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Is foliar Cl^- concentration the cause of photosynthetic decline in grapevine during mild salinity?

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ABSTRACT

Moderate levels of Cl^- have been associated with grapevine salt tolerance. The hypothesis to be tested in this work is: photosynthesis in grapevine is negatively correlated with foliar Cl^- concentration. To further test this hypothesis, multiple mild salinity experiments on four different *Vitis* genotypes (Cabernet-Sauvignon, Riparia Gloire, Ramsey and SC2) were conducted and photosynthesis, ion concentrations and gene expression responses were quantified. The salt-tolerant rootstock Ramsey had greater Cl^- exclusion capabilities than *V. vinifera* cultivars both during rooted cutting greenhouse experiments and three years of field-grafted experiments; SC2 also excluded Cl^-. Differential gene expression indicated that salinity affected transcript abundance more in salt-sensitive genotypes (97.7 % of DEGs in the dataset), especially chloroplast-related transcripts. The transcript abundances of known anion transporters were determined and a family of putative B transporters was associated with the Cl^- exclusion phenotype. Photosynthesis and growth were maintained in Ramsey and SC2 under mild salinity. However, photosynthesis declined in Cabernet-Sauvignon with isosmotic 20 mM salt concentrations of NaCl, KCl or $NaNO_3$, independent of the salt type. While foliar Cl^- concentrations did correlate with salt tolerance during control and NaCl conditions, it was not found to be the cause of photosynthetic decline in *Vitis* during mild salinity.

KEYWORDS

Vitis, grapevine, salinity, mild, tolerance, chloride, photosynthesis

Supplementary data can be downloaded through: https://oeno-one.eu/article/view/4795

INTRODUCTION

Salinity is a prevalent abiotic stress affecting agricultural land throughout the world (Rengasamy, 2006). It is estimated that approximately 20 % of cultivated land and 33 % of irrigated land is affected by salinity throughout the globe (Shrivastava and Kumar, 2015). Salinity stress is characterised by high ion concentrations in soils. The USDA defines salinity as soils with an electrical conductivity (EC) above 4 dS m^{-1}, an exchangeable Na^+ percentage below 15 and a soil pH below 8.5 (Allison *et al.,* 1954). While ions such as Mg^{2+}, Ca^{2+}, K^+, SO_4^{2-} and HCO_3^- are known to contribute to salinity, the majority of research into the subject has involved Na^+ and Cl^-. This is not only because they are two of the most soluble and widespread salts, but also because many crops are particularly sensitive to these ions (Munns and Tester, 2008).

High levels of salt in soils trigger both osmotic and ionic disruption in plant function (Munns, 2002; Munns and Tester, 2008). The initial osmotic effect is similar to the symptoms of water deficit (Munns, 2002), characterised by reduced water potential in the soils due to the increased solute concentration. This reduction in soil water potential inhibits water uptake into the plant, decreasing growth and nutrient uptake (Acosta-Motos *et al.,* 2017). To overcome this initial osmotic effect, many plants respond through the closure of stomates, increasing accumulation of organic osmolytes and hormonal regulation (Munns and Tester, 2008). All of these mechanisms are energetically costly and can lead to slowed or stunted growth (Munns and Gilliham, 2015). Another response is to increase the uptake of salt ions, for use as osmoticums, from the soil, lowering the water potential in the plant and driving water uptake (Munns and Gilliham, 2015). Uncontrolled ion uptake over time will exasperate the secondary effect of salinity–ionic toxicity (Munns and Tester, 2008). Ionic toxicity occurs when cellular ionic concentrations exceed the optimal range and lead to detrimental effects on plant function such as reduced ribosomal function, photosynthetic inhibition and decreased uptake of other essential macro and micronutrients (Geilfus, 2018). The symptoms of ion toxicity are not observed until the ion concentrations rise to toxic levels for the specific plant. This is dependent on the individual plant's tolerance and developmental stage, the ion concentrations in the soil, as well as the length of exposure (Greenway and Munns, 1980). Crops have different sensitivities to salinity in their growth environment, while some are tolerant to high levels, others, such as *Vitis*, are considered salt-sensitive (Greenway and Munns, 1980; Munns and Tester, 2008).

Vitis vinifera is a relatively drought-tolerant plant species (Cardone *et al.,* 2019), but one of the few crop species considered to be sensitive to Cl^-, along with citrus (*Citrus* ssp. L.), avocado (*Persea americana* L.), soybean (*Glycine max* L.) and faba bean (*Vicia faba* L.) (Li *et al.,* 2017). Particularly, photosynthetic decrease in *V. vinifera* during salinity exposure has been associated with increased Cl^- concentrations in leaves (Downton, 1977a; Downton *et al.,* 1990; Prior *et al.,* 1992; Walker *et al.,* 1981; Walker *et al.,* 1997). Researchers have shown in *Vitis* that Cl^- concentrations in various tissues (leaf, petiole, stems and roots) reach higher concentrations than Na^+ when exposed to equal concentrations in the growth medium (Downton, 1977a; Martin *et al.,* 2020; Walker *et al.,* 1981; Walker *et al.,* 2010). Notably, in *V. vinifera* cultivars (the primary grapevine species used for wine production (Zhou-Tsang *et al.,* 2021)), there exists a prevalence of the dominant HKT1;1 allele controlling high rates of Na^+ exclusion from the shoot (Henderson *et al.,* 2018), potentially explaining the underlying reason why *Vitis* shows a higher accumulation of Cl^- than Na^+ when the two ions are present in equal concentrations in the growth medium. Within the genus of *Vitis*, there are different tolerances and ion accumulation patterns during saline conditions among genotypes (Downton, 1977b; Fort *et al.,* 2015; Heinitz *et al.,* 2020; Sykes, 1987).

To investigate if grapevine's growth inhibition is caused by Cl^- toxicity (not osmotic effects) at low saline concentrations: two genotypes considered salt sensitive, Cabernet-Sauvignon (CS) [*V. vinifera*] (Vincent *et al.,* 2007) and Riparia Gloire (RI) [*V. riparia*] (Ismail *et al.,* 2014; Zhou-Tsang *et al.,* 2021), as well as two genotypes considered salt-tolerant, Ramsey (RA) [*V. champinii*] (Southey and Jooste, 1991; Stevens *et al.,* 1999; Suarez *et al.,* 2019; Sykes, 1985; Walker *et al.,* 1997, 2010; Zhou-Tsang *et al.,* 2021) incorrectly known as 'Salt Creek' (Fort *et al.,* 2015; Lowe and Walker, 2006) and SC2 (SC) [*V. vinifera* × *V. girdiana* hybrid] (Heinitz *et al.,* 2020) were studied in greenhouse conditions. Greenhouse plants were control and salinity (20 mM) treated. These plants were then examined for tissue ion concentration, leaf area, root length, lateral shoot length, gene expression,

stomatal conductance and photosynthesis. In field conditions, self-grafted Syrah (SY) [*V. vinifera*], also known as 'Shiraz', and Colombard (CO) [*V. vinifera*] were compared with scions (SY and CO) grafted onto both RA and RI rootstocks for leaf Cl^- accumulation.

MATERIALS AND METHODS

1. Plant material

Four grapevine genotypes were used for the greenhouse experiments: Cabernet-Sauvignon clone 8 (*V. vinifera)* abbreviated: CS, Riparia Gloire (*V. riparia*) abbreviated: RI, Ramsey (*V. champinii*) abbreviated: RA, SC2 (*V. vinifera* × *V. girdiana*) abbreviated: SC. Original vines used for clonal propagation and grafted vines for the field experiment were obtained from Dr Andrew Walker and the Foundation Plant Services at the University of California, Davis, CA, USA. Single nodal vegetative cuttings were rooted in water aerated by stone diffusion aeration pumps (referred to as rooted cuttings throughout). When cuttings were rooted, plants were transferred to pots as described below.

2. Greenhouse salinity experiments

Greenhouse salinity experiments were performed at the Nevada Agricultural Experiment Station at the University of Nevada, Reno located in Reno, NV, USA. Rooted cuttings were planted in small black plastic pots (73 × 229 mm, Anderson Die & MFG. CO. Portland, OR, USA) with ~50 g Hydrokorrels Hydroton lightweight expanded clay aggregate (Netherlands) at bottom of the pot, ~80 g fritted clay (Turface® Athletics MVP®, Buffalo Grove, IL, USA) on top of the expanded clay aggregate and filled to 1.2 kg final weight with sand (commercial grade medium, Quikrete®, Atlanta, GA, USA) or in large black plastic pots (229 × 381 mm Treepot, Stuewe and Sons, INC, Corvallis, OR, USA) with ~0.75 kg Hydrokorrels Hydroton lightweight expanded clay aggregate (Netherlands) at bottom of the pot, topped with ~1 kg fritted clay (Turface® Athletics MVP®, Buffalo Grove, IL, USA) and filled to 13.3 kg final weight with sand (commercial grade medium, Quikrete®, Atlanta, GA, USA). Plants were irrigated with Cramer's solution (1.5 mM $Ca(NO_3)_2 \times 4H_2O$; 2 mM KNO_3; 0.6 mM $Mg(SO_4) \times 7H_2O$; 1 mM KH_2PO_4; 1.5 mM $CaCl_2 \times 2H_2O$; 36 µM Fe (Sprint 330); 1 µM $Mn(SO_4) \times H_2O$; 0.5 µM $CuSO_4 \times 5H_2O$; 20 µM $ZnSO_4 \times 7H_2O$; 20 µM H_3BO_3; 0.01 µM $(NH_4)_6Mo_7O_{24} \times 4H_2O$) (Cochetel *et al.,* 2020) daily until experiments began, then all control groups were irrigated every day with Cramer's Solution for the entirety of the experiments. All salt-treated plants were irrigated daily with their respective salinity solutions described below.

All greenhouse experiments (one-week, two-week and big-pot photosynthesis), except the multiple salt photosynthesis experiment, were conducted with four genotypes (CS, RA, RI and SC) and two treatments (control and salt) in a completely randomised design. The one-week experiment (n (number of replicates) = 5–8 individual vines) was conducted first, and the two-week experiment (n = 10 individual vines) was designed following the one-week experiment. In these experiments, the control group plants were irrigated daily with 100 mL Cramer's Solution for the entire treatment period. Salt group plants were irrigated with 100 mL of Cramer's Solution + 20 mM NaCl. Leaf area and root length were measured at each harvest. At the harvest date (day 7 or 14), shoot tissue (leaf, petiole and stem) was harvested and then dried for 48 hours in a 60 °C oven.

For the large pot photosynthesis experiment (n = 5 individual vines), the control group plants were irrigated with 2 L of Cramer's Solution daily for the treatment period. Salt-treated plants were irrigated with 2 L of Cramer's Solution + 20 mM NaCl. The experiment was conducted over 16 days with 4 days of pre-treatment observation. Lateral shoot length was measured every odd day in the morning with a meter stick. Photosynthesis and stomatal conductance were measured every even day starting at ~10:00 AM. At harvest, leaf tissue (without petiole) was collected and then dried for 48 hours in a 60 °C oven.

The multiple salt photosynthesis experiment was conducted with one genotype (CS) and four treatments (control, NaCl, KCl or $NaNO_3$) in a completely randomised design. For the multiple salt photosynthesis experiment (n = 3 individual vines), salt treatments included either NaCl, KCl, or $NaNO_3$ at 20 mM solution added to the Cramer's Solution; the control group was irrigated only with Cramer's Solution. All plants received 400 mL of Cramer's Solution daily, an excess amount to flush the pots to prevent salt accumulation. The experiment was conducted over 26 days, with 4 days of pre-treatment analysis each morning, measuring photosynthesis and lateral shoot length on alternative days. Auxiliary shoot length was measured every odd day in the morning with a meter stick. Photosynthesis measurements were taken every even day starting at ~10:00 AM. At harvest, leaf tissue (without petiole) was sampled and then dried for 48 hours in a 60 °C oven.

3. Field site

Grafted vines were planted in the Las Vegas Cooperative Extension Research Center and Orchard in the Spring of 2016 in Las Vegas, NV, USA. These vines were grown in the same location of planting, in mildly saline soil with no additional application of salinity during the experimentation period (2017, 2018 and 2019). Two scion materials were used: Colombard (*V. vinifera*) abbreviated: CO and Syrah (*V. vinifera* L.) abbreviated: SY. Graft combinations include the following scion/rootstock (6 graft types) combinations: CO/CO, CO/RA, CO/RI, SY/SY, SY/RA and SY/RI planted in a completely randomised design (n = 6–12 individual vines). The control vines grafted onto their own genotype's rootstocks (CO/CO and SY/SY) are referred to as self-grafted throughout. During the Fall seasons of 2017, 2018 and 2019, one mature healthy front-facing leaf (without petiole), free of insect pressure, was collected from each of the four cordons of each vine. The four leaves from each year from individual plants were grouped as one composite biological replicate. Leaves were dried for 48 hours in a 60 °C oven. Leaves (without petioles) were ground and then analysed for Cl^-. The soil at the vineyard planting site in Las Vegas, NV, USA were analysed for soil salinity and ionic concentrations using 10 sub-samplings equally spaced along each outer row compiled for a single composite sample for each side.

3. Greenhouse growth measurements

Fresh and dry weights of plant tissue were measured at the time of harvest and after drying, respectively. The tissue was dried in an oven at 60 °C for 48 hours before weighing dry plant tissue. Root lengths were obtained by scanning with an Epson Perfection V800 Photo and quantified in WinRHIZO™ from Regent Instruments Inc. (Quebec, Canada). Excised leaves (without petioles) were photographed and leaf area was quantified using the ImageJ software (Schneider *et al.,* 2012). Mean relative growth rate, net assimilation rate and leaf area ratio equations used are from Hunt, 1982. Leaf area ratio is calculated using the variables: L_A (leaf area) and W (whole plant dry weight). Mean relative growth rate and net assimilation rate are calculated from the one- and two-week data using the variables: W_1 (whole plant dry weight of the first time point), W_2 (whole plant dry weight of second time point), T_1 (day of first time point), T_2 (day of second time point), L_{A1} (leaf area of first time point) and L_{A2} (leaf area of second time point).

$$\textbf{Leaf Area Ratio}\ (cm^2 \cdot g^{-1}) = \frac{L_A}{W}$$

$$\textbf{Mean Relative Growth Rate}\ (g \cdot g^{-1} \cdot day^{-1}) = \frac{\log_e W_2 - \log_e W_1}{T_2 - T_1}$$

$$\textbf{Net Assimilation Rate}\ (g \cdot cm^{-2} \cdot day^{-1}) = \frac{W_2 - W_1}{T_2 - T_1} * \frac{\log_e L_{A2} - \log_e L_{A1}}{L_{A2} - L_{A1}}$$

4. Lateral shoot length

All vines in the big-pot photosynthesis and multiple-salt photosynthesis experiments were trained as a single cane on a bamboo stake. One lateral shoot at the top of the vine was allowed to grow, all other lateral buds were removed daily. Every other day, the lateral shoot length was measured in the morning (starting at ~10:00 AM) with a meter stick from the base of the cane to the tip of the lateral meristem. The growth rate was calculated as growth change from the previous measurement over the day. In the big-pot photosynthesis experiment, one SC control and one SC salt lateral shoots broke during treatment and were not included in lateral shoot length or lateral shoot growth rate analysis (n = 4), all other genotypes (CS, RI and RA) and their treatment groups (control and salt) had all replicates included in the analysis (n = 5).

5. Photosynthesis measurements

Photosynthetic measurements were performed every other day in the morning (starting at ~10:00 AM) using the LiCOR (model Li-6400XT) Portable Photosynthesis system from LI-COR Biosciences (Lincoln, NE, USA) using the following settings: Flow Rate = 400 µmol s^{-1}, Reference CO_2 = 400 µmol mol^{-1}, PAR = 1200 µmol m^{-2} s^{-1}, Leaf Temperature = 27 °C, Soda Lime = full scrub, Desiccant = full scrub, Fan = Fast. Measurements were recorded once photosynthesis stabilised (~2 min).

6. Cl^- titrations

Depending on tissue type Cl^- concentration relative to an instrument detection threshold, ~0.5–2 grams of tissue were weighed into 50 mL conical tubes. A solvent of 20 mL of 0.5 M HNO_3 was added to the tube. Tubes were vortexed for 3 min. Then tubes were incubated for 30 min in a 65 °C water bath. Tubes were re-vortexed for 3 min. Tubes were then centrifuged for 5 min at 3500 × *g*. The supernatant was filtered through Whatman™ #1 filter paper. Finally, 10 mL of filtered supernatant was transferred to the titration cup and diluted with distilled-deionised H_2O to 30 mL. Titrations were performed using a Mettler Toledo (Columbus, OH, USA) EasyPlus™ Titrator equipped with a Silver Ring Sensor (EM45-BNC) using 0.1 M $AgNO_3$ as titrant.

7. Soil and ion analysis

Soil analysis was performed by A & L Western Laboratories (Modesto, CA, USA) using the North American Proficiency Testing (NAPT) procedures. The saturation percentage and pH were quantified using the saturated paste procedure (NAPT S-1.00 and 1.10). The EC, Cl^-, CO_3^{2-}, HCO_3^- and B were analysed using the saturated paste extract procedure (NAPT S-1.20, 1.30, 1.40 and 1.50). The Ca^{2+}, Mg^{2+} and Na^+ were measured using the saturated paste extract–inductively coupled plasma spectrometry procedure (NAPT S-1.60). The Na^+ absorption ratio and exchangeable Na^+ percentage were calculated from the collected data.

Foliar Na^+ and K^+ ion analysis was performed by A & L Western Laboratories (Modesto, CA, USA) through HNO_3/HCl digestion using a microwave and analysis by inductively coupled plasma spectrometry (NAPT P-4.30). NO_3^- was quantified by A & L Western Laboratories (Modesto, CA, USA) using automated Cd^{2+} reduction (NAPT P-3.10).

8. Transcriptomics

Small potted plants from all four genotypes were exposed to either two weeks of salinity (20 mM NaCl + Cramer's solution) or control (Cramer's solution) conditions. Harvests were conducted after one and two weeks of treatment of root and leaf tissue (n = 2–5 individual vines). The RNA extraction, sequencing, processing, quantification, differential expression, gene ontology and principal component analysis were performed as described in Cochetel *et al.* (2020) except the V2/V3 assembly and annotation of the *Vitis* genome were used (Canaguier *et al.,* 2017).

9. Graphing and statistics

Graphing of transcriptomic data was performed using the R software (R Core Team, 2018). RNA-Seq data is available at the Sequence Read Archive (SRA) database from NCBI (https://www.ncbi.nlm.nih.gov/) as BioProject: PRJNA516950. All non-transcriptomic figures were created in Prism GraphPad (Version 6.0h). Statistics were done in Prism GraphPad (Version 6.0h) or using the R software (when performing three-way repeated-measures ANOVAs) (R Core Team, 2018). Statistics were done using multiple t-tests, linear regressions and ANOVAs. The fixed effects were based on the experiment (*i.e.*, genotype, treatment, time, rootstock and year). Succeeding the ANOVAs, multiple comparisons, followed by either Tukey's (when comparing every mean with every other mean) or Sidak's (when comparing only control *versus* salt within genotype) post hoc tests were used. All ANOVA tables are included in Supplementary Table 1. All other statistical tests and their results for the figures are contained in Supplementary Table 2. The symbols used for statistical significance are: ns, *, **, *** and **** refer to $P > 0.05$, $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ and $P \leq 0.0001$, respectively. The letters used for statistical significance within figures denote statistical significance of $P \leq 0.05$ (a is significantly different than b). Overlapping letters within figures denote statistical significance of $P > 0.05$ (ab is not significantly different than b). Letters without an apostrophe (a) and letters with an apostrophe (a') within a single figure are the results of separate statistical comparisons (a is not comparable to a').

RESULTS

1. Salt sensitivity was associated with elevated shoot Cl^-

The small pot salinity experiments were conducted in the greenhouse to test the hypothesis that: RA and SC are salt-tolerant and will maintain growth while reducing shoot Cl^- accumulation relative to the salt-sensitive CS during mild salinity. To test this hypothesis, one and two-week mild salinity (20 mM NaCl) experiments were performed with plant growth measurements (tissue weight, leaf area and root length), transcriptomic expression analysis and shoot Cl^- quantified after the experiments.

TABLE 1. Physiological and growth data from the two-week experiment**.**

		Salt % of control							
Genotype		CS		RA		RI		SC	
Time Point		W1	W2	W1	W2	W1	W2	W1	W2
Root Length	%	80.8**	61.5**	85.6	110.7	92.4	80.0	138.8	116.9
	SD	13.2	33.6	26.8	31.0	37.3	26.0	78.3	40.5
Leaf Area	%	97.9	81.1	77.8	124.5	91.3	80.9	110.8	96.7
	SD	25.8	18.7	16.8	30.0	30.6	46.8	45.7	30.9
Leaf Area Ratio	%	91.9	97.5	102.5	103.9	99.5	108.3	92.8	86.2*
	SD	18.2	8.2	6.8	11.9	26.1	33.1	16.0	6.6
Mean Relative Growth Rate (%)		71*		180*		61		86	
Net Assimilation Rate (%)		75		172*		56		98	

*Presentation of physiological data (root length, leaf area, leaf area ratio) and growth data (mean relative growth rate and net assimilation) from the two-week experiment. Salt treatment is normalised for week one (W1) and week two (W2) time points by percent of control. Physiological and growth data are from plants whose Cl^- data is presented in Figure 1B. Asterisks indicate significance between salt and control treatment per respective genotype and week by multiple t-tests. Data are mean and SD (standard deviation), n = 10 individual small potted vines.

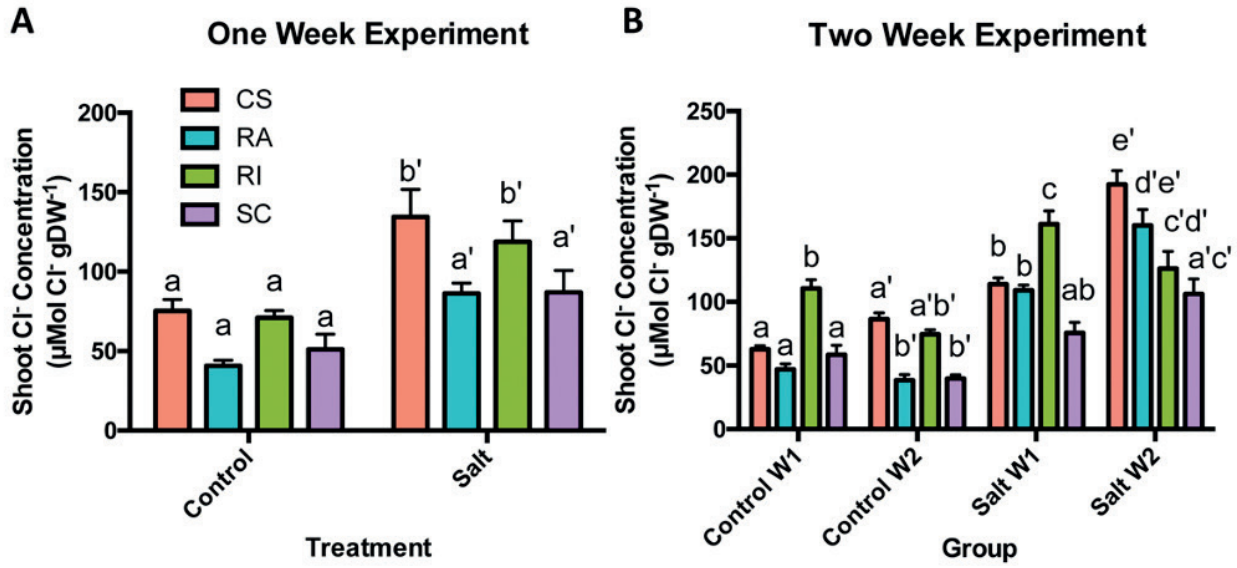

FIGURE 1. Shoot Cl^- concentration in control (Cramer's solution) and salt-treated (Cramer's solution + 20 mM NaCl) plants grown in greenhouse conditions.

Figure 1A was a one-week experiment and Figure 1B was a separate two-week experiment. Week 1 (W1) and Week 2 (W2) groups were harvested after 7 and 14 days of treatment respectively. Significance calculated by A) two-way ANOVA or B) three-way ANOVA multiple comparisons with a Tukey's post hoc test. Letter differences denote statistical significance of $P \leq 0.05$ within respective experiment. Letters with and without apostrophes are results from separate statistical comparison. Data are means ± SE (standard error of the mean), A) n = 5–8, B) n = 10, individual small potted vines.

From the plant tissue growth data, leaf area ratio, mean relative growth rate and net assimilation rate were calculated (Table 1).

CS's root length decreased under salt treatment relative to control-treated plants during week one (19.2 %) and two (38.5 %), as well as the decreased mean relative growth rate (29 %) over the treatment period (Table 1). Leaf area, leaf area ratio and net assimilation rate for salt-treated CS were no different than the control group. The salt-tolerant RA was the only genotype to have significant increases with net assimilation rate increasing 72 % and mean relative growth rate increasing 80 % in salt *versus* control for this genotype while having no significant changes in root length, leaf area, or leaf area ratio (Table 1). During one week of salinity treatment, no differences were observed in any growth measurements for RI, RA or SC. Following two weeks of salinity treatment, no significant decreases were observed in RI, relative to the control (Table 1). SC decreased leaf area ratio (13.8 %) at the week two harvest, with all other plant growth measurements unaffected by the treatment.

In Figure 1A there was a significant increase in shoot Cl^- concentrations from control to salt that was equal for all genotypes, shown by the interaction coefficient being non-significant (Figure 1A, Supplementary Table 1 and Supplementary Table 2). In the control group of the one-week experiment, the two salt-sensitive genotypes: CS and RI had mean shoot Cl^- concentrations of 75.2 ± 7.2 (mean ± SE) and 70.8 ± 4.5 µMol Cl^- • gDW^{-1}, respectively, and the two salt-tolerant genotypes: RA and SC were 40.7 ± 3.5 and 51.2 ± 9.4 µMol Cl^- • gDW^{-1}, respectively (Figure 1A). The salt-treated plants, in the one-week experiment, had significantly increased shoot Cl^- for CS, RI and RA but not SC, relative to the control plants of each genotype (Supplementary Table 2). In this experiment, salt-treated CS, RI, RA and SC had a shoot Cl^- concentration of 134.4 ± 17.4, 118.8 ± 13.1, 86.3 ± 6.5 and 86.9 ± 13.8 µMol Cl^- • gDW^{-1}, respectively (Figure 1A). In the salt-treated group, both RA and SC had significantly lower Cl^- than CS, while RI did not (Supplementary Table 2).

In Figure 1B, genotype, time, salt and all of the interactions between them significantly impacted shoot Cl^- concentration (Supplementary Table 1). In the first week, RI had the only significant difference (an increase) in control shoot Cl^- from CS, with 110.6 ± 6.7 (RI) *versus* 62.8 ± 2.7 (CS) µMol Cl^- • gDW^{-1}. During the second week, RA (38.5 ± 4.3 µMol Cl^- • gDW^{-1}) and SC (39.7 ± 3.0 µMol Cl^- • gDW^{-1}) maintained a lower Cl^- concentration than CS (86.4 ± 4.9 µMol Cl^- • gDW^{-1}) which had a relative increase from week one (Figure 1B). In the first week of salinity, the RI shoot Cl^- concentration was at its highest with a Cl^- concentration of 161.1 ± 10.4 µMol Cl^- • gDW^{-1}, consistent with the increased shoot Cl^- concentration in the control at this time point, compared to 113.9 ± 5.0, 109.1 ± 4.2 and 75.5 ± 8.5 µMol Cl^- • gDW^{-1} for CS, RA and SC, respectively (Figure 1B). In CS there was a significant increase in Cl^- from week one (113.9 ± 5.0 µMol Cl^- • gDW^{-1}) to week two (192.4 ± 10.9 µMol Cl^- • gDW^{-1}) coupled with a decrease in mean relative growth rate (29 % decrease) in the salt treatment group relative to the control (Table 1, Figure 1B and Supplementary Table 2). SC was the only genotype with lower Cl^- relative to CS in the week one salt group, although this was not significant (Figure 1B and Supplementary Table 2). During the second week, RI's Cl^- concentration lowered to 126.2 ± 13.5 µMol Cl^- • gDW^{-1}, leading RI to have significantly lower shoot Cl^- relative to CS along with SC (Figure 1B and Supplementary Table 2). While RI's shoot Cl^- lowered from week one to week two in the salt group, the opposite trend can be seen in RA. During the two weeks of salt treatment, RA's shoot Cl^- concentration significantly increased from 109.1 ± 4.2 µMol Cl^- • gDW^{-1} in week one to 160.1 ± 12.5 µMol Cl^- • gDW^{-1} in week two (Figure 1B and Supplementary Table 2).

2. Salt sensitivity was associated with increased differential gene expression

The transcriptomic experiment was conducted to test the hypothesis: mild salinity (20 mM) will induce a transcriptomic response in salt-sensitive *Vitis* genotypes. To test this hypothesis, CS, RA, RI and SC were treated with control or salt (20 mM NaCl) conditions and root and leaf tissue were harvested after one and two weeks with evaluation by RNA-sequencing. The transcriptomic analysis detected significant expression differences among the four genotypes during salinity treatment (Supplementary Figure 1). A principal component analysis (PCAs) of the roots and leaves in both week one and week two, indicated that genotype explained the majority of the variation in the data set (Supplementary Figure 1A, 1B, 1E, 1F). Only RI in the leaves had separation based on treatment in the PCAs (Supplementary Figure 1B and F).

Overall, there were low numbers of differentially expressed genes (DEGs) in all four genotypes in the root tissue at week one (47) and week two (357) (Supplementary Figure 1C, 1G). There were more DEGs in the leaf tissue, compared to the root tissue, during week one (2824) and week two (3383), with the majority in RI (1644) and CS (1187) in week one and RI (3369) in week two (Supplementary Figure 1D, 1H). Very low numbers of DEGs in root and leaf tissue during both harvests were found in RA (20) and SC (134) (Supplementary Figure 1C, 1D, 1G, 1H).

Salt-sensitive genotypes had more DEGs than salt-tolerant genotypes (97.7 % of DEGs). In the DEGs shared by the salt-sensitive genotypes (CS and RI) in the week one leaf harvest, there was an enrichment of DEGs with a gene ontology of chloroplast-related transcripts. Gene ontology enrichment identified two significant enrichments in the week one leaf harvest for increased DEGs shared by the salt-sensitive (CS and RI) genotypes as chloroplast accumulation movement and chloroplast avoidance movement (P adjusted values = 0.0069). Additional analyses of known anion transporters amongst species or induced by salinity did not identify any clear candidates for Cl^- exclusion. However, differences in constitutive expression of putative B transporters (*VIT_04s0008g04980, VIT_11s0016g04740, VIT_09s0002g05810, VIT_07s0005g02060, VIT_17s0000g08530, VIT_05s0020g00440*) were associated with the Cl^- exclusion phenotype (data not shown), with higher Cl^- exclusion associated with higher B transporter transcript abundance. These transporters are all members of the ion transporter BOR family (Takano *et al.,* 2002). One putative B transporter (*VIT_11s0016g04740*) had very high levels of gene expression in SC roots (~600 TPM [transcripts per million]) during both weeks in salt and control vines with minimal to no expression measured for the other three genotypes; there was no expression of this gene in the leaves for any genotype. Another putative B transporter (*VIT_04s0008g04980*) was significantly expressed (~10 TPM) in the roots in SC and RA, and only expressed in the leaves of RA, but with little or no expression in CS or RI in roots or leaves.

3. RA rootstock decreased foliar Cl^- accumulation regardless of scion in field conditions

The field site experiment was conducted to test the hypothesis: commercially used RA decreases foliar Cl^- concentrations in grafted scions (CO and SY) under field conditions relative to self-grafted (CO or SY) and salt-sensitive (RI) rootstocks. To test this hypothesis, SY and CO scions grafted onto either their own genotype's roots, RA, or RI were evaluated for foliar Cl^- concentrations over three years at the Las Vegas Field site.

The EC from the four composite soil samples collected at the field site were 7.7, 11.4, 5.3 and 3.5 dS • m^{-1}, the exchangeable Na^+ percentages were 2.9, 10.4, 1.2 and 1.2 and the pH of each was 7.4, 7.8, 7.4 and 7.7, respectively (Supplementary Table 2). For Cl^-, the four soil samplings had values of 19.5, 26.3, 7.5 and 3.2 meq • L^{-1}. For Na^+ the four soil samplings had concentrations of 17.3, 51.0, 8.6 and 7.3 meq • L^{-1} (Supplementary Table 3). Based on the USDA classification of saline soils (EC > 4.0 dS • m^{-1}, exchangeable Na^+ % < 15 and pH < 8.5) (Allison *et al.,* 1954), the field site would be considered saline soil.

In Figure 2, the graft type and year both had significant impacts on the foliar Cl^- concentrations, but the interaction between the two was not significant (Supplementary Table 1). The scions grafted to RA rootstocks had decreased foliar Cl^- concentrations *versus V. vinifera* self-grafted rootstocks (CO and SY) over three years, consistent with the decreased accumulation of shoot Cl^- observed in rooted cuttings of RA *versus* CS in the greenhouse (Figures 1 and 2).

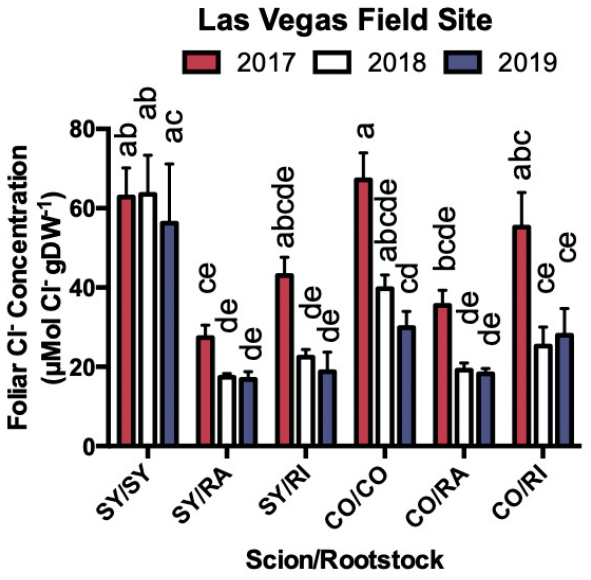

FIGURE 2. Foliar Cl^- concentrations in grafted vines over three years in field conditions.

Scion/rootstock grafting combinations are clustered and depicted by year. Statistics performed through two-way ANOVA multiple comparisons with a Tukey's post hoc test. Different letters denote statistical significance of $P \leq 0.05$. Data are means ± SE n = 6–12 individual grafted vines.

This lower Cl^- concentration was significant for all years for SY/RA and in 2017 for CO/RA. In 2018 and 2019 CO/RA and CO/CO had no significant differences, with foliar Cl^- concentrations in CO/CO of 19.2 ± 1.7 and 18.2 ± 1.4 µMol Cl^- • gDW^{-1} *versus* 39.7 ± 3.5 and 29.9 ± 4.1 µMol Cl^- • gDW^{-1}, respectively (Figure 2). Scions on RI also had decreased foliar Cl^- concentrations during 2018 and 2019 for SY/RI *versus* SY/SY, but no significant decreases for any years for CO/RI *versus* CO/CO (Figure 2).

4. Genotypes varied in photosynthetic response to mild salinity

This experiment was conducted to test the hypothesis: mild (20 mM) salinity decreases photosynthesis, stomatal conductance and lateral shoot growth in salt-sensitive *Vitis* genotypes (CS and RI), but not in salt-tolerant (RA and SC). To test this hypothesis, all four genotypes (CS, RI, SC and RA) had their photosynthesis and lateral shoot growth measured over 16 days of daily irrigation treatment with either mild salinity (Cramer's solutions + 20 mM NaCl) or control conditions (Cramer's solution) in five replicated big pots. In Figure 3A, the genotype, salt, time and genotype × time interaction had significant impacts on photosynthesis, while the other interactions did not (Supplementary Table 1). During 16 days of salinity treatment in big pots, CS had the only significant decrease in photosynthesis, starting the day of salinity treatment (Figure 3A). RA and SC had no significant differences in photosynthesis over the 16 days of 20 mM NaCl treatment relative to their controls. At the end of treatment (day 16), photosynthesis was lowered for CS 47 % in the salt treatment relative to the control treatment; RI had a lowered photosynthesis of 40 % that was not statistically significant (Figure 3A). Stomatal conductance was affected by genotype, time and genotype × time interaction, with the salt treatment only having a significant effect in CS with no significant differences between control and salt-treated RA, SC or RI (Supplementary Table 1 and Supplementary Figure 2A). During the experiment, no genotype had a significant difference in lateral shoot length or growth rate over the 15 days of treatment, and only time and the genotype × time interaction had a significant effect on growth rate (Supplementary Table 1, Supplementary Figure 2B, 2C). Foliar Na^+ concentration in Supplementary Figure 2D was increased by the salt treatment with a genotype × salt interaction, but no genotype-specific effect (Supplementary Table 1). Na^+ was increased two-fold in SC salt-treated relative to its control, with no significant increases observed in CS, RI or RA (Supplementary Figure 2D). No significant increase was observed in any genotype

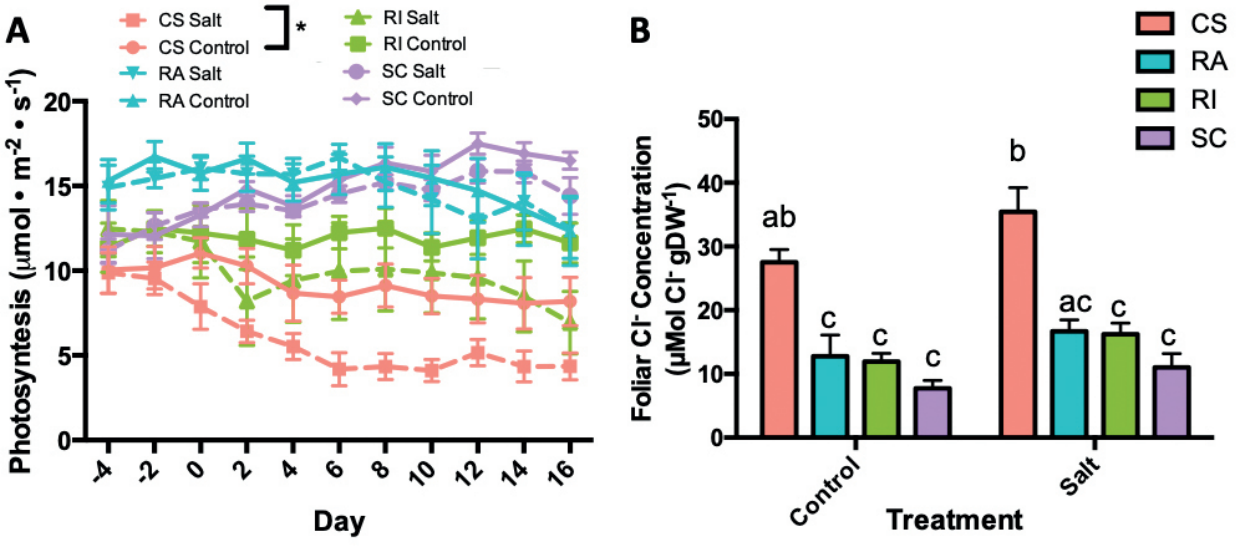

FIGURE 3. Photosynthesis and Cl^- data from big pot photosynthesis experiment.

A) Photosynthesis was measured during every other day of treatment and B) foliar Cl^- was quantified after harvest. A) The solid lines correspond to the control treatment (Cramer's solution) and the dash to the salinity treatment (Cramer's solution + 20 mM NaCl). A) Day 0 is when treatment was started, prior to day 0 all groups were irrigated with Cramer's solution. Significance calculated by A) repeated measures three-way ANOVA multiple comparisons followed by a simple main effect comparison against control treatment per genotype for days 2–16 and displayed as treatment effect between salt and control or B) by two-way ANOVA with multiple comparisons and a Tukey's post hoc test displayed using letters identifying significant differences of $P \leq 0.05$. Data are means ± SE, $n = 5$ individual big potted vines.

for Cl^- in salt-treated groups relative to their control but RI, RA and SC all had significantly lower Cl^- than CS in the control and salt treatment groups (Figure 3B and Supplementary Table 2).

5. Isosmotic salts with and without Cl^- decreased photosynthesis in CS equally

This experiment was conducted to test the hypothesis: Cl^--based salinity is the cause of the photosynthetic decrease in *Vitis*. To test this hypothesis, CS were treated in triplicate with either salt-containing Cl^- (NaCl or KCl), a salt solution without Cl^- ($NaNO_3$) but of equal isosmotic molarity (20 mM salt + Cramer's solution), or Cramer's solution control. In Figure 4A and Supplementary Figure 3A, photosynthesis and stomatal conductance were affected by time and the time x salt interaction, but salt alone did not have a significant effect (Supplementary Table 1). While there was a significant effect for all three salt treatments (NaCl, KCl and $NaNO_3$) relative to the control for photosynthesis on the final day of treatment (day 26), there were no observable differences between the salt treatment groups (Figures 4A and Supplementary Table 2). The only significant difference in stomatal conductance over the treatment period for any salt treatment (NaCl, KCl and $NaNO_3$) relative to the control, existed on day 20 for the $NaNO_3$ treatment (*), with no other significant differences (Supplementary Figure 3A).

The NaCl, KCl and $NaNO_3$ treatment groups led to decreases in photosynthesis of 32 %, 31 % and 35 % (Figure 4A) relative to the control group on day 26, respectively. The growth rate in Supplementary Figure 3B was significantly affected by salt but not the time or the salt × time interaction (Supplementary Table 1). The growth rate was significantly different from the control for the $NaNO_3$ treatment after two weeks of treatment (day 15–27) but not for the NaCl or KCl treatments (Supplementary Figure 3B). On day 27, for the $NaNO_3$ treatment, the growth rate decreased 73 %, relative to the control (Supplementary Figure 3B). Lateral shoot length was significantly different from the control for NaCl and $NaNO_3$ but not for the KCl treatment (Figure 4B). The decrease in lateral shoot length at day 27 was 18 % and 49 % of the control for NaCl and $NaNO_3$ treatments, respectively (Figure 4B). The KCl treatment was the same as the control for lateral length over the treatment period, with significant differences from both the NaCl and $NaNO_3$ treatments (Figure 4B and Supplementary Table 2). The multiple salt treatments had significant effects on Na^+, K^+ and Cl^- but not NO_3^- (Supplementary Table 1). Na^+ concentrations increased two-fold in the $NaNO_3$ treatment relative to the control, while NaCl and KCl were not significantly different in Na^+ concentration from the control (Supplementary Figure 4A). K^+ concentrations increased in the KCl and $NaNO_3$ treatments but not in the NaCl

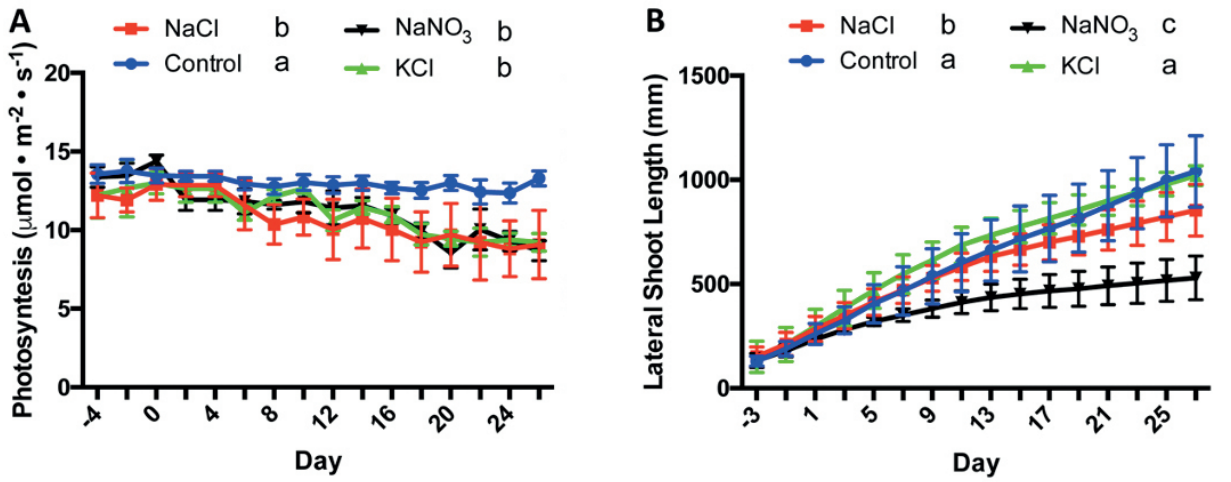

FIGURE 4. Photosynthesis and lateral shoot length during multiple salt (NaCl, KCl and $NaNO_3$) photosynthesis experiment.

A) Photosynthesis and B) lateral shoot length were measured alternatively every other day of treatment. Day 0 is when treatment was started, prior to day 0 all groups were irrigated with Cramer's solution. Significance calculated by A) two-way ANOVA using repeated measures multiple comparisons with a Tukey's post hoc test (day 2–26) and displayed using letters as final day (26) significance B) or linear regression (day -3–27) displayed as slope's significance using different letters relating to a $P \leq 0.05$ within respective figures. Data are means ± SE, $n = 3$ individual small potted vines.

relative to the control (Supplementary Figure 4B). Cl^- concentrations increased three-fold over the control in the KCl treatment, with no significant increases in the other treatments (NaCl or $NaNO_3$) (Supplementary Figure 4C). NO_3^- concentrations were not significantly changed in any of the treatments (Supplementary Figure 4D).

DISCUSSION

In this study, we found genotypic differences in foliar Cl^- concentrations as well as photosynthetic, transcriptomic and growth responses to salinity; however, not all of the responses were as expected. The relationship between Cl^- and photosynthetic decline in *Vitis* genotypes under mild salinity treatment was not present as in previous studies of *V. vinifera* (Downton, 1977a; Downton *et al.,* 1990; Prior *et al.,* 1992; Walker *et al.,* 1981, 1997). It was expected that increased foliar Cl^- concentrations would co-occur with decreased photosynthesis during mild salinity and only Cl^- containing salts would negatively impact the shoot. Osmotic effects of the different salts (NaCl, $NaNO_3$ and KCl) were not as previously shown under water deficit (Cochetel *et al.,* 2020), and this was validated by the 20 mM KCl treatment having no effect on lateral shoot length or growth rate. SC and RA were found to be good Cl^- excluders, which was as expected due to previous reporting (Heinitz *et al.,* 2020; Southey & Jooste, 1991; Stevens *et al.,* 1996; Walker *et al.,* 1997).

1. Salt sensitive genotypes had higher shoot Cl^- and differential gene expression during mild salinity

The 20 mM salinity treatment significantly decreased root length, as well as the mean relative growth rate in the salt-sensitive CS while stimulating differential gene expression relative to other grapevine species (Table 1 and Supplementary Figures 1C, 1D, 1G, 1H). Of particular intrigue, was the enrichment of chloroplast-related transcripts shared by CS and RI in the week one leaf harvest. This finding first indicated an effect on photosynthesis in the salt-sensitive genotypes during mild salinity. In the salt-tolerant RA, this level of salinity significantly stimulated growth, the net assimilation rate and mean relative growth rate in the salt relative to the control treatment, with minimal differential gene expression (Table 1 and Supplementary Figures 1C, 1D, 1G, 1H). This clear separation between the known salt-sensitive genotype's (CS) growth inhibition and high transcriptomic response *versus* the salt-tolerant genotype's (RA) growth stimulation and low transcriptomic response, as well as its agronomic relevance highlighted by the comparability to the field site (Supplementary Table 3), indicated that the mild salinity (20 mM) level was sufficient to identify genotypic differences for these experiments herein. These salinity concentrations represent realistic salt levels found in Nevada and California vineyards.

Dozens of papers have been published about salinity in grapevines (Cramer *et al.,* 2007; Downton, 1977b; Henderson *et al.,* 2014; Walker *et al.,* 2018; to name just a few). To date, only one other publication has examined SC under saline conditions (Heinitz *et al.,* 2020). Our findings are consistent with this study and demonstrate that SC consistently showed patterns of Cl^- exclusion throughout all experiments (Figures 1 and 3B); SC appeared to be a good Cl^- excluder. The association of B transporters with the Cl^- exclusion phenotype was of particular interest, especially the high constitutive expression of *VIT_11s0016g04740* and *VIT_04s0008g04980* in SC and RA. Overall, the differential gene expression was lower than what was observed in Cochetel *et al.* (2020), with only 23 % of the differential gene expression in the mild salinity dataset *versus* the water deficit dataset presented in that work. Multiple studies have examined the Cl^- exclusion capabilities of CS, RI and RA (Downton, 1977b; Ismail *et al.,* 2014; Southey and Jooste, 1991; Stevens *et al.,* 1999; Suarez *et al.,* 2019; Sykes, 1985; Tregeagle *et al.,* 2006; Vincent *et al.,* 2007; Walker *et al.,* 1997; Walker *et al.,* 2010). While CS, like many other *V. vinifera* cultivars, are shown to have a higher accumulation of aerial tissue Cl^-, there are varying results for RI and RA that have been reported.

In this study, RA and SC (the salt-tolerant genotypes) had a shoot Cl^- concentration range lower than CS and RI (the salt-sensitive genotypes) during control conditions in week 1 (Figure 1) and this was consistent in week 2. Shoot Cl^- was used in the small pot experiments due to minimum dry tissue requirements for analysis, foliar Cl^- was not possible for all genotypes. During one week of saline conditions, RA and SC had a Cl^- concentration range below that of CS and RI (Figure 1). Following two weeks of salinity, CS, RA and SC's Cl^- concentration increased while RI's decreased (Figure 1B and Supplementary Table 2). In RA, the increased Cl^- concentration was likely used as an osmolyte driving water flow into the plant, which would be consistent with the increased net assimilation rate and mean relative growth

rate seen in salt *versus* control for this genotype (Table 1). It has been shown previously that RI can, during certain conditions, have lower foliar Cl^- concentrations than RA (Downton, 1977b), while RA is consistently described as salt-tolerant (Southey and Jooste, 1991; Stevens *et al.,* 1999; Suarez *et al.,* 2019; Sykes, 1985; Walker *et al.,* 1997; Walker *et al.,* 2010; Zhou-Tsang *et al.,* 2021) and RI is described as salt-sensitive (Ismail *et al.,* 2014; Zhou-Tsang *et al.,* 2021). Also of note is that it has been shown that Cl^- exclusion capabilities of RA can break down over prolonged exposure to salinity (Tregeagle *et al.,* 2006), though this is likely not the case in our experiments due to the relatively short-term exposure to salinity. It is important to mention that experimental design, irrigation solution concentration, time of salinization and age of plants can all affect Cl^- accumulation. In the two-week experiment, the increased accumulation of Cl^- in RA, relative to RI, during the second-week harvest of the salt group could have resulted in the increased plant growth in RA relative to RI. While RI had no significant changes in any growth measurements (root length, leaf area, leaf area ratio, mean relative growth rate and net assimilation rate) in salt-treated groups relative to the controls, RA had statistically significant increases (Table 1). As carbon assimilation, and ultimately growth, depend on water uptake and transpiration, which can result in increased uptake of ions, the higher Cl^- concentration in RA over RI may be a trade-off of increased growth in RA during these short-term exposures to mild salinity.

2. RA was a good Cl^- excluder in field-grafted conditions

RA was observed to be the best Cl^- excluding rootstock in the field experiments *versus* RI, SY and CO in the mildly saline soil. The soil Cl^- and Na^+ concentrations were similar to the levels used in the greenhouse experiments, with average Cl^- and Na^+ concentrations of 14.1 ± 5.3 and 21.1 ± 10.2 meq • L^{-1} at the Las Vegas Field Site, respectively (Supplementary Table 3). During each year of sampling, RA grafted-types, both SY/RA and CO/RA, had two of the lowest Cl^- levels for that year compared to all other graft types (Figure 2). Even though CO/RA foliar Cl^- concentration was at least 39 % lower than CO/CO in 2018 and 2019, due to biological variation and the low levels of Cl^- in the CO/CO as well as the CO/RA, significant differences were not observed in these years. This salt tolerance of CO grafted to RA presented here in regards to foliar Cl^- exclusion corroborated the findings of Southey and Jooste (1991), showing the salt tolerance of this graft type in regards to vine performance in mildly saline field conditions in South Africa (Southey and Jooste, 1991). Similarly, when Sultana (*V. vinifera*) scions are grafted to RA rootstocks under saline conditions in Australia (Walker *et al.,* 1997), Cl^- exclusion, photosynthesis and fruit yield in the grafted plants are improved compared to the rooted cutting of Sultana (Walker *et al.,* 1997). In field-grown grafted plants in Australia, Stevens *et al.* (1996) also found that RA was able to decrease foliar Cl^- in grafted SY scions *versus* rooted cuttings of SY during both saline and non-saline conditions (Stevens *et al.,* 1996).

The field experiments were consistent across scions and years, RA decreased the Cl^- transported to the leaf tissue when compared to *V. vinifera* rooted plants (Figure 2). While RI also reduced the foliar Cl^- concentrations of both scions relative to *V. vinifera* rooted plants, RI never reduced concentrations lower than RA for a given scion during the same year (Figure 2). Based on the results of the greenhouse experiments and the findings of the field site, our results confirm that RA is a good rootstock for reducing foliar Cl^- concentrations in mildly saline soils.

3. Photosynthetic decline was not associated with foliar Cl^- concentration during mild salinity

It has been noted in multiple publications in the grapevine that there is a correlation between increasing foliar Cl^- concentrations and decreasing photosynthesis (Downton, 1977a; Downton *et al.,* 1990; Prior *et al.,* 1992; Walker *et al.,* 1981; Walker *et al.,* 1997). This observed correlation has led to the predominant hypothesis in the field of grapevine salinity that specific ion toxicity, through increased foliar Cl^- concentration, is the cause of photosynthetic decline during salinity in grapevines (Zhou-Tsang *et al.,* 2021). Our results did not indicate that this correlation was untrue, but did indicate that the foliar Cl^- concentration was not the cause of the observed photosynthetic decline. The lowered photosynthesis in CS on day 0 (the initial day of salinity treatment) (Figure 3A), first indicated that foliar Cl^- concentration may not have caused the photosynthetic decrease in this genotype. Ion toxicity was unlikely to occur in such a short period. Munns (2002) notes that ionic toxicity typically occurs in days to weeks depending on the salinity level and other environmental factors that affect transpiration (Munns, 2002).

The foliar ion analysis of Cl^- and Na^+ at the end of this experiment, supported this hypothesis (Figures 3B and Supplementary Figure 2D). CS did not have a significantly increased Cl^- concentration during the treatment period in the salinity group relative to the control (Figure 3B and Supplementary Table 2). RI and RA Cl^- concentrations in both control groups and salt groups were equivalent, with RA having no decrease in photosynthesis and RI having an observable, although non-significant lowered photosynthesis after 16 days (Figure 3 and Supplementary Table 2). These findings again indicated that the foliar Cl^- concentration was not the cause of the photosynthetic decrease during mild (20 mM) salinity in these experiments. The Na^+ concentrations also could not be the cause of the photosynthetic decreases, as the ion only increased in the SC salt-treated group (a 2-fold increase), but not in CS, RI or RA. Thus, it is clear that the decline in photosynthesis is more complicated than originally hypothesised. Instead, we postulate that the ionic strength of the irrigation solution or in the plant tissue (including cell or organelle compartmentation), rather than a specific ion toxicity, along with an osmotic adjustment in response to the salinity, may be responsible for the photosynthetic decrease occurring or not in a given genotype. While mild osmotic potential decreases in the soil solution could have caused declines in photosynthesis (*e.g.*, decrease in stomatal conductance), this would most likely have been a temporary effect on the order of hours and not days or weeks. Supporting this argument, maize leaf elongation declines rapidly but recovers within hours due to osmotic adjustment with mild to moderate salinity (Cramer & Bowman, 1991), but photosynthesis is unaffected by salinities at this level (Cramer *et al.,* 1994).

4. Na^+ containing salts reduced growth rate of CS

The largest and most rapid decreases in lateral shoot length and growth rate in CS occurred with Na^+ containing salts, with the biggest decreases seen with $NaNO_3$. NaCl salinity reduced shoot growth in another *V. vinifera* cultivar (Sultana) (Prior *et al.,* 1992). The K^+ and Cl^- ions each may have a stimulatory effect on growth, particularly in the short term, due to their use in osmoregulation by the plant (Munns and Gilliham, 2015). However, it appears that Na^+ had an inhibitory effect on growth in CS during mild salinity conditions. This could be due to the compartmentalization of Na^+, an ionic strength effect, the energetic cost of Na^+ exclusion, or the production of compatible counter anions during Na^+ uptake down its electrochemical gradient (Munns & Gilliham, 2015). In the case of the $NaNO_3$ treatment, the NO_3^- taken up would likely be assimilated into NH_4^+ and amino acids and thus osmoregulation may be limiting (Supplementary Figure 4D). Further investigation into the cause of Na^+ induced shoot growth decrease is needed to support or reject these hypotheses.

5. Photosynthetic decline occurred in CS during isosmotic salinity regardless of Cl^-

The largest decline in photosynthesis for all three salt-treated groups (NaCl, KCl and $NaNO_3$) began on day 14 of treatment and reached a new steady state of decreased photosynthesis, relative to the control group, on day 20 (Figure 4A). This new steady-state photosynthesis for the three salt-treated groups remained steady for the rest of the experiment. While the decrease that occurred after two weeks of treatment could be an indication of ion toxicity, the occurrence in all three salt treatment groups diminishes the possibility that this decrease was Cl^--specific. If the decreases in photosynthesis were Cl^--specific such as has been previously suggested (Downton, 1977a; Downton *et al.,* 1990; Prior *et al.,* 1992; Walker *et al.,* 1981; Walker *et al.,* 1997), the photosynthetic decrease should have been observed in the NaCl and KCl treatments, but not the $NaNO_3$ treatment. This was not what occurred, instead, all treatment groups declined in photosynthesis together and had significant differences from the control at the final day of treatment (day 26) with no significant differences between the salt treatments. This finding that foliar Cl^- concentration was not the likely cause of the photosynthetic decrease in this experiment was again corroborated by the fact that the foliar Cl^- concentration only significantly increased in the KCl treatment. These findings in conjunction with the findings of the big-pot photosynthesis experiment clearly indicated that foliar Cl^- concentration was not the cause of the photosynthetic decrease in *Vitis* species during mild salinity and takes it further in showing that 20 mM salinity, regardless of Cl^-, resulted in a photosynthetic decrease in CS.

CONCLUSION

Genotypic differences in responses to mild salinity (20 mM) were observed in growth rate, ion accumulation, gene expression, stomatal conductance and photosynthesis. Supporting previous studies, our findings herein confirmed that both SC and RA had inherent Cl^- exclusion

capabilities and RA may be considered an effective Cl^- excluding rootstock for the scion; RA was more salt-tolerant than the other genotypes tested. The Cl^- exclusion capabilities of RA rootstock observed in the greenhouse over multiple experiments, relative to *V. vinifera* (cv. CS) were reflected in the Las Vegas field site over all three years and with both grafted scions, *V. vinifera* (cvs. CO and SY). While Cl^- accumulation, may be a useful proxy for salt tolerance in *Vitis* species, one must be careful to not overinterpret Cl^-'s role in the decline of salt sensitivity-related photosynthesis. Cl^- was not attributed as the cause of photosynthetic decrease during mild salinity (20 mM) in the *Vitis* genotypes studied in this project. Instead, the decreased photosynthesis during salinity appeared to be more complicated and may be the result of the effects of ionic strength or osmotic adjustment of the tissue, cell type or organelle, regardless of the particular salt involved (NaCl, KCl or $NaNO_3$).

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