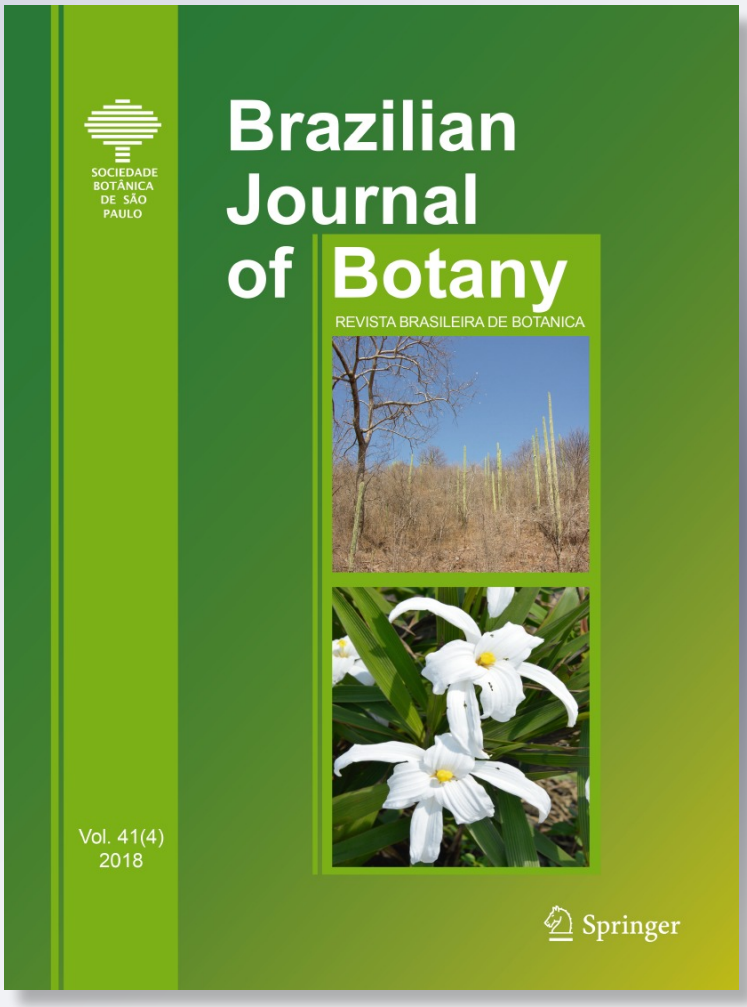
*Differential physiological responses of Tunisian wild grapevines (*Vitis vinifera *L. subsp.* sylvestris*) to NaCl salt stress*

Hend Askri, Fatma Gharbi, Saloua Rejeb, Ahmed Mliki & Abdelwahed Ghorbel

Brazilian Journal of Botany

ISSN 0100-8404
Volume 41
Number 4

Braz. J. Bot (2018) 41:795-804
DOI 10.1007/s40415-018-0500-x



Springer

Brazilian Journal of Botany (2018) 41:795–804
https://doi.org/10.1007/s40415-018-0500-x

ORIGINAL ARTICLE

Differential physiological responses of Tunisian wild grapevines (*Vitis vinifera* L. subsp. *sylvestris*) to NaCl salt stress

Hend Askri[1] · Fatma Gharbi[2] · Saloua Rejeb[1] · Ahmed Mliki[3] · Abdelwahed Ghorbel[3]

Received: 23 March 2018 / Accepted: 6 September 2018 / Published online: 24 September 2018

Abstract

The effects of salt stress on growth, organic and inorganic solute accumulation and chlorophyll florescence were studied on 3-month-old plants of six Tunisian wild grapevine accessions in order to identify salt tolerance mechanisms and select tolerant genotypes. Potted plants were grown under controlled conditions and irrigated for 14 days with 0, 100 and 150 mM NaCl Long Ashton nutrient solution. Salt begins to adversely affect plant growth and plant nutrition at 100 mM NaCl. Compared to control, shoot growth rates were 21.5% less for *Khedhayria*, *Tebaba* and *Ouchteta*, 33% for *Djebba* and *Zouarâa* and 49% for *Houamdia*. They were assigned to stomatal and non-stomatal factors. Stomatal conductance decreased after 1 day at 150 mM NaCl in all accessions in response to reduced leaf water potential. Leaves in tolerant accessions were well hydrated through efficient osmotic adjustment, sufficient potassium flux and selectivity of K^+ versus Na^+. In addition, salt tolerance of wild grapes was related to limiting Na^+ transport to lamina and to compartmentalization of Cl^- on root and leaf vacuoles, improved in *Tebaba* and *Khedhayria* by the uptake of K^+. At the same time, disturbances of the PSII have been noted as non-stomatal factors, and the most important photoprotective mechanism against photosynthetic damages was non-photochemical energy dissipation. However, chlorophyll fluorescence parameters were more stable in *Tebaba* compared to *Khedhayria,* thus showing better salt tolerance. Based on our results, wild grapevine accessions could be classified from most tolerant to most sensitive as follows: *Tebaba* > *Khedhayria* > *Ouchteta* > *Zouarâa* > *Djebba* > *Houamdia*.

Keywords NaCl stress · Osmotic adjustment · Photochemistry · Plant growth · *Vitis sylvestris*

1 Introduction

Salinity adversely affects growth (Shani and Ben-Gal 2005; Li et al. 2006; Tunçturk et al. 2008; Ashraf 2009), mineral content and photosynthetic activity of grapevines as the result of osmotic and ionic effects (Dardeniz et al. 2006; Urdanoz and Aragüés 2009; Jogaiah et al. 2014). In Tunisia, the progression of soil and water salinization is a serious concern for sustaining production of vineyard grapevines (Askri 2014). Glycophytes have developed different mechanisms to cope with these effects. Osmotic adjustment in both roots and leaves through solute accumulation has been at least partially involved in the salt tolerance of many plant genotypes (Munns and Tester 2008). The accumulation of inorganic ions like potassium and calcium (Hadi and Karimi 2012) and compatible organic solutes such as soluble carbohydrates, amino acids, proline, betaines and others contributes to maintaining water uptake and cell turgor, thus allowing for physiological processes such as stomatal opening, photosynthesis and cell expansion (Negrao et al. 2017). According to Fisarakis et al. (2001), salt tolerance in *Sultana* grapevines was related not only to their ability to limit the transport of toxic ions to laminas but also to their ability to increase K^+ concentration in leaf tissues. In this context, Upreti and Murti (2010) associated salt tolerance in grape rootstocks firstly to their capacity to limit the increase in Na^+ in roots

✉ Hend Askri
askrihend.inrgref@gmail.com

[1] Institut National de Recherches en Génie Rural, Eaux et Forêts INRGREF, BP 10, 2080 Ariana, Tunisia

[2] Faculté des Sciences de Tunis FST, Campus Universitaire, 2092 Tunis, Tunisia

[3] Centre de Biotechnologie de Borj Cedria CBBC, B.P. 901, 2050 Hammam-Lif, Tunisia

and secondly to their capacity to decrease K^+ concentration and Na^+/K^+ ratio in root tissues. Recently, Jogaiah et al. (2014) associated salt tolerance of the same genus to phenol accumulation in tolerant genotypes as a cellular adaptive mechanism for scavenging free radicals of oxygen and preventing subcellular damage during stress. Baker and Rosenqvist (2004) considered chlorophyll fluorescence as a rapid and non-destructive analytical tool recommended for selection for plant tolerance to environmental stress. Zribi et al. (2009) proposed some chlorophyll fluorescence parameters such as maximum quantum efficiency of PSII (Fv/Fm), photochemical quenching (qP), non-photochemical quenching (NPQ) and linear electron transport rate (ETR) as criteria to select salt tolerant genotypes of tomato. Wild grapevines showed more tolerance than cultivated grapevines to many biotic (Yingqiang et al. 2012) and abiotic stresses (Askri et al. 2012; Daldoul et al. 2010). They will be used through genetic transformations for future selection and breeding of grapevines (Arnold et al. 2010). Increasing salt tolerance of target species might be more successful if wild genotypes selections were based on physiological mechanisms which confer tolerance (Gupta and Huang 2014). However, little is known about wild grapevine responses to salt stress and the physiological mechanisms employed to cope with salinity. The main objective of this investigation was to evaluate the effects of NaCl salt stress on growth, water relations, mineral nutrition, organic and inorganic compound accumulation and chlorophyll fluorescence of different wild grapevine accessions in order to correlate these effects for a better understanding of the physiological mechanisms of salt tolerance.

2 Materials and methods

Growth conditions and treatments – The experiment was set up with six accessions of wild grapevines (*Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi 1966; Arnold et al. 2010) which were: *Tebaba, Djebba, Houamdia, Khedhayria, Ouchteta* and *Zouarâa.* They were collected from different localities in the northwest of Tunisia and were identified by prospection by Dr. Zoghlami (Zoghlami et al. 2003). Woody cuttings were cultivated in peat for 2 months. Single two- month-old plants were transferred into 10-l pots of inert sandy soil and grown for two additional months in a greenhouse (16 h light period, PAR of 300 µmol m^2s^{-1}, minimum and maximum temperatures of 19.9 and 29.5 °C, respectively, and an average humidity of 74.2%). The pots were filled with inert sandy soil and irrigated for 30 days with a Long Ashton nutrient solution (3.5 mM $(CaNO_3)_2$, 3 mM KNO_3, 2 mM NH_4NO_3, 0.6 mM K_2HPO_4, 1.5 mM $MgSO_4$, 1.6 mM KH_2PO_4, 90 µM Fe-EDTA, 9.1 µM $MnCl_2$, 0.76 µM $ZnSO_4$, 0.7 µM $CuSO_4$, 46.3 µM H_3BO_3 and 0.21 µM $(NH_4)_6MO_7O_{24}$ with pH 6.0, electrical conductivity (EC) 2 dS/m, Hewitt and Smith 1975). Salinization started in selected homogenous plants at the vegetative stage with an average length of 0.8 m and a total leaf area of 10 dm^2. Salt was added progressively in increments of 25 mM NaCl to avoid osmotic shock, until reaching concentrations of 100 and 150 mM NaCl. Plants under all treatments (0, 100, 150 mM NaCl) were grown under the same conditions for 14 days.

Growth measurements – Growth was characterized by relative growth rate RGR (Hunt 1990). RGR is the increase in plant material per unit of time. It was calculated for leaves (RGR_L), stems (RGR_S) and roots (RGR_R) using the following equation:

$$\mathrm{RGR}\left(\mathrm{day}^{-1}\right) = \frac{\mathrm{LnWf} - \mathrm{LnWi}}{\mathrm{tf} - \mathrm{ti}}$$

LnWi and LnWf are the neperian log of initial and final dry weights, respectively, measured when salinization was first initiated (ti) and 42 days later (tf).

Predawn leaf water potential, leaf osmotic potential and stomatal conductance – 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). These measurements were taken at predawn (4 a.m.) within 14 days of exposure to 150 mM NaCl. Care was taken to minimize water loss during transfer of the leaf to the chamber by enclosing it in a plastic bag immediately after excision. Leaf osmotic potential (Ψ_S) was determined by an osmometer (Herman Roebling, Type 13/13 DR, Berlin, Germany), using the leaves of the Ψ_{PD} measurements. After freezing the leaf blades with N_2 liquid, cell sap was pressed on by a syringe (Moutinho-Pereira et al. 2001). Stomatal conductance (g_s, mol H_2O m^2 s^{-1}) was measured with an AP4 Delta-T Devices porometer on leaves similar to those used for the predawn potential measurements. Leaf and root water content (ml g^{-1} dry weight) were calculated by the ratio of fresh weight minus dry weight to dry weight.

Chlorophyll fluorescence measurements – Chlorophyll fluorescence emission from the upper leaf surface of intact plants was measured with a modulated fluorometer (Mini PAM Photosynthesis Yield analyser, Walz, Effeltrich, Germany). The minimal (F_0) and maximal fluorescence (F_m) emissions were assessed in leaves at predawn. Leaves were continuously illuminated with a white actinic light in order to measure maximal steady fluorescence (F_s) and maximal fluorescence (F'_m). The parameter F'_0 (minimal

fluorescence level in light adapted leaves was estimated following Baker and Rosenqvist (2004) as $F'_0 = F_0/(F_v/F_m + F_0/F'_m)$. The quantum yield of PSII electron transport (Φ_{PSII}) was calculated as $\Phi_{PSII} = ((F'_m - F_s)/F'_m)$ (Schreiber et al. 1995). 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 (qP) was calculated as $qP = (F'_m - F_s)/(F'_m - F'_0)$ (Schreiber et al. 1986) and the intrinsic efficiency of open PSII (Φ_{exc}) as $\Phi_{exc} = (F'_m - F'_0)/F'_m$ (Genty et al. 1989).

Determining plant organic and inorganic solute – At the end of the experiment, dry and fresh weights were determined of the leaves (lamina and petioles), shoots and roots. The organs were analysed for their concentration of chloride, sodium and potassium. They were dried at 60 °C for 48 h, then ground and extracted with dilute HNO_3 (0.5%). Sodium and potassium concentrations were determined using a flame photometer, while chloride was measured by silver iron titration with a Buchler–Cotlove chloridometer. Sodium, chloride and potassium fluxes during nutrient absorption by plants were calculated based on the equation used by Johansen et al. (1970):

$$\text{Flux}\left(\text{mmol dry weight root}^{-1}\right) = \frac{\text{Ln}\,(\text{WRf} - \text{LnWRi})(\text{Qf} - \text{Qi})}{(\text{tf} - \text{ti})(\text{WRf} - \text{WRi})}$$

WRi and WRf are the dry weights of roots at initialization (ti) and 42 days after salinization (tf), respectively. Qi and Qf are the total quantities of ion in the plant at ti and tf, respectively. Selectivity of K^+ versus Na^+ was determined for the whole plant as the ratio of the amount of potassium absorbed by the sum of the amounts of K^+ and Na^+. The concentrations of Na^+ and Cl^- ($mmols.g^{-1}$) in laminas and roots were expressed in $mmols.l^{-1}$ using the water content in dry weight matter of the organs ($ml.g^{-1}$). Total soluble carbohydrates were extracted in buffered 80% ethanol from dried material at 70 °C and quantified by the Anthrone method according to Staub (1963). Free leaf proline was extracted from fresh weights according to Bates et al. (1973).

Statistical analysis – The experiment was arranged in a randomized complete block design (RB) with 5–10 replicates. Data were expressed as mean ± SD and means compared by using the one-way and multivariate analysis of variance (ANOVA) followed by Fisher's LSD tests. Differences between individual means were significant at $P < 0.05$. All analyses were performed using the software Statistica version 9.0, from Statsoft, Inc., Tulsa Oklahoma USA, www.statsoft.com. Figures are illustrated using SigmaPlot version 10.0, from Systat Software, Inc., San Jose California USA, www.systatsoftware.com.

3 Results

Growth analysis – Saline treatments of 100 and 150 mM NaCl had a depressive effect on total plant growth, particularly on leaves and stems (Table 1). Moreover, significant variability in biomass was dependent on accession. Three tolerance groups were obtained at 100 mM NaCl, based on reductions in RGR_L and RGR_S: (1) *Khedhayria*, *Tebaba* and *Ouchteta*, (2) *Djebba* and *Zouarâa* and (3) *Houamdia*. Similar reductions were observed in both leaves and stems; however, there were some variations among the accessions which were calculated at 25, 34 and 49% for groups (1), (2) and (3), respectively. The effect of salt on RGR_L increased with the NaCl level. At 150 mM, accessions *Ouchteta* and *Djebba* were further affected by salt resulting in the following ranking: *Khedhayria* +*Tebaba* > *Ouchteta* > *Zouarâa* > *Djebba* > *Houamdia*. Contrary to RGR, the root/shoot ratio (DW_R/DW_S) increased significantly with NaCl, especially for accessions *Djebba*, *Houamdia* and *Zouarâa*. At 100 mM, the increase in NaCl compared to the control was about 66.2% for the sensitive accession *Houamdia* and varied between 10 and 21% for tolerant accessions *Khedhayria* and *Tebaba*.

Mineral and organic analysis – Regardless of accession, increasing salinity in the growth medium significantly raised both Na^+ and Cl^- content in laminas and roots, but more particularly the chloride content (Table 2). Furthermore, chloride and sodium contents in roots were always higher than those in laminas. Both 100 and 150 mM NaCl induced a decrease in potassium content in roots and an increase in laminas. Sodium and chloride flux increased significantly in treated plants of all accessions compared to control plants (Fig. 1a, b). Simultaneously, significant decreases were observed in potassium flux compared to the control and the intensity of salt was elevated with salinity level and depended on accession (Fig. 1c). At 150 mM NaCl, K^+ flux was reduced by 33% in *Tebaba* and *Khedhayria*, followed by *Ouchteta* (47%), *Zouarâa* (55%) and finally, *Houamdia* and *Djebba* (70%). Selectivity in the whole plant of K^+ versus Na^+ ($S_{K/Na}$) also decreased with salinity treatments and was more pronounced with 150 mM NaCl (Fig. 1d). *Houamdia* and *Djebba* were the most affected at the highest salinity; reductions were about 60% of those of the control. Adding 150 mM NaCl for 14 days significantly increased leaf proline content in all accessions (Fig. 2a). The most significant change compared to the control was in *Houamdia* (438%), followed by *Ouchteta* and *Djebba* (≈ 250%), and then *Khedhayria* and *Tebaba* (≈ 200%). The least affected was *Zouarâa* with an increase of about 138%. Total leaf soluble carbohydrates increased slightly in all accessions (Fig. 2b). The most affected accessions were *Tebaba* (18.5%) and *Khedhayria*

Table 1 Changes in relative growth rate (days^{-1}) of leaves (RGR_L), stems (RGR_S), roots (RGR_R) and root/shoot dry weight ratio (DW_R/DW_S) of six wild grapevines accessions (Acc.) under control, 100 and 150 mM NaCl

Acc./mMNaCl	RGR_L	RGR_S	RGR_R	DW_R/DW_S
Khedhayria				
0	0.049^{a}	0.058^{a}	0.049	0.179^{cd}
100	0.039^{b}	0.048^{b}	0.046	0.197^{c}
150	0.032^{b}	0.041b^{c}	0.039	0.192^{c}
Houamdia				
0	0.038^{b}	0.056^{a}	0.034	0.139^{d}
100	0.023c	0.036^{c}	0.029	0.231^{bc}
150	0.019d	0.029^{d}	0.021	0.211^{c}
Zouarâa				
0	0.041^{ab}	0.052^{a}	0.041	0.137^{e}
100	0.028^{c}	0.040^{bc}	0.035	0.209^{b}
150	0.026^{c}	0.039^{c}	0.030	0.180^{d}
Tebaba				
0	0.050^{a}	0.055^{a}	0.042	0.208^{bc}
100	0.040^{b}	0.046^{b}	0.038	0.251^{ab}
150	0.036^{b}	0.042^{b}	0.031	0.220^{b}
Ouchteta				
0	0.048^{a}	0.059^{a}	0.052	0.201^{bc}
100	0.037^{b}	0.044^{bc}	0.048	0.276^{a}
150	0.028^{c}	0.033^{c}	0.039	0.225^{bc}
Djebba				
0	0.034^{b}	0.049^{a}	0.027	0.139^{e}
100	0.022^{c}	0.034^{c}	0.022	0.202^{bc}
150	0.016^{d}	0.025^{d}	0.017	0.211^{c}
F values				
Accession (Acc)	86.5**	23.2**	30.3**	7.7**
Salinity (S)	161.0**	112.4**	26.8**	15.0**
Interaction (Acc × S)	0.9**	1.9**	0.9^{ns}	1.7**

Values are mean (±SD) of at least 10 replications. Data labelled with different letters are significantly different at $*P < 0.05$; $**P < 0.01$

(13.3%), followed by *Ouchteta* and *Zouarâa* (8.2%), while the least affected were *Houamdia* (6.2%) and *Djebba* (4.3%).

Water relations and osmotic adjustment – Concomitant decline in stomata conductance (g_s, Fig. 3a, b) and pre-dawn leaf water potential (Ψ_{PD}, Fig. 3c, d) was observed after 1 day and 14 days of exposure to 150 mM NaCl in both tolerant accessions *Tebaba* and *Khedhayria* and sensitive ones *Houamdia* and *Djebba*. Ψ_{PD} values recorded after 1 day of stress in tolerant, and sensitive accessions were above -0.8 MPa, the threshold reported by Choné et al. (2001) under which grapevines are submitted to severe water stress. Advancing in salt stress, Ψ_{PD} depressed in sensitive accessions and exceeded critical values, while it was steady in tolerant ones. Salt stress simultaneously reduced leaf osmotic potential in both tolerant and sensitive accessions (Ψ_S, Fig. 3e, f). However, depressions of Ψ_S compared to the control were significantly more pronounced in *Khedhayria* and Tebaba (90%) than in *Houamdia* and *Djebba* (35%).

Chlorophyll fluorescence responses – The light parameters of chlorophyll fluorescence were affected differently in wild grapevine accessions after 15 days salinization at 150 m M NaCl. Φ_{PSII} and Φ_{exc} decreased significantly compared to the control, except for Tebaba which recorded slight increases (Fig. 4a, b). Of all of the accessions affected, *Khedhayria* was the least. Moreover, photochemical quenching (qP) was not significantly affected in tolerant accessions such *Tebaba*, but *Khedhayria* was (Fig. 4c). Decrease in Φ_{PSII}, which is the product of Φ_{exc} and qP, could be explained by the decrease in Φ_{exc} resulting from an increase in energy dissipation (NPQ) by antennas (Fig. 4d) on one hand and, on the other hand, by a decrease in qP.

Table 2 Changes in Na^+, Cl^- and K^+ contents in lamina and roots of six wild grapevines after 14 days salinization at 100 and 150 mM NaCl

	Lamina (meq g^{-1}DW)			Roots (meq g^{-1}DW)		
	Na^+	Cl^-	K^+	Na^+	Cl^-	K^+
NaCl level (mM)						
0	0.030^{a}	0.036^{a}	0.427^{a}	0.084^{a}	0.101^{a}	0.820^{a}
100	0.169^{b}	0.408^{b}	0.614^{b}	0.873^{b}	0.874^{b}	0.519^{b}
150	0.331^{c}	0.624^{c}	0.686^{bc}	0.972^{c}	0.932^{c}	0.427^{c}
Accessions						
Khedhayria	0.131^{c}	0.358^{b}	0.551^{b}	0.537^{d}	0.568^{c}	0.552^{c}
Houamdia	0.198^{a}	0.323^{b}	0.602^{a}	0.606^{c}	0.539^{d}	0.553^{c}
Zouarâa	0.147^{c}	0.304^{b}	0.562^{b}	0.577^{cd}	0.596^{c}	0.621b
Tebaba	0.176^{b}	0.343^{b}	0.556^{ab}	0.568^{cd}	0.600^{c}	0.551^{c}
Ouchteta	0.298^{a}	0.500^{a}	0.616^{a}	0.767^{b}	0.739^{a}	0.524^{c}
Djebba	0.110^{d}	0.309^{b}	0.567^{ab}	0.802^{a}	0.772^{b}	0.729^{a}
F values (ANOVA)						
Accession	16.9**	5.8**	5.06**	22.1**	4.7**	18.4**
Salinity	158.1**	167.7**	251.9**	652.3**	220.7**	229**
Accession × salinity	5.7**	2.9**	2.5**	7.5**	1.4^{ns}	9.4**

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

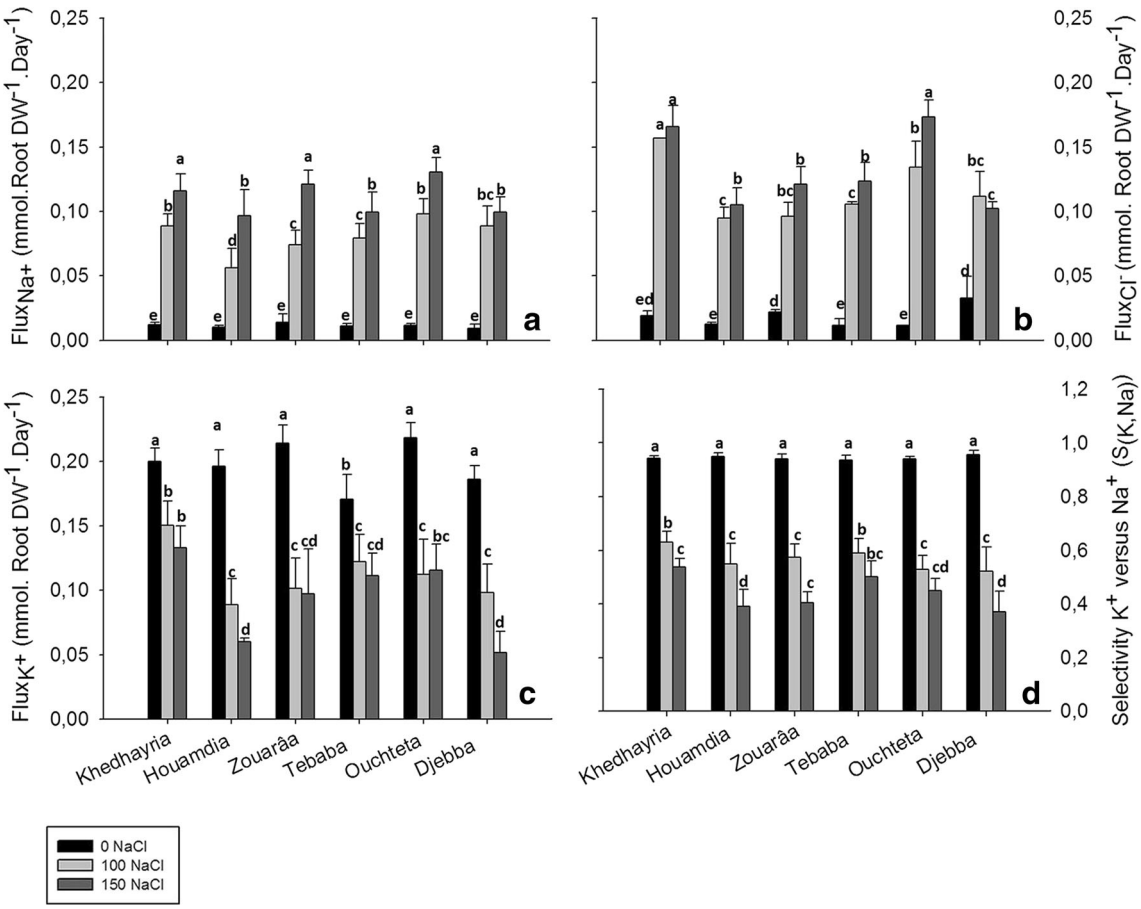


Fig. 1 Changes in root flux of sodium $Flux_{Na+}$ (**a**), chloride $Flux_{Cl-}$ (**b**), potassium $Flux_{K+}$ (**c**) and selectivity K^+ versus Na^+ $S_{(K/Na)}$ (**d**) of six wild grapevines under control (black), 100 mM NaCl (light grey) and 150 mM NaCl (dark grey). Values are mean (± SD) of at least 10 replications. Data labelled with different letters are significantly different at $P < 0.05$

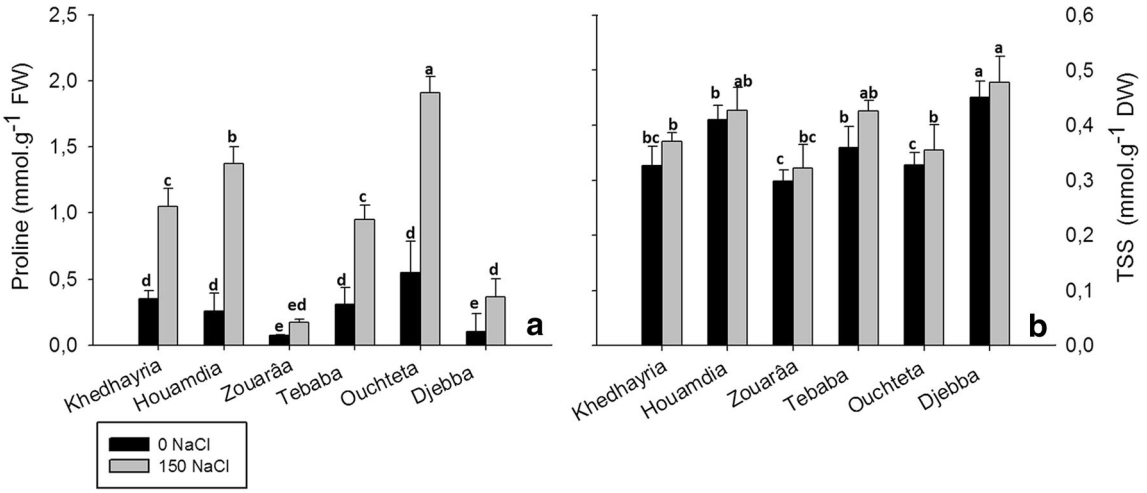


Fig. 2 Changes in leaf contents of proline (**a**) and total soluble sugars TSS (**b**) of six wild grapevines under control (black), 100 mM NaCl (light grey) and 150 mM NaCl (dark grey). Values are mean (± SD) of at least 10 replications. Data labelled with different letters are significantly different at $P < 0.05$

4 Discussion

According to our results, high concentrations of NaCl had deleterious effects on plant growth and changed the distribution of assimilates between shoot and root organs of wild vines. This led to decreases in relative growth rates (RGR) and in root/shoot ratios. Changes in growth and root to shoot dry weight ratios under salt stress have been previously reported on grapevines (Upreti and Murti 2010; Sivritepe et al. 2010; Hamrouni et al. 2011; Mohammadkhani et al. 2014) as well as on other ligneous species such as pear (Wu and Zou 2009), amaranth (Omamt et al. 2006), olive (Melgar et al. 2008) and citrus (Syvertsen and Bandaranayake 2011). In this study, we used the parameters RGR_L and RGR_S and tow salinity levels to rank accessions according to salt tolerance: *Khedhayria* + *Tebaba* > *Ouchteta* > *Zouarâa* > *Djebba* > *Houamdia*. Water relations and osmotic adjustment parameters showed that tolerant accessions, *Tebaba* and *Khedhayria*, had the lowest osmotic potential values (Fig. 3e) but at the same time, the highest water potentials (Fig. 3a). These latter values were greater than those reported for grapevines under severe stress (Choné et al. 2001; Patakas et al. 2005). We associated this behaviour to higher leaf osmotic adjustment in tolerant accessions compared to sensitive ones. Our results were in agreement with those of Patakas et al. (2002) who attributed lower leaf osmotic potential in grapevines to active osmotic potential.

Moreover, salinity caused mineral disturbances through increases in Na^+ and Cl^- content in laminas and roots, decreases in roots' K^+ content and increases in lamina K^+ content (Table 2). These results corroborated those of Munns (2005), implying that sequestering Na^+ and Cl^- in cell vacuoles causes K^+ to accumulate and organic solutes in cytoplasm and organelles to balance the osmotic pressure. Differences between accessions in potassium flux (Fig. 1c) could explain the improved osmotic adjustment observed in tolerant accessions, *Khedhayria* and *Tebaba,* and better control of the stomata opening (Fig. 3c). Indeed, increases in K^+ concentration in the guard cell vacuoles lead to greater vacuolar osmotic pressure and ostioles opening (Wang et al. 2013; Shabalaa and Pottosin 2014). In addition, maintaining higher levels of transport and absorption of K^+ in tolerant accessions of wild vines is attributed to higher potassium flux (Fig. 1d) and overall selectivity of K^+ versus Na^+ $S_{(K/Na)}$. Our results agreed with Fisarakis et al. (2005) who reported that the ability of vines to maintain high K^+ levels in leaves may act as the major monovalent cationic osmoticum in the presence of high external salt concentrations. In the same context, Sivritepe et al. (2010) mentioned a significant increase in Na^+, K^+, Ca^{2+}, N, PO_4^{3-}, Mg^{2+}, Fe^{2+}, Mn^{2+} and Zn^{2+} root contents and a decrease in K^+ in roots when 5.45 NaCl was applied for 60 days. They assumed that the increase in the transport of inorganic ions up to the leaves was the major component of osmotic adjustment in *Sultana* vines. In our study, salinity levels of 100 and 150 mM NaCl increased root fluxes of Na^+ and Cl^- (Fig. 1a, b) and were more pronounced with the *Khedhayria* and *Tebaba* accessions compared to sensitive accessions such as *Houamdia* and *Djebba*. *Ouchteta* and *Zouarâa*, however, had intermediate responses. Mohammadkhani et al. (2014) related the tolerance of grape genotypes to their capacity to restrict Na^+ and Cl^- transport to shoots. In our experiment, high concentrations of Na^+ and Cl^- in roots compared to those in laminas confirmed that their transport was limited and/or they were excluded from these photosynthetic organs, this

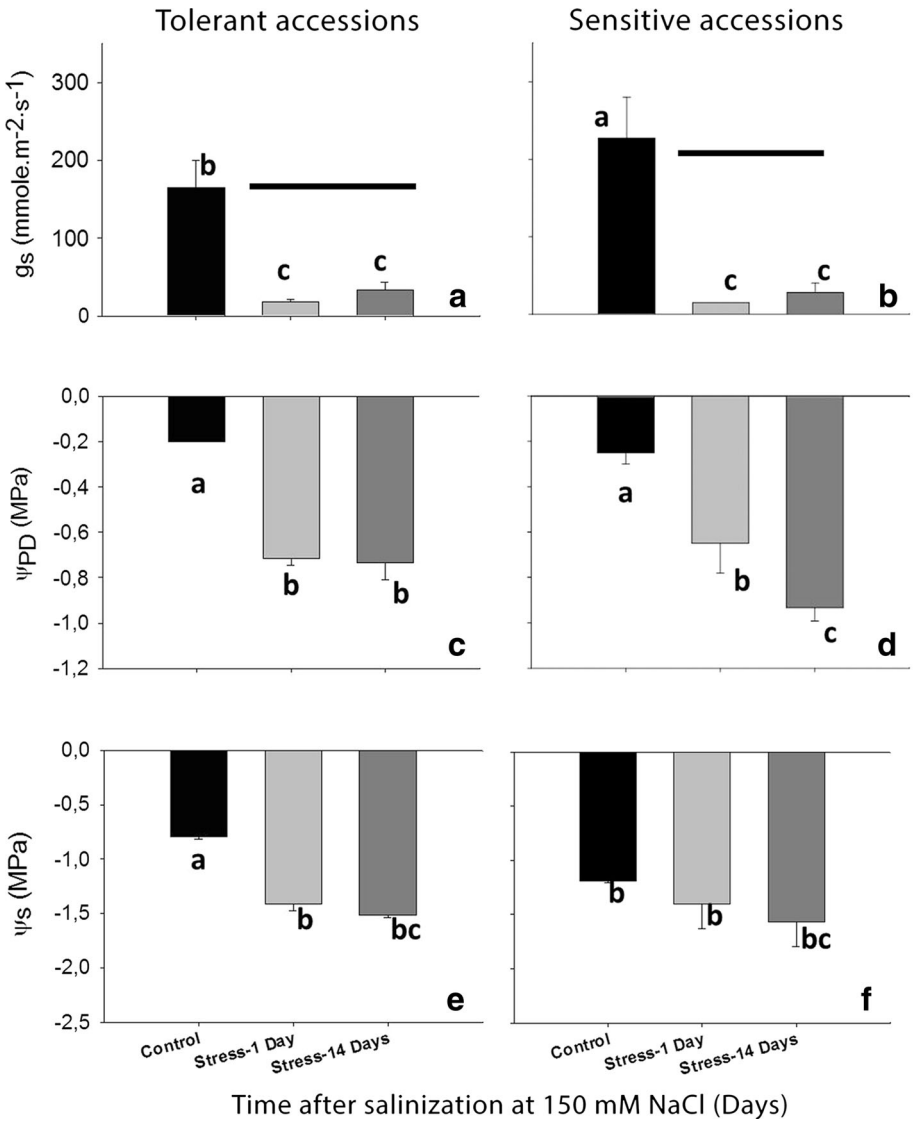


Fig. 3 Average changes in stomatal conductance g_S (**a**, **b**), leaf predawn water potential Ψ_{PD} (**c**, **d**), leaf osmotic potential Ψ_S (**e**, **f**), of tolerant (*Khedhayria*, *Tebaba*) and sensitive (*Houamdia*, *Djebba*) wild grapevines accessions at control (black), 1 day (light grey) and 14 days (dark grey) after salinization at 150 mM NaCl. Values are mean (± SD) of at least 10 replications. Data labelled with different letters are significantly different at $P < 0.05$

behaviour being more observable with Na^+. These results suggested that salt tolerance in wild vines was not correlated with limiting absorption of toxic ions but with their ability to exclude them or limit their transport to laminas. Comparing Na^+ and Cl^- cytosolic concentrations in laminas and roots to the upper limit 100 mmol.l^{-1} reported in adapted or not adapted cells (Singh et al. 2013) revealed that at 100 mM NaCl wild vine accessions recorded low Na^+ cytosolic concentrations in their laminas (Askri 2014). In laminas, Cl^- contents were between 103 and 158 mmol.l^{-1}. In roots Na^+ and Cl^- contents ranged between 137 and 177 mmol.l^{-1}. Although these estimated values represented average concentrations in vacuole and cytoplasm, the hypothesis can be put forward that wild grapevine genotypes exclude Na^+ from laminas and compartmentalize Cl^- in lamina vacuoles and Na^+ and Cl^- in root cell vacuoles. Azevedo-Neto et al. (2004) reported that soluble organic solutes induced by relatively high levels of salt in the roots of the tolerant genotypes contributed to maintaining of root water uptake and shoot water flow by maintaining high water potential. We observed that tolerant accessions, *Khedhayria* and *Tebaba,* were more effective in excluding Na^+ from roots and lamina cytoplasm than sensitive ones, resulting in better absorption of K^+. According to our results, tolerance to salinity in wild vines could be partly attributed to a better adaptation of laminas and roots to osmotic adjustment. This hypothesis is supported by better overall selectivity of potassium versus sodium. Proline appears to play an organic osmotic role, but the rate increases were not positively correlated to accession tolerance, whereas total soluble sugars probably played a minor role in the process of leaf osmotic adjustment. According to Fisarakis et al. (2005), greater tolerance of *vitis* genotypes is associated with decreased metabolic

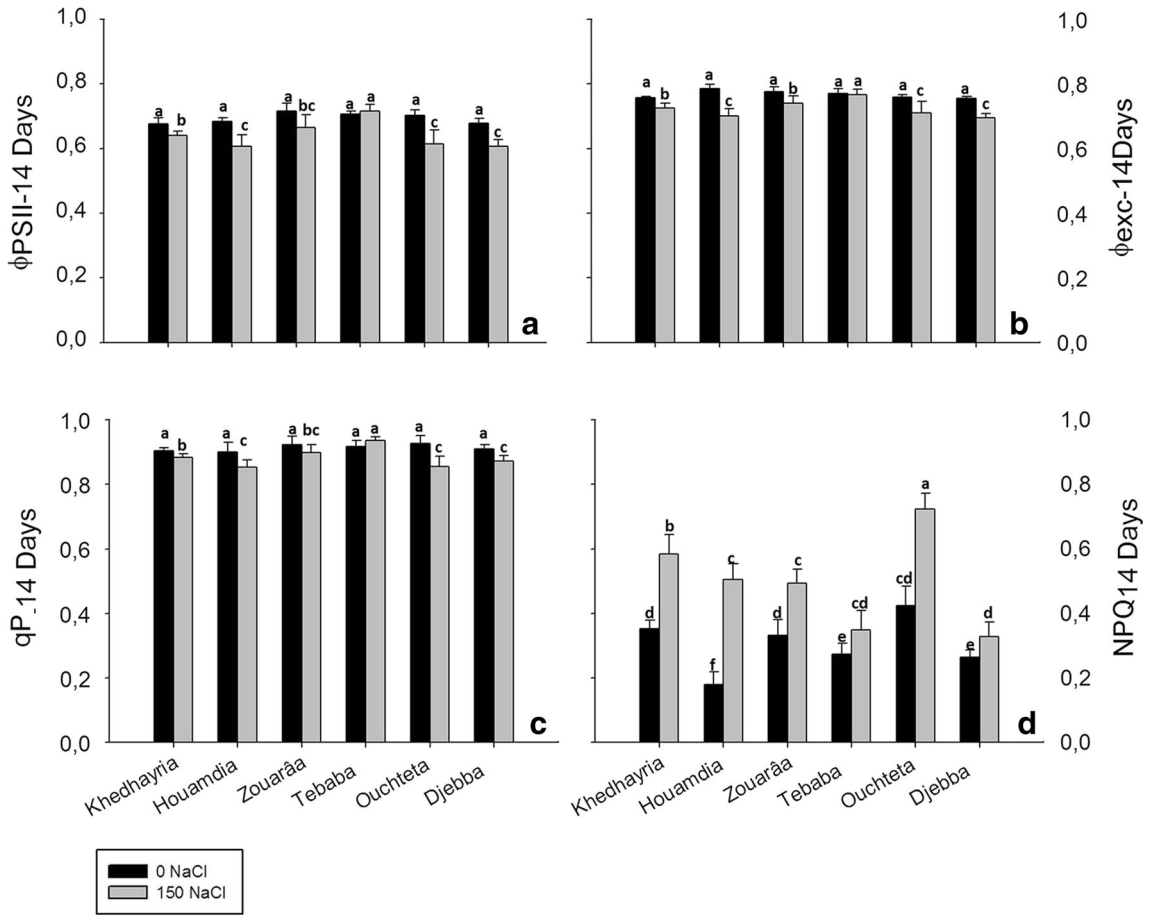


Fig. 4 Changes in chlorophyll fluorescence parameters: Φ_{PSII} (**a**), Φ_{exc} (**b**), qP (**c**) and NPQ (**d**) of six wild grapevines under control (black) and after 14 days salinization at 150 mM NaCl (light grey). Values are mean (± SD) of at least 10 replications. Data labelled with different letters are significantly different at $P < 0.05$

cost with osmoregulation and/or effective compartmentalization of inorganic solutes in vacuoles and organic solutes in the cytosol. According to our results, not severe damages occurred to plant growth, especially for *Tebaba* and *Khedhayria* accessions. Growth reductions may be associated with photosynthetic inhibitions through metabolic disturbances resulting from ionic imbalance or toxicity of ions (Singh et al. 2013). Decreases in photosynthetic assimilation may be associated with stomatal (Ouerghi et al. 2000) or non-stomatal limitation (Fisarakis et al. 2001). In the case of our experiments, significant and concomitant reductions in growth and stomatal conductance observed in six accessions from the first day at 150 mM NaCl illustrated the contribution of the stomatal component to photosynthetic reduction. We can attribute the decrease in stomatal conductance to a concomitant decrease in water potential. Nevertheless, it is due to osmotic adjustment that the plant maintained an adequate level of leaf hydration. Later, with the increasing salinity of the external environment, disturbances of the PSII were noted and could be considered non-stomatal factors. According to Fisarakis et al. (2001), they may be attributed to high accumulation of Cl^- that inhibits net photosynthesis by reducing root uptake of NO^{3-} ions, strongly correlated with reduced net photosynthesis.

With reference to our results on chlorophyll fluorescence, two mechanisms were identified as protecting the PSII system from oxidative damages under saline conditions: (1) the non-photochemical energy dissipation and (2) the photochemical regulation mechanism. However, these photoprotective mechanisms were associated with a decrease in plant photosynthetic yield. De Lucena et al. (2012) reported reductions in photosynthetic yields of mango as a response to NaCl stress to balance electron transport and carbon metabolism. In our study, non-photochemical energy dissipation was the most active mechanism. The sensitive accession *Houamdia* recorded an increase of 181% in non-photochemical quenching (NPQ) compared to the control. However, tolerant accessions *Tebaba* and *Khedhayria* were less affected by NaCl salt and recorded reductions in NPQ of about 27 and 65%, respectively. The second photochemical mechanism was

involved in the decrease in the proportion of open reaction centres, demonstrated by Φ_{PSII} and Φ_{exc} reductions compared to the control. Furthermore, the tolerant accessions *Tebaba* and *Khedhayria* were less affected by high salinity levels than accessions *Houamdia, Djebba and Zouarâa*. In addition, fewer variations of Φ_{PSII} and Φ_{exc} parameters recorded in accession *Tebaba* compared to accession *Khedhayria,* justified the best tolerance of *Tebaba* to NaCl. For that, a final classification was proposed for the six wild grapevine accessions ranging from the most tolerant to the most sensitive to salt as follows: *Tebaba* > *Khedhayria* > *Ouchteta* > *Zouarâa* > *Djebba* > *Houamdia*. Salt tolerance in many species was positively correlated to photosystem II stability, and chlorophyll fluorescence parameter variations have been used to rank barley lines (Kalaji et al. 2011) and chickpea lines (Çiçek et al. 2018) according to their tolerance to salt stress.

In conclusion, salt tolerance of wild grapes was not correlated to the absorption of Na^+ and Cl^- but to the accession ability to limit sodium transport to lamina and to chloride compartmentalization in root and leaf vacuoles, which is improved by sufficient uptake of K^+ that acts as the main osmoticum in response to massive influx of Cl^-. Tolerant accessions maintained better vigour, tissue hydration and ensured efficient osmotic adjustment. These characteristics imparted to tolerant accessions were explained by a better supply of potassium ($Flux_K$, S (K/Na)), a greater stomata aperture and more efficient osmotic adjustment. Furthermore, photoprotective mechanisms developed by wild grapevines were more efficient in tolerant accessions in regulating photochemistry, thus ensuring better stability of light reactions of photosynthesis. The photosynthesis process is mainly responsible for growth reduction explained by significant stomatal closure, one day after salt stress and 14 days later by alterations of photochemistry (Φ_{exc}, Φ_{PSII} and qP) and increased energy dissipation (NPQ) to avoid damage of PSII. Finally, altering cationic nutrition, including that of potassium is also involved in the decrease in growth.

Acknowledgements The authors thank Dr. Zoghlemi Nejia from the biotechnology centre of Borj Cedria for her valuable assistance in selecting the accessions of wild vines studied and sampling them in their natural site.

Authors' contributions AH designed and carried out the experiment. FG performed fluorescence chlorophyll measurements. AH wrote the manuscript with the support from FG, SR, AM and AG.

Declaration We attest to the fact that all authors listed on the title page have contributed significantly to the work, have read the manuscript, attest to the validity and legitimacy of the data and its interpretation and agree to its submission to Brazilian Journal of Botany.

References

Arnold C, Schnitzler A, Parisot C, Maurin A (2010) Historical reconstruction of a relictual population of wild grapevines (*Vitis vinifera* L. ssp. *sylvestris*, Gmelin, Hegi) in a flood plain forest of the upper seine valley, France. River Res Appl 26:904–914

Ashraf M (2009) Biotechnological approach of improving plant salt tolerance using antioxidants as markers. Biotechnol Adv 27:84–93

Askri H (2014) Réponses écophysiologiques et moléculaires de la vigne sauvage (*Vitis vinifera* L. ssp. *sylvestris*) tunisienne au stress salin. Dissertation, Faculté des Sciences de Tunis, Tunisie

Askri H, Daldoul S, Ben Ammar A, Rejeb S, Jardak R, Rejeb MN, Mliki A, Ghorbel A (2012) Short-term response of wild grapevines (*Vitis vinifera* L. ssp. *sylvestris*) to NaCl salinity exposure: changes of some physiological and molecular characteristics. Acta Physiol Plant 34:957–968

Azevedo-Neto AD, Tarquinio-Prisco J, Enéas-Filho J et al (2004) Effects of salt stress on plant growth, stomatal response and solute accumulation of different maize genotypes. Braz J Plant Physiol 16:31–38

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

Bates LS, Waldren RP, Teare D (1973) Rapid determination of free proline for water stress studies. Plant Soil 39:205–207

Björkman O, Demmig-Adams B (1994) Regulation of photosynthetic light energy capture, conversion, and dissipation in leaves of higher plants. In: Schulze E-D, Caldwell MM (eds) Ecophysiology of photosynthesis. Springer, Berlin, pp 17–47

Choné X, Van Leeuwen C, Dubourdieu D, Gaudille JP (2001) Stem water potential is a sensitive indicator of grapevine water status. Ann Bot 87:477–483

Çiçek N, Oukarroum A, Strasser RJ, Schansker G (2018) Salt stress effects on the photosynthetic electron transport chain in two chickpea lines differing in their salt stress tolerance. Photosynth Res 136:291–301

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

Dardeniz A, Müftuoglu NM, Altay H (2006) Determination of salt tolerance of some American rootstocks. Bangladesh J Bot 35:143–150

De Lucena CC, De Siqueira DL, Prieto Martinez HE, Cecon PR (2012) Salt stress change chlorophyll flourescence in Mango. Rev Bras Frutic 34:1245–1255

Fisarakis I, Chartzoulakis J, Stavrakas D (2001) Response of Sultana vines (*Vitis vinifera* L.) on six rootstocks to NaCl salinity exposure and recovery. Agric Water Manag 51:13–27

Fisarakis I, Nikolaou N, Tsikalas P, Therios I, Stavrakas D (2005) Effect of salinity and rootstock on concentration of potassium, calcium, magnesium, phosphorus, and nitrate-nitrogen in Thompson seedless grapevine. J Plant Nutr 27:2117–2134

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

Gupta B, Huang B (2014) Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. Int J Genom. https://doi.org/10.1155/2014/701596

Hadi MR, Karimi N (2012) The role of calcium in plants' salt tolerance. J Plant Nutr 35:2037–2054

Hamrouni L, Hanana M, Abdelly C, Ghorbel A (2011) Exclusion du chlorure et inclusion du sodium: deux mécanismes concomitants

de tolérance à la salinité chez la vigne sauvage *Vitis vinifera* subsp. *sylvestris* (var.Séjnène). Biotechnol Agron Soc Environ 15:387–400

Hegi G (1966) Illustrierte Flora von Mittel-europa. Band V, Teil I, Verlag, Hamburg

Hewitt EJ, Smith TA (1975) Plant mineral nutrition. English Universities Press Ltd, London

Hunt R (1990) Basic growth analysis. Unwin Hyman Press, London

Jogaiah S, Ramteke SD, Sharma J, Kumar UA (2014) Moisture and salinity stress induced changes in biochemical constituents and water relations of different grape rootstock cultivars. Int J Agron. https://doi.org/10.1155/2014/789087

Johansen C, Edwards DG, Loneragan JF (1970) Potassium fluxes during potassium absorption by intact barley plants of increasing potassium content. Plant Physiol 45:601–603

Kalaji HM, Govindjee Bosac K, Koscielniak J, Zuk-Gołaszewskae K (2011) Effects of salt stress on photosystem II efficiency and CO_2 assimilation of two Syrian barley landraces. Environ Bot 73:64–72

Li X, An P, Inanaga S, Eneji AE, Tanabe K (2006) Salinity and defoliation effects on soybean growth. J Plant Nutr 29:1499–1508

Melgar JC, Syvertsen JP, Martinez V, Garci-Sanchez F (2008) Leaf gas exchange, water relations, nutrient content and growth in citrus and olive seedlings under salinity. Biol Plant 52:385–390

Mohammadkhani N, Heidari R, Abbaspour N, Rahmani F (2014) Evaluation of salinity effects on ionic balance and compatible solute contents in nine grape (*Vitis* L.) genotypes. J Plant Nutr 37:1817–1836

Moutinho-Pereira JM, Magalháes N, Torres De Castro LF, Chaves MM, Torres-Pereira JM (2001) Physiological responses of grapevine leaves to Bordeaux mixture under light stress conditions. Vitis 40:117–121

Munns R (2005) Genes and salt tolerance: bringing them together. New Phytol 167:645–663

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681

Negrao S, Schmocke SM, Tester M (2017) Evaluating physiological responses of plants to salinity stress. Ann Bot 119:1–11

Omamt EN, Hammes PS, Robbertse PJ (2006) Differences in salinity tolerance for growth and water use efficiency in some amaranth (*Amaranthus* spp.) genotypes. N Z J Crop Hortic Sci 34:11–22

Ouerghi Z, Cornic G, Roudani M, Ayadi A, Brulfert J (2000) Effect of NaCl on photosynthesis of two wheat species (*Triticum durum* and *T. aestivum*) differing in their sensitivity to salt stress. J Plant Physiol 156:335–340

Patakas A, Nikolaou N, Zioziou K, Radoglou K, Noitsakis B (2002) The role of organic solute and ion accumulation in osmotic adjustment in drought-stressed grapevines. Plant Sci 163:361–367

Patakas A, Noitsakis B, Chouzouri A (2005) Optimization of irrigation water use in grapevines using the relationship between transpiration and plant water status. Agric Ecosyst Environ 106:253–259

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 E-D, Caldwell MM (eds) Ecophysiology of photosynthesis. Springer, Berlin, pp 49–70

Shabalaa S, Pottosin I (2014) Regulation of potassium transport in plants under hostile conditions: implications for abiotic and biotic stress tolerance. Physiol Plant 151:257–279

Shani U, Ben-Gal A (2005) Long-term response of grapevines to salinity: osmotic effects and ion toxicity. Am J Enol Vitic 56:148–154

Singh PK, Mondal S, Bandana Bose (2013) Cross-talk signaling and survival strategies in plants under changing environment. In: Hemantaranjan A (ed) Advances in plant physiology. Scientific Publishers, Jodhpur, pp 296–313

Sivritepe N, Sivritepe OH, Çelik H, Katkat AV (2010) Salinity responses of grafted grapevines: effects of scion and rootstock genotypes. Not Bot Hort Agrobot Cluj 38:193–201

Staub AM (1963) Extraction, identification et dosages des glucides dans les extraits d'organes et les corps bactériens. In: Masson et Compagnie (ed) Techniques de laboratoire, Tome 1 et 2, Paris, pp 1307–1366

Syvertsen JP, Bandaranayake W (2011) Salinity tolerance of Cleopatra mandarin seedlings and two of its Trifoliata hybrids, US-897 and X639. Proc Fla State Hort Soc 124:47–51

Tunçturk M, Tunçturk R, Yasar F (2008) Changes in micronutrients, dry weight and plant growth of soybean (*Glycine max* L. Merrill) cultivars under salt stress. Afr J Biotechnol 7:1650–1654

Upreti KK, Murti GSR (2010) Response of grape rootstocks to salinity: changes in root growth, polyamines and abscisic acid. Biol Plant 54:730–734

Urdanoz V, Aragüés R (2009) Three-year field response of drip-irrigated grapevine (*Vitis vinifera* L., cv. Tempranillo) to soil salinity. Plant Soil 324:219–230

Wang M, Zheng Q, Shen Q, Guo S (2013) The critical role of potassium in plant stress response. Int J Mol Sci 14:7370–7390

Wu QS, Zou YN (2009) Adaptive responses of birch-leaved pear (*Pyrus betulaefolia*) seedlings to salinity stress. Not Bot Horti Agrobot 37:133–138

Yingqiang W, Xiping W, Shunyuan X, Yuejin W (2012) Ectopic expression of *VpALDH2B4*, a novel aldehyde dehydrogenase gene from Chinese wild grapevine (*Vitis pseudoreticulata*), enhances resistance to mildew pathogens and salt stress in *Arabidopsis*. Planta 236:525–539

Zoghlami N, Mliki A, Ghorbel A (2003) Occurrence and discrimination of spontaneous grapes native to Tunisia by RAPD markers. Acta Hortic 603:157–166

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 120:367–372