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RESEARCH ARTICLE

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Effects of salt stress on plant growth, abscisic acid and salicylic acid in own-rooted cultivars of *Vitis vinifera* L.

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Abstract

Aim of study: In most areas of vineyards worldwide, cultivars are frequently grafted on specific rootstocks to avoid *Daktulosphaira vitifoliae* pest attack. Nevertheless, the absence of this pest in Canary Islands allowed the chance to conserve and cultivate traditional or new own-rooted genotypes without the requirement of the rootstocks. To investigate the responses of own-rooted genotypes of *Vitis vinifera* L. to salt stress conditions, 'Castellana Negra' ('CN') and 'Negramoll' ('Ne') were used with the aim to characterize their morphological and physiological responses.

Area of study: Canary Islands, Spain.

Material and methods: The effects of NaCl stress on growth, abscisic acid (ABA), salicylic acid (SA) and proline were assessed in 'CN' and 'Ne' under greenhouse conditions.

Main results: In 'CN', the decrease of leaf number in stressed plants was lower and started eleven days later than in 'Ne'. Salt stress also reduced stomatal conductance (gs), although such decrease took place earlier in 'CN' than in 'Ne'. ABA and SA concentrations in 'CN' leaves were 2-fold higher than those of 'Ne'. Salt stress increased leaf ABA and SA content in both genotypes, compared to control. In conclusion, ABA and SA appear to be involved in grapevines responses to salinity and suggest that exogenous SA could be useful to mitigate the stress impacts.

Research highlights: 'CN' exhibited a better response than 'Ne' through the delay of salt injury establishment, and the dissimilar responses between 'CN' and 'Ne' seem to be associated to the higher accumulation of ABA and SA under salt stress.

Additional key words: growth rate; leaf biomass; phytohormones; proline; stomatal closure.

Abbreviations used: ABA (abscisic acid); CN (Castellana Negra); DAT (days after treatment); gs (stomatal conductance); Ne (Negramoll); ROS (reactive oxygen species); SA (salicylic acid); SOS5 (*Salt Overly Sensitive*); VvNAC17 (NAC *transcription factor*).

Authors' contributions: SJAM collected and performed data, did statistical and laboratory analyses and revised the paper. AUG collected and performed data and revised the paper. VVP participated in laboratory analyses, data discussion and paper revision. RMPC contributed to data discussion and paper revision. AGC revised and improved discussion and the current manuscript. JM conceptualized the research, analysed and discussed data and wrote the paper. All authors read and approved the manuscript.

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Introduction

Grapevines (*Vitis vinifera* L.) often grow in semiarid areas subjected to serious problems of drought and salinity (Cramer *et al.*, 2007). This species has been considered moderately sensitive to salt stress (Downton *et al.*, 1990; Prior *et al.*, 1992; Stevens *et al.*, 1999), and it has been described that salinity induces a decrease of net CO_2 assimilation, leaf expansion, organ dry matter, whole plant growth, and berry development expressed as bunch number, small size, and yield (Downton *et al.*, 1990; Fisarakis *et al.*, 2001; Walker *et al.*, 2008). Likewise, it has also been reported that NaCl (100 mM) greatly inhibits the growth of grapevines and decreases

the content of pigments and photosynthesis (Qin *et al.*, 2016).

The effects of the salinity stress on the plant responses has been reviewed from physiological and molecular perspectives (Tang *et al.*, 2015). In general, salinity considerably affects plant growth through the alteration of metabolic processes and photosynthetic efficiency. Firstly, NaCl induces osmotic stress and sequentially the buildup of Na^+ and Cl^- ions, which are toxic for the cell (Tester & Davenport, 2003). Precisely, salinity declines soil water potential leading to turgor loss in non-acclimated plants. To conserve water uptake, plants tend to adjust their osmotic potential through a process involving various osmolytes, such as amino acids, sugars, organic and inorganic acids, etc. (Hasegawa *et al.*, 2000; Munns, 2002).

At the hormonal level, the role of abscisic acid (ABA) on the modulation of plant stress responses under salt stress has been well-documented (De Costa *et al.*, 2007; Arbona *et al.*, 2010; Raghavendra *et al.*, 2011; Dilukshi-Fernando & Schroeder, 2016). Therefore, the mechanisms that confer stress responses through ABA are known, as well as the way in which ABA-regulated gene products act in salt tolerance at different stages of the life cycle; furthermore, the modulation of the reactive oxygen species (ROS) and the involvement of ABA in the regulation of stomatal closure and the subsequent dehydration tolerance have also been reported (Dilukshi-Fernando & Schroeder, 2016; Brunetti *et al.*, 2019; Qi *et al.*, 2020). Transcriptome analysis revealed that many of ABA-regulated genes were induced by drought and salt stress conditions (Seki *et al.*, 2002). It has also been reported that salt stress increases ROS synthesis through the activation of the transcription of reduced *nicotinamide adenine dinucleotide phosphate* (NADPH) *oxidase* genes, and that *ABA-insensitive* 4 with *RbohD* and *vitamin C defective 2*, regulate ROS metabolism throughout seed germination (Luo *et al.*, 2021). Besides, under salinity stress, *salt overly sensitive* (SOS5) gene encoding cell wall adhesion protein is needed for normal cell expansion, cell wall integrity and structure. Through a synergy with ABA, SOS5 scavenge ROS by inducing antioxidant system and increasing related gene expressions, and therefore improve salt responses (Acet & Kadıoğlu, 2020). It should be also remarked that exogenous ABA application improved salt tolerance in plants through a decrease in leaf Cl^- concentration (Gómez-Cadenas *et al.*, 2002), and salt-induced endogenous ABA increase mediated the inhibition of leaf growth and limited the accumulation of Na^+ and Cl^- in leaves (Montero *et al.*, 1997).

As ABA, salicylic acid (SA) is another agent that has been implicated in the regulation of several plant physiological processes (Shakirova *et al.*, 2003) such as growth, photosynthesis, ion uptake, and membrane permeability (Khan *et al.*, 2003; Khodary, 2004). It is also considered a signal molecule that modulates plant responses to drought (Singh & Usha, 2003) and salinity (Borsani *et al.*, 2001; Gunes *et al.*, 2005). In particular, SA could mitigate the negative effects of salinity in grapevine through the decrease in the accumulation of detrimental ions and the improvement of the absorption of essential and beneficial elements (Amiri *et al.*, 2014). The exogenous application of SA also alleviated the deleterious effects of salinity in *Arabidopsis thaliana* (Borsani *et al.*, 2001). Under drought conditions, exogenous application of SA alleviated oxidative damage in *Oryza sativa* L. seedlings through the upregulation of antioxidant enzymes, and hence protected photosynthetic pigments, which might improve photosynthesis under the imposed stress (Sohag *et al.*, 2020). Recently, it has been shown that the novel NAC transcription factor (*VvNAC17*) is expressed in grapevine tissues under environmental constraints such as drought, high temperature, and freezing, or by exogenous treatments through SA and ABA. In addition, the *VvNAC17* gene was overexpressed in *A. thaliana* under salt stress, being the germination rates and the root lengths of the *VvNAC17*-overexpression plants higher in comparison to wild-type (Ju *et al.*, 2020).

To explore the contribution of compatible solutes to plant stress responses, many experimental systems reported that plants accumulate proline and glycine betaine to enable water uptake and protect cells against increased levels of ROS under salinity stress conditions (Hare & Cress, 1997; Ashraf & Foolad, 2007). Proline protects plants from various stresses and helps them to recover from stress more rapidly. Under salt stress conditions, proline synthesis could alleviate cytoplasmic acidosis and maintain *nicotinamide adenine dinucleotide phosphate* (NADP) at values compatible with plant metabolism (Miller *et al.*, 2010). Exogenous application of proline to plants subjected to stress enhances growth, influences plant-water relations through the maintenance of cells turgidity, and increases the rate of photosynthesis (Hayat *et al.*, 2012). In *Olea europaea* L. plants, the application of proline seems to increase salt tolerance through the better performance of antioxidant enzymatic activities, photosynthetic activity, plant growth and the preservation of a suitable plant water status (Ben Ahmed *et al.*, 2010). Proline production also depends on plant species and salt concentration in the medium as shown in *Beta vulgaris* spp. where proline increased significantly under long-term severe salinity (250 mM NaCl) and not altered under lesser concentrations (75, 100 and 150 mM NaCl) (Tahjib-Ul-Arif *et al.*, 2019).

To investigate the responses of *V. vinifera* L. species to salt stress conditions, 'Castellana Negra' ('CN') and 'Negramoll' ('Ne') were used in this work to assess their morphological and physiological responses to salinized irrigation water during a relatively short-term period. Both cultivars were selected because they are

widely settled in Canary Islands and they differ in their vigor and growth features (Rodríguez-Torres, 2017), and also to explore their agronomical potential as own-rooted genotypes in areas free of phylloxera pest (*Daktulosphaira vitifoliae*). Plant physiological variables such as phytohormones, proline, stomatal closure and plant growth were characterized throughout the imposed stress stage.

Material and methods

Plant material

V. vinifera L. 'CN' and 'Ne' cultivars are well adapted to subtropical Canary Island conditions. Due to the lack of phylloxera pest (*D. vitifoliae*) in this location, no rootstocks are required, and own-rooted cultivars were used. Three-month-old plantlets (30-cm tall) were transplanted and grown in plastic pots (5 L) containing peat substrate (Leader potting soil, Germany) under greenhouse conditions. At nutrient level, the composition of the substrate was N (200 mg L^{-1}), P_2O_5 (200 mg L^{-1}), and K_2O (300 mg L^{-1}), and was enriched before transplanting with 50 g for each pot of granular fertilizer (Osmocote Pro, NPK fertilizer containing Mg with trace elements: 18-9-10+2MgO+-Te). Throughout the experimental period, temperature oscillated between 18 and 30 ºC, relative humidity was 60-90%, and maximum photosynthetically active radiation (PAR) was 1200 µmol m^{-2} s^{-1}. The whole experimental system was repeated during three consecutive seasons (spring-summer 2017, 2018 and 2019) and similar results were obtained.

Salinity stress conditions

To investigate at what extent the studied genotypes are able to tolerate salt stress, both cultivars were subjected to the same salt concentration during a variable period of time (depending on the damage symptoms). Thus, since cultivars differ in their responses to the stress condition, length of the experiments were different for each of them. Sampling was performed when plants reached a defined symptom threshold based on the number of abscised leaves per plant and the intensity of leaf injury. Consequently, the following experimental design was established. Thirty days after transplanting, salinity stress was applied by adding a concentration of 80 mM of NaCl to the irrigation water and maintained until severe specific salt symptoms such as leaf necrosis and abscission were evident, *i.e.*, 39 and 49 days after treatment (DAT) for 'Ne' and 'CN', respectively. At these dates, around 35-50% of leaves per plant and genotype suffered serious salt injuries and finally abscised. Irrigation of plants with saline or non-saline water (control) was performed three times a week at field capacity. Plants of both cultivars were arranged in a randomized complete block design with three blocks and three plants per treatment and block.

Plant growth measurements and sampling

Plant growth was periodically determined as leaf number and stem length throughout the experimental period. At the end the trial time, samples of three leaves (3^{rd}-5^{th} leaf from the apex) per plant and three plants per treatment were collected and their fresh weights were recorded. Samples were frozen in liquid nitrogen, freeze-dried, weighted and used for further analyses. Relative growth rate (RGR) was calculated from dry weights according to the Hunt & Cornelissen (1997) formula: $RGR = (\ln W_f - \ln W_0)/(t_f - t_0)$, where t_f and t_0 respectively signify the final and initial sampling dates, and W_f and W_0 represent dry weight per plant at the end and at the beginning of the experiment, respectively. As mentioned above, the sampling dates at the end of the experiment were established accordingly to the intensity of salt injury in each genotype. Thus, although plant growth and stomatal conductance (gs) measurements were conducted regularly throughout the experimental period (39 days), those biochemical parameters that need collect material (destructive method) were determined both at the beginning and at the end of the experimental period.

Stomatal conductance

Stomatal conductance was measured by using a steady-state porometer (SC-1 Leaf Porometer, Meter Group, Inc. USA). Measurements were performed on fully expanded leaves, generally among the third and the fifth leaf counting from plant apex. Determinations were made between 8:00 and 10:00 a.m., temperature within the leaf chamber registered 23 ± 3 ºC and leaf to-air vapor pressure deficit was 1.8 ± 0.2 kPa.

Determination of phytohormones

ABA and SA were analyzed by liquid chromatography coupled to tandem mass spectrometry with some modifications (Durgbanshi *et al.*, 2005). In brief, 25 μL of a mixture of internal standards containing 100 ng of [$^2H_6$] ABA and 100 ng of [$^{13}C_6$] SA was added to 0.05 g of fine powdered leaves. The triturated plant tissues were homogenized in 2 mL of ultrapure water and subsequently centrifuged at 5,000 × g for 10 min to pellet debris as described previously (Durgbanshi *et al.*, 2005; Mahouachi *et al.*, 2014). Afterward, pH of the

supernatant was tuned to 2.8 with a 15% v/v aqueous solution of glacial acetic acid and it was partitioned twice against an equal volume of diethyl ether. The aqueous phase was discarded, the organic layer was evaporated in vacuum at room temperature and the solid residue resuspended in 1 mL of a 10% v/v aqueous solution of methanol, which was filtered through a 0.22 µm cellulose acetate filter (Durgbanshi *et al.*, 2005; Mahouachi *et al.*, 2014). A 20 μL aliquot of this solution was then directly injected in the Ultra Performance Liquid Chromatography (UPLC) system coupled to a Triple Quadrupole Mass Spectrometer (TQD Mass Spectrometer coupled to an Acquity LC, Waters Milford, MA, USA) through an orthogonal Z-spray electrospray interface. Known amounts of pure standard samples were employed to prepare calibration curves, allowing thus the determination of the concentrations of plant hormones. The separation was achieved with a reverse phase C18 column (Gravity, 50 × 2.1 mm 1.8 µm particle size, Macherey–Nagel GmbH, Germany), using a methanol:water gradient, both supplemented with 0.1% acetic acid at a flow rate of 0.3 mL min^{-1} (Durgbanshi *et al.*, 2005).

Proline determination

Ground leaves (0.05 g) were extracted in 5 mL of an aqueous 3% w/v sulfosalicylic acid (Sigma-Aldrich, Madrid, Spain) solution by the use of a homogenizer (Ultra-Turrax, IKA-Werke, Staufen, Germany). The extracts were centrifuged (4,000 × g, 40 min, 4 ºC) and proline determination was carried out following the procedure of Bates *et al.* (1973). Briefly, 1 mL of the supernatant was mixed with 2 mL of a 50% w/v solution of ninhydrin reagent (Sigma-Aldrich, Madrid, Spain) in glacial acetic acid. The resulting mixture reacted in a water bath (100 ºC, 1 h) and then was cooled into an ice bath for 15 min. Absorbance was read in the organic phase at 520 nm through a Genova Plus Spectrophotometer (Jenway, Bibby Scientific, Chelmsford, UK). Tissue proline content was calculated by interpolation employing a standard curve prepared with commercial proline (Sigma-Aldrich, Madrid, Spain) as reported previously (Bates *et al.*, 1973; Mahouachi *et al.*, 2012).

Statistical analyses

Statistical analyses were performed by using the software IBM® SPSS® Statistics version 25 for Windows (IBM Corporation, Armonk, NY, United States). A two-way ANOVA was chosen to examine the effects of cultivar and treatment and their interactions on the measured parameters at the significance level indicated in each case ($p < 0.05$, 0.01 or 0.001).

Results

Plant growth

Total leaf number per plant in control plants varied approximately between 9 to 19 leaves in the studied cultivars throughout the experimental period (Fig. 1a). NaCl (80 mM) treatment significantly reduced the number of leaves about 25% (17 DAT) and 39% (28 DAT) compared to control, and that decrease reached 52% at the end of the experiment (39 DAT) in 'Ne' (Fig. 1a). However, 'CN' only reached a 31%

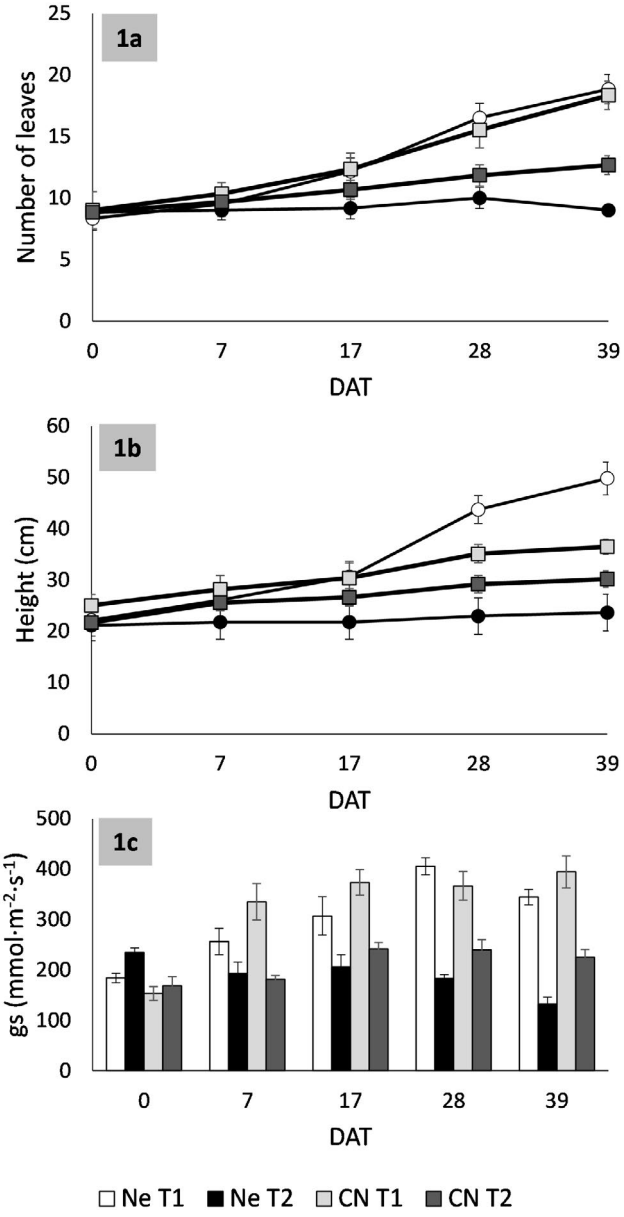

Figure 1. Leaf number (1a), stem height (1b) and stomatal conductance (gs, 1c) in 'Negramoll' (Ne) and 'Castellana Negra' (CN) *Vitis vinifera* L. plants subjected to saline (T2, 80 mM NaCl) and non-saline (T1, control) treatments for 39 days. Data are means ± SE and each value was determined in three different plants with three replicates per treatment (n = 9).

of reduction with respect to control at 39 DAT (Fig. 1a), and a significant interaction cultivar × treatment was found at this date (Table 1), illustrating a dissimilar behaviour of the two cultivars against salinity treatment. Hence, salt treatment caused damage in the edges of 'Ne' leaves, whereas 'CN' leaves remain healthier (Fig. 2). For this reason, the experiment with 'CN' plants was extended up to 49 DAT, when 19.50 ± 1.09 and 12.67 ± 1.05 leaves were measured in control and treated plants, respectively.

The stem height in 'Ne' plants reached a plateau from the beginning of salt stress imposition which lasted throughout the experimental period as shown in Fig. 1b, the average height changed from 21.75 ± 3.32 cm (*i.e.*, 17% of decrease compared to control 7 DAT) to 23.63 ± 3.56 (*i.e.*, 53% 39 DAT). In contrast, the difference between treated 'CN' plants regarding control increased from 9% at 7 DAT to 17% at 39 DAT (Fig. 1b). Again, this finding was consistent with the significant interaction cultivar × treatment shown in Table 1.

Salt stress also reduced leaf fresh weight at the end of stress period by approximately 79% and 32% in 'Ne' and 'CN', respectively compared to control (Table 2). Similar trend was observed in leaf dry weight, which was reduced, by about 83% and 42% in 'Ne' and 'CN', respectively (Table 2). In the same vein, Fig. 3 shows the RGR calculated for both cultivars from the final and initial leaf dry weight. Keeping the initial data as reference, it should be highlighted the increase of the final leaf dry weight of control plants from both cultivars, whereas treated 'CN' plants values barely varied and 'Ne' plants values were smaller than the initial ones as consequence of the salinity.

Stomatal conductance

Salt stress induced a continuous decrease of gs from 17 DAT until the end of the experiment in both 'Ne' and 'CN' plants (Fig. 1c, Table 1). Such reduction reached 61% for 'Ne' and 43% for 'CN' 39 DAT in comparison to their respective controls.

Plant hormone changes

Levels of foliar ABA in grapevine plants differ between cultivars regardless of the treatments (Table 3). Thus, ABA concentration in 'CN' leaves was 2-fold higher than that of 'Ne' in absence of salt stress. Salt stress imposition significantly increased leaf ABA content by 97 and 106% in 'Ne' and 'CN', respectively compared to control. Regarding leaf concentration of SA, it showed the same trend as ABA in control plants of the studied cultivars, being SA concentration 2-fold higher in 'CN' than in 'Ne' leaves (Table 3). The addition of NaCl to the irrigation water considerably increased SA levels, which were 390 and 345% higher than the control in the leaves of 'Ne' and 'CN', respectively. Overall, statistical analyses of ABA and SA changes revealed significant differences between cultivars, treatments and interaction cultivar × treatment.

Proline concentration

Foliar proline content was also determined at the initial and the end of the experimental period in 'Ne' and 'CN' plants. Meanwhile no changes in proline concentration were observed between control and stressed plants in 'CN', NaCl stress increased leaf proline concentration about 72% with respect to control in 'Ne' (Table 3). In addition, data showed statistical differences between cultivars and interaction cultivar × treatment at the end of trial.

Table 1. Results of two-way ANOVA for the effects of cultivar (Ne: 'Negramoll', CN: 'Castellana Negra'), treatment (T1: control, T2: 80 mM NaCl) and their interaction on the leaf number, stem height, and gs.

		DAT 0	DAT 7	DAT 17	DAT 28	DAT 39
Leaf number	Cultivar	nsd	nsd	nsd	nsd	nsd
	Treatment	nsd	nsd	*	***	***
	Cultivar × Treatment	nsd	nsd	nsd	nsd	*
Stem height	Cultivar	nsd	nsd	nsd	nsd	nsd
	Treatment	nsd	nsd	*	***	***
	Cultivar × Treatment	nsd	nsd	nsd	nsd	**
gs	Cultivar	**	nsd	nsd	nsd	**
	Treatment	*	***	**	***	***
	Cultivar × Treatment	nsd	nsd	nsd	*	nsd

nsd = not significant difference, asterisks denote significant differences at $p < 0.05$ (*), $p < 0.01$ (**) or $p < 0.001$ (***). DAT = days after treatment.

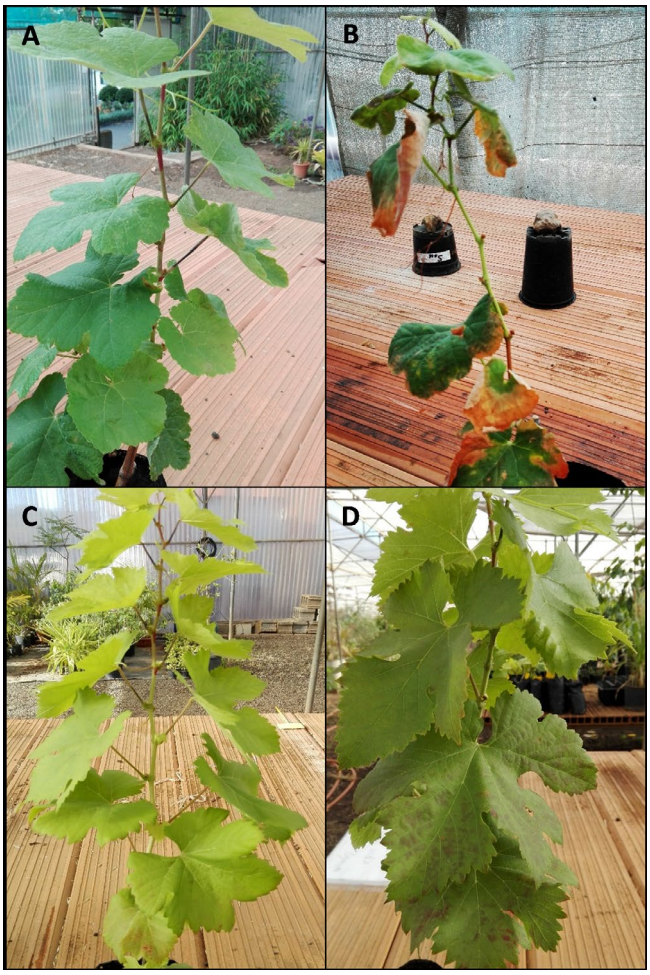

Figure 2. Aspect of aerial parts of representative 'Negramoll' (Ne) and 'Castellana Negra' (CN) *Vitis vinifera* L. plants after 39 days of being subjected to saline (T2, 80 mM NaCl) and non-saline (T1, control) treatments: Ne-T1 (A); Ne-T2 (B); CN-T1 (C); CN-T2 (D).

Discussion

The responses of *V. vinifera* L. 'CN' and 'Ne' to NaCl stress were assessed from the onset of salt treatments until the appearance of severe symptoms of leaf necrosis and abscission, typical of salt toxicity. Under the same NaCl concentration (80 mM) in the irrigation water, 'CN' exhibited a relative better response than 'Ne' through the delay in the appearance of salt toxicity symptoms in plants. In this context, NaCl treatment significantly reduced the number of leaves in 'Ne' from 28 DAT, and such decrease reached 52% at the end of the experiment (39 DAT) compared to control. However, in 'CN', leaf number in stressed plants was significantly reduced from 39 DAT (11 days later than 'Ne') and reached 31% of decrease with respect to control. Interestingly, a significant interaction cultivar × treatment was found 39 DAT (Table 1), pointing out the different behaviour of the two cultivars against salinity treatment. This is consistent with the sharp visual difference found between treated plants of both cultivars 39 DAT: salt treatment caused damage in the edges of 'Ne' leaves, whereas 'CN' leaves remain healthier (Fig. 2). Regarding plant growth, the stem height has barely grown in 'Ne' plants under salt stress imposition throughout the experimental period, and decreased around 53% compared to control 39 DAT. In contrast, respect to control, treated 'CN' plants evolved from a 9% of difference 7 DAT to a 17% 39 DAT (Fig. 1b). This result was also concomitant with the significant interaction cultivar × treatment shown in Table 1. Concerning leaf biomass, salt stress reduced leaf fresh and dry weights in both cultivars but by different behaviour between them, and as well significant interaction cultivar × treatment occurred at 39 DAT

Table 2. Initial and final leaf fresh and dry weight (g) in 'Negramoll' (Ne) and 'Castellana Negra' (CN) *Vitis vinifera* L. plants subjected to saline (T2, 80 mM NaCl) and non-saline (T1, control) conditions. Final samplings correspond to DAT 39 for Ne and DAT 49 for CN. Data are means ± standard errors and each value was determined in three different plants with three replicates per treatment (n = 9). Results of two-way ANOVA show the effects of cultivar, treatment and their interaction on the shown data.

Cultivar	Treatment	Fresh weight (g)		Dry weight (g)	
		Initial sampling	Final sampling	Initial sampling	Final sampling
Ne	T1	13.85 ± 0.12	21.04 ± 1.06	3.05 ± 0.08	6.88 ± 0.39
	T2		4.40 ± 0.65		1.15 ± 0.20
CN	T1	17.57 ± 1.38	22.24 ± 2.39	3.30 ± 0.29	6.96 ± 0.84
	T2		15.07 ± 1.88		4.03 ± 0.52
Summary of two-way ANOVA results					
Cultivar		nsd	**	nsd	*
Treatment		-	***	-	***
Cultivar × Treatment		-	**	-	*

nsd = not significant difference, asterisks denote significant differences at $p < 0.05$ (*), $p < 0.01$ (**) or $p < 0.001$ (***). DAT = days after treatment.

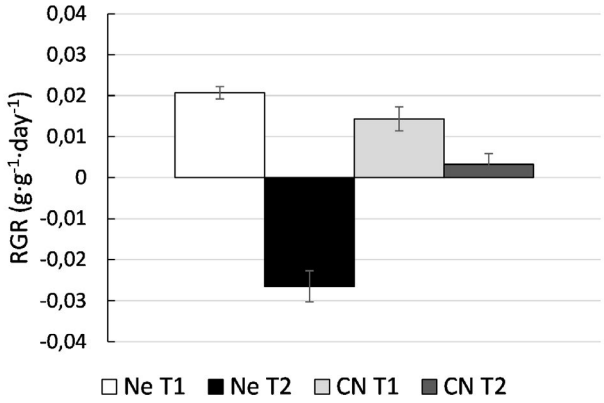

Figure 3. Relative growth rate (RGR) calculated from leaf dry weight of 'Negramoll' (Ne) and 'Castellana Negra' (CN) *Vitis vinifera* L. plants subjected to saline (T2, 80 mM NaCl) and non-saline (T1, control) conditions. Final samplings correspond to DAT 39 for Ne and DAT 49 for CN. Data are means ± SE and each value was determined in three different plants with three replicates per treatment (n = 9).

(Table 2). Thus, the salt excess in water solution induced a marked decrease of leaf fresh and dry weights in 'Ne' and at a lesser extent in 'CN' plants (Fig. 3). It has previously been reported that salinity decreases the ability of susceptible plant species to uptake water, and this quickly induces reductions in growth rate (Munns, 2002; Munns *et al.*, 2006). The decrease of plant growth performance induced by NaCl stress conditions has been reported in several plant species, such as fruit crops *Dimocarpus longan* L. (Mahouachi *et al.*, 2013) and *Mangifera indica* L. (Mahouachi, 2018) or legumes such as *Vigna angularis* L (Ahanger *et al.*, 2020), among others. In the present work, data reveal a clear differential response at growth level under salt stress between both cultivars, which involve a better agronomic potential for 'CN' than for 'Ne' under salinity conditions.

In addition, the reduction of the gs induced by salt stress reached 61% for 'Ne' and 43% for 'CN' 39 DAT compared to their respective controls. In other experimental systems, salinity induced a decrease of net CO_2 assimilation, dry matter accumulation in organs, and a whole growth of grapevines (Downton *et al.*, 1990; Walker *et al.*, 2008; Qin *et al.*, 2016). Under high salinity conditions, the own-rooted 'Sultana' grapevines exhibited a higher photosynthetic rate and better growth parameters than those grafted onto several rootstocks, and such results may be linked to the capacity of this genotype to withstand the major accumulation of ion content and the ability for osmotic adjustment (Fisarakis *et al.*, 2001). In other species, NaCl stress decreased several parameters related to photosynthetic machinery, such as gas exchange parameters, chlorophyll synthesis, and photochemical efficiency (Fv/Fm) (Ahanger *et al.*, 2020).

Regarding plant hormones, data showed that the levels of foliar ABA and SA in grapevine plants differ between cultivars irrespective of the treatments. Thus, ABA and SA concentrations in 'CN' leaves doubled those of 'Ne'. The addition of NaCl to the irrigation water considerably increased ABA and SA levels, which were higher than the control in both studied genotypes. The role of ABA on mediating plant responses to high salinity and drought conditions is well known in fruit crops (Gómez-Cadenas *et al.*, 2002; Mahouachi *et al.*, 2012; Munns *et al.*, 2006; Mahouachi *et al.*, 2013); however, the involvement of SA in plant response to salt stress in own-rooted grapevine has not been addressed so far. ABA-mediated stress responses, such as stomatal closure and the subsequent

Table 3. Initial and final abscisic acid (ABA), salicylic acid (SA) and proline content (ng per g of dry weight for ABA and SA, (±mol per g of dry weight for proline) in leaves of 'Negramoll' (Ne) and 'Castellana Negra' (CN) *Vitis vinifera* L. plants subjected to saline (T2, 80 mM NaCl) and non-saline (T1, control) conditions. Final samplings correspond to DAT 39 for Ne and DAT 49 for CN. Data are means ± standard errors and each value was determined in three different plants with three replicates per treatment (n = 9). Results of two-way ANOVA show the effects of cultivar, treatment and their interaction on the shown data. show the effects of cultivar, treatment and their interaction on the shown data.

Cultivar	Treatment	ABA (ng·g^{-1} DW)		SA (ng·g^{-1} DW)		Proline (µmol·g^{-1} DW)	
		Initial sampling	Final sampling	Initial sampling	Final sampling	Initial sampling	Final sampling
Ne	T1	633.57 ± 47.01	579.17 ± 103.32	572.75 ± 85.81	1860.50 ± 344.75	0.91 ± 0.22	4.47 ± 0.42
	T2		1139.74 ± 131.19		9122.78 ± 54.81		7.69 ± 0.70
CN	T1	1290.05 ± 46.07	1083.39 ± 143.30	701.71 ± 42.56	3696.39 ± 600.65	1.18 ± 0.18	5.08 ± 0.54
	T2		2230.50 ± 158.32		16441.68 ± 824.35		3.87 ± 0.27
Summary of two-way ANOVA results							
Cultivar		***	***	***	***	nsd	**
Treatment		-	***	-	***	-	nsd
Cultivar × Treatment		-	*	-	***	-	***

nsd = not significant difference, asterisks denote significant differences at $p < 0.05$ (*), $p < 0.01$ (**) or $p < 0.001$ (***). DAT = days after treatment.

dehydration tolerance, ABA-regulated gene expression at different stages of the plant cycle, and the detoxification of ROS have been previously discussed (Dilukshi-Fernando & Schroeder, 2016; Brunetti *et al.*, 2019; Qi *et al.*, 2020). Our data showed that the increase of ABA was concomitant with the decrease of gs and consequently with the stomatal closure in 'CN' and 'Ne', indicating a close relationship between ABA signal and stomatal movement in both genotypes in response to salt stress. In the same way, the contribution of SA to plant stress tolerance is getting clearer nowadays. Drought stress increased SA accumulation in *Solanum lycopersicum* L. plants, suggesting that this phytohormone could play an important role on the early response to drought (Muñoz-Espinoza *et al.*, 2015). It has also been indicated that a crosstalk between jasmonic acid (JA) and SA could enhance the tolerance of these species to water stress (Muñoz-Espinoza *et al.*, 2015). In this context, Ghaffari *et al.* (2020) reported that JA can improve water deficit tolerance of sugar beet through the upregulation of antioxidant enzyme activities. Exogenous application of SA enhanced growth and photosynthesis, increased levels of N, K^+ and Ca^{2+}, and reduced Na^+ and Cl^- concentration in *Vigna angularis* L. (Ahanger *et al.*, 2020). The SA supply also induced the protection of Photosystem II activity via the up-regulation of the antioxidant system in *Triticum aestivum* L. plants (Chen *et al.*, 2016). In addition, under drought conditions, SA increased antioxidant enzymes, proline and total soluble sugars as defense responses of *T. aestivum* L. cultivars. Exogenous SA promoted growth and stress priming effects of this species and hence alleviated yield restriction. SA also regulated several physiological and metabolic processes related to photosynthesis, protein and amino acid metabolism, and signal transduction (Sharma *et al.*, 2017). Data presented here suggest that the major accumulation of SA in 'CN' compared to 'Ne' under natural and specially salinity conditions could be involved in its better adaptive response to the imposed stress. Therefore, the increase of endogenous content of SA in 'CN' could enhance its performance at least at physiological level, such as the minor reduction of stomatal closure or the delay in the decrease of leaf number.

Concerning proline production, it has been previously reported an increase of its levels in several plant species subjected to salt or drought stress conditions under diverse experimental systems (Mahouachi *et al.*, 2012, 2013; Argamasilla *et al.*, 2014; Sharma *et al.*, 2017). In the current study, proline concentration increased and subsequently functioned as a stress marker in 'Ne'; however, did not play such role in 'CN' plants subjected to salinity conditions. Based on the results presented here and on earlier studies, it seems that the response of proline under salt stress is not common and may depend on the degree of plant species and/or genotypes tolerance to the stress period and on the salt concentration in the medium, as shown in *Beta vulgaris* spp. (Tahjib-Ul-Arif *et al.*, 2019), *D. longan* L. (Mahouachi *et al.*, 2013), and *M. indica* L. (Mahouachi, 2018).

In conclusion, the dissimilar pattern of changes exhibited by the studied genotypes in terms of plant growth, number of leaves, stomata behaviour, proline production or plant hormone levels may suggest that the major tolerance of 'CN' to salt stress in comparison to 'Ne' seems to be associated to the higher accumulation of ABA and SA under both salt stress and natural conditions. These findings suggest that exogenous application of SA should be explored in future experiments to improve the responses of *V. vinifera* L. genotypes sensitive to salt/abiotic stress.

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