150 mM NaCl, respectively, suggesting that no inhibition of photosynthesis occurred at high salt levels (Kafi 2009). This situation was so far reported in other plant species such as wheat (Abdeshahian et al. 2010). In fact, F_v/F_m is almost constant for different plant species measured under non-stressed conditions, with $0.8 \leq F_v/F_m \leq 0.86$ (Scarascia-Mugnozza et al. 1996). For the most severe stress, F_v/F_m decreases to 0.588 ± 0.019 (Jiang et al. 2006). Φ_{PSII} , which estimates the efficiency of light use for electron transport by PSII, depends on the ability of the leaf to remove electrons from the quinone acceptors of PSII (q_p) (Baker and Rosenqvist 2004) and the efficiency of excitation capture of PSII (Φ_{exc}) (Schreiber et al. 1995). For photochemical quenching (q_p) there was no statistically significant change of values with saline exposure for the tolerant accession Khédhayria, an indication that proportion of reaction centres remaining open were similar under control and saline conditions (James et al. 2002), whereas for the sensitive accession, a slight but significant decrease in q_p was recorded. In this study, the decrease of Φ_{PSII} recorded after 14 days of exposure at 150 mM NaCl could be essentially due to the decrease of Φ_{exc} resulting from the increase of energy dissipation through the antennae (NPQ).

Table 4 Minimal Chl a fluorescence (F_0), Maximal Chl a fluorescence (F_m), maximum quantum efficiency of PSII photochemistry (F_v/F_m), intrinsic efficiency of open PSII (Φ_{exc}), photochemical quenching (q_p) and non photochemical quenching (NPQ) of stressed and control plants of Khédhayria and Houamdia accessions after 1-day and 14-day exposure to 150 mM NaCl

	F_0	F_m	F_v/F_m	Φ_{PSII}	Φ_{exc}	q_p	NPQ
1 day after 150 mM NaCl							
<i>V. sylvestris</i> acc. <i>Khédhayria</i>							
0 mM NaCl	0.24 ± 0.20 ^a	1.29 ± 0.09 ^a	0.82 ± 0.00 ^a	0.71 ± 0.03 ^a	0.77 ± 0.03 ^{ab}	0.92 ± 0.02 ^a	0.40 ± 0.10 ^a
150 mM NaCl	0.22 ± 0.15 ^a	1.22 ± 0.04 ^a	0.82 ± 0.01 ^a	0.72 ± 0.03 ^a	0.75 ± 0.02 ^b	0.93 ± 0.01 ^a	0.48 ± 0.13 ^a
<i>V. sylvestris</i> acc. <i>Houamdia</i>							
0 mM NaCl	0.24 ± 0.17 ^a	1.21 ± 0.11 ^a	0.80 ± 0.00 ^a	0.71 ± 0.01 ^a	0.78 ± 0.01 ^a	0.91 ± 0.01 ^{ab}	0.20 ± 0.04 ^b
150 mM NaCl	0.23 ± 0.22 ^a	1.22 ± 0.10 ^a	0.81 ± 0.01 ^a	0.71 ± 0.02 ^a	0.79 ± 0.02 ^a	0.90 ± 0.02 ^b	0.22 ± 0.10 ^b
<i>F</i> values							
Accession (A)	0.75 ^{ns}	1.66 ^{ns}	27.72 ^{**}	0.03 ^{ns}	13.66 ^{**}	9.4 ^{**}	30.67 [*]
Salinity (S)	2.01 ^{ns}	1.18 ^{ns}	1.81 ^{ns}	0.63 ^{ns}	0.02 ^{ns}	0.2 ^{ns}	1.65 ^{ns}
Interaction (S × T)	1.16 ^{ns}	1.84 ^{ns}	0.14 ^{ns}	1.42 ^{ns}	2.60 ^{ns}	4.4 ^{ns}	0.47 ^{ns}
14 days after 150 mM NaCl							
<i>V. sylvestris</i> acc. <i>Khédhayria</i>							
0 mM NaCl	0.27 ± 0.35 ^a	1.38 ± 0.15 ^a	0.80 ± 0.00 ^a	0.68 ± 0.05 ^a	0.76 ± 0.01 ^a	0.89 ± 0.04 ^a	0.35 ± 0.02 ^b
150 mM NaCl	0.26 ± 0.12 ^a	1.34 ± 0.08 ^a	0.81 ± 0.01 ^a	0.61 ± 0.04 ^b	0.72 ± 0.01 ^b	0.87 ± 0.03 ^{ab}	0.58 ± 0.06 ^a
<i>V. sylvestris</i> acc. <i>Houamdia</i>							
0 mM NaCl	0.28 ± 0.16 ^a	1.31 ± 0.16 ^a	0.80 ± 0.01 ^a	0.67 ± 0.04 ^a	0.75 ± 0.02 ^a	0.90 ± 0.03 ^b	0.18 ± 0.04 ^c
150 mM NaCl	0.26 ± 0.16 ^a	1.40 ± 0.11 ^a	0.81 ± 0.01 ^a	0.61 ± 0.04 ^b	0.71 ± 0.01 ^b	0.85 ± 0.07 ^a	0.50 ± 0.05 ^a
<i>F</i> values							
Accession (A)	0.05 ^{ns}	1.37 ^{ns}	10.77 ^{ns}	0.05 ^{ns}	0.68 ^{ns}	0.08 ^{ns}	85.69 ^{**}
Salinity (S)	12.52 ^{ns}	0.25 ^{ns}	28.71 ^{ns}	8.52 [*]	3.83 [*]	6.69 [*]	414.58 ^{**}
Interaction (S × T)	0.42 ^{ns}	6.66 ^{ns}	20.05 ^{ns}	0.32 ^{ns}	0.74 ^{ns}	1.08 ^{ns}	11.92 [*]

Values are mean (±SD) of at least five replications. Data labelled with different letters are significantly different at * $P < 0.05$; ** $P < 0.01$

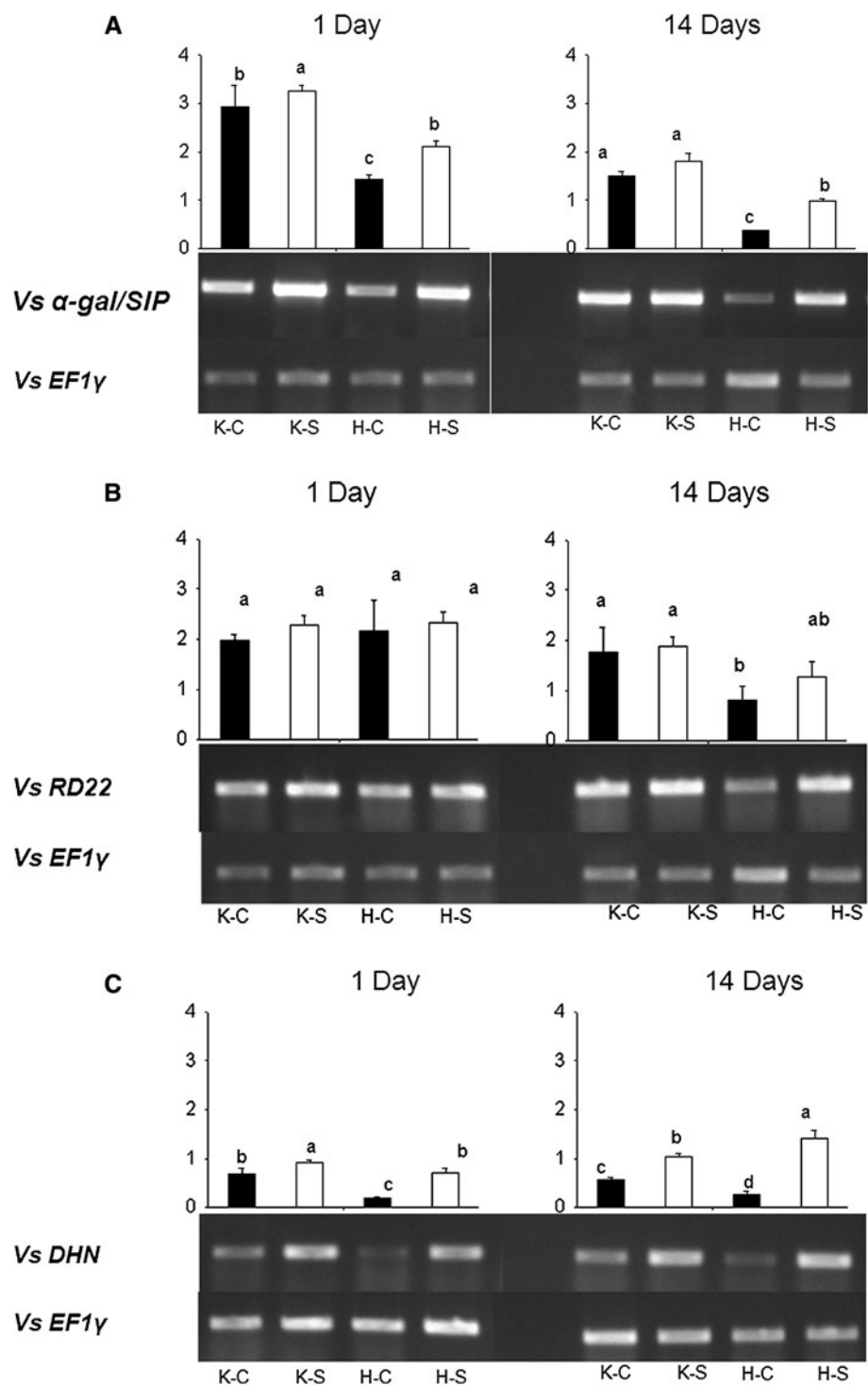
The major process involved in the protection against photo damage is probably the energy dissipation which reduces the relative yield to maintain an adequate balance between photosynthetic electron transport and carbon metabolism (Ashraf 1999). Our results revealed that the sensitive accession Houamdia displayed a higher increase of the NPQ (177.8%) compared with the tolerant accession Khédhayria (65.7%). In barley (Jiang et al. 2006) differences for fluorescence parameters (Φ_{exc} and q_p) were found for salt-sensitive lines while salt-tolerant lines remained relatively unaffected. In wheat (Abdeshahian et al. 2010) NPQ increased and q_p decreased as the salt level increased independently of the salt tolerance of the four studied genotypes. In rape, Atalassi et al. (2009) reported high rates of q_p in salt-tolerant genotypes in relation to the efficiency of light use for PSII and the ability of these lines to maintain QA (primary electron acceptor) partially oxidized. For the authors, this parameter is efficient for screening of salt-tolerant genotypes in rape.

At least under our experimental conditions and for this developmental stage of wild grape we found a relationship between the sensitivity of wild grape accessions and two parameters of chlorophyll-fluorescence (NPQ and q_p). Our

findings on the relevance of chlorophyll fluorescence for screening tolerance to salinity are in agreement with those made for wheat (*Triticum aestivum*, Abeshahian et al. 2010), tomato (*Lycopersicum esculentum*, Zribi et al. 2009) and rape (*Brassica napus*, Atalassi et al. 2009). Such parameters could be adequate indicators of salt stress in grapevine. For example, NPQ and q_p have been used for screening for salt-tolerant wheat genotypes (Zair et al. 2003).

Genotype-specific differences detected at the physiological level were paralleled at the mRNA level as well. The effect of salt-stress treatment was further investigated in leaf derived RNA of Khédhayria and Houamdia accessions. Transcript levels of three genes known to be regulated by salt stress: alkaline alpha galactosidase-seed imbibition protein (*Vv α-gal/SIP*, GenBank accession no. HS102333), response dehydration 22 (*Vv RD22*, GenBank accession no. HS102336) and dehydrin (*Vv DHN*, accession no. HS102334) were evaluated by semi-quantitative RT-PCR (Fig. 2). The analysed transcripts were characterized as important genes responding to NaCl either in cultivated grapevines as referred by (Daldoul et al. 2010; Hanana et al. 2008) or in other species such as wild grapes (Xiao and Nassuth, 2006), cereals (Sivamani et al. 2000; Xu et al.

Fig. 2 Changes in *Vs* α -gal/SIP (a), *Vs* RD22 (b) and *Vs* DHN (c) mRNA abundance level under control (C) and 150 mM NaCl treatment in two accessions [Houamdia (H) and Khédhayria (K)] under control (C) and stress (S) conditions. Salt-stress treatment was applied for 1 day and 14 days. Transcript accumulation level was analysed by RT-PCR with gene-specific primers. Representative gel images from at least three independent experiments are shown in a–c. Transcript profile of EF1 γ was used as internal control. Graphs over each figure show the relative value of genes (*Vs* α -gal/SIP, *Vs* RD22, *Vs* DHN) against *Vs* EF1 γ . Values are mean ($\pm$ SD) of three replications. Data labelled with different letters are significantly different at * $P < 0.05$; ** $P < 0.01$



1996), tobacco (Hara et al. 2003) or New Zealand's spinach (Hara et al. 2008). In fact, the present report represents the first attempt to analyse the expression of these candidate genes in wild Tunisian grapevine. The use of three candidate genes (*Vv* α -gal/SIP, *Vv* RD22, *Vv* DHN) could allow as having a better view of the effect of NaCl on wild grapevine.

In addition, we were interested to see at what time point the most significant changes in mRNA could occur. mRNA corresponding to the *Vs* α -gal/SIP gene was significantly increased early as 24 h after salt treatment. However, the expression level of this gene was always higher in the tolerant accession Khédhayria. Similar results were obtained by

Daldoul et al. (2010). Stress-associated expression of an alkaline α -galactosidase gene has mainly been reported in monocots (Lee et al. 2004; Zhao et al. 2006). Recently a stress-responsive gene encoding an alkaline α -galactosidase was identified in New Zealand's spinach (*Tetragonia tetragonioides*), a perennial seashore plant subjected to drought stress (Hara et al. 2008) and from a Tunisian salt-tolerant grapevine (*Vitis vinifera*) var. Razegui, (Daldoul et al. 2010). It was speculated to be involved in galactosyl-saccharide degradation for enhancing carbohydrate utilisation under abiotic stresses.

For the *VsRD22* gene the differential gene expression was observed only in the sensitive Houamdia after 14 days of salt treatment. In addition, the gene expression level of *Vs RD22* remains always higher in the tolerant accession. The expression of *Vs RD22* was not genotype-dependent, particularly at early time point. In contrast, results of Daldoul et al. (2010) showed a differential gene expression of the *RD22* gene between salt and sensitive cultivated grapevines under the hydroponic system. The *RD22* gene was recently characterized as related to the response of grapevine to salt and drought stresses in cultivated grapevines (Hanana et al. 2008). Its expression was found to be ABA-dependent and activated by *MYB* and *MYC* transcription factors under drought stress (Abe et al. 1997).

The expression pattern of the *Vs DHN* increases upon salt treatment showing a late stress response in both accessions with a higher level in Houamdia (sensitive accession). A differential gene expression of *DHN* was also reported in wild grapes (*Vitis riparia*) under various abiotic stresses (Lopez et al. 2003; Xiao and Nassuth 2006). *DHN* protein, which belongs to LEA family, has been postulated to play a protective role under different abiotic stresses (Dalal et al. 2009). This function was demonstrated by genetic transformation in several plant species such as wheat and rice (Sivamani et al. 2000; Xu et al. 1996), tobacco (Hara et al. 2003), yeast cells (Imai et al. 1996; Miyamoto and Hatano 1999) and bacteria (*Escherichia coli*, Liu and Zheng 2005).

We have combined the physiological and molecular studies for a global approach to characterize salt stress in wild grapevine plants. The up-regulation of the candidate genes studied under stress condition was in accordance with the salt stress status of the plants detected at the physiological level. Furthermore, the gene expression of *Vs DHN* was paralleled by the different behaviour of the leaf water potential of two wild grapevine accessions (as shown in Table 3). The abundance of *Vs DHN* under stress treatment was concomitant with the decline of leaf water and osmotic potentials in both accessions (Table 3). Similar results were observed in cultivated grapevines (Daldoul et al. 2010). We concluded that the moderate salt-stress treatment was suitable to induce specific alterations at the physiological level (Ψ_{leaf}) as well as at the level of gene expression.

To our knowledge this is the first report on physiological and molecular response of wild grapevine (*Vitis sylvestris*) to salt stress. Taking into account both accessions Khédhayria and Houamdia and according to the current data, we assume that the SGR could be suggested as a useful salt-tolerance indicator in characterizing wild grapevine accessions towards their tolerance to salinity. However, the chlorophyll fluorescence alteration, via significant increase of the NPQ and a decrease of the q_p as it was observed in the sensitive accession Houamdia, would be rather an indicator of salt sensitivity. At the molecular level, different gene expressions took place in the contrasting accessions; thus, early induction of gene expression was observed for *Vs α -gal/SIP*, whereas late induction was recorded for *Vs DHN* gene. The expression of *Vs RD22* was not genotype-dependent, particularly at an early time point.

Acknowledgments The authors thank Dr. Fatma Gharbi from University of science (Tunis) for her help in chlorophyll a fluorescence measurements.

References

- Abdeshahian M, Nabipour M, Meskarbashee M (2010) Chlorophyll fluorescence as criterion for the diagnosis salt stress in wheat (*Triticum aestivum*) plants. *Int J Chem Biol Eng* 3(4):184–186
- Abe H, Yamaguchi-Shinozaki K, Urao T, Iwasaki T, Hosokawa D, Shinozaki K (1997) Role of *Arabidopsis* MYC and MYB homologs in drought- and abscisic acid-regulated gene expression. *Plant Cell* 9:1859–1868
- Agaglu YS, Ergul A, Aras S (2004) Molecular characterization of salt stress in grapevine cultivars (*Vitis vinifera* L.) and rootstocks. *Vitis* 43(3):107–110
- Alexander D, Groot Obbink J (1971) Effect of chloride in solution culture on growth and chloride uptake of Sultan and Salt Creek grape vines. *Aust J Exp Agric Animal Hus* 11:357–361
- Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25:3389–3402
- Antcliff AJ, Newman HP, Barrett HC (1983) Variation in chloride accumulation in some American species of grapevine. *Vitis* 22: 357–362
- Arnold C, Gillet F, Gobat M (1998) Situation de la vigne sauvage *Vitis vinifera* ssp. *silvestris* en Europe. *Vitis* 37(4):159–170
- Arzani A (2008) Improving salinity tolerance in crop plants: a biotechnological view. *In Vitro Cell Dev Biol Plant* 44:373–383
- Ashraf M (1999) Interactive effect of salt (NaCl) and nitrogen form on growth, water relations and photosynthetic capacity of sunflower (*Helianthus annuus* L.). *Ann Appl Biol* 135(2):509–513
- Askri H, Rejeb S, Nahdi H, Rejeb MN, Miki A, Ghorbel A (2010) Indicateurs de tolérance au stress salin chez la vigne sauvage (*Vitis vinifera* L. ssp. *silvestris*). *Annales de l'INRGREF* Numéro special:11–20
- Atlassi V, Nabipour, Mesarbashee M (2009) Effect of salt stress on chlorophyll content, fluorescence, Na⁺ and K⁺ ions content in rape plants (*Brassica napus* L.). *Asian J Agric Res* 3(2):28–37
- Baker E, Rosenqvist NR (2004) Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. *J Exp Bot* 55:1607–1621

- Björkman O, Demmig-Adams B (1994) Regulation of photosynthetic light energy capture, conversion, and dissipation in leaves of higher plants. In: Schulze, Caldwell MM (eds) Ecophysiology of Photosynthesis. Springer, Berlin, pp 17–47
- Cavagnaro JB, Ponce MT, Guzman J, Cirrincione MA (2006) Argentinean cultivars of *Vitis vinifera* grow better than European ones when cultured in vitro under salinity. Biocell 30(1):1–7
- Cramer GR, Ergül A, Grimplet J, Tillett RL, Bohlman MC, Vincent D, Sonderegger J, Evans J, Osborne C, Quilici D, Schlauch KA, Schooley DA, Cushman JC (2006) Water and salinity stress in grapevines: early and late changes in transcript and metabolite profiles. Funct Integr Genomics 7(2):111–134
- Dalal M, Deepti T, Chinnusamy V, Bansal KC (2009) Abiotic stress and ABA-inducible Group 4 LEA from *Brassica napus* plays a key role in salt and drought tolerance. J Biotechnol 139(2):137–145
- Daldoul S, Chenenanou S, Mliki A, Höfer M (2009) Improvement of an RNA purification method for grapevine (*Vitis vinifera* L.) suitable for cDNA library construction. Acta Physiol Plant 31:871–875
- Daldoul S, Guillaumie S, Reustle GM, Krczal G, Ghorbel A, Delrot S, Mliki A, Höfer M (2010) Isolation and expression analysis of salt induced genes from contrasting grapevine (*Vitis vinifera* L.) cultivars. Plant Sci 179:489–498
- Direction générale de l'aménagement et de la conservation des terres agricoles (DG/ACTA) (2005) Examen et évaluation de la situation actuelle de la salinisation des sols et préparation d'un plan d'action de lutte contre ce fléau Dans les périmètres irrigués en Tunisie. Programme PISEAU, phase 1. Ministère de l'Agriculture
- Downton WJS, Loveys BR (1981) Abscissic acid content and osmotic relations of salt-stressed grape vine leaves. Aust J Pysiol 8:443–452
- Downton WJS, Loveys BR, Grant WJR (1990) Salinity effects on the stomatal behavior of grapevine. New Phytol 16:499–503
- Fisarakis I, Chartzoulakis J, Stavarakas D (2001) Response of Sultana vines (*Vitis vinifera* L.) on six rootstocks to NaCl salinity exposure and recovery. Agri Water Manage 51:13–27
- Garcia M, Charbaji T (1993) Effect of sodium chloride salinity on cation equilibria in grapevine. J Plant Nutr 16:2225–2237
- Genty B, Briantais JM, Baker NB (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta 99:87–92
- Grimplet J, Wheatley MD, Jouira HB, Deluc LG, Cramer GR, Cushman JC (2009) Proteomic and selected metabolite analysis of grape berry tissues under well-watered and water-deficit stress conditions. Proteomics 9(9):2503–2508
- Hamrouni L (2009) Evaluation de la tolérance au sel chez la vigne en Tunisie. Thèse en sciences biologiques, 230 p
- Hanana M, Deluc L, Fouquet R, Daldoul S, Léon C, Barrieu F, Ghorbel A, Mliki A, Hamdi S (2008) Identification et caractérisation d'un gène de réponse à la déshydratation *rd22* chez la vigne (*Vitis vinifera* L.). C R. Biologies 331:569–578
- Hara M, Terashima S, Fukaya T, Kuboi T (2003) Enhancement of cold tolerance and inhibition of lipid peroxidation by citrus dehydrin in transgenic tobacco. Planta 217:290–298
- Hara M, Tokunaga K, Kuboi T (2008) Isolation of a drought-responsive alkaline a-galactosidase gene from New Zealand spinach. Plant Biotechnol 25:497–501
- Hatami E, Ashari ME, Javadi T (2010) Effect of salinity on some gas exchange characteristics of grape (*Vitis vinifera*) cultivars. Int J Agric Biol 12(2):308–310
- Heywood V, Zohary D (1991) A catalogue of the wild relatives of cultivated plants native to Europe. Flora Mediterranea 5:375–415
- Hunt R (1990) Basic growth analysis. Unwin Hyman, London, p 110
- Imai R, Chang L, Ohta A, Bray EA, Takagi M (1996) A lea-class gene of tomato confers salt and freezing tolerance when expressed in *Saccharomyces cerevisiae*. Gene 170:243–248
- James RA, Rivelli AR, Munns R, Von Caemmerer S (2002) Factors influencing CO₂ assimilation, leaf injury and growth in salt-stressed durum wheat. Funct Plant Biol 29:1393–1403
- Jellouli N, Ben Jouira H, Skouri H, Gargouri A, Ghorbel A, Mliki A (2008) Proteomic analysis of Tunisian grapevine cultivar Razegui under salt stress. J Plant. Physiol 165:471–481
- Jellouli N, BenJouira H, Daldoul S, Chenennaoui S, Ghorbel A, Ben Salem A, Gargouri A (2010) Proteomic and transcriptomic analysis of grapevine PR10 expression during salt stress and functional characterization in yeast. Plant Mol Biol Rep 28:1–8. doi:10.1007/s11105-009-0116-1
- Jiang Q, Roche D, Monaco TA, Durham S (2006) Gas exchange, chlorophyll parameters and carbon isotope discrimination of 14 barley genetic lines in response to salinity. Field Crops Res 96:269–278
- Kafi M (2009) Effect of salinity and light on photosynthesis respiration and chlorophyll fluorescence in salt sensitive wheat (*Triticum aestivum*) cultivars. J Agric Sci Tech 11:547–555
- Lee RH, Lin MC, Chen SCG (2004) A novel alkaline alpha-galactosidase gene is involved in rice leaf senescence. Plant Mol Biol 55:281–295
- Levadoux LD (1956) Wild and cultivated populations of *Vitis vinifera* L. Annales de l'Amelioration des Plantes 6:59–118
- Liu Y, Zheng Y (2005) PM2, a group 3 LEA protein from soybean, and its 22-mer repeating region confer salt tolerance in *Escherichia coli*. Biochem Biophys Res Commun 313:25–332
- Lopez CG, Banowetz GM, Peterson CJ, Kronstad WE (2003) Dehydrin expression and drought tolerance in seven wheat cultivars. Crop Sci 43:577–582
- Maas EV, Hoffman GJ (1977) Crop salt tolerance current assessment. J Irrig Drain 103:115–134
- Miyamoto R, Hatano T (1999) Introduction of the *h1C6* gene, which encodes a homologue of a late embryogenesis abundant (LEA) protein, enhances freezing tolerance of yeast. J Plant Physiol 155:509–512
- Moutinho-Pereira JM, Magalhães N, Torres De Castro LF, Chaves MM, Torres-Pereira JM (2001) Physiological responses of grapevine leaves to Bordeaux mixture under light stress conditions. Vitis 40(3):117–121
- Munns R (2002) Comparative physiology of salt and water stress. Plant Cell Environ 25:239–250
- Ocete R, Deltio R, Lara M (1995) Les parasites des populations de la vigne sylvestre, *Vitis vinifera* silvestris (Gmelin) Hegi des Pyrenées Atlantiques (France). Vitis 34(3):191–192
- Plenchette C, Furlan V, Fortin JA (1982) Effect of different endomycorrhizal fungi on five host plants grown on calcined montmorillonite clay. J Am Soc Hortic Sci 107:535–538
- Prior LD, Grieve AM, Cullis BR (1992) Sodium chloride and soil texture interactions in irrigated field grown sultana grapevines, I Yield and fruit quality. Aust J Agric Res 43:1051–1066
- Raymond W, Fung M, Wenping Q, Yingcai S, Schachtman DP, Huppert K, Csaba F, László GK (2007) Gene expression variation in grapevine species *Vitis vinifera* L. and *Vitis aestivalis* Michx. Gen Resour Crop Evol 54(7):1541–1553
- Raymond WM, Fung MG, Csaba F, Laszlo GK, Yan H, Marsh E, McIntyre LM, Schachtman DP, Wenping Q (2008) Powdery mildew induces defense-oriented reprogramming of the transcriptome in a susceptible but not in a resistant grapevine. Plant Physiol 146(1):236–249
- Scarascia-Mugnozza G, De Angelis P, Matteucci G, Valentini R (1996) Long term exposure to to elevate CO₂ in a natural *Quercus ilex* L. community: net photosynthesis and

- photochemical efficiency of PSII at different levels of water stress. *Plant Cell Environ* 19:643–654
- Schreiber U, Schliwa U, Bilger W (1986) Continuous recording of photochemical and non-photochemical fluorescence quenching with a new type of modulation fluorometer. *Photosynth Res* 10:51–62
- Schreiber U, Bilger W, Neubauer C (1995) Chlorophyll fluorescence as a non invasive indicator for rapid assessment of in vivo photosynthesis. In: Schulze ED, Caldwell MM (eds) *Ecophysiology of photosynthesis*. Springer, Berlin, pp 49–70
- Shani U, Ben-Gal AI (2005). Long-term response of grapevines to salinity: osmotic effects and ion toxicity. *Am J Enol Vitic* 56(2):148–154
- Sivamani E, Bahieldin A, Wraith JM, Al-Niemi T, Dyer WE, Ho T-HD, Qu R (2000) Improved biomass productivity and water use efficiency under water deficit conditions in transgenic wheat constitutively expressing the barley *HVA1* gene. *Plant Sci* 155:1–9
- Snoussi H, Harbi Ben Slimane M, Ruiz-García L, Martínez-Zapater JM, Arroyo-García R (2004) Genetic relationship among cultivated and wild grapevine accessions from Tunisia. *Genom* 47:1211–1219
- Stevens RM, Harvey G, Partington DL, Coombe BG (1999) Irrigation of grapevines with saline water at different growth stages I Effects on soil, vegetative growth, and yield. *Aust J Agric Res* 50:343–355
- Storey R, Schachtman DP, Thomas MR (2003) Root structure and cellular chloride, sodium and potassium distribution in salinized grapevines. *Plant Cell Environ* 26:789–800
- Tattersall EAR, Ergül A, Grimplet J, Deluc L, Bohlman MC, Vincent D, Lomen E, Osborne C, Wheatley MD, Blank R, Schlauch KA, Cushman JC, Cramer GR (2007) Gene expression profiling of grapevine responses to salt, osmotic and cold shock stresses. *Funct Integr Genomics* 7(4):317–333
- Walker RR, Blackmore DH, Clingeleffer PR, Correll RL (2002) Rootstock effects on salt tolerance of irrigated field-grown grapevines (*Vitis vinifera* L. cv. *Sultana*). Yield and vigour interrelationships. *Aust J Grape Wine Res* 8:3–14
- Walker RR, Blackmore DH, Clingeleffer PR, Correll RL (2004) Rootstock effects on salt tolerance of irrigated field-grown grapevines (*Vitis vinifera* L. cv. *Sultana*). Ion concentration in leaves and juice. *Aust J Grape Wine Res* 10:90–99
- Xiao H, Nassuth A (2006) Stress and development-induced expression of spliced and unspliced transcripts from two highly similar dehydrin 1 genes in *V. riparia* and *V. vinifera*. *Plant Cell Rep* 25(9):968–977
- Xu D, Duan X, Wang B, Hong B, Ho T-HD, Wu R (1996) Expression of a late embryogenesis abundant protein gene, *HVA1*, from barley confers tolerance to water deficit and salt stress in transgenic rice. *Plant Physiol* 110:249–257
- Zair I, Chlyah A, Sabounji K, Tittahsen M, Chlyah H (2003) Salt tolerance improvement in some wheat cultivars after application of *in vitro* selection pressure. *Plant Cell Tissue Organ Cult* 73:237–244
- Zhao TY, Willis CJ, Mullen J, Meeley RB, Helentjaris T, Martin D, Downie B (2006) An alkaline alpha-galactosidase transcript is present in maize seeds and cultured embryo cells, and accumulates during stress. *Seed Sci Res* 16:107–121
- Zoghalmi N, Mliki A, Ghorbel A (2003) Occurrence and discrimination of spontaneous grapes native to Tunisia by RAPD markers. *Acta Hortic* 603:157–166
- Zoghalmi N, Riahi L, Laucou V, Lacombe T, Ghorbel A, This P (2009) Origin and genetic diversity of Tunisian grapes as revealed by microsatellite markers. *Scientia Horticultura* 120:479–486
- Zribi L, Gharbi F, Rezgui F, Rejeb S, Nahdi H, Rejeb MN (2009) Application of chlorophyll fluorescence for the diagnosis of salt stress in tomato *Solanum lycopersicum* (variety Rio Grande). *Sci Hortic* 120(3):367–372

The Impact Factor for Acta Physiologiae Plantarum increased in 2011!

Springer

A. Moi

636



2011 IMPACT FACTOR

The Impact Factor for Acta Physiologiae Plantarum increased in 2011

Visit us at springer.com

Dear Dr. Askri,

As you have recently published in [Acta Physiologiae Plantarum](#) we would like to share the good news with you: The 2011 Impact Factor for the journal has just been released (Thomson Reuters Journal Citation Reports® 2011) and is **1.639**. In 2010, the Impact Factor was: 1.344.

We would like to thank you and your colleagues for contributing to this positive development! Please feel free to share this information with your fellow researchers interested in [Acta Physiologiae Plantarum](#).



As you know, the Impact Factor is just one measure used for evaluating a journal. Should you be interested in more information on the Impact Factor in general please [click here](#).

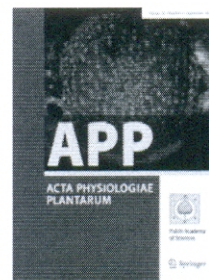
We hope you enjoyed your publishing experience with [Acta Physiologiae Plantarum](#) and will consider also publishing your future articles with us.

Best regards,

[Marika Stauch](#)

for

Your Springer Marketing team



[Acta Physiologiae Plantarum](#)
on SpringerLink.com



[Author Academy](#)

Questions on writing and publishing your paper or on how to do peer reviewing? The free Springer Author Academy offers detailed advice.