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Research Article

A Whole Plant Analysis of Chloride and Sodium Exclusion Using a Range of Grapevine Rootstock Genotypes

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Background and Aims. Salt exclusion is an important attribute for wine grapes since many countries have limits to the concentration of sodium (Na^+) and/or chloride (Cl^-) tolerated in wine. The aim was to investigate whole plant capacity for Na^+ and Cl^- exclusion and the within-plant partitioning of accumulated ions to better understand these important salt tolerance traits. *Methods and Results*. Rooted cuttings of 140 Ruggeri and K51-40 (good and poor shoot Cl^- excluders, respectively) and five hybrids from a cross between the two genotypes were used. When challenged with salinity, 140 Ruggeri limited the accumulation of Cl^- and Na^+ in the stem, petioles, and laminae and had a significantly lower whole plant concentration of Cl^- and Na^+ when compared to K51-40. The latter indicates that 140 Ruggeri accumulates less Cl^- and Na^+ than K51-40 by a lower uptake or a potentially greater efflux by roots, or both. While K51-40 accumulated significantly more Na^+, it was able to retrieve it from the xylem; store it in the roots, stem, and petiole; and keep the lamina concentration comparable to that of 140 Ruggeri. Petioles of all genotypes appeared to play a role in limiting Cl^- accumulation in laminae and particularly for K51-40, to limit Na^+ accumulation in laminae. *Conclusions*. The grapevine capacity for Cl^- and Na^+ exclusion can be defined primarily as the lower net accumulation on a whole plant basis, reflecting the difference between the uptake and any efflux that may occur. Lower root to shoot transport is a key factor in shoot Cl^- and Na^+ exclusion. Petiole accumulation assists in limiting the Cl^- and Na^+ accumulation in the laminae. *Significance of the Study*. The study addressed the knowledge gap by examining Cl^- and Na^+ exclusion on a whole plant basis, highlighting a range of within-plant mechanisms that act in limiting the accumulation of both ions in the laminae.

1. Introduction

Factors that contribute to salt tolerance in grapevine include Na^+ and Cl^- exclusion, vacuolar sequestration of Na^+ and Cl^- ions that have entered cells, osmotic adjustment involving accumulated Na^+ and Cl^- ions and/or organic osmolytes, and reactive oxygen species (ROS) signaling and detoxification [1]. Na^+ and Cl^- exclusion is particularly important for wine grapes because in many countries there are limits to the concentration of either or both ions that are tolerated in wine [2]. In Australia, the limit is 1 g/L of soluble chlorides expressed as NaCl or 607 mg/L Cl^- [3].

Cl^- exclusion has been defined by Teakle and Tyerman [4] as "the ability of plants to prevent root uptake of Cl^- from the soil and subsequent transport in the xylem to the shoot." The same would apply for Na^+. Different mechanisms are known to regulate the processes of Na^+ and Cl^- exclusion in grapevines [1]. The primary capability for Na^+ and Cl^- exclusion resides in the root [5]. There are, however, examples of a scion influence on the total ion accumulated, particularly for Cl^- [6].

Studies on the shoot Cl^- exclusion process in grapevine have been facilitated by two contrasting rootstocks. Rootstock 140 Ruggeri, a good excluder, is derived from a cross between *Vitis berlandieri* Boutin B and *V. rupestris* du Lot [7]. Rootstock DAVIS K 51_40 (hereafter referred to as K51-40), on the other hand, a poor excluder, is derived from a cross between *V. champinii* Planchon and *V. riparia* Michaux [7]. The difference in the capacity for shoot Cl^- exclusion between 140 Ruggeri and K51-40 is consistent across field trials, short term [6] and long term [8], glasshouse trials involving plants grown in potting mix or hydroponics [9, 10], and

glasshouse trials involving rooted leaves [9–11]. A common observation among the experimental systems has been a lower concentration of Cl^- in petioles and/or laminae of salt-treated 140 Ruggeri relative to that of K51-40. Salt-treated rooted leaves, however, appear to respond differently to plants propagated from cuttings by having a significantly higher concentration of Cl^- in roots of 140 Ruggeri than in K51-40 but no difference between the two genotypes in the whole plant (rooted leaf) concentration of Cl^- [11].

Crosses between the two genotypes led to F1 progeny showing continuous distribution in the concentration of Cl^- in laminae and petioles of hybrids grown in potting mix and solution culture [10], indicating the control of Cl^- accumulation by multiple genes. In contrast, there was a skewed distribution in the concentration of Na^+ in laminae of hybrids grown in potting mix and solution culture [12, 13]. The parents, K51-40 and 140 Ruggeri, accumulated similar concentrations of Na^+ in the laminae [12]. The skewed transgressive pattern of Na^+ exclusion in the F1 progeny is largely associated with the inheritance of a single major locus involving HKT Group 1 transporters [14].

The major mechanism for shoot Na^+ exclusion in *Vitis* is now known to be linked to the *VisHKT1;1* gene, shown to be transcribed in cells associated with the root vasculature and to encode a Na^+ selective plasma membrane transporter [14]. There, in the root vasculature, it is proposed to be involved in the retrieval of Na^+ from root xylem vessels into surrounding cells [14, 15], such as xylem parenchyma and pericycle cells.

The best excluders of Cl^- from shoots are known to limit the concentration of Cl^- in the xylem [9, 10]. Shoot Cl^- exclusion has been hypothesized to be controlled by an unidentified gene that encodes a transport protein on the plasma membrane of cells surrounding xylem vessels in the roots, e.g., xylem parenchyma and/or pericycle cells. Various candidate genes have been proposed [1, 4]. For example, Cubero-Font et al. [16] proposed the involvement of SLAH1 in conjunction with SLAH3 in mediating Cl^- efflux from pericycle cells into the root xylem vessels. There is also some evidence for extrusion of Cl^- from the root [17, 18], for the recirculation of accumulated Cl^- from the shoot to root [19–21], and for the involvement of environmental factors [10]. These multiple processes may explain why there is evidence for both single gene [22] and multiple gene [10, 23, 24] control of shoot Cl^- exclusion in grapevine.

The root stele is the primary location of the mechanism for shoot Na^+ exclusion [15] and also likely for shoot Cl^- exclusion; however, other factors appear to be involved but are yet to be identified. For example, the *VisHKT1;1* gene expressed in roots accounts for approximately 70% of the variation in the lamina Na^+ concentration in the K51-40 × 140 Ruggeri hybrid population [14]. Mechanisms operating within the shoot, e.g., retrieval from the xylem within the petiole and within-petiole storage, together with environmental influences [10] may account for some, or all, of the remaining variation.

While there have been previous studies that have measured concentrations of Cl^- and Na^+ in petioles and laminae of plants propagated from cuttings, including those involving 140 Ruggeri and K51-40 [10], and others involving own-rooted and grafted grapevine cultivars [25], those studies have not reported on the comparison between genotypes of whole plant Cl^- and Na^+ concentrations. This study was designed to address that gap.

The objective was to undertake a complete analysis of the concentrations of Na^+ and Cl^- in the whole plant and its component parts (the organs root and stem and the leaf tissues, lamina, and petiole), to better understand the Na^+ and Cl^- exclusion processes from a whole plant perspective. We used K51-40 and 140 Ruggeri and five hybrids from the family of a cross between them. The study involved potted plants in a glasshouse environment treated with control (no applied salt) and salt (50 mmol/L Cl^- with mixed cations) for a 3-week period followed by destructive harvest and analysis. The dry mass (dm) and concentration of Cl^-, Na^+, K^+, Ca^{2+}, and Mg^{2+} of the whole plant and its component parts were measured and compared between the genotypes.

2. Materials and Methods

Two parent rootstocks, known to differ significantly in capacity for Cl^- exclusion, K51-40 (poor excluder) and 140 Ruggeri (good excluder), and five F1 hybrids (HB 13, HB 28, HB 30, HB 76, and HB 78) were chosen for the experiment. The five F1 hybrids were chosen for their range in the capacity to exclude Cl^- and Na^+ based on the observations of Dunlevy et al. [13] and Gong et al. [10, 12].

2.1. Plant Establishment. Sixteen cuttings of K51-40 and 140 Ruggeri and the five F1 hybrids were collected from vines maintained at CSIRO, Irymple, Victoria. They were trimmed at the basal end, dipped in Clonex Hardwood Rooting Hormone Gel (Yates, Padstow, NSW, Australia), and inserted into a 50 : 50 sand:perlite mix in Heat and Grow Propagation Trays (Sage Horticultural, Hallam, Vic., Australia) within a mist house and irrigated with rainwater. Once roots had established, the rooted cuttings were transferred to 2.8 L pots filled with coarse sand topped with perlite. The pots were lined at the base with frost cloth to prevent the sand falling through the drainage holes. The plants were established in a glasshouse maintained at 22–28°C, including daily watering with a quarter strength special Hoagland's solution [26] containing the following nutrients with the concentration (mmol/L) in parentheses: KNO_3 (1.5), $Ca(NO_3)_2.4H_2O$ (1.0), $MgSO_4.7H_2O$ (0.5), KH_2PO_4 (0.25), H_3BO_3 (4.6×10^{-2}), $MnCl_2.4H_2O$ (9.1×10^{-3}), $ZnSO_4.7H_2O$ (7.6×10^{-4}), $CuSO_4.5H_2O$ (3.2×10^{-4}), $Na_2MoO_4.2H_2O$ (2.4×10^{-4}), and EDTA-Fe-Na (7.1×10^{-2}). In the following spring, all plants were pruned above the first node and allowed to reshoot.

2.2. Experimental Layout. Once plants had established new shoots to around 4–5 nodes, they were selected for uniformity and four of each genotype were allocated to control and four to the salt treatment. The seven genotypes were placed in four statistical blocks in a randomized complete block design to account for potential environmental

variation within the glasshouse. There were four replicates per genotype within each treatment (one per block). The plants were maintained as single leader shoots for the duration of the experiment.

2.3. Salt Treatment Applications. A 25 mmol/L Cl^- treatment with mixed cations was prepared in the one-quarter strength special Hoagland's solution as described above and applied by hand watering on day one of the experiment. The salt solution was composed of NaCl (15 mmol/L), $CaCl_2.2H_2O$ (2.5 mmol/L), and $MgCl_2.2H_2O$ (2.5 mmol/L) to achieve a concentration of 25 mmol/L mixed Cl^- (Na^+:Ca^{2+}:Mg^{2+} = 6:1:1). The control was one-quarter strength special Hoagland's solution without added salt, also applied daily by hand watering. The salt concentration was stepped-up to 50 mmol/L mixed Cl^- on day two of the experiment. The 50 mmol/L mixed Cl^- treatment and no-salt control treatment were then applied daily by hand watering for a further three weeks. Volumes of the salt and control treatment applied to pots were sufficient to permit a substantial flow of watering solution through the pot to prevent the build-up of salts.

2.4. Plant Harvest and Sampling. All plants were destructively harvested after 3 weeks of salt treatment. Prior to separating each plant into roots, stem, petioles, and laminae, the roots were dipped in the treatment solution (control or salt) and any adhering sand particles were removed. The stem comprised the original cutting used to propagate the rooted cutting and the new stem structure developed from the cutting and carrying the leaves. Each harvested plant component was weighed, briefly dipped in deionized water, and then blotted dry with paper towel, before being placed in labelled paper bags. The bags were placed in an oven and dried at 60°C for at least 72 h before dm was obtained. Total plant dm was the sum of the dm for each of the harvested plant components.

The dried mass of each plant component was ground to a fine powder using a BenchTop Ring Mill, head type Chrome 40 steel (Rocklabs, Auckland, New Zealand). The dried powdered material was then stored for ion analysis.

2.5. Chloride Analyses. The dried powdered samples of roots, stem, petioles, and laminae were analysed in duplicate by silver ion titration [27] with a digital chloridometer model 4-2502 (Labconco-Buchler Instruments, Kansas City, MO, USA). Briefly, the dried samples (50 mg for laminae and 25 mg for each of petioles, stem, and roots) were weighed into 5 mL glass vials, followed by addition of 4 mL of acid reagent (100 mL of glacial acetic acid and 6.4 mL of 70% nitric acid made up to 1 L with MilliQ water), and then allowed to extract for 30 min. Four drops of gelatin reagent (Labconco Corporation, Kansas City, MO, USA) were added just prior to the analysis.

The Cl^- concentration (% dm) in each plant component was determined for each replicate plant. To convert to a Cl^- concentration in the whole plant, the content (μmoles) of Cl^- in the total dm for each plant component was calculated and then summed for all plant components to obtain a Cl^- content in the whole plant dm. The summed value of the Cl^- content in all plant components, i.e., in the whole plant, was then used with the summed value of dm for the plant components (whole plant) to obtain Cl^- concentration (g Cl^-/100 g dm) for the whole plant expressed as % dm.

2.6. Cation Analyses. The cations (Na^+, K^+, Ca^{2+}, and Mg^{2+}) were determined as total concentration (% dm) of each mineral element in each plant component for each replicate plant by inductively coupled plasma-optical emission spectrometry (ICP-OES) following the procedure and calibration process described by Walker et al. [27]. The concentration of each cation (% dm) in the whole plant was determined as described above for Cl^-.

2.7. Statistical Methods. General Linear Model (Systat Software, San Jose, CA, USA) was used to determine if there was a significant block effect within the randomized complete block design. Analysis of variance (ANOVA) was also performed with Systat. Since no significant block effect was found, a block factor was not included in the ANOVA. Where there was a bimodal data distribution for ion concentration between treatments, data for control and salt-treated plants were analysed separately by one-way ANOVA. Data for Cl^- and Na^+ concentration were in this category. For consistency with data analysis for the Cl^- and Na^+ concentration, the data for dm and the K^+, Ca^{2+}, and Mg^{2+} concentration were also treated separately for control and salt treatment; however, two-way ANOVA was used to compare treatment means for dm, K^+, Ca^{2+}, and Mg^{2+} concentration. Pairwise comparisons between predicted mean values for genotype were made using Fisher's protected 5% LSD.

3. Results

3.1. Dry Mass of the Whole Plant and Its Component Parts. Data were distributed normally with no interaction between salt treatment and genotype for the dm of any plant component (P = 0.08, 0.32, 0.77, and 0.24 for roots, stem, petiole, and lamina, respectively) or the whole plant (P = 0.22). Based on means across all rootstocks for control and salt-treated plants, salt treatment resulted in a lower dm of petiole, lamina, and whole plant; however, there was no effect on that of roots and stem (Table 1). Genotype had no effect on the dm of all plant components and whole plant of the control treatment but significantly affected the dm of all plant components and whole plant of the salt treatment (Table 1).

The results demonstrate that the parental lines, K51-40 and 140 Ruggeri, had a similar dm for the root, stem, and whole plant of the salt treatment. The exceptions were petioles and laminae of salt-treated plants, where the dm of K51-40 petioles and laminae was, respectively, 47% and 15% higher than that for 140 Ruggeri (Table 1). The whole plant dm of salt-treated 140 Ruggeri and K51-40 was significantly higher than that of all hybrids except HB 78 (Table 1).

Table 1: Plant component and whole plant dm of 140 Ruggeri, K51-40, and 5 hybrids from the control (C) and salt (S) treatment.

	Dry mass (g)				
	Root	Stem	Petiole	Lamina	Whole plant
Control					
140 Ruggeri	12.06	14.38	0.94	8.53	35.91
K51-40	14.45	13.33	0.97	6.81	35.56
HB 13	6.47	7.59	0.76	5.77	20.59
HB 28	10.79	10.40	1.01	6.98	29.18
HB 30	10.68	11.28	0.74	5.32	28.02
HB 76	9.92	11.10	1.00	8.00	30.02
HB 78	12.01	15.13	0.91	8.23	36.28
Mean ± se	10.40 ± 0.92	11.65 ± 0.83	0.88 ± 0.04	7.00 ± 0.36	29.93 ± 1.84
Significance (GC)	$P = 0.080$	$P = 0.103$	$P = 0.630$	$P = 0.393$	$P = 0.119$
Salt					
140 Ruggeri	16.10c	11.80bc	0.55a	6.27b	34.72c
K51-40	13.61bc	13.56c	0.81c	7.21c	35.19c
HB 13	11.49b	7.66ab	0.50a	4.91ab	24.56ab
HB 28	8.19ab	10.29b	0.69b	6.48b	25.65b
HB 30	7.11a	7.39a	0.40a	3.60a	18.50a
HB 76	6.44a	6.16a	0.42a	4.14a	17.16a
HB 78	11.29b	11.57bc	0.59ab	6.42b	29.87bc
Mean ± se	9.77 ± 1.16	9.14 ± 0.90	0.53 ± 0.05	5.21 ± 0.45	24.65 ± 2.35
Significance (GS)	$P < 0.001$	$P = 0.001$	$P < 0.001$	$P = 0.001$	$P < 0.001$
Significance (T)	$P = 0.331$	$P = 0.309$	$P < 0.001$	$P < 0.001$	$P = 0.005$

Different letters indicate significant differences between genotype means with P values obtained from one-way ANOVA; P values for comparison of treatment means were obtained from a two-way ANOVA involving salt treatment and genotype; g: gram; GC: genotype control; GS: genotype salt; se: standard error. P values describing significant effects of genotype (G) for each of C and S and significant effects of treatment (T) are included.

3.1.1. Chloride Concentration. There was a bimodal distribution for data on the Cl^- concentration for whole plant and all plant components except roots, for which data were distributed normally and where the interaction between treatment and genotype was not significant ($P = 0.35$). Data for control and salt treatment for the whole plant and all plant components including roots were treated separately.

For plants receiving the control treatment, there were no differences among any of the genotypes in the concentration of Cl^- accumulated in roots or in the whole plant. There were, however, genotype effects on the concentration of Cl^- accumulated in the stem, petiole, and lamina (Table 2). For example, the concentration of Cl^- accumulated in the stem, petiole, and lamina of the K51-40 control was significantly higher than that of 140 Ruggeri (Table 2).

Salt treatment increased the concentration of Cl^- in all plant components and in the whole plant, based on means ± standard error across all genotypes for each of the control and salt-treated plants. For each plant component and for the whole plant, there were significant differences between genotypes (Table 2). The Cl^- concentration in the petiole and lamina of salt-treated K51-40 was, respectively, 5.89- and 6.49-fold higher than that for 140 Ruggeri, while in the stem and whole plant, it was, respectively, 2.22- and 2.28-fold higher. The Cl^- concentration in the roots was not significantly different between the two genotypes. The stem and petiole of all hybrids had concentrations of Cl^- higher than that for 140 Ruggeri. For the salt-treated whole plant, the Cl^- concentration of K51-40 was higher than that for all other genotypes, while for hybrid HB 13, it was not significantly different from that of 140 Ruggeri (Table 2).

When the concentration of Cl^- in the root, stem, and petiole of salt-treated plants for all genotypes was compared with the concentration of Cl^- in lamina, different relationships were evident. There was no relationship ($R^2 = 0.01$) between the concentration of Cl^- in laminae and the concentration of Cl^- in roots (Figure 1). There were, however, strong positive relationships between the concentration of Cl^- in laminae with that in stem ($R^2 = 0.87$) and with that in petioles ($R^2 = 0.97$), with a much greater slope of the relationship with petioles (1.79) than that with stem (0.19) (Figure 1).

3.1.2. Sodium Concentration. There was a bimodal distribution for data on the Na^+ concentration for the whole plant and all plant components; hence, data for control and salt treatment were treated separately.

For plants receiving the control treatment, the concentration of Na^+ in the petiole and lamina was low (≤0.02% dm) with no significant differences among any of the genotypes (Table 3). There were, however, genotype differences in the Na^+ concentration for root, stem, and whole plant. K51-40 had a higher concentration of Na^+ than that for 140 Ruggeri in the stem and whole plant of controls; however, there was no difference between the two genotypes in the Na^+ concentration of root, petiole, and lamina (Table 3).

Salt treatment increased the concentration of Na^+ in all plant components and whole plant, based on means ± standard error across all genotypes for each of the control and salt-treated plants (Table 3). For each plant component and for the whole plant, there were significant differences between

Table 2: Plant component and whole plant Cl^- concentration of 140 Ruggeri, K51-40, and 5 hybrids from the control (C) and salt (S) treatment.

	Cl^- concentration (% dm)				
	Root	Stem	Petiole	Lamina	Whole plant
Control					
140 Ruggeri	0.45	0.09a	0.14a	0.06a	0.21
K51-40	0.41	0.30c	0.49e	0.20d	0.33
HB 13	0.56	0.16ab	0.19ab	0.06a	0.25
HB 28	0.54	0.09a	0.15a	0.04a	0.25
HB 30	0.37	0.17ab	0.25bc	0.08ab	0.23
HB 76	0.55	0.20b	0.37d	0.14c	0.29
HB 78	0.52	0.18b	0.29d	0.10b	0.27
Mean ± se	0.47 ± 0.03	0.18 ± 0.02	0.28 ± 0.03	0.10 ± 0.02	0.26 ± 0.01
Significance (GC)	$P = 0.740$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.403$
Salt					
140 Ruggeri	0.71a	0.41a	0.87a	0.43a	0.57a
K51-40	0.67a	0.91c	5.12d	2.79d	1.30d
HB 13	0.65a	0.66b	2.16b	1.27b	0.83ab
HB 28	0.90ab	0.73bc	1.80b	1.17ab	0.92b
HB 30	0.61a	0.75bc	2.69bc	1.62b	0.89b
HB 76	0.85ab	0.71bc	2.36b	1.53b	1.02bc
HB 78	1.08b	0.79bc	3.18c	2.01c	1.21c
Mean ± se	0.79 ± 0.06	0.72 ± 0.05	2.58 ± 0.44	1.56 ± 0.24	0.97 ± 0.08
Significance (GS)	$P = 0.015$	$P = 0.001$	$P < 0.001$	$P = 0.001$	$P = 0.005$

Different letters indicate significant differences between genotype means with P values obtained from one-way ANOVA; control and salt treatment means are compared using standard errors; dm: dry mass; GC: genotype control; GS: genotype salt; se: standard error. For each treatment, P values for significant genotype (G) effects are included.

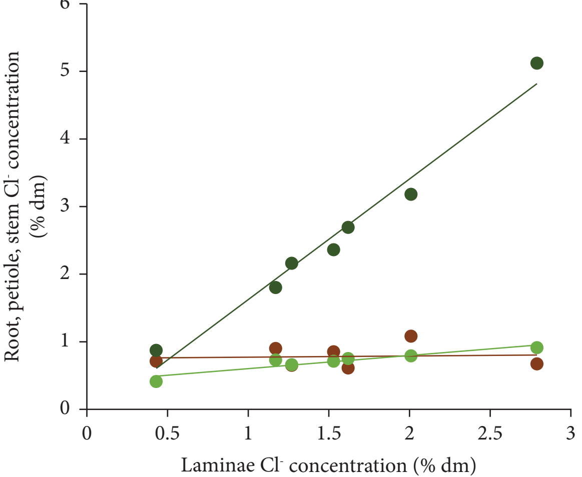

Figure 1: Root (●—●), petiole (●—●), and stem (●—●) Cl^- concentration of all salt-treated genotypes plotted against the lamina Cl^- concentration. Regression analysis of the salt-treated vines shows significant correlations between the Cl^- concentration in the laminae and that in the petioles ($P < 0.0001$, $R^2 = 0.97$, slope = 1.79) and between the Cl^- concentration in the laminae and that in the stem ($P = 0.002$, $R^2 = 0.87$, slope = 0.19) but not between the Cl^- concentration in the laminae and that in the roots ($P = 0.87$).

genotypes of salt-treated plants (Table 3). The Na^+ concentration in the lamina was low (≤0.06% dm) for all genotypes except HB 30, which was marginally but significantly higher (0.13% dm). For the salt-treated whole plant and all plant components except lamina, the concentration of Na^+ was significantly higher in K51-40 than in 140 Ruggeri. Hybrid HB 76 had a concentration of Na^+ that was not significantly different from that of 140 Ruggeri in the whole plant and in all plant components except roots, where it was higher in HB 76 than in 140 Ruggeri by 29%. Hybrid HB 78 had a concentration of Na^+ that was not significantly different from that of K51-40 in the root, stem, and lamina; however, its concentration in the petiole and whole plant was lower by 54% and 12%, respectively, relative to that of K51-40 (Table 3).

3.1.3. Potassium Concentration. Data were distributed normally; however, there was an interaction between the salt treatment and genotype for the K^+ concentration in the lamina ($P = 0.02$) and petiole ($P = 0.04$) but not for the roots ($P = 0.25$), stem ($P = 0.08$), and whole plant ($P = 0.32$). Based on means across all genotypes for each of the control and salt treatments, salt treatment had no effect on the K^+ concentration in the lamina, increased the K^+ concentration in the petiole, and reduced the concentration in the root, stem, and whole plant. The genotype had no effect on the K^+ concentration in the root, stem, lamina, and whole plant of controls nor in the stem and whole plant of the salt-treated plants (Table 4).

For plants receiving the control treatment, the K^+ concentration in the petiole was significantly higher for K51-40 than for 140 Ruggeri with no significant difference between 140 Ruggeri and the other hybrids (Table 4).

For the salt-treated plants, there was no significant difference between 140 Ruggeri and K51-40 in the K^+ concentration of the whole plant and of all plant components except petiole, where K51-40 was 11% higher than that for 140 Ruggeri. The K^+ concentration in the petiole of HB

Table 3: Plant component and whole plant Na^+ concentration of 140 Ruggeri, K51-40, and 5 hybrids from the control (C) and salt (S) treatment.

	Na^+ concentration (% dm)				
	Root	Stem	Petiole	Lamina	Whole plant
Control					
140 Ruggeri	0.15ab	0.03a	0.02	0.02	0.07a
K51-40	0.19b	0.06b	0.01	0.01	0.11b
HB 13	0.14a	0.03a	0.01	0.01	0.06a
HB 28	0.19b	0.03a	0.02	0.01	0.09ab
HB 30	0.12a	0.05b	0.01	0.01	0.07a
HB 76	0.18ab	0.03a	0.01	0.01	0.07a
HB 78	0.20b	0.04ab	0.01	0.01	0.08ab
Mean ± se	0.18 ± 0.01	0.04 ± 0.01	0.02 ± 0.002	0.01 ± 0.002	0.08 ± 0.01
Significance (GC)	$P = 0.010$	$P = 0.001$	$P = 0.225$	$P = 0.769$	$P = 0.036$
Salt					
140 Ruggeri	0.31a	0.09a	0.03a	0.01a	0.18a
K51-40	0.45c	0.33c	0.74b	0.04ab	0.33d
HB 13	0.30a	0.16ab	0.08a	0.06b	0.21a
HB 28	0.37b	0.19b	0.13a	0.05ab	0.22ab
HB 30	0.29a	0.31c	0.33a	0.13c	0.27bc
HB 76	0.40bc	0.09a	0.01a	0.01a	0.17a
HB 78	0.42bc	0.28c	0.34a	0.06ab	0.29c
Mean ± se	0.40 ± 0.02	0.23 ± 0.03	0.26 ± 0.09	0.05 ± 0.01	0.26 ± 0.02
Significance (GS)	$P < 0.001$	$P = 0.001$	$P = 0.012$	$P = 0.004$	$P < 0.001$

Different letters indicate significant differences between genotype means with P values obtained from one-way ANOVA; control and salt treatment means are compared using standard errors; dm: dry mass; GC: genotype control; GS: genotype salt; se: standard error. For each treatment, P values for significant genotype (G) effects are included.

Table 4: Plant component and whole plant K^+ concentration of 140 Ruggeri, K51-40, and 5 hybrids from the control (C) and salt (S) treatment.

	K^+ concentration (% dm)				
	Root	Stem	Petiole	Lamina	Whole plant
Control					
140 Ruggeri	0.75	1.39	4.09a	1.59	1.25
K51-40	0.60	1.63	5.19b	1.46	1.25
HB 13	0.83	1.70	4.24a	1.48	1.44
HB 28	0.80	1.67	3.73a	1.19	1.30
HB 30	0.65	1.51	4.34a	1.60	1.25
HB 76	0.67	1.64	4.31a	1.49	1.38
HB 78	0.61	1.61	4.20a	1.33	1.26
Mean ± se	0.69 ± 0.02	1.60 ± 0.03	4.36 ± 0.17	1.46 ± 0.03	1.32 ± 0.03
Significance (GC)	$P = 0.210$	$P = 0.103$	$P = 0.032$	$P = 0.528$	$P = 0.525$
Salt					
140 Ruggeri	0.51ab	1.27	4.46a	1.13a	0.93
K51-40	0.47ab	1.14	4.94b	1.44ab	1.02
HB 13	0.41a	1.01	4.32a	1.55b	0.90
HB 28	0.58b	1.26	4.42a	1.33ab	1.13
HB 30	0.47ab	1.03	3.89a	1.64b	0.98
HB 76	0.48ab	1.31	5.51c	1.72b	1.20
HB 78	0.55b	1.24	4.27a	1.60b	1.08
Mean ± se	0.48 ± 0.02	1.18 ± 0.04	4.66 ± 0.17	1.58 ± 0.07	1.06 ± 0.04
Significance (GS)	$P = 0.031$	$P = 0.497$	$P = 0.001$	$P < 0.001$	$P = 0.315$
Significance (T)	$P < 0.001$	$P < 0.001$	$P = 0.023$	$P = 0.093$	$P < 0.001$

Different letters indicate significant differences between genotype means with P values obtained from one-way ANOVA; P values for comparison of treatment means were obtained from a two-way ANOVA involving salt treatment and genotype; dm: dry mass; GC: genotype control; GS: genotype salt; se: standard error. P values describing significant effects of genotype (G) for each of C and S and significant effects of treatment (T) are included.

76 was significantly higher than that for K51-40, which in turn was higher than that for 140 Ruggeri and all other hybrids (Table 4).

3.1.4. Calcium and Magnesium Concentration. Data were distributed normally with no interaction between the salt treatment and genotype for the Ca^{2+} and Mg^{2+} concentration in any of the plant components or whole plant, with interaction P values for the roots, stem, petiole, lamina, and whole plant of 0.99, 0.08, 0.98, 0.29, and 0.98, respectively, for Ca^{2+} and 0.86, 0.35, 0.15, 0.51, and 0.64, respectively, for Mg^{2+}.

For the Ca^{2+} concentration, based on means across all genotypes for each of the control and salt treatments, the salt treatment resulted in a marginally but significantly lower concentration in roots; however, there was no effect on that of the stem, petiole, lamina, or whole plant (Table S1). The genotype had no effect on the Ca^{2+} concentration in roots of control and salt-treated plants; however, it significantly affected the concentration in all other plant components and the whole plant of controls but only of the stem of the salt-treated plants (Table S1).

For plants receiving the control treatment, the Ca^{2+} concentration of K51-40 was significantly higher than that of 140 Ruggeri for the lamina and the whole plant only. For the salt-treated plants, however, the only differences between the genotypes were recorded for the Ca^{2+} concentration in the stem, where HB 13 had a higher concentration than that of all other genotypes except HB 30 (Table S1).

For the Mg^{2+} concentration, based on means across genotypes for each of the control and salt treatments, the salt treatment significantly increased the Mg^{2+} concentration of the whole plant and of all plant components except roots (Table S2). The genotype had no effect on the Mg^{2+} concentration in roots of control plants nor in the stem, lamina, and whole plant of salt-treated plants but significantly affected the concentration in all other plant components and the whole plant of controls and of the roots and petioles of the salt-treated plants (Table S2).

For both the control and salt-treated plants, the petiole Mg^{2+} concentration of K51-40 was significantly higher than that of 140 Ruggeri by 58% and 88%, respectively, whereas there was no difference between the two genotypes for all other plant components and the whole plant. HB 78 had the highest Mg^{2+} concentration in roots of the salt-treated plants (Table S2).

4. Discussion

The better capacity of the salt-treated 140 Ruggeri for shoot Cl^- exclusion relative to K51-40 and all other hybrids assessed is demonstrated by its significantly lower concentration of Cl^- in the stem, petiole, and lamina. Radiotracer studies with $^{36}Cl^-$ have confirmed lower root to shoot transport of Cl^- in 140 Ruggeri relative to K51-40 [9, 10], which was associated with a significantly lower concentration of Cl^- in the xylem of 140 Ruggeri than that of K51-40 [9]. Hence, the better capacity of 140 Ruggeri than K51-40 for shoot Cl^- exclusion appears to involve a process in the stele of 140 Ruggeri that limits the accumulation of Cl^- in xylem vessels [9, 10].

Assuming that the whole plant Cl^- concentration reflects the end result of the difference between the unidirectional Cl^- uptake by the plant and any Cl^- effluxed by the plant, referred to here as net accumulation, the lower concentration of Cl^- in the whole plant of 140 Ruggeri relative to K51-40 indicates a significantly lower net accumulation by 140 Ruggeri. Radiotracer studies by Gong et al. [10] demonstrated no significant difference between K51-40 and 140 Ruggeri in either unidirectional $^{36}Cl^-$ flux (10 min) or $^{36}Cl^-$ uptake (3 h) into roots of rooted leaves exposed to Cl^- concentrations of 5, 10, or 25 mM, indicating similar rates of Cl^- entry to roots of the two genotypes. Abbaspour et al. [17], on the other hand, found that the initial influx of Cl^- to the root of a good Cl^- excluding genotype (1103 Paulsen) was higher than that of K51-40. They also presented evidence showing greater efflux of Cl^- to the vacuole of roots and to the outside medium by 1103 Paulsen relative to K51-40. Greater efflux to the vacuole suggests a higher concentration of Cl^- in roots of 1103 Paulsen; however, the root Cl^- concentration did not appear to be measured in that study. Rootstock 140 Ruggeri has a similar or better capacity for Cl^- exclusion than 1103 Paulsen [6, 8]; hence, it is possible that 140 Ruggeri has a similar capacity to efflux Cl^- out of roots. In the study of Gong et al. [10], the root Cl^- concentration of the good excluder (140 Ruggeri) was equal or less than that of the poor excluder (K51-40) depending on the plant growth medium used. In this study, there was no difference in the root Cl^- concentration between the genotypes. Further evidence for the Cl^- efflux from roots comes from Wu et al. [18], who proposed that the gene *VviNPF2.2*, when expressed in the root epidermis and cortex, could function in passive anion efflux from root cells and from Li et al. [28] who proposed that *AtNPF2.5* modulates the Cl^- efflux from roots of *Arabidopsis thaliana*.

Petioles of all salt-treated genotypes had the highest concentration of Cl^- relative to all other organs sampled, ranging from 54% (HB 28 and HB 76) to 102% (140 Ruggeri) higher than in laminae. The study showed that for every unit increase in the lamina Cl^- concentration, there was a 1.8 unit increase in the petiole Cl^- concentration. This apparent protective role of the petiole was also observed by Downton [25] who suggested that petioles act as a reservoir for surplus ions, enabling the maintenance of lower concentrations in the laminae. This could occur by the retrieval of Cl^- from the xylem in petioles. Further work will be required to investigate the potential mechanism involved.

The lower net uptake of Cl^- by salt-treated rooted cuttings of 140 Ruggeri relative to K51-40, shown by the significantly lower whole plant concentration of Cl^-, was different to the response of rooted leaves of 140 Ruggeri subjected to salt treatment [11]. In that study, the rooted leaves of 140 Ruggeri, compared with those of K51-40, had a lower concentration of Cl^- in the petiole and lamina and a higher concentration of Cl^- in roots, but there was no difference between the genotypes in the concentration of Cl^- in the whole rooted leaf [11]. The current study involved plants established in a coarse

sand medium, whereas the rooted leaf study involved a hydroponics system with recirculating solution. While it is possible that the hydroponics medium could have impacted the rate of any Cl^- efflux from roots, a further difference is that rooted cuttings have both a growing root system and growing shoot, whereas rooted leaves have a growing root system but no growing shoot. Root to shoot transport of Cl^- is lower in 140 Ruggeri than in K51-40 for both rooted leaves and rooted cuttings [9, 10]. However, if the whole "plant" concentration of Cl^- is lower in 140 Ruggeri than in K51-40 for rooted cuttings but not different between the genotypes for rooted leaves [11], the growing shoot of rooted cuttings could play a role in the lower whole "plant" net uptake of Cl^- relative to that in rooted leaves.

One can only speculate on how a growing shoot can contribute to the lower net uptake of Cl^- by 140 Ruggeri relative to K51-40. Shoot to root signals are known to regulate the root growth and nutrient uptake in response to light, with sugars as an example of a translocated substance able to modulate both the nutrient uptake and root growth [29]. The rooted cuttings of K51-40 and 140 Ruggeri pooled across the control and salt treatments had a mean lamina and root dm of 7.21 and 14.33 g, respectively. Rooted leaves of K51-40 and 140 Ruggeri pooled across control and salt treatments in the study of Walker et al. [11] had a mean lamina and root dm of 2.56 and 1.22 g, respectively. While the lamina dm of rooted cuttings was greater than that for rooted leaves by 2.8-fold, the root dm was greater by 11.7-fold. This indicates that translocated sugars had a much greater impact on the root growth of rooted cuttings than that of rooted leaves. The impact, if any, of translocated sugars on membrane ion transport processes in roots, however, is unknown.

Recirculation of Cl^- from leaves to other parts of the plant has previously been observed in grapevines [19] although in that case it involved plants that had been removed from a prior salt stress. Further evidence for recirculation of Cl^- from shoot to root has been provided in studies involving the salt-treated plants of pistachio [20] and soybean [21]. If recirculation of Cl^- from shoot to root was a factor in limiting the accumulation of Cl^- in the shoot of the salt-treated 140 Ruggeri, there would have to be a link between the growing shoot and greater efflux of Cl^- from roots since, if not, the concentration of Cl^- in roots of 140 Ruggeri would likely increase. In support of a linked shoot to root recirculation possibility, there was no increase in the root Cl^- concentration of salt-treated rooted cuttings (containing a growing shoot) of 140 Ruggeri compared with that of K51-40, as shown in this experiment, whereas there was a significant increase in the root Cl^- concentration of salt-treated rooted leaves (with no growing shoot) of 140 Ruggeri compared with that of K51-40 [11]. More work would be required to investigate this possibility.

Shoot Na^+ exclusion in grapevine rootstocks is largely controlled by the *VisHKT1;1* gene [14]. It appears to play a role similar to that of HKT in other species [15] where it actively retrieves Na^+ from the xylem with retention in the lower plant, particularly in roots [30]. Our data for both the control and salt-treated plants demonstrate significant retention of Na^+ in roots, where, depending on the genotype, the concentration was 7.5- to 20-fold higher than that of the laminae for the controls and 2.2- to 40-fold higher than that of the laminae for the salt-treated plants.

Both the petiole and lamina of the salt-treated plants of the best shoot Na^+ excluders, 140 Ruggeri and hybrid HB 76, had a Na^+ concentration that was comparable with that of control plants. This highlights the primary role of the root and stem in minimizing Na^+ accumulation in the petiole and lamina. The petioles of the salt-treated K51-40, on the other hand, had a Na^+ concentration of 0.74% dm, which was 74-fold higher than that of the K51-40 control (0.01% dm) and 74-fold and 25-fold higher, respectively, than that of the salt-treated HB 76 (0.01% dm) and 140 Ruggeri (0.03% dm). The Na^+ concentration in the laminae of the salt-treated K51-40, 140 Ruggeri, and HB 76 was, however, not significantly different (range 0.01 to 0.04% dm). This demonstrates the effectiveness of the K51-40 petiole in retrieving Na^+ and keeping the lamina concentration low. It further suggests that Na^+, which may have "escaped" the retrieval mechanism in the root and lower plant, is translocated to the shoot and potentially retrieved and stored in the petiole, thus minimizing accumulation in the laminae. While *VisHKT1;1* is involved in the retrieval of Na^+ in the root, it is possible that other members of the HKT family are involved in the retrieval of Na^+ from the xylem in the petioles. While Wu et al. [31] investigated the potential roles of *VviHKT1;6, VviHKT1;7*, and *VviHKT1;8* in grapevine, the study did not include petioles.

Our study also shows that 140 Ruggeri is a better net Na^+ excluder than K51-40 when challenged by salinity, shown by the significantly lower concentration of Na^+ accumulated in the whole plant. Previously it was thought that the shoot Na^+ exclusion capacity of 140 Ruggeri and K51-40 was the same [12, 14], but that conclusion was based on concentrations accumulated in the petiole/laminae only. In the case of durum wheat, two contrasting lines with respect to shoot Na^+ exclusion had a similar unidirectional plasma membrane influx of Na^+ to root cells; however, the better Na^+ excluder had a significantly lower shoot uptake of Na^+ [32]. In our study, 140 Ruggeri and hybrid HB 76 had the least net Na^+ accumulation in the whole plant. Furthermore, both had the ability to store Na^+ in the roots, thus keeping the concentration low in the other plant components. It is not known if efflux was a factor in the difference in net Na^+ accumulation between 140 Ruggeri and K51-40. Evidence for active Na^+ efflux from root tips of sweet lime and Cleopatra mandarin was provided by Greenshapan and Kessler [33], while Britto and Kronzucker [34] concluded that the efflux of Na^+ from the root most likely occurs from root tips where the Na^+ efflux transporter SOS1 has been localized.

The nonsignificant difference between the salt-treated 140 Ruggeri and K51-40 in the concentration of K^+ in the root, stem, laminae, and whole plant was similar to the results reported by Walker et al. [35] for laminae and roots. The concentration of K^+, however, in roots of all genotypes assessed was notably higher for plants grown in a hydroponic system with recirculating solution for the Walker et al. [35] study, relative to the plants grown in coarse sand in the current study.

5. Summary

The study has enabled salt (Cl^- and Na^+) exclusion in grapevines to be re-examined from a whole plant perspective. It can be defined primarily as lower net accumulation on a whole plant basis, reflecting the balance between the uptake and any efflux that may occur. Lower root to shoot transport is a key factor in shoot Cl^- and Na^+ exclusion. Petiole accumulation assists in limiting the Cl^- and Na^+ accumulation in laminae. While our study was limited to rooted cuttings, without bunches (fruits), it is recommended that future studies include bunches to enable an investigation of Cl^- and Na^+ concentrations in rachis and fruits since rachis may play a similar role to petioles in limiting accumulation in fruits.

While previous studies [10, 14] demonstrated no difference in the shoot Na^+ exclusion capacity between the salt-treated 140 Ruggeri and K51-40 based on the lamina Na^+ concentration, this study has shown that 140 Ruggeri had a better capacity to limit the net accumulation of Na^+ to the whole plant. 140 Ruggeri also had the best capacity among the genotypes to limit the net accumulation of Cl^- to the whole plant. Hybrid 13 was the only genotype with an equivalent capacity to 140 Ruggeri for limiting the net accumulation of both Cl^- and Na^+ to the whole plant and hence may be useful for future studies. Furthermore, while K51-40 takes up more Na^+, it has the capacity to retrieve it from the xylem; store it in the roots, stem, shoots, and petiole; and keep lamina concentrations low.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

Supplementary Table 1 (S1): plant component and whole plant Ca^{2+} concentration of 140 Ruggeri, K51-40, and 5 hybrids from the control and salt treatment. Supplementary Table 2 (S2): plant component and whole plant Mg^{2+} concentration of 140 Ruggeri, K51-40, and 5 hybrids from the control and salt treatment. (*Supplementary Materials*)

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