Delivering chloride and sodium excluding rootstocks for quality wine production



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Delivering Chloride and Sodium Excluding Rootstocks for Quality Wine Production

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This study was undertaken from July 2010 to April 2014. It involved collaboration with Australian Wine Research Institute to address salty taste in wine and with University of Adelaide to investigate the chloride (Cl^-) exclusion trait in grapevine rootstocks.

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1. Abstract

Salt in irrigation water and soil is a concern in some Australian wine regions. Strategies for maintaining long term performance of grapevines in saline environments include use of salt excluding rootstocks to which wine varieties of choice are grafted. Performance of eight different rootstocks with a range in vine vigour was assessed in a salt affected region with Shiraz as scion. The rootstocks were 140 Ruggeri, 1103 Paulsen, 110 Richter, Ramsey, 101-14, Merbein 5489, Merbein 5512 and Merbein 6262 (hereafter referred to as M 5489, M 5512 and M 6262). Rootstock M 6262 was a relatively poor chloride (Cl^-) excluder. Wines of M 6262 contained 482 mg/L Cl^- in 2012 and were characterised as having *Salty* taste and a perceived thickness of the wine on the palate (*Viscosity*), whereas wines of M 6262 contained 384 mg/L Cl^- in 2013 and were not characterised as having *Salty* taste or *Viscosity*. Sodium (Na^+) concentrations in wine from all rootstocks were relatively low (under 40 mg/L). Hence, *Salty* taste detection for red wines, based on the methods used in this study, occurred at wine Cl^- concentrations of between 384 and 482 mg/L. The other seven rootstocks were similar in capacity for Cl^- exclusion from grape juice, but there were differences between the rootstocks in final concentrations in wine (all under 175 mg/L Cl^-). A salt tolerance index (STI) for the various rootstocks was calculated based on yield, leaf area index, grape juice Cl^- and Na^+ concentrations and wine colour density. Best STI was obtained for M 5489, 110 Richter and 140 Ruggeri rootstocks and the lowest for M 6262.

Rootstocks with different capacity for Cl^- and Na^+ exclusion were used to obtain a natural series of salt concentrations in wines. Chardonnay wines containing 407 mg/L Cl^- and 374 mg/L Na^+ were characterised as having *Salty* taste and high *Viscosity* by descriptive analysis, but neither *Salty* taste nor *Viscosity* were detected in Shiraz wines containing 274 mg/L Cl^- and 131 mg/L Na^+. These values are compared with Best Estimate Thresholds (BET) for *Salty* taste in white and red wines as determined by AWRI in other studies.

The Cl^- exclusion phenotype in rooted leaves was shown to be associated with differences in the partitioning of Cl^- between roots and shoot (petiole and lamina), rather than differences in total plant Cl^- accumulation. Good Cl^- excluders sequester

more Cl^- in roots, with lower Cl^- concentrations in xylem and in petiole and lamina. The number of candidate genes potentially linked with Cl^- exclusion was initially identified at 23, but reduced to 7 as the project progressed. Two putative markers for Cl^- exclusion were identified.

2. Executive Summary

The interaction between genotype, environment and management, including scion variety of choice, is a major factor determining the performance of rootstocks (Walker and Clingeleffer 2009). In development of new rootstocks, it is not possible to assess performance in all regions, in all soil types with all scion varieties. Hence, trials with new rootstocks, after limited release, are required in order to build information on their performance (Clingeleffer and Walker 2011). This study compared three new low to medium vigour rootstocks (M 5489, M 5512 and M 6262) with five standard rootstocks (Ramsey, 1103 Paulsen, 101-14, 110 Richter and 140 Ruggeri) in a region with good winter rainfall (approximately 450 mm), shallow soils over a calcareous layer, and limited available irrigation water (100 mm limit) which was moderately saline (1.8 dS/m). Vines planted in 2007 were assessed for juice ion concentrations in 2010-11. This was followed by a full analysis of vine performance over two seasons (2011-12 and 2012-13), including yield, yield components, leaf area index, juice and wine ion concentrations and wine sensory quality. The aim was to enable recommendations to be communicated to industry at the conclusion of the project.

A further objective in 2010-11 was to utilise differences in salt exclusion capacity of various rootstocks to create a ‘natural salt series’ in Chardonnay and Shiraz wines enabling examination of the sensory detection threshold for *Salty* taste in the wines. Information on rootstock effects on sensory attributes of Chardonnay and Shiraz wines was a secondary output of these studies.

The Cl^- exclusion mechanism in the good Cl^- excluding rootstock, 140 Ruggeri, was further characterised in comparative studies with rootstock K 51-40, a poor Cl^- excluder. A third variety, Cabernet Sauvignon, with Cl^- exclusion capacity intermediate between that of 140 Ruggeri and K 51-40 was also used. Rooted

leaves were used for these studies. A range of candidate genes were identified from reviews of the literature and from microarray studies. Quantitative PCR expression analyses were undertaken using RNA obtained from root samples taken from plants (rooted leaves) of the three varieties grown in the presence and absence of salt.

The field trial comparing the three new rootstocks with five industry standard rootstocks found a wide range in leaf area index and time to reach target grape juice total soluble solids (TSS), with a 5 to 12 day gap between first and last rootstocks to mature fruit to the target value (24ºBrix). Yield, laminae and grape juice Cl^- concentrations at harvest, and wine ion concentrations, spectral parameters and sensory attributes also varied depending on rootstock type. The increase in Cl^- concentrations in progressing from grape juice to wine was on average 2.2 fold over the two years, whereas the increase in Na^+ concentrations was 1.1 fold.

Yield and growth have been standard ways to assess salt tolerance of plants under saline conditions (Walker 1994). An alternative way to characterise salt tolerance, termed salt tolerance index (STI), was tested. The STI takes into account yield, leaf area index, grape juice Cl^- and Na^+ concentrations and wine colour density. Best STI was obtained for M 5489 (7.60), 110 Richter (7.03) and 140 Ruggeri rootstock (6.96) and least for M 6262 (0.54). Rootstocks 1103 Paulsen, Ramsey, 101-14 and M 5512 were intermediate (range 4.83 to 5.65).

In studies to characterise *Salty* taste detection in wine, it was found that Chardonnay wine with 407 mg/L Cl^-, 374 mg/L Na^+ and 470 mg/L K^+ was assessed as having *Salty* and *Viscous* attributes and that Shiraz wine with 482 mg/L Cl^- but only 26 mg/L Na^+ and 1344 mg/L K^+ was similarly assessed as having *Salty* and *Viscous* attributes. On the other hand, Shiraz wine with 274 mg/L Cl^-, 131 mg/L Na^+ and 1110 mg/L K^+ was not assessed as having *Salty* taste or *Viscous* attributes. Similarly, Shiraz wine with 384 mg/L Cl^- , 30 mg/L Na^+ and 1173 mg/L K^+ was not assessed as having *Salty* taste or *Viscous* attributes. This suggests that relatively high concentrations of K^+ (in this case, 1110 to 1173 mg/L), even in the presence of up to 384 mg/L Cl^- or up to 131 mg/L Na^+, are insufficient for *Salty* taste detection using descriptive analysis, but that *Salty* taste becomes detectable in the range 384

482 mg/L Cl^-. These values are also compared with best estimate thresholds for *Salty* taste in white and red wines as determined in other studies.

Experiments with rooted leaves comparing Cl^- exclusion capacity of 140 Ruggeri, Cabernet Sauvignon and K 51-40 provided evidence that the Cl^- exclusion phenotype is associated with differences in partitioning of Cl^- to various parts of the plant. The good Cl^- excluder, 140 Ruggeri, sequestered higher Cl^- concentrations in roots and had lower Cl^- concentrations in xylem and less Cl^- in petiole and lamina of the rooted leaves. The accumulation of Cl^- in laminae of K 51-40 was accompanied by higher accumulation of K^+, compared with 140 Ruggeri. Possible reasons for the link between Cl^- and K^+ accumulation in K 51-40 were either passive accumulation of K^+ for ion balance to accompany the higher accumulation of Cl^-, or a more direct link through an ion transporter, for example, cation coupled Cl^- co-transporter (CCC).

The non-specific Cl^- transport inhibitor, DIDS, changed the Cl^- exclusion phenotype of 140 Ruggeri from a situation where roots of 140 Ruggeri accumulated higher concentrations of Cl^- than K 51-40 to a situation where root Cl^- concentrations were similar for both genotypes. This provided evidence for a role of Cl^- transporters in determining the Cl^- exclusion phenotype of 140 Ruggeri. High nitrate (NO_3^-) concentrations (12 mM relative to 3 mM) in the nutrient solution also resulted in 21% and 13% reductions in Cl^- accumulation in roots of 140 Ruggeri and K 51-40, respectively. The high NO_3^- treatment caused 140 Ruggeri to accumulate 88 % more Cl^- in laminae, resulting in similar Cl^- concentrations in laminae of the two genotypes (0.49 and 0.52% dry weight, respectively), i.e. the difference in Cl^- exclusion between the genotypes was eliminated when high NO_3^- was present in the root medium. This suggested the presence of a Cl^-/ NO_3^- ion transporter in roots of both genotypes.

Studies aimed at development of a marker for Cl^- exclusion to assist early selection of Cl^- excluding genotypes in the rootstock breeding program initially identified 23 candidate genes. This was reduced to seven after analysing the results of a microarray study using high quality RNA from roots of the three genotypes. Two putative markers were identifed for further study.

3. Background

Drought, with consequent water restrictions, limits the availability of water for leaching and increases soil salinity. Climate change is expected to result in hotter, drier summers, placing additional pressure on limited water supplies. A consequence, in some regions, e.g. Langhorne Creek, has been high irrigation water salinities, combined with limited water supplies, resulting in very high concentrations of Cl^- and Na^+ in grape juice and wine. Red wines are particularly at risk, since on average there is a 1.7 fold increase in Cl^- in going from must to wine due to extraction from skins (Walker et al. 2010).

The Australian Food Standards Code currently imposes a limit of 607 mg/L of Cl^- in wine. Whilst there is no current limit for Na^+ in Australian wine, the maximum permissible levels in wine vary widely among countries, ranging from 60 mg/kg (Switzerland) to 500 mg/L (Canada) (Stockley and Lloyd Davies 2001). Some Australian wine companies are imposing limits on permissible Cl^- and Na^+ in grape juice at grape intake.

Little information is available, however, that relates upper limits of Cl^- and Na^+ in wine and *Salty* taste. The study of Walker, Godden and Francis et al. (2003) demonstrated that there were statistically significant and substantial differences among Shiraz wines made from six rootstocks in '*Salty*' ratings, which correlated with the Cl^-, Na^+ and K^+ concentrations of the wines. Wines with '*Salty*' taste were described as *Soapy*, which was defined as a *Slimy* or soft mouth-feel character. In addition, a high '*Salty*' score was related to relatively low scores for perceived *Acidity*, *Fruit Flavour*, *Astringency* and *Fruit Flavour* persistence. That study did not address *Salty* taste in white wine, nor did it address detection thresholds for *Salty* taste. This study attempted to better characterise the '*Salty*' taste attribute and the question of detection thresholds.

Adoption of salt excluding, salt tolerant rootstocks with capacity for quality wine production presents a significant opportunity to mitigate the impact of reduced water supply and quality, and enhance environmental sustainability and adaption to climate change.

Vitis vinifera varieties of grapevine are classified as moderately salt tolerant (Maas and Hoffman 1970). Some (not all) rootstocks derived from American *Vitis* species have higher salt tolerance than *V. vinifera* varieties (Zhang et al. 2000).
Salt tolerance of grapevine has generally been measured by growth and yield under saline conditions (Walker 1994). The higher salt tolerance of *V. vinifera* varieties, e.g. Sultana, on rootstocks such as Ramsey and 1103 Paulsen relative to their salt tolerance on own roots has been linked to greater capacity for salt exclusion and higher conferred vine vigour (Walker et al. 2002 and 2004). The rootstocks Ramsey and 1103 Paulsen not only conferred higher vigour and higher yields to Sultana scion, but also demonstrated good capacity for Cl^- exclusion from laminae and grape juice (Walker et al. 2002 and 2004). It is not clear, however, if rootstocks with good capacity for Cl^- and Na^+ exclusion, but low inherent vigour, are also able to perform well in terms of yield under saline conditions. This study attempted to address that question through a field trial involving rootstocks with a range in inherent vigour.

It is apparent that classifying rootstocks for salt tolerance based solely on Cl^- and/or Na^+ exclusion capacity or solely on yield performance under saline conditions has limitations. Clearly, there is a requirement to keep Cl^- and Na^+ concentrations below imposed limits for domestic and export markets and below detection thresholds for *Salty* taste, but there is also a need to ensure that wine spectral and sensory attributes meet market expectations. Hence, in designing this study, the concept of a salt tolerance index, that incorporates these factors, was included for an initial investigation.

New rootstocks developed by CSIRO that confer improved grape juice composition to scions, including lower juice K^+ concentrations and pH, and therefore reduced need for pH adjustment during winemaking (Walker and Clingeleffer 2009), have yet to be tested under saline field conditions. These rootstocks have been included in this study and are compared with the performance of standard rootstocks in a moderately saline environment.

Salt exclusion by the grapevine is an effective strategy to reduce Na^+ and Cl^- concentrations in wines, but little is known about the capacity of certain rootstocks to maintain high Cl^- and Na^+ exclusion over an extended period. Most trials have been

of three to five years duration, with few trials conducted for a longer period. Observations of a diminution in salt exclusion capacity over several years by some standard rootstocks, e.g. 1103 Paulsen, have been made (Tregeagle et al. 2006). Ideally, in order to combat future challenges associated with salinity, rootstocks must exhibit the highest possible Na^+ and Cl^- exclusion capacity and show good performance over an extended period.

High capacity for Cl^- and Na^+ exclusion are required traits, in addition to other traits, when breeding for improved rootstocks. Selection for high capacity for Cl^- and Na^+ exclusion has involved use of glasshouse and field based screening for accumulation of Cl^- and Na^+ in laminae and grape juice. These studies are generally long term, and smarter, faster methods are needed. Understanding the method of Cl^- and Na^+ exclusion and the key genes involved is essential in order to provide a basis for identifying markers that might be used in a breeding program for rapid and early selection of new genotypes with capacity for Cl^- and Na^+ exclusion. This study aimed to further characterise the Cl^- exclusion mechanism as a basis for identifying potential markers for the Cl^- exclusion trait.

Two studies, both under glasshouse conditions, one involving hybrids of a cross between cultivars Ramsey (*V. champinii*) and Sultana (*Vitis vinifera*) and another involving hybrids from a cross between cultivars K-51-40 (*Vitis champinii* 'Dogridge' x V. riparia 'Gloire') and 140 Ruggeri (*V. cinerea* var. *helleri* 'Resseguier #2 x *V. rupestris* 'St. George') have reported continuous variation in Cl^- accumulation between the hybrids, suggesting quantitative, heritable Cl^- exclusion (Sykes 1985; Gong et al. 2011). In a separate study (Sykes 1987), involving hybrids and backcrosses of *V. cinerea* var. *helleri (formerly V. berlandieri*) and *V. vinifera*, also under glasshouse conditions, the ratio of genotypic to phenotypic variance was greater for backcross families than for F1 hybrids (*V. cinerea* var*. helleri* x *V. vinifera*), which suggested that a dominant gene for Cl^- exclusion may be derived from *V. cinerea* var*. helleri*.

The study by Tregeagle et al. (2010) reported significantly higher concentrations of Cl^- in xylem of a poor salt excluder, K 51-40, compared with a good salt excluder, 140 Ruggeri, which indicates either reduced loading of Cl^- into xylem or greater

retrieval of Cl^- from the xylem by the better salt excluder. Gong et al. (2011) and Abbaspour et al. (2014) using the apoplastic tracer PTS both found no evidence that higher apoplastic transport was contributing to the higher concentrations of Cl^- accumulating in the shoot of K 51-40. This suggests the exclusion process is regulated at one or more points in the symplastic pathway in roots. The symplastic pathway involves transport of ions across plant cell membranes, and this includes the plasma membrane and tonoplast, potentially of different cell types, for example, root cortical cells, where higher vacuolar loading of Cl^- may play a role in the Cl^- exclusion process of good Cl^- excluding genotypes (Gong et al. 2011), and xylem parenchyma cells, where plasma membrane transport may control release of ions into the xylem. Recent reviews (Teakle and Tyerman 2010) have identified a range of genes that regulate Cl^- uptake and transport in plants. Several ion transporter genes may be involved in Cl^- exclusion from the shoot. Examples include *CLC, ALMT, CCC, NRT, SLAC and SLAH* (Teakle and Tyerman 2010).

This study will further characterise the Cl^- exclusion mechanism using rooted leaves as a model system and undertake experiments to identify candidate genes that may be involved as a basis to development of possible markers for Cl^- exclusion.

4. Project Aims and Performance Targets

4.1 Project Aims:

- Using an established field trial in the Padthaway region, compare Shiraz grafted to new low to medium vigour rootstocks (M 5489, M 5512 and M 6262) with Shiraz on industry standard rootstocks (Ramsey, 1103 Paulsen, 140 Ruggeri, 101-14 and 110 Richter) under saline conditions (irrigation water EC 1.8 dS/m) for impacts on juice and wine salt concentrations, wine sensory characteristics and vine performance.
- Develop a 'salt tolerance index' for specific rootstocks based on yield, pruning weight and grape juice Cl^- and Na^+ concentrations.
- Examine thresholds for *Salty* taste detection in relation to concentrations of Na^+, Cl^- and K^+ in wine.
- Propagate the best CSIRO bred rootstocks from long term (14 year) salinity trials at Merbein (EC_{iw} = 2.1 dS/m) and establish a test site with industry input for comparison with standard rootstocks. Final evaluation (2014-2017) of these rootstocks will carry forward into a subsequent project.
- Using characterised rootstocks, advanced rootstock hybrids and a population of progeny showing continuous variation (from high to low) in Cl^- exclusion capacity, develop a marker for Cl^- exclusion for use in rapid screening for salt tolerance.

4.2 Outputs and Performance Targets:

2010-11

A Field performance of Shiraz, grafted on CSIRO and standard rootstocks assessed under saline field conditions. *Laminae samples collected at harvest for mineral element analyses. Berry samples collected at harvest for berry size and basic fruit composition and salt analyses. Small scale wines of Shiraz and Chardonnay on standard rootstocks made for Salty taste characterisation*.

B Grape juice maturation analyses completed. *pH, total soluble solids and titratable acids determined in processed samples.*

C Laminae and grape juice ion analyses completed. *Chloride and cation concentrations determined in processed samples.*

D Selections (2) from long term field trials propagated. *Cuttings taken, heat treated and transferred to SA for propagation.*

E Candidate genetic markers for chloride exclusion identified*. Microarray and PCR screening of candidates in vinifera and non-vinifera rootstocks*.

2011-12

A Sensory assessment and compositional analyses of wines from 2011 harvest completed. *Wine profiling, ion analyses, difference tests and Salty taste assessments conducted.*

B Field performance of vines grafted on CSIRO and standard rootstocks assessed under saline field conditions. *Laminae sampes collected at harvest for mineral element analyses. Berry samples collected at harvest for berry size and basic fruit composition. Yield and its components determined at harvest. Fruit harvested and small scale wines made.*

C Grape juice maturation analyses completed. *pH, total soluble solids and titratable acids determined in processed samples.*

D Laminae and grape juice ion analyses completed. *Chloride and cation concentrations determined in processed samples.*

E Selections (2) from long term field trials propagated. *Additional cuttings transferred to SA and vines nursery propagated for spring 2012 planting.*

F List of candidate chloride exclusion markers refined. *Robustness of candidates further assessed using microarrays, PCR and sequencing.*

2012-13

A Sensory assessment and compositional analyses of wines from 2012 harvest completed. *Wine profiling, ion analyses, difference tests and Salty taste assessments conducted.*

B Field performance of vines grafted on CSIRO and standard rootstocks assessed under saline field conditions. *Laminae samples collected at harvest for mineral element analyses. Berry samples collected at harvest for berry size and basic fruit composition. Yield and its components determined at harvest. Fruit harvested and small scale wines made.*

C Grape juice maturation and laminae and grape juice ion analyses completed. *Grape juice pH, total soluble solids and titratable acids determined. Chloride and cation concentrations determined in processed samples.*

D Selections (2) from long term field trials established. *Vines trained and established.*

E Marker for chloride exclusion. *Markers trialled using material obtained from field sites; all reliable markers reported.*

2013-14

A Data collated on field performance in season 2013 under saline conditions of vines grafted on CSIRO and standard rootstocks. *Analyses completed on laminae and petiole samples (chloride and cations) and soil samples (ECe, SAR, pH, chloride and cations) taken in season 2013 from rootstock trial*.

B Data available on composition of wines made from fruit grown under saline conditions and harvested in season 2013 from vines grafted on CSIRO and standard

rootstocks. *Ion analyses and spectral parameters measured for Shiraz wines made from wine company rootstock trial.*

C Data available on the sensory properties of wines made from fruit grown under saline conditions and harvested in season 2013 from vines grafted on CSIRO and standard rootstocks. *Sensory assessments completed, data collated and analysed for Shiraz wines made from wine company rootstock trial.*

D Communication of project outcomes completed through Final Report. *All data collated and analysed and Final Report completed and submitted.*

5. Comparison of Shiraz grafted to low to medium vigour rootstocks with Shiraz on industry standard rootstocks

5.1 Objectives

- Using an established field trial in the Padthaway region, compare Shiraz grafted to new low to medium vigour rootstocks (M 5489, M 5512 and M 6262) with Shiraz on industry standard rootstocks (Ramsey, 1103 Paulsen, 140 Ruggeri, 101-14 and 110 Richter) under saline conditions (irrigation water EC 1.8 dS/m) for impacts on juice and wine salt status, wine sensory characteristics and vine performance.
- Develop a 'salt tolerance index' for specific rootstocks based on yield, pruning weight and grape juice Cl^- and Na^+ concentrations.

5.2 Materials and Methods

5.2.1 Trial design

The trial was established on a commercial vineyard at Padthaway, SA, in 2007. A commercial clone of Shiraz was grafted to the following eight rootstocks: M 5489, M 5512, M 6262, Ramsey, 1103 Paulsen, 140 Ruggeri, 101-14 and 110 Richter. Each rootstock was planted in a single row, varying in length from 144 vines to 91vines, with 2.0 m spacing between vines. Spacing between rows was 3.5 m. There were 72 rows in total. Rootstock rows were arranged in clusters of 8, with one row for each rootstock within the cluster, and specific rootstock rows randomised within the 8 rows of each cluster. A fully randomised block design was superimposed with 22 replicate vines (blocks) of each rootstock. The 22 blocks were selected to cover the full spatial variability within the trial site using the 8 row clusters.

5.2.2 Soil type and soil sampling

Soil type was a sandy loam over clay changing from a red to yellow mottle at around 45 cm depth. Soil samples were taken during the post harvest period in March 2012

and 2013, respectively. Samples were taken at three depths with a hand-held auger, including a surface sample at 1-10 cm, then samples at 25-35 cm and at 55-65 cm. There were 24 sampling sites across the site in 2012 and 30 in 2013. A 1:5 soil:water extract was prepared from each sample and electrical conductivity, soluble ion (Cl^-, Ca^{2+}, K^+, Mg^{2+} and Na^+) concentrations and sodium adsorption ratio (Richards 1954) were determined by CSIRO Land and Water Analytical Services.

5.2.3 Trellis and pruning

The vines were established on a 75 cm T-trellis and trained as quadrilateral cordons. The T was situated 120 cm above ground level. The vines were spur pruned, retaining approximately 80 buds per vine.

5.2.4 Weather data

Weather data were obtained from the closest Australian Bureau of Meteorology station, which was approximately 2.5 km away from the Padthaway site. Rainfall totals for the period July to June in 2011-12 and 2012-13 were 440.8 and 394.0 mm, respectively.

5.2.5 Irrigation practices

Irrigation was applied via drippers. The dripper line was situated along the vine row at 30 cm above ground level. Drippers were spaced 1.0 m apart with flow rate of 2 L/h. The site was irrigated with bore water. Total amounts of water applied in 2011-12 and 2012-13 were 0.88 and 0.98 ML/ha, respectively.

Irrigation scheduling was based on weather conditions, vine condition, vine health and data from GBug soil moisture probes (Measurement Engineering Australia, Magill, South Australia). GBug sensor depths varied slightly between rootstocks. For rootstock 140 Ruggeri, sensors were at 15, 30 and 45 cm. For rootstocks Ramsey and M 5489 sensors were at 15, 40 and 65 cm. For rootstocks 110 Richter, 101-14, 1103 Paulsen, M 5512 and M 6262 sensors were at 15, 40 and 70 cm depth. Regardless of the difference in sensor depths between rootstocks, when irrigations were on, all rows/rootstocks received the same irrigation duration. First irrigation in

season 2011-12 was applied on 21 November 2011 and in 2012-13 it was applied on 5 December 2012. With the exception of a period of regulated deficit irrigation between fruit set and veraison, irrigations were applied once the Readily Available (soil) Water (RAW) had been used. Winter rainfall was relied on to provide adequate leaching of salts from the soil profile.

5.2.6 Electrical conductivity and composition of irrigation water

Mean total soluble salts in the irrigation water in season 2011-12 was 1160 mg/L and in 2012-13 it was 1057 mg/L. These values equated to electrical conductivities of 1.81 and 1.65 dS/m, respectively. Mean concentrations of Cl^-, Na^+, K^+, Mg^{2+} and Ca^{2+} in the irrigation water were respectively 12.2, 9.2, 0.1, 1.4 and 3.4 mM. Sodium adsorption ratio of the irrigation water was 4.2.

5.2.7 Harvest scheduling

The trial was harvested in each year when the grape juice soluble solids concentrations of Shiraz grape juice were as close as possible to 24^{o}Brix. Harvest dates were different depending on the rootstock, as shown in Table 1. Rootstocks 101-14 and 1103 Paulsen were earliest in both seasons. Rootstocks M 5489 and M 5512 were latest in both seasons, with 12 days difference between maturation of first and last rootstocks in 2012 and 5 days difference in 2013 (Table 1).

Table 1: Harvest dates for Shiraz on the 8 rootstocks.

Rootstock	Harvest dates	
	Season 2011-12	Season 2012-13
101-14	2 Mar	20 Feb
1103 Paulsen	2 Mar	20 Feb
140 Ruggeri	2 Mar	21 Feb
110 Richter	7 Mar	20 Feb
Ramsey	7 Mar	21 Feb
M 6262	7 Mar	21 Feb
M 5489	14 Mar	25 Feb
M 5512	14 Mar	25 Feb

Samples of 80 berries per vine were taken at all sites on the day of harvest. Each 80-berry sample was obtained by taking 20 berries from each 'quarter' of the vine (left and right of the main trunk, and on each side); five berries were sampled from four bunches in each 'quarter', making a total of 16 bunches sampled per vine; for each bunch, two berries were sampled from the 'shoulders' of the bunch, two from the middle and one from the tail. All berry samples were placed in plastic bags and held in an ice-cooled insulated container for transport to the laboratory. Berry samples were gently crushed with mortar and pestle to extract juice. The juice samples were then centrifuged (Beckman J25i; Beckman Coulter Inc., Fullerton, CA, USA) for 15 min. at 4^{o}C @ 5930 g. The clear juice was decanted and used immediately for measurement of total soluble solids, pH and titratable acidity. The samples were then frozen for later analysis of ions.

5.2.8 Bunch numbers, yield and leaf area index

Bunch numbers were counted post flowering and again at harvest. Bunch thinning was undertaken post flowering, thinning to one bunch per shoot. Yield per vine was measured at harvest. Leaf area index (LAI) was also measured on the day of harvest using a LiCor LAI 2000 leaf area meter.

5.2.9 Total soluble solids, pH and titratable acidity

Fresh grape juice samples were analysed for total soluble solids (^{o}Brix) with a digital refractometer (PR-1, Atago Co Ltd, Japan). Measurements of pH and titratable acidity were made using an autotitrator (Metrohm Ltd., Herisau, Switzerland).

5.2.10 Fermentation

Small-scale wines were made from Shiraz on all eight rootstocks. Triplicate fermentations were made for each rootstock. Fruit from each rootstock vine was harvested into plastic buckets. After weighing, to determine yield per vine, buckets of fruit from all 22 vine replicates were assembled. Fruit from each replicate vine was distributed evenly to three sets of three trays to result in triplicate fruit lots for winemaking. Each winemaking replicate consisted of approximately 30 kg of fruit. Wines were made at the UA Hickinbotham Roseworthy Wine Science Laboratory

winemaking facility, Waite Campus, Adelaide. The triplicate fruit consignments for each rootstock were delivered to the winery and placed in cold storage for approximately 12 hours to remove field heat and minimize oxidation. Fruit for each winemaking replicate was weighed prior to crushing.

Crushing and de-stemming were performed using a combined crusher/destemmer (Enoitalia, ENO-15, Florence, Italy) and placed in suitably sized fermenters awaiting inoculation. Must samples were taken for pH, titratable acidity and Baume readings. Must pH was adjusted ideally to within 3.4-3.6 and SO_2 added to achieve 20-25 ppm as free SO_2. Inoculation was carried out with rehydrated yeast (EC 1118) at the rate of 0.25 g/L, calculated at 600 mL/kg of fruit weight. Fermentation on skins was initiated and temperature controlled at 18 -20ºC with diammonium phosphate (DAP) added as required up to a maximum of 400 mg/L total and minimum TSS of 4 ºBaume. Desired rate of fermentation was based on a decrease of 1 - 2 ºBaume per day, with temperature adjusted accordingly. Fermentation lasted for approximately 14 days, with caps managed by plunging twice daily, and residual sugar checked daily using a Density Meter (Anton Paar, DMA 35, Graz, Austria). Skins were pressed off at 2 ºBaume using a Diemme 130 L membrane press (Diemme Enologia, Lugo, Ravenna, Italy). The must was fermented to dryness, with Baume and temperature readings taken daily. Once sugars were less that 2 g/L (measured using enzymatic tests), wine was racked off gross lees. Wine was then cold stabilised at 0ºC for a minimum of 21 days.

After settling time to allow dead yeast to drop out, a second racking was performed to remove potassium bi-tartrate lees and measurements were made of pH, titratable acidity, % alcohol, acetic acid, free and total SO_2 with SO_2 adjusted to 80 mg/L as free SO_2. No fining agents were added. Instead, the wines spent 21 days at 0ºC to aid with settling. The wines were filtered through an Ezyquipe Z6 pad, followed by Millipore Durapore 0.45 µm membrane filter for sterile filtration. Wines were bottled into 750 mL bottles (2012 vintage) and into 375 mL bottle (2013 Vintage), using N_2 in the ullaged headspace. CO_2 was used as a pre-fill gas at all times during processing, and all vessels and equipment were fully cleaned and sanitized. Bottles were closed using Stelvin closures.

5.2.11 Ion concentrations in laminae, grape juice at harvest and in wines

Chloride: Laminae and grape juice samples at harvest and wines were examined in duplicate for Cl^- concentrations by silver ion titration with a chloridometer (Model 442-5150, Digital Chloridometer, Lenexa, Kansas, USA). Juice samples of 1 mL were used for Cl^- determination, along with 3 mL of acid reagent (100 mL glacial acetic acid and 6.4 mL concentrated nitric acid per litre) and four drops of gelatine added just prior to analysis. Laminae samples (50 mg) were weighed into 4 mL glass vials and 3 mL of acid reagent (as above) added and allowed to extract for 30 minutes, then four drops of gelatine reagent were added prior to analysis.

Cations, phosphorous and sulphur: Dried laminae, grape juice and wine samples were examined for cation (K^+, Na^+, Ca^{2+} and Mg^{2+}), phosphorous and sulphur concentrations.

(a) *Laminae*: Extracts of approximately 100 mg powdered laminae samples were weighed into 15 mL polypropylene digestion vessels (SC415) (Environmental Express, Mt Pleasant, SC, USA) and 2 mL of 15 M nitric acid was added. They were held in a fumehood overnight at room temperature. A disposable polypropylene (SC417) watch glass was added to each tube. The tubes were sequentially heated on a 96 tube HotBlock ^{TM} Pro Digestion System (SC189-240) with a Pro Controller-Touch Screen (SC180-240) (Environmental Express, Mt Pleasant, SC, USA) increasing the temperature at 3ºC/min initially to 60ºC, with a holding period at 60ºC for 20 minutes, then at 85ºC for 60 minutes and subsequently at 85ºC for 210 minutes. A disposable polypropylene (SC417) watch glass was added to each tube. Ninety six vessels were heated per batch. The tubes were allowed to cool, then made up to 15 mL with MilliporeR reverse osmosis water and then inverted to mix thoroughly.

(b) *Grape juice and wine*: The details are as above except that samples (2 mL) were dispensed into 25 mL pre-treated glass test tubes and 2 mL of 15 M nitric acid was added. After holding in a fume hood overnight at room temperature, the tubes were sequentially heated on a 96 tube aluminium block HotBlockTM Pro Digestion System (as above) initially at 60ºC for 120 minutes then at 70ºC for 60 minutes and 115ºC for 240 minutes, with disposable polypropylene watch glass, as above. Fifty tubes

were allowed to cool, then made up to 20 mL with Millipore reverse osmosis water as above. The solutions were then filtered through Whatman No 42 (ashless) filter paper 125 mm. Aliquots of approximately 10 mL were transferred to polypropylene tubes for cation, phosphorous and sulphur analyses.

Cation, phosphorous and sulphur concentrations were measured by inductively coupled plasma-optical emission spectrometry (Spectro ARCOS, Spectro Analytical Instruments, Kleve, Germany, and Thermo Scientific iCAP 6000 Series, Thermo Electron Limited, Cambridge, United Kingdom). Calibration of the ICP was carried out at the commencement of each analysis run using 1% HNO_3 acid blank solution, and eight multi element standards (Standards 1-8) in 1 % HNO_3 acid.

During the run, after every 12 samples, the instrument was verified using Standard 8. When the instrument failed set criteria, the verification was repeated and if it failed a second time the instrument was recalibrated. At the beginning and end of each batch run, independent multi element certified standards were analysed as additional quality control checks capturing each element being analysed for a range of concentrations.

5.2.12 Red wine colour and phenolics measures

Spectral characteristics of the red wines were evaluated in October 2012 and 2013, respectively, which was approximately five weeks after bottling. Measurements were according to the method of Somers and Evans (1977) using a GBC 918 UV/Vis spectrophotometer (GBC Scientific Equipment Pty. Ltd., Dandenong Australia).

5.2.13 Sensory analysis

A preliminary tasting of the wines was carried out in November of 2012 and 2013 for the wines from the respective seasons. The aim of the preliminary tasting was to assess the wines for appearance, aroma and palate, to determine whether the wines were suitable for formal sensory analysis and to assess whether any replicates should be excluded from analysis. In each case it was decided that the wines were suitable for formal descriptive analysis.

Formal descriptive analysis of the wines was conducted in February 2013 and 2014 for wines from the respective seasons. A panel of at least 11 assessors was convened for the studies. Panellists attended three training sessions to determine appropriate descriptors for rating in the formal sessions. Wines were assessed by appearance, aroma and palate. During these sessions, panellists generated and refined terms. Following the three training sessions, tasters participated in a practice session in the sensory booths under the same conditions as those for the formal sessions. After the practice session, any terms which needed adjustment were discussed and the final list of terms was determined. All attributes were defined and reference standards were used.

For the formal sensory assessment, samples were presented to panellists in 30 mL aliquots in 3-digit-coded, covered, ISO standard wine glasses at 22-24°C, in isolated booths under daylight-type lighting, with randomised presentation order following an incomplete Williams latin square design. In formal booth sessions, the assessors were presented with five trays of three wines per tray, except in the final session, where assessors evaluated four trays of three wines per tray. The assessors were forced to have a 30 second rest between samples and a ten minute rest between trays. During the ten minute break, assessors were requested to leave the booths. Samples were evaluated over five days of formal sessions.

Twenty four wines (from eight rootstocks with triplicate winemaking replicates) were evaluated during the study. Wines were presented to assessors three times. Hence, the assessors saw 72 samples in total. The intensity of each attribute was rated using an unstructured 15 cm line scale coded from 0 to 10, with indented anchor points of ‘low’ and ‘high’ placed at 10% and 90%, respectively. Data were acquired using Fizz sensory software (version 2.46 and 2.47B Biosystemes, Couternon, France).

5.2.14 Statistical methods

One way analysis of variance was applied to the grape juice and wine data for each scion variety with rootstocks as the main source of variation at each site using Genstat 10 Release 12.1-13.1(VSN International, Hemel Hempstead, UK) (Payne et

al. 2009). Where significant (P<0.05) within site rootstock effects were found, comparison between means at that site were made using the Fisher's protected least significant difference (LSD) test with 5% level of significance. Regression techniques were applied to examine specific relationships.

For sensory assessment, panel performance was assessed using Fizz, Senstools (OP&P, The Netherlands) and PanelCheck (Matforsk) software, and included analysis of variance for the effect of sample, judge and presentation replicate and their interactions, degree of agreement with the panel mean and degree of discrimination across samples. All judges were found to be performing to an acceptable standard. ANOVA was carried out in 2013 using JMP 5.0.1a (SAS Institute, Cary, NC) and in 2014 using Genstat 14.1.0, for the effects of rootstock, judge and rootstock*judge as main effects, with the model including the nested effects of winemaking replicate and presentation replicate. Following ANOVA, Fisher's protected least significant difference (LSD) value was calculated (P=0.05). Principal component analysis (PCA) was conducted on the mean values averaged over panellists and replicates, using the correlation matrix,.

5.3 Results

With the exception of soil variables, leaf area index, bunch weight, berry weight, yield and laminae ion concentrations, which are averaged for vintages 2012 and 2013, all other data are presented separately for each vintage.

Electrical conductivity of the 1:5 soil: water extract increased with depth, from 0.14 dS/m in the surface 10 cm to 0.32 dS/m at 60 cm. Soluble Na^+ concentrations (range 21.4 - 53.2 mg/L) exceeded soluble Cl^- concentrations (range 11.6 - 34.0 mg/L) by 56 to 84% in the 1:5 soil:water extracts. Soluble calcium (Ca^{2+}) concentrations (range 13.4 - 23.6 mg/L) exceeded soluble magnesium (Mg^{2+}) concentrations (mean 4.2 mg/L) range by 3.6 to 5.0 fold. Soluble potassium (K^+) concentrations were similar at the various depths (mean 4.4 mg/L). Sodium adsorption ratios increased with depth, from 1.30 in the surface 10 cm to 2.92 at 60 cm, and were under 3 at all depths. Values above 3 are indicative of a sodic soil (Cass 2002).

Based on means for the two seasons, leaf area index (LAI) values were high for Ramsey (1.32) and 140 Ruggeri (1.30) and low for M 6262 (0.71). LAI was moderate to high for 110 Richter (1.08) and moderate for 1103 Paulsen, 101-14, M 5489 and M 5512 (range 0.94 - 0.97). Bunch weights were high for Ramsey (124 g) and 140 Ruggeri (118 g), moderate to high for 1103 Paulsen (87 g), 110 Richter (92 g) and M 5489 (0.93 g), moderate for 101-14 (75 g) and M 5512 (76 g) and low for M 6262 (0.62 g).

Berry weights were higher for Ramsey (1.21 g) and 101-14 (1.24 g), with all other rootstocks giving berry weights in the range 1.07 to 1.15 g. Yield was highest for Ramsey (10.0 kg/vine), moderately high for 140 Ruggeri (8.1 kg/vine), moderate for 110 Richter (6.6 kg/vine), M 5489 (6.8 kg/vine) and M 5512 (5.7 kg/vine), moderately low for 1103 Paulsen (4.9 kg/vine) and 101-14 (4.6 kg/vine) and low for M 6262 (3.9 kg/vine).

Laminae ion concentrations at harvest are shown in Table 2. The best Cl^- excluders were 140 Ruggeri (0.19 % dw), 110 Richter (0.21 % dw), 101-14 (0.22 % dw) and 1103 Paulsen (0.23 % dw), followed by M 5489 and M 5512 (0.26 % dw). Rootstocks 140 Ruggeri, 110 Richter and 101-14 were all better Cl^- excluders than Ramsey (0.32 % dw). Shiraz on M 6262 accumulated significantly more Cl^- (0.99 % dw) than Shiraz on the other rootstocks (range 0.19 to 0.32 % dw) (Table 2). Shiraz laminae Na^+ concentrations were all low (range 0.13 to 0.15 % dw) (Table 2).

There was a clear distinction between rootstocks M 5489, M 5512 and M 6262 and the other rootstocks with regard to K^+ and Ca^{2+} concentrations in laminae. Lamina K^+ concentrations were lower in these rootstocks (range 0.60 to 0.69 % dw) relative to the other rootstocks (range 0.86 to 0.94 % dw), while lamina Ca^{2+} concentrations were higher in these rootstocks (range 3.40 to 3.60 % dw) than in the other rootstocks (range 2.55 to 2.94 % dw) (Table 2). Shiraz laminae Na^+ concentrations were all low (range 0.13 to 0.15 % dw) (Table 2). Laminae Mg^{2+} concentrations were within a relatively narrow range, from 0.25 % dw (140 Ruggeri, 110 Richter) to 0.33 % dw (M 6262) (Table 2).

Table 2: Mean Shiraz laminae ion concentrations at harvest. Values are means for two seasons 2012 and 2013; n = 22.

Rootstock	Specific ion (% dw)				
	Cl^-	Na^+	K^+	Ca^{2+}	Mg^{2+}
140 Ruggeri	0.19^{a}	0.13^{a}	0.92^{cd}	2.83^{cd}	0.25^{a}
1103 Paulsen	0.23^{a}	0.15^{c}	0.92^{cd}	2.61^{ab}	0.28^{b}
110 Richter	0.21^{a}	0.15^{c}	0.91^{cd}	2.55^{a}	0.25^{a}
Ramsey	0.32^{b}	0.13^{a}	0.86^{c}	2.94^{d}	0.29^{bc}
101-14	0.22^{a}	0.13^{a}	0.94^{d}	2.70^{bc}	0.28^{b}
M 5489	0.26^{ab}	0.14^{ab}	0.60^{a}	3.69^{f}	0.29^{bc}
M 5512	0.26^{ab}	0.14^{ab}	0.61^{a}	3.40^{e}	0.31^{cd}
M 6262	0.99^{c}	0.14^{ab}	0.69^{b}	3.62^{f}	0.33^{d}
$LSD_{Rootstock}$	0.07 ***	0.01 **	0.06 ***	0.14 ***	0.02 ***

** = $P< 0.01$; *** = $P< 0.001$; different superscript letters indicate significant differences between means.

5.3.1 Vintage 2012

Grape juice soluble solids at harvest ranged from slightly under target (24 ºBrix) for Ramsey, M 5489, 110 Richter, M 6262, M 5512 and 140 Ruggeri (23.1 to 23.9 ºBrix) to slightly over target (24.4 to 25.6 ºBrix) for 1103 Paulsen and 101-14 (Table 3). Grape juice pH was in the range 3.62 (110 Richter) to 3.76 (140 Ruggeri) (Table 3). Grape juice pH of Shiraz on 110 Richter and M 5489 (3.62 and 3.64, respectively) was lower than for Shiraz on Ramsey, 101-14 and 140 Ruggeri (range 3.74 to 3.76) Table 3). Grape juice titratable acidity was relatively high for 1103 Paulsen (5.49 g/L), lower for Ramsey (4.97 g/L) and intermediate for all other rootstocks (5.08 to 5.26 g/L) (Table 3).

Table 3: Shiraz grape juice total soluble solids (TSS), pH and titratable acidity (TA) at harvest in 2012; n = 22.

Rootstock	Parameter		
	TSS (°Brix)	pH	TA (g/L)
140 Ruggeri	23.9^{ab}	3.76^{b}	5.09^{a}
1103 Paulsen	24.4^{b}	3.68^{ab}	5.49^{b}
110 Richter	23.3^{a}	3.62^{a}	5.08^{a}
Ramsey	23.1^{a}	3.74^{b}	4.97^{a}
101-14	25.6^{c}	3.75^{b}	5.26^{ab}
M 5489	23.1^{a}	3.64^{a}	5.21^{ab}
M 5512	23.7^{ab}	3.68^{ab}	5.08^{a}
M 6262	23.6^{ab}	3.69^{ab}	5.14^{a}
$LSD_{Rootstock}$	0.9 ***	0.08 **	0.30 *

* = $P< 0.05$; ** = $P< 0.01$; *** = $P< 0.001$; different superscript letters indicate significant differences between means.

Grape juice Cl^- concentrations at harvest were high for M 6262 (293.8 mg/L) but relatively low for all other rootstocks (range 32.4 to 51.2 mg/L) (Table 4). There was no effect of rootstock on grape juice Na^+ concentrations (range 27.3 to 49.2 mg/L) or K^+ concentrations (range 1667 to 1829 mg/L) at harvest (Table 4).

Grape juice soluble Ca^{2+} concentrations varied over a wide range from 83.8 - 95.5 mg/L (140 Ruggeri and Ramsey) to 181.9 mg/L (M 6262), with M 5512 having a moderately high concentration (147.8 mg/L) and all other rootstocks intermediate (range 104.3 to 135.3 mg/L) (Table 4).

Grape juice Mg^{2+} concentrations also varied over a wide range from 107.8 - 113.7 mg/L (M 5489 and Ramsey) to 132.7 - 147.1 mg/L (140 Ruggeri, 1103 Paulsen and 101-14), with the other rootstocks intermediate (range 118.6 to 123.0 mg/L) (Table 4).

All wines were pH adjusted during fermentation to ideally be between approximately 3.55 and 3.65. There were no significant differences between wines from the various rootstocks in final wine pH (range 3.56 to 3.66) (Table 5). Similarly, rootstock type had no significant effect on final wine titratable acidity, with concentrations in the range 5.5 to 6.7 g/L (Table 5). Wine alcohol concentration was highest for Shiraz on 101-14 rootstock (14.5%) (Table 5), reflecting the highest grape juice total soluble solids for that rootstock (25.6 ºBrix) (Table 3). Similarly, second highest wine alcohol concentration was obtained with rootstock 1103 Paulsen (14.0%) (Table 5), which had the second highest grape juice total soluble solids at harvest (24.4 ºBrix) (Table 3). Wine from all other rootstocks had alcohol concentrations in the range 12.9 to 13.7% (Table 5).

Shiraz wine from rootstock M 6262 reached a relatively high Cl^- concentration of 482.4 mg/L. This was an increase of 1.65-fold over the Cl^- concentration in grape juice. Wine from Ramsey rootstock was next highest (171.6 mg/L), which was higher than for 1103 Paulsen, M 5489 and M 5512 (range 131.3 to 147.2 mg/L), while wine from 140 Ruggeri and 110 Richter were significantly lower (95.5 and 107.5 mg/L, respectively).

Shiraz wine Na^+ concentrations were all relatively low, in the range 26.4 to 36.5 mg/L. Shiraz on M 6262 had the highest wine K^+ concentration (1344 mg/L), while for the other rootstocks there was no significant difference in concentrations (range 911 to 1076 mg/L).

There was no effect of rootstock on wine Ca^{2+} concentrations (range 55.9 to 68.7 mg/L). Wine Mg^{2+} concentrations were highest from rootstock 101-14 (146.9 mg/L) and lowest from rootstock M 5489 (107.8 mg/L), with all other rootstocks intermediate (range 113.7 to 132.3 mg/L).

Table 4: Shiraz grape juice ion concentrations at harvest in 2012; n = 22.

Rootstock	Specific ion (mg/L)				
	Cl^-	Na^+	K^+	Ca^{2+}	Mg^{2+}
140 Ruggeri	32.4[a]	30.8	1829	83.8[a]	132.7[d]
1103 Paulsen	45.5[a]	37.3	1742	119.6[cd]	140.6[e]
110 Richter	41.5[a]	27.3	1717	104.3[bc]	123.0[c]
Ramsey	51.2[a]	30.0	1764	95.5[ab]	113.7[ab]
101-14	40.0[a]	31.4	1708	128.3[d]	147.1[f]
M 5489	43.7[a]	33.6	1667	135.3[de]	107.8[a]
M 5512	46.1[a]	36.3	1708	147.8[e]	118.6[bc]
M 6262	293.8[b]	49.2	1790	181.9[f]	122.1[c]
$LSD_{Rootstock}$	41.9 ***	ns	ns	17.8 ***	6.10 ***

*** = $P < 0.001$, ns = not significant; different superscript letters indicate significant differences between means.

Wine of Shiraz on 101-14 rootstock had highest colour density (11.64 au), total phenolics (47.95 au), total anthocyanins (661.8 mg/L) and ionised anthocyanins (113.6 mg/L) (Table 6). Colour densities of Shiraz on 101-14 and 1103 Paulsen (11.64 and 9.79 au, respectively) were higher than for the other rootstocks (range 7.68 - 8.89 au), probably reflecting riper fruit at harvest (Table 3). Apart from the higher values for Shiraz on 101-14, there were few major differences between the other rootstocks for total phenolics (range 38.01 to 42.25 au), total anthocyanins (range 562.9 to 596.6 mg/L) and ionised anthocyanins (range 70.5 to 92.1 mg/L) (Table 6). Wine colour hue was not affected by rootstock type (range 0.50 to 0.56 au) (Table 6).

Table 5: Shiraz wine pH, titratable acidity (TA) and alcohol in 2012; n = 3.

Rootstock	Parameter		
	pH	TA (g/L)	Alcohol (% v/v)
140 Ruggeri	3.58	6.6	13.7^{c}
1103 Paulsen	3.60	6.7	14.0^{d}
110 Richter	3.66	5.8	13.4^{b}
Ramsey	3.57	6.1	13.7^{c}
101-14	3.56	6.6	14.5^{e}
M 5489	3.62	5.8	13.7^{c}
M 5512	3.61	5.8	13.6^{bc}
M 6262	3.66	5.5	12.9^{a}
$LSD_{Rootstock}$	ns	ns	0.2 ***

*** = $P < 0.001$, ns = not significant; different superscript letters indicate significant differences between means.

Regarding the sensory analytical data, from Figure 1 it can be seen that Shiraz wine from rootstock M 6262 was scored highest for the *Salty* and *Viscosity* attributes and lowest for *Oak/wood* aroma, *Overall Fruit* flavour, *Red Fruits* (palate), *Hotness* and *Fruit Aftertaste*. The palate attribute, *Salty*, was scored significantly higher for M 6262 than for all other rootstocks (Figure 1).

Table 6: Shiraz wine colour density, colour hue, total phenolics and total and ionised anthocyanins in 2012; n = 3.

Rootstock	Colour density	Colour hue (abs. units)	Total Phenolics	Total Antho. (mg/L)	Ionised Antho. (mg/L)
140 Ruggeri	8.89^{bc}	0.54	40.44^{ab}	584.6^{a}	82.8^{ab}
1103 Paulsen	9.79^{c}	0.53	42.25^{b}	582.3^{a}	92.1^{b}
110 Richter	7.93^{ab}	0.56	41.29^{b}	596.6^{a}	70.5^{a}
Ramsey	8.03^{ab}	0.53	38.01^{a}	579.8^{a}	74.9^{a}
101-14	11.64^{d}	0.50	47.95^{c}	661.8^{b}	113.6^{c}
M 5489	8.74^{abc}	0.54	42.22^{b}	581.9^{a}	80.5^{ab}
M 5512	8.01^{ab}	0.55	39.20^{ab}	562.9^{a}	73.8^{a}
M 6262	7.68^{a}	0.56	41.58^{b}	589.2^{a}	71.0^{a}
$LSD_{Rootstock}$	1.16 ***	0.04 ns	3.05 ***	34.7 ***	16.6 ***

*** = $P< 0.001$, ns = not significant; different superscript = significant differences.

Wines from 101-14 rootstock were rated highest for *Oak/wood*, *Overall Fruit Flavour*, *Astringency*, *Hotness* and *Fruit Aftertaste* and lowest for *Red Fruits* aroma attributes. For *Red Fruits* (aroma and palate), 140 Ruggeri was rated highest (Figure 1). For *Opacity* (colour intensity), 101-14 wines were rated highest, followed by 1103 Paulsen, while Ramsey and M 5512 wines were rated lowest (Figure 1). For *Dark Fruits* aroma and palate attributes, 101-14 wines were rated highest, while for *Dark Fruits* aroma only, Ramsey wines scored lowest (Figure1).

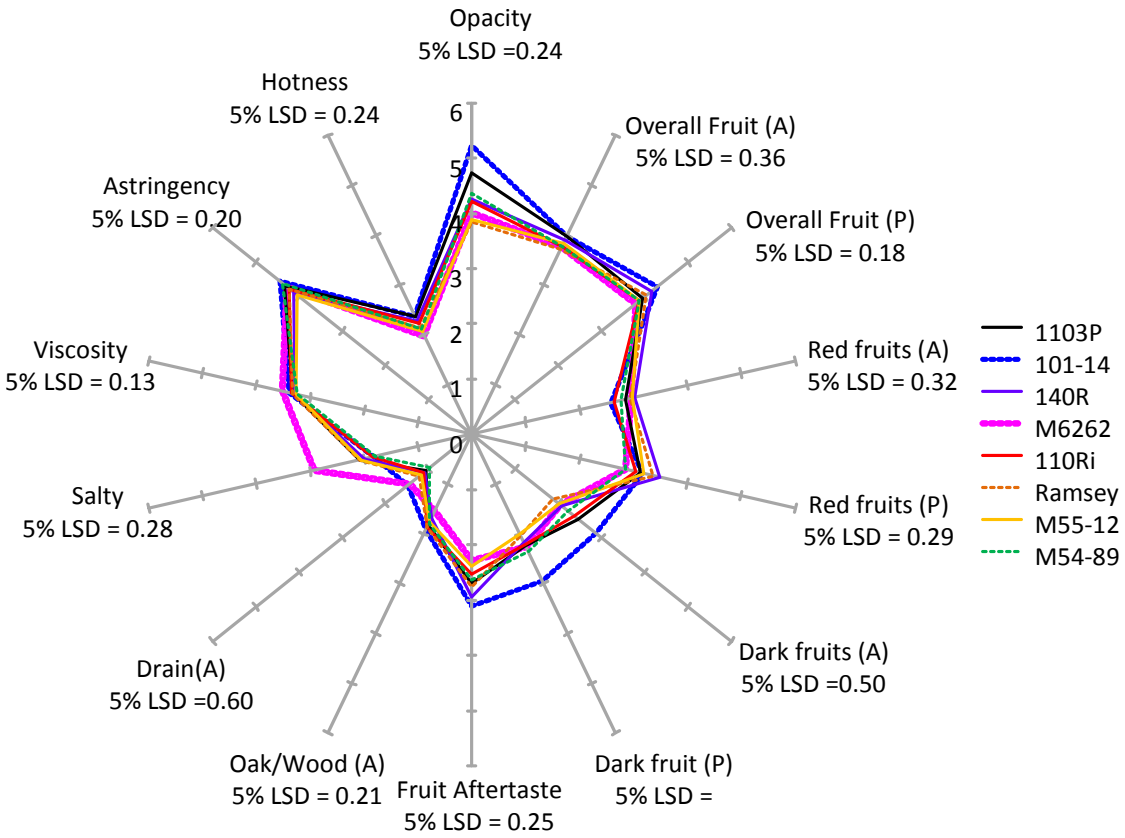


Figure 1: Mean scores for the significant sensory attributes for the Shiraz 2012 wines (n= 11 judges x 3 winemaking replicates x 3 presentation replicates).

5.3.2 Vintage 2013

Grape juice total soluble solids at harvest ranged from slightly under target (24 ºBrix) for M 6262 (23.1°Brix) to on target for M 5512 (24.1°Brix), slightly over target for M 5489 (24.4 °Brix) and 140 Ruggeri (24.6 °Brix) and over target for 110 Richter, Ramsey, 1103 Paulsen and 101-14 (25.1 to 26.2 ºBrix) (Table 7).

Grape juice pH was in the range 3.47 (110 Richter) to 3.59 (140 Ruggeri) (Table 7). Grape juice titratable acidity was relatively high for 110 Richter and 1103 Paulsen (6.25 to 6.40 g/L), lower for M 5512, Ramsey, M 5489 and 140 Ruggeri (5.27 to 5.42 g/L) and intermediate for M 6262 and 101-14 (5.86 to 5.90 g/L) (Table 7).

Table 7: Shiraz grape juice total soluble solids (TSS), pH and titratable acidity (TA) at harvest in 2013; n = 22.

Rootstock	Parameter		
	TSS (°Brix)	pH	TA (g/L)
140 Ruggeri	24.6^{cd}	3.59^{c}	5.42^{a}
1103 Paulsen	25.6^{ef}	3.48^{a}	6.40^{c}
110 Richter	25.1^{de}	3.47^{a}	6.25^{c}
Ramsey	23.5^{ab}	3.58^{c}	5.37^{a}
101-14	26.2^{f}	3.56^{bc}	5.90^{b}
M 5489	24.4^{c}	3.51^{ab}	5.40^{a}
M 5512	24.1^{bc}	3.56^{bc}	5.27^{a}
M 6262	23.1^{a}	3.50^{a}	5.86^{b}
$LSD_{Rootstock}$	0.62 ***	0.05 ***	0.22 ***

*= P<0.05; **=P<0.01; ***=P<0.001, ns=not significant; different superscript letters indicate significant differences between means. TSS= total soluble solids.

Grape juice Cl^- concentrations at harvest were high for M 6262 (141.3 mg/L) but relatively low for all other rootstocks (range 30.1 to 43.7 mg/L) (Table 8). Grape juice Na^+ concentrations were low, in the range 15.6 to 23.4 mg/L (Table 8). There was no effect of rootstock on grape juice K^+ concentrations at harvest (range 1063 to 1173 mg/L) (Table 8). Grape juice soluble Ca^{2+} concentrations varied over a wide range from 58.6 mg/L (140 Ruggeri) to 115.2 mg/L (M 6262), with all other rootstocks intermediate (range 64.9 to 95.5 mg/L) (Table 8). Grape juice Mg^{2+} concentrations also varied over a wide range, from M 5512 (79.7 mg/L), M 5489 (80.7 mg/L) and Ramsey (80.6 mg/L) all at the lower end, to 1103 Paulsen (100.5 mg/L) and 101-14 (105.9 mg/L) at the higher end, while all other rootstocks were intermediate (range 88.9 to 93.5 mg/L) (Table 8).

Table 8: Shiraz grape juice ion concentrations at harvest in 2013; n = 22.

	Specific ion (mg/L)				
Rootstock	Cl^-	Na^+	K^+	Ca^{2+}	Mg^{2+}
140 Ruggeri	30.3[a]	23.4[c]	1173	58.6[a]	88.9[b]
1103 Paulsen	34.1[a]	20.3[ab]	1063	88.8[bc]	100.5[c]
110 Richter	31.9[a]	19.1[ab]	1141	71.8[ab]	93.5[b]
Ramsey	42.7[a]	20.9[bc]	1112	64.9[a]	80.6[a]
101-14	33.0[a]	15.6[a]	1074	94.1[c]	105.9[c]
M 5489	30.1[a]	21.6[c]	1090	95.5[c]	80.7[a]
M 5512	43.7[a]	15.8[ab]	1142	86.7[b]	79.7[a]
M 6262	141.3[b]	23.3[c]	1109	115.2[d]	89.6[b]
$LSD_{Rootstock}$	23.5 ***	5.1 **	ns	17.4 ***	6.7 ***

** = $P < 0.001$; *** = $P < 0.001$; ns = not significant; different superscript letters indicate significant differences between means.

All wines were pH adjusted during fermentation, and ranged from 3.50 (M 6262) to 3.83 (1103 Paulsen), with all other rootstocks intermediate (range 3.66 to 3.77) (Table 9). Titratable acidities (TA) were in a slightly higher range (6.3 to 6.9 g/L) in 2013 than in 2012 (5.5 to 6.7 g/L) (Table 9). TA values in 2013 were at the lower end for M 5512 (6.3 g/L) and higher end for 140 Ruggeri (6.9 g/L), while other rootstocks were intermediate (range 6.5 to 6.7 g/L) (Table 9).

Wine alcohol concentration reflected grape juice TSS concentrations, with high values for 101-14 (16.0 %) and 1103 Paulsen (16.1 %) and a relatively low value for M 6262 (13.4 %). Wine from all other rootstocks had alcohol concentrations in the range 13.7 to 15.2% (Table 9).

Table 9: Shiraz wine pH, titratable acidity (TA) and alcohol in 2013; n = 3.

Rootstock	Parameter		
	pH	TA (g/L)	Alcohol (% v/v)
140 Ruggeri	3.77^{c}	6.9^{d}	15.2^{e}
1103 Paulsen	3.83^{d}	6.7^{c}	16.1^{f}
110 Richter	3.76^{c}	6.6^{b}	15.0^{e}
Ramsey	3.69^{b}	6.5^{b}	13.9^{c}
101-14	3.82^{d}	6.6^{b}	16.0^{f}
M 5489	3.66^{b}	6.5^{b}	14.5^{d}
M 5512	3.66^{b}	6.3^{a}	13.7^{b}
M 6262	3.50^{a}	6.7^{c}	13.4^{a}
$LSD_{Rootstock}$	0.04 ***	0.1 ***	0.2 ***

***=P<0.001; different superscript letters indicate significant difference between means

Shiraz wine from rootstock M 6262 reached a relatively high Cl^- concentration of 383.7 mg/L. This was an increase of 2.72-fold over the Cl^- concentration in grape juice, significantly higher than the increase (from grape juice to wine) recorded for the 2012 vintage (1.65-fold). Wine Na^+ concentrations were all relatively low, in the range 25.2 to 35.7 mg/L.

Shiraz on M 6262, M 5512 and M 5489 had lower wine K^+ concentrations (1074 to 1173 mg/L) than Shiraz on the other rootstocks (range 1259 to 1425 mg/L). There was no effect of rootstock on wine Ca^{2+} concentrations (range 58.8 mg/L to 72.9 mg/L).

Wine Mg^{2+} concentrations were highest from rootstocks 101-14 (132.9 mg/L) and 1103 Paulsen (126.4 mg/L) and lowest from rootstocks M 5489, M 5512 and Ramsey (94.4 to 97.2 mg/L), with all other rootstocks intermediate (range 101.2 to 108.2).

Wine colour density was highest for rootstocks 1103 Paulsen (12.75 au), 101-14 (12.60 au) and M 5489 (12.62 au) and lowest for M 6262 (7.90 au) (Table 10). The other rootstocks were intermediate (range 8.96 to 11.74 au) (Table 10).

Total phenolics were highest for 1103 Paulsen (55.97 au) and 101-14 rootstocks (56.74 au) and lowest for M 6262 (35.73 au), while all other rootstocks were intermediate (range 39.63 to 48.61 au) (Table 10).

Total anthocyanins were high for 101-14 (758.1 mg/L), 1103 Paulsen (740.6 mg/l), 140 Ruggeri (738.6 mg/L) and M 5489 (697.8 mg/L) and low for M 6262 (554.3 mg/L), with other rootstocks intermediate (622.5 to 672.0 mg/L) (Table 10).

Ionised anthocyanins were high for M 5489 (122.3 mg/L) and low for M 6262 (83.2 mg/L), Ramsey (86.3 mg/l) and 140 Ruggeri rootstocks (87.2 mg/L), with other rootstocks intermediate (range 105.4 to 112.0 mg/L) (Table 10).

Wine colour hue was relatively high (less bright) for 110 Richter (0.57 au), 140 Ruggeri (0.58 au), 101-14 (0.59 au) and 1103 Paulsen (0.60 au) and lower (brighter) for M 6262 (0.49 au), M 5512 (0.51 au), M 5489 (0.52 au) and Ramsey (0.53 au) (Table 10).

Table 10: Shiraz wine colour density, colour hue, total phenolics and total and ionised anthocyanins in 2013; n = 3.

Rootstock	Colour density	Colour hue (abs units)	Total Phenolics	Total Antho. (mg/L)	Ionised Antho.
140 Ruggeri	10.04^{c}	0.58^{de}	48.19^{c}	738.6^{ef}	87.2^{a}
1103 Paulsen	12.75^{f}	0.60^{f}	55.97^{d}	740.6^{ef}	110.1^{b}
110 Richter	11.74^{e}	0.57^{d}	48.61^{c}	672.0^{cd}	105.4^{b}
Ramsey	8.96^{b}	0.53^{c}	39.63^{b}	622.5^{b}	86.3^{a}
101-14	12.60^{f}	0.59^{ef}	56.74^{d}	758.1^{f}	110.8^{b}
M 5489	12.62^{f}	0.52^{bc}	47.79^{c}	697.8^{de}	122.3^{c}
M 5512	10.90^{d}	0.51^{b}	41.84^{b}	632.5^{bc}	112.0^{b}
M 6262	7.90^{a}	0.49^{a}	35.73^{a}	554.3^{a}	83.2^{a}
$LSD_{Rootstock}$	0.78 ***	0.01 ***	3.29 ***	48.7 ***	8.6 ***

***=*P*<0.001, ns=not significant; different superscript letters indicate significant differences between means .

Regarding the sensory data, from Figure 2 it can be seen that there were no significant differences between the rootstocks in the *Salty* taste of the Shiraz wines. This was despite Cl^- concentration in wine of Shiraz from M 6262 rootstock reaching 383.7 mg/L.

Viscosity of Shiraz wine from M 6262 rootstock was lower than for Shiraz wines from the other rootstocks (Figure 2).

Clear differences were observed among the rootstocks in wine *Opacity* (colour intensity) with 1103 Paulsen, highest, followed by 101-14 and 110 Richter. Fruit from these rootstocks was the ripest (grape juice TSS values in the range 25.1 to 26.2 ºBrix (Table 10) and with wine alcohol contents in the range 15.0 to 16.1(Table 12). Wine from these rootstocks also received higher ratings for the *Hotness*, *Viscosity*, *Bitter Taste*, *Dark Fruits* (aroma and palate), *Overall Fruit* intensity (aroma and palate), *Fruit Aftertaste* and *Vanilla* attributes, relative to the other rootstocks. Wine from Merbein 6262 was notably lower for *Opacity*, *Overall Fruit* intensity (aroma and palate), *Dark Fruits* (aroma and palate), *Fruit Aftertaste* and *Jammy/Cooked Fruit* (Figure 2**).**

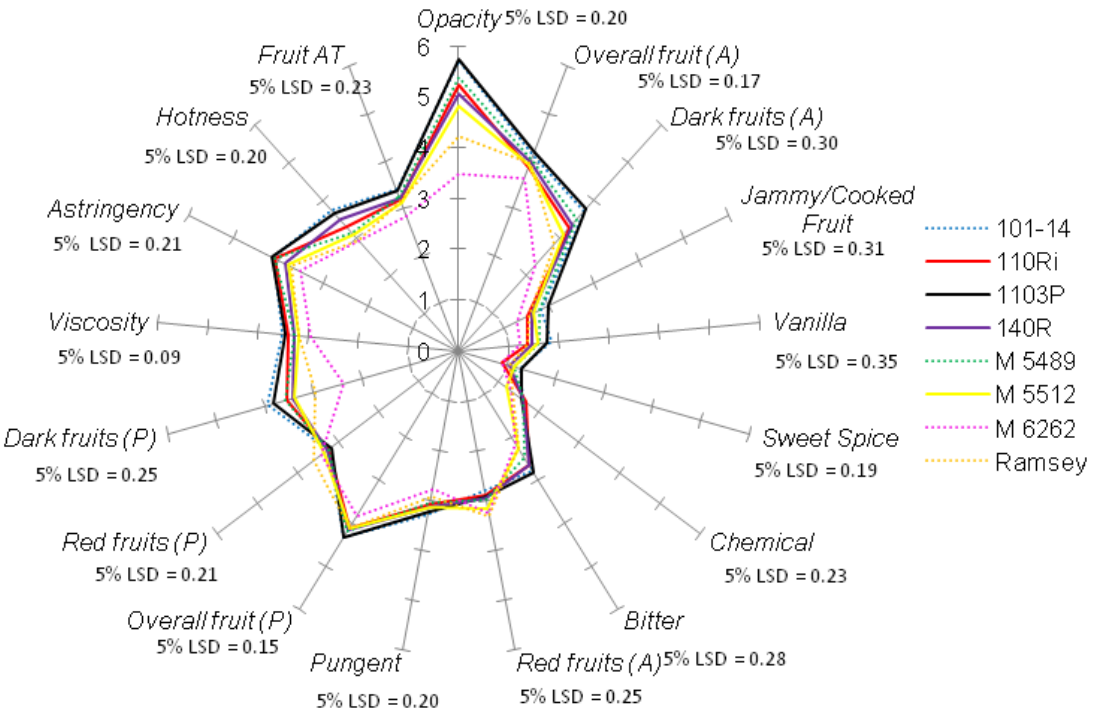


Figure 2: Mean scores for the significant sensory attributes for the Shiraz 2013 wines (n= 11 judges x 3 winemaking replicates x 3 presentation replicates).

5.4 Discussion

For the soil type at the site (sandy loam over clay), electrical conductivities of the 1:5 soil water extracts were moderate and not expected to result in significant reductions in available soil water (Lanyon 2011). Further, SAR of the 1:5 soil water extracts were below 3 and indicative of a non-sodic soil (Cass 2002). It is notable that while Na^+ concentrations in soil water extracts were higher than Cl^- concentrations, the reverse occurred in laminae and grape juice, i.e. higher Cl^- than Na^+ concentrations.

There was consistency of earlier ripening of Shiraz on rootstocks 101-14 and 1103 Paulsen and later ripening of Shiraz on M 5489 and M 5512 in both seasons, with a gap of approximately 12 days in 2012 and 5 days in 2013. These differences did not appear to be related to crop load. Krstic and Hannah (2000) reported early ripening characteristics of Shiraz, Chardonnay and Cabernet Sauvignon on rootstock 101-14 in the first year of assessment in a rootstock trial in Sunraysia. However, this was just one year of results and the following years may have differed. Furthermore, there was no consistent evidence for early ripening of Shiraz on 101-14 at Koorlong or Merbein, Victoria (Walker et al. 2000). The relatively low amounts of applied irrigation water with a shallow rooted rootstock variety such as 101-14 (Nicholas 1997) may be a factor in its earlier ripening and relatively low yields at the Padthaway site at which the current work was conducted.

Contrary to expectations, grape juice K^+ and pH was not markedly different between rootstocks M 5489, M 5512 and M 6262 and the 5 other rootstocks in both years of the study. The three rootstocks M 5489, M 5512 and M 6262 had been selected for capacity to accumulate lower K^+ in grape juice compared with other rootstocks, based on trials conducted in a warm irrigated region (Walker and Clingeleffer 2009). However, laminae of these three rootstocks when sampled at harvest contained significantly less K^+ and more Ca^{2+} than the other rootstocks. Thus the lower K phenotype was expressed in the laminae but not in the grape juice in this trial. Reasons for the difference are not known, but the result highlights that there is not necessarily a strong link between lamina K^+ and grape juice K^+ at harvest.

On the other hand there is a strong link between lamina Cl^- and grape juice Cl^- concentration at harvest, as shown by the consistently higher concentrations of Cl^- in laminae and grape juice of Shiraz on Merbein 6262 in both seasons. This rootstock was a poor Cl^- excluder relative to the other rootstocks. The increase in Cl^- concentration in progressing from grape juice to wine of Shiraz from this rootstock ranged from 1.65-fold (in 2012) to 2.72-fold (in 2013) and reflects extraction of Cl^- from grape skins during the fermentation process. On-average, this is higher than the value obtained (1.7-fold) in earlier studies (Walker et al. 2010). The increase in concentration of Na^+ in progressing from grape juice to wine was (on average) 1.1 fold, significantly lower than the increase in Cl^- concentration. This lower increase in Na^+ concentration (grape juice to wine) relative to Cl^- concentration reflects the lower concentrations of Na^+ relative to Cl^- in grape skin (Gong et al. (2010), with the consequence that less Na^+ than Cl^- is extracted into must during fermentation of red wine varieties.

Wine Cl^- concentration of 482 mg/L in 2012 was sufficient for detection of *Salty* taste, while wine Cl^- concentration of 384 mg/L in 2013 was insufficient for detection of *Salty* taste. Wine Na^+ and K^+ concentrations were not greatly different between the seasons, with values of 26.4 and 1344 mg/L, respectively, in 2012, and 30.4 and 1173 mg/L, respectively, in 2013. This suggests a relatively small change in concentration of wine Cl^- from 384 to 482 mg/L was the primary determinant of *Salty* taste in these wines. Wine Na^+ concentrations were relatively low for Shiraz on all rootstocks in both seasons, indicating that in this case there was a tighter link between Cl^- and *Salty* taste than with Na^+. K^+ concentrations in wine of Shiraz on M 6262 were also higher than in Shiraz wine from all other rootstocks in 2012 and may have contributed to *Salty* taste in that year. However, K^+ concentrations in wine of M 6262, M 5489 and M 5512 were lower than in wines from the other rootstocks in 2013, indicating that wine K^+ concentrations are influenced by a wide range of factors (Walker et al. 2012).

Salt tolerance of rootstocks is generally assessed on the basis of scion yield, and there were marked differences in yield performance for the various rootstocks. Ramsey rootstock led to highest yields followed by 140 Ruggeri, with 1103 Paulsen, 101-14 and M 6262 having relatively low yields. In general, higher yields were

associated with higher leaf area index (LAI), for example, for Ramsey and 140 Ruggeri, and lower yield with lower LAI, for example, for M 6262. Previous studies (Walker et al. 2002; Walker et al. 2010) have similarly demonstrated a link between yield and vine size as measured by pruning weight.

For the first time we explored a new way of assessing the performance of rootstocks under saline situations. We calculated a salt tolerance index (STI) as follows:

$$STI = \frac{\text{Yield x Wine colour density}}{\text{LAI x Juice } Cl^- \text{ x Juice } Na^+} \times 100$$

While yield and LAI were linked in this study, we used yield divided by LAI on the basis that good yield without excessive canopy cover was a desirable attribute, with high wine colour density a further desirable attribute, and high juice Cl^- and Na^+ concentrations as least desirable attributes. Using this method, three rootstocks 140 Ruggeri, 110 Richter and M 5489 had a relatively high STI values (6.96 - 7.60). Rootstock M 6262 had a very low STI (0.54) due mainly to its relatively high accumulation of Cl^- in grape juice. Rootstocks 1103 Paulsen, Ramsey, 101-14 and M 5512 had moderate to high STI values (range 4.83 - 5.65).

Wines from the various rootstocks were clearly different in sensory attributes in both seasons. In 2012, the riper fruit from 101-14 and 1103 Paulsen rootstocks generally resulted in wine that had higher colour intensity, *Dark Fruit*, *Hotness*, *Overall Fruit* and *Fruity Aftertaste*. Similarly, the riper fruit of 1103 Paulsen and 101-14 in 2013 produced wine that had higher colour densities, *Dark Fruit*, *Opacity*, *Hotness*, *Overall Fruit*, *Overall Fruit Intensity* and *Fruity Aftertaste* in comparison with wine from the other rootstocks. It was notable that for 2013 wines, when adjusted for alcohol content, many of the significant rootstock effects on sensory attributes were no longer significant. The only remaining attributes significantly affected by rootstock were *Astringency*, *Stalky*, *Dark Fruits* aroma and *Red Fruits* aroma.

6. Thresholds for salty taste detection in wine in relation to concentrations of Na^+, Cl^- and K^+.

6.1 Objective

- Examine thresholds for salty taste detection in relation to concentrations of Na^+, Cl^- and K^+ in wine.

6.2 Materials and Methods

6.2.1 Plant material and experimental design

The Chardonnay and Shiraz sites used in this study were located in a commercial vineyard in South Australia at Padthaway (36º38'45"S, 140º31'0"E – Shiraz; 36º38'44"S, 140º30'42"E – Chardonnay) (Walker et al. 2010). The trials were planted in 1992 as complete randomised blocks with 1-year old vines. Main treatments were scion varieties i.e. Shiraz and Chardonnay (*Vitis vinifera*) on their own roots or grafted to rootstocks 140 Ruggeri (*V. cinerea* var. *helleri* 'Resseguier #2 *x V. rupestris* 'St. George'), K51-40 (*V. champinii* 'Dogridge' x *V. riparia* 'Gloire'), Schwarzmann (*V. rupestris* x *V. riparia*), 1202 Couderc (*V. vinifera* 'Mourvedre'x *V. rupestris* 'Martin'), referred to in the text as 1202C, and Kober 5BB (*V. cinerea* var. *helleri* 'Resseguier #2 x *V. riparia* 'Gloire de Montpellier'). Chardonnay clone I10V1 was used, and for Shiraz, clone BGVSS Cl.30 was used. There were ten blocks. Each treatment was replicated once and randomised within each block.

6.2.2 Trellis type and spacings between rows and vines

Trellis type was vertical with fruiting wires at 1.2 and 1.5 m, with 2.75 m between rows and 1.8 m between vines. All vines were trained as quadrilateral cordons and mechanically pre-pruned with hand spur finish to maintain approximately 100 buds per vine.

6.2.3 Soil

Soil at Padthaway was uniform shallow brownish gravelly calcareous mottled clay (Alf Cass and Cliff Hignett, unpublished data, 1999).

6.2.4 Weather data

Weather data were obtained from the Australian Bureau of Meteorology station at Padthaway, which was approximately 5 km away from the vineyard. Rainfall (July 2010 to June 2011) was 700.5 mm, well above the long term average of 462 mm.

6.2.5 Irrigation practices

Irrigation was applied via drippers with flow rate of 3.5 L/h. Spacing between drippers was 60 and 90 cm for Chardonnay and Shiraz, respectively.

Soil water monitoring to assist irrigation scheduling for Chardonnay and Shiraz vines was carried out using a capacitance probe (EnviroSCAN RT-5, Sentek Sensor Technologies, Stepney, South Australia, Australia) with the lowest sensor at 90 cm depth (Chardonnay) and 100 cm depth (Shiraz) within the drip line. For Chardonnay vines, irrigation was not normally applied until approximately the middle of November and thereafter once the Readily Available Water (RAW) in the soil profile had been used (Table 2, Tregeagle et al. 2006). For Shiraz vines, irrigation was not normally applied until the middle of December, and with the exception of a period of regulated deficit irrigation between fruit set and veraison, irrigations were applied thereafter once the RAW had been used. Winter rainfall was relied on at Padthaway to provide adequate leaching of salts from the soil profile. The sites were irrigated with bore water, with total irrigation water applied at the Chardonnay site being 1.70 ML/ha, and for the Shiraz site it was 0.70 ML/ha.

6.2.6 Composition of irrigation water

The irrigation water in 2010-11 had a mean EC of 1.5 dS/m and a SAR of 4.3. Mean concentrations of Cl^-, Na^+, K^+, Mg^{2+} and Ca^{2+} in the irrigation water were 10.87, 9.18, 0.12, 1.36 and 3.18 mM, respectively.

6.2.7 Harvest scheduling

Trials were harvested when juice soluble solids concentrations were as close as possible to 22 ^{o}Brix for Chardonnay and as close as possible to 24 ^{o}Brix for Shiraz. Harvest dates were 7 March 2011 for Chardonnay and 21-22 March 2011 for Shiraz. Samples of 80 berries per vine were taken at all sites on the day of harvest. Each 80-berry sample was obtained by taking 20 berries from each 'quarter' of the vine (left and right of the main trunk, and on each side); five berries were sampled from four bunches in each 'quarter', making a total of 16 bunches sampled per vine; for each bunch, two berries were sampled from the 'shoulders' of the bunch, two from the middle and one from the tail. All berry samples were placed in plastic bags and held in an ice-cooled insulated container for transport to the laboratory.

Berry samples were gently crushed with mortar and pestle to extract juice. The juice samples were then centrifuged (Beckman J25i; Beckman Coulter Inc., Fullerton, CA, USA) for 15 min. at 4^{o}C @ 5930 g. The clear juice was decanted and used immediately for measurement of total soluble solids, pH and titratable acidity. The samples were then frozen for later analysis of ions.

6.2.8 Total soluble solids, pH and titratable acidity

Fresh grape juice samples were analysed for total soluble solids (^{o}Brix) with a digital refractometer (PR-1, Atago Co Ltd, Japan). Measurement of pH and titratable acidity was made using an autotitrator (Metrohm Ltd., Herisau, Switzerland).

6.2.9 Fermentation

Small-scale wines were made from Chardonnay and Shiraz on own roots and on 140 Ruggeri, K51-40, 1202C and Kober 5BB rootstocks. Small scale wines were included for Chardonnay on Schwarzmann, but not for Shiraz on Schwarzmann. Duplicate fermentations were made.

Fruit from each rootstock was harvested into plastic trays. Winemaking replicates consisted of approximately 10 kg of fruit. Wines were made at the experimental winemaking facility at CSIRO, Merbein, Victoria. Fruit for each treatment was delivered to the winery and cooled to 1 ºC overnight prior to crushing to minimize

oxidation. A crusher/de-stemmer was used (Zambelli Enotech s.r.l, Italy) with adjustable rollers, and must transferred to suitably sized glass fermenters. Must pH was adjusted to 3.4-3.6 and potassium metabisulphite added to give 20-25 ppm free SO_2. For Chardonnay, wines were immediately pressed off skins using a small batch Willmes centre membrane pneumatic press and a pectinase addition from *Aspergillus aculeatus* supplied by Sigma was made to assist juice settling. Inoculation was carried out with rehydrated yeast (Lalvin EC 1118, Lallemand Australia) at the rate of 0.25 g/L for Chardonnay and 0.20 g/L for Shiraz, calculated at 600 mL/kg of fruit weight. Fermentation was initiated and temperature controlled at 18 ºC, with diammonium phosphate (DAP) added at the rate of 0.15 g/L. Fermentation lasted 14 days, with caps (Shiraz) managed by plunging twice daily, and fermentation progress checked daily using an Anton Parr Density Meter. For Shiraz ferments, skins were pressed off at approximately 2 ºBaume using a small batch 50 kg air bag press (Willmes Australia) with a pneumatic center membrane, with pressure reaching 6 bar for one 'press without crumble' cycle. Once sugars were less than 2 g/L, measured using dextrose tablets, wine was racked off gross lees and SO_2 adjusted to 30 mg/L as free SO_2. Copper sulphate (0.4% solution) was also added at a rate of 1 mL/L post first racking into full glass containers sealed with a bubble trap. After two weeks of settling at 18ºC, a second racking was performed and SO_2 and pH checked and further adjusted as required. Wines were subsequently cold stabilised (1 ºC) for a minimum of 3 weeks prior to being racked and filtered through a Millipore filter housing (125 mm). The wines were filtered through a series of fine pads (3.1 µm, 2.5 µm, 1.1 µm and 0.7 µm, for Chardonnay and Shiraz, plus 0.45 µm for Chardonnay only) followed by bottling into screw cap sealed 375 ml bottles, using N_2 in the ullaged headspace. CO_2 was used as a pre-fill gas at all times during processing, and all vessels and equipment were fully cleaned and sanitized. Copper sulphate additions were made at bottling as required based on copper fining sensory tests prior to bottling. Wines were stored at 15-16ºC until required.

6.2.10 Ion concentrations in grape juice and wine

Chloride: Silver ion titration was conducted with a chloridometer (Model 442-5150, Digital Chloridometer, Lenexa, Kansas, USA), using juice and wine samples (1 mL) following addition of 3 mL of acid reagent (100 mL glacial acid and 6.4 mL concentrated nitric acid per litre) and four drops of gelatin. The gelatin reagent was added just prior to analysis.

Cations, phosphorous and sulphur: Samples were processed and analysed as described in Section 5.2.11.

6.2.11 Red wine colour and phenolics measures

Spectral characteristics of the red wines were evaluated approximately five months after bottling in October 2011 according to the method of Somers and Evans (1977) using a GBC 918 UV/vis spectrophotometer (GBC Scientific Equipment Pty Ltd, Dandenong Australia).

6.2.12 Descriptive sensory analysis

Preliminary assessment of the wines from 2011 by experienced personnel, including AWRI and CSIRO Plant Industry personnel, indicated that the wines were suitable for formal descriptive analysis and that replicates from each treatment would be included in the study, being free of winemaking related off-flavours.

Descriptive sensory analysis of the wines using a consensus based approach was undertaken by members of the AWRI descriptive sensory panel in March 2012, with 12 assessors, four male, eight female for Chardonnay wines and five male, seven female for the Shiraz wines. All assessors had extensive wine sensory descriptive experience. For each study, assessors attended three training sessions to determine and define appropriate descriptors for formal assessment of the wines. During these sessions the assessors evaluated wines from the study which represented the full range of sensory properties. Wines were initially assessed by appearance, aroma and palate, however for the Chardonnay study, the differences in appearance were minimal, and no appearance attribute was assessed formally. Following the first training session, standards for aroma attributes and basic tastes were presented.

Aroma standards were also available for assessors during the formal assessment sessions. Additional training in basic tastes was provided for the assessors. It included ranking of salty, sweet, acid and bitter solutions, as well as tasting of wines with varying chloride concentrations, including some from the study. To avoid bias in their responses, assessors were not informed that salt was the focus of the study. A practice rating session was conducted for assessors. Attribute lists were then refined. Formal sessions were conducted as described above.

6.2.13 Statistical methods

One way analysis of variance was applied to the grape juice and wine data for each scion variety with rootstocks as the main source of variation at each site using Genstat 10 Release 12.1 - 13.1 (VSN International, Hemel Hempstead, UK) (Payne et al. 2009). Where significant (P<0.05) within site rootstock effects were found, comparison between means at that site was made using the Fishers protected least significant difference (LSD) test with 5% level of significance, or better. Regression techniques were applied to examine specific relationships.

6.3 Results

The 2011 season was impacted by very high rainfall, July 2010 to June 2011, of 700.5 mm, which was 52% higher than the long term average of 462 mm. Significant rainfall was experienced during the harvest period and bunch rot was a significant threat. Both the Chardonnay and Shiraz were harvested a little earlier than usual because of the bunch rot threat.

The wet season also resulted in greater leaching of root zones than would normally be the case. Despite this, the primary objective of achieving a ‘natural series’ of salt concentrations in grape juice from relatively low (under 50 mg/L Cl^-) to relatively high (around 270 mg/L Cl^-) was achieved.

6.3.1 Chardonnay

Chardonnay on K 51-40 and 140 Ruggeri rootstocks had higher berry weights (1.37 and 1.35 g, respectively) than on own roots (1.28 g) and on 1202C rootstock (1.29 g), but had similar berry weights to Chardonnay on Schwarzmann and Kober 5BB rootstocks (1.33 and 1.34 g, respectively) (Table 11). Yield of Chardonnay on K 51-40 (2.5 kg/vine) was significantly lower than for Chardonnay on own roots and on the other rootstocks (range 11.6 to 15.8 kg/vine), which were not significantly different from each other (Table 11).

Grape juice total soluble solids, pH and titratable acidity were not significantly different between grafted and ungrafted Chardonnay, with means of 21.4 ºBrix, 3.37 and 7.61 g/L, respectively (Table 12).

Grape juice Cl^- concentration was highest for Chardonnay on K 51-40 rootstock (272.4 mg/L), intermediate for Chardonnay on Kober 5BB rootstock (100 mg/L), while Chardonnay on own roots (51.5 mg/L), 140 Ruggeri (29.6 mg/L) and Schwarzmann (42.2 mg/L) were significantly lower (Table 12). Grape juice Na^+ concentration was also highest for Chardonnay on K 51-40 rootstock (197.4 mg/L), with no significant differences between Chardonnay on own roots and the remaining rootstocks (range 26.3 to 109.7 mg/L) (Table 12).

Table 11: Chardonnay berry weight and yield at harvest in 2011; n = 10.

	Parameter	
Rootstock	Berry wt. (g)	Yield (kg/vine)
Own roots	1.28^{a}	14.4^{b}
140 Ruggeri	1.35^{c}	11.6^{b}
Schwarzmann	1.33^{abc}	13.3^{b}
K51-40	1.37^{c}	2.5^{a}
1202C	1.29^{ab}	15.8^{b}
Kober 5BB	1.34^{bc}	11.7^{b}
LSD_R	0.05	4.4
	*	***

* = $P< 0.05$; *** = $P< 0.001$; ns = not significant; different superscript letters indicate significant differences between means.

Table 12: Chardonnay grape juice total soluble solids (TSS), pH, titratable acidity (TA), Cl^- and Na^+ concentrations at harvest in 2011; n = 10.

Scion	Rootstock	TSS (°Brix)	pH	TA (g/L)	Cl^- (mg/L)	Na^+ (mg/L)
Chardonnay	Own roots	21.2	3.39	7.81	51.5[a]	26.3[a]
	140 Ruggeri	21.4	3.35	7.59	29.6[a]	37.3[a]
	K51-40	21.7	3.39	7.63	272.4[c]	197.4[b]
	Kober 5BB	21.6	3.35	8.03	100.0[b]	109.7[ab]
	Schwarzmann	21.5	3.41	7.34	42.2[a]	66.5[a]
	1202C	21.2	3.33	7.25	59.8[ab]	54.5[a]
	LSD_R	ns	ns	ns	40.7	98.5
					***	*

* = *P*< 0.05; *** = *P*< 0.001; ns = not significant; different superscript letters indicate significant differences between means.

Table 13: Chardonnay wine pH, titratable acidity (TA), alcohol, volatile acidity, free and total SO_2 at bottling in 2011; n = 2.

Rootstock	pH	TA (g/L)	Alcohol (% v/v)	Volatile Acidity (g/L)	Free SO_2 (mg/L)	Total SO_2 (mg/L)
Own Roots	3.27	8.15	12.87[a]	0.40	31.0	113.5
140 Ruggeri	3.25	7.81	12.85[a]	0.39	31.5	125.0
K51-40	3.26	7.92	12.58[b]	0.37	31.0	117.5
Kober 5BB	3.21	7.93	12.85[a]	0.40	31.0	116.0
Schwarzmann	3.26	8.16	12.97[a]	0.37	32.5	116.0
1202C	3.18	7.54	12.86[a]	0.40	29.5	106.0
Significance	ns	ns	*	ns	ns	ns
5% LSD			0.15			

* = *P*< 0.05; ns = not significant; different superscript letters indicate significant differences between means.

Wine alcohol concentration was lower for Chardonnay on K 51-40 rootstock (12.58 %) relative to Chardonnay on own roots and on each of the other rootstocks (range 12.85 to 12.97%) (Table13). There were no differences between Chardonnay on own roots and Chardonnay on each of the rootstocks for wine pH (mean 3.24), titratable acidity (mean 7.92 g/L), volatile acidity (mean 0.39 g/L), free SO_2 (mean 31.1 mg/L) and total SO_2 (mean 115.7 mg/L) (Table 13).

Wine of Chardonnay from K 51-40 rootstock had the highest wine Cl^- concentration (407.4 mg/L) with Kober 5BB intermediate (127.5 mg/L), while wine of Chardonnay from vines on own roots and on all other rootstocks had lower concentrations, in the range 41.8 to 72.8 mg/L.

Chardonnay on K 51-40 also had highest wine Na^+ concentration (374.2 mg/L), with Kober 5BB, Schwarzmann and 1202C intermediate (range 90.6 to 116.9 mg/L) and Chardonnay on own roots and 140 Ruggeri significantly lower (44.7 to 64.3 mg/L).

Chardonnay on K 51-40 similarly had highest wine Ca^{2+} concentration (73.9 mg/L) with Chardonnay on own roots and on other rootstocks in the range (51.8 to 62.8 mg/L). Furthermore, Chardonnay on K51-40 had a higher Mg^{2+} concentration (86.5 mg/L) than Chardonnay on own roots and on the other rootstocks (range 75.0 to 80.0 mg/L), with 140 Ruggeri intermediate (82.6 mg/L).

Chardonnay on own roots had highest wine K^+ concentration (584.3 mg/L) with Chardonnay on all rootstocks lower (range 427.6 to 507.4 mg/L). There were no significant differences between grafted and ungrafted vines for wine P and S concentrations (mean values of 92.0 and 139.1 mg/L, respectively).

Palate and aroma sensory attributes for the Chardonnay wines showed clear rootstock effects. For the *Salty* taste and *Viscosity* attributes, the K 51-40 wines were scored significantly higher than all others. Previous sensory studies have indicated that wines with elevated Cl^- and Na^+ concentrations can be *Viscous* or *Soapy* in mouth-feel (Walker et al. 2003).

For the *Citrus* attribute, Chardonnay on own roots and on two rootstocks (Schwarzmann and 1202C) were scored significantly higher than the wines from the other three rootstocks.

For *Acidity*, all wines were scored quite highly, with own roots scored highest and K 51-40 lowest. Since titratable acidity values were not different among the rootstocks, it is likely that the *Salty* taste in K 51-40 wine had a masking effect on *Acidity*.

Scores for *Honey* aroma and flavour were closely correlated, with an individual wine replicate of Kober 5BB rated highest. This attribute may be related to oxidative influences during winemaking or storage.

Principal component analysis (Figure 3) shows high PC2 scores for *Salty* taste and *Viscosity* and low *Acidity* for both replicates of K 51-40 wines. The high scores for *Salty* taste and *Viscosity* were linked with high wine Cl^-, Na^+, Ca^{2+} and Mg^{2+}, but not with wine K^+ (Figure 3).

Overall, the pairs of replicates for 140 Ruggeri, Kober 5BB and 1202C were separated from each other, while each of the other rootstock pairs were rated similarly (Figure 3).

For *Overall Fruit* aroma, Chardonnay on K 51-40 and Schwarzmann rootstocks were scored significantly higher than the other rootstocks. Schwarzmann was rated significantly higher in *Overall Fruit* flavour than all other rootstocks.

Wine of Chardonnay from own roots was scored significantly higher for *Acidity* than Chardonnay from grafted vines (Figure 3).

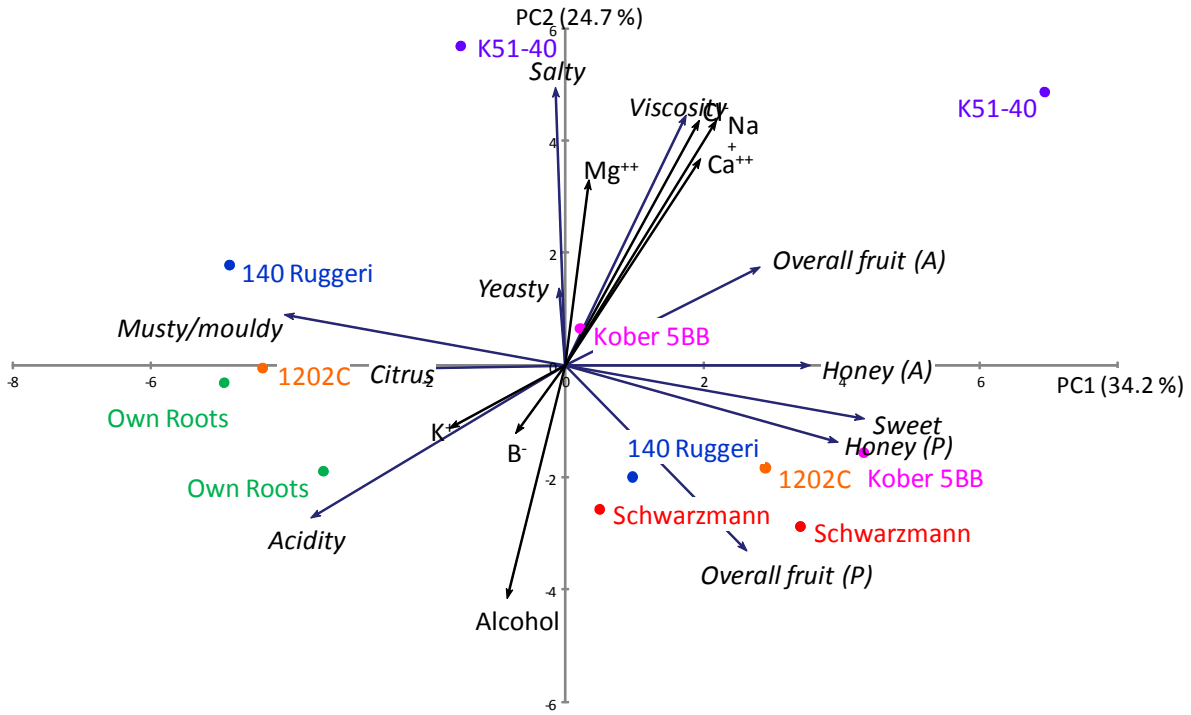


Figure 3: PCA biplot (PC1 and 2) for the winemaking replicates of the Chardonnay wines from the six rootstocks: sensory attributes with compositional measures included as supplementary variables.

6.3.2 Shiraz

Yield of Shiraz on K 51-40 (3.0 kg/vine) was similar to yield of Shiraz on own roots (3.5 kg/vine) and on Kober 5BB rootstock (3.4 kg/vine), but significantly lower than on 1202C and 140 Ruggeri rootstocks (both 5.3 kg/vine) (Table 14). Berry weight was similar between grafted and ungrafted vines (mean 1.54 g) (Table 14).

Shiraz grape juice soluble solids at harvest were lower for rootstock Kober 5BB (21.2 ºBrix) than for the other rootstocks (range 22.1 to 22.6 ºBrix) (Table 15). Wine of Shiraz from 140 Ruggeri rootstock had a higher pH (3.57) than wine from own roots, K 51-40 and 1202C rootstocks (range 3.49 to 3.52), while Kober 5BB had a similar wine pH (3.53) to 140 Ruggeri (3.57) (Table 15). Wine of Shiraz on 1202C rootstock had the highest titratable acidity (6.93 g/L), while Shiraz on 140 Ruggeri had the lowest (6.09 g/L), with other rootstocks in the range 6.31 to 6.74 g/L (Table 15).

Table 14: Shiraz berry weight and yield at harvest in 2011; n = 10

	Parameter	
Rootstock	Berry wt. (g)	Yield (kg/vine)
Own roots	1.55	3.5^{ab}
140 Ruggeri	1.44	5.3^{b}
K51-40	1.44	3.0^{a}
1202C	1.66	5.3^{b}
Kober 5BB	1.62	3.4^{ab}
LSD_R	0.20	1.9
	ns	*

* = *P*< 0.05; ns = not significant; different superscript letters indicate significant differences between means.

Table 15: Shiraz grape juice total soluble solids (TSS), pH, titratable acidity (TA) and Cl^- and Na^+ concentrations at harvest in 2011; n = 10.

		Parameter				
Scion	Rootstock	TSS (°Brix)	pH	TA (g/L)	Cl^- (mg/L)	Na^+
Shiraz	Own roots	22.3^{b}	3.49^{a}	6.31^{ab}	47.7^{ab}	56.8^{bc}
	140 Ruggeri	22.1^{b}	3.57^{b}	6.09^{a}	30.4^{a}	33.0^{ab}
	K51-40	22.1^{b}	3.52^{a}	6.74^{cd}	111.5^{c}	84.5^{c}
	Kober 5BB	21.2^{a}	3.53^{ab}	6.48^{bc}	30.9^{a}	17.2^{a}
	1202C	22.6^{b}	3.50^{a}	6.93^{d}	71.3^{b}	42.2^{ab}
	LSD_R	0.67	0.04	0.36	24.6	35.8
		*	*	*	**	*

* = *P*< 0.05; ** = *P*< 0.01; ns = not significant; different superscript letters indicate significant differences between means.

Shiraz on K 51-40 had highest concentration of grape juice Cl^- (111.5 mg/L) and Na^+ (84.5 mg/L), while for the other rootstocks, the range in concentrations was 30.4 to 71.3 mg/L for Cl^- and 17.2 to 56.8 mg/L for Na^+ (Table 15).

There was no difference between grafted and ungrafted vines in pH (mean 3.44), titratable acidity (mean 8.44 g/L), free SO_2 (mean 38.5 mg/L) or total SO_2 (mean 82.9 mg/L) of wines from ungrafted and grafted Shiraz vines (Table 16). Wine alcohol concentrations (Table 16) were significantly lower for Shiraz on 140 Ruggeri (12.14%) and Kober 5BB (11.65%) compared with the other rootstocks (range 12.66 to 12.95%).

Volatile acidity was slightly lower for Shiraz on own roots and 140 Ruggeri (both 0.18 g/L) compared with K 51-40, Kober 5BB and 1202C rootstocks (range 0.20 to 0.21 g/L) (Table 16).

Chloride concentration in Shiraz wine was highest from K 51-40 rootstock (274.1 mg/L). Next highest concentrations were from 1202C rootstock (177.2 mg/L) and Shiraz on own roots (154.4 mg/L). Concentrations in wine from 140 Ruggeri and Kober 5BB rootstocks were significantly lower (52.5 – 53.7 mg/L).

Sodium concentration in Shiraz wine was highest from K 51-40 rootstock (130.5 mg/L), followed by Shiraz on own roots (70.0 mg/L), while wine from rootstocks 140 Ruggeri, Kober 5BB and 1202C had much lower concentrations (20.5 to 44.5 mg/L).

There was no difference between grafted and ungrafted vines in concentrations of K^+ (mean 1084 mg/L), Ca^{2+} (mean 85.6 mg/L), Mg^{2+} (101.4 mg/L) and S (120.8 mg/L) in Shiraz wines. Concentrations of P in Shiraz wine were highest from 1202C rootstock (100.9 mg/L), intermediate for vines on own roots and on 140 Ruggeri rootstock (60.1 to 61.9 mg/L), and lowest for vines on Kober 5BB and K 51-40 rootstocks (33.3 to 44.6 mg/L).

Table 16: Shiraz wine pH, titratable acidity (TA), alcohol, volatile acidity, free and total SO^2 (TA) at bottling in 2011; n = 2.

Rootstock	pH	TA (g/L)	Alcohol (% v/v)	Volatile Acidity (g/L)	Free SO_2 (mg/L)	Total SO_2 (mg/L)
Own Roots	3.40	8.75	12.86^{a}	0.18^{b}	46.0	96.0
140 Ruggeri	3.49	8.17	12.14^{b}	0.18^{b}	32.0	73.0
K51-40	3.42	8.11	12.66^{a}	0.21^{a}	40.0	85.0
Kober 5BB	3.45	8.19	11.65^{b}	0.21^{a}	34.0	78.0
1202C	3.44	9.00	12.95^{a}	0.20^{ab}	40.5	82.5
Significance	ns	ns	**	*	ns	ns
5%LSD			0.38	0.012		

* = $P< 0.05$; ** = $P< 0.01$; ns = not significant; different superscript letters indicate significant differences between means.

Shiraz on Kober 5BB had lowest wine colour density (5.80 au), total anthocyanins (263 mg/L), ionised anthocyanins (43.1 mg/L) and total phenolics (28.5 au) and the highest colour hue (0.63 au) relative to vines on own roots and the other rootstocks, where mean values were 8.16 au (colour density), 461 mg/L (total anthocyanins), 68.3 mg/L (ionised anthocyanins), 39.9 au (total phenolics) and 0.54 au (colour hue) (Table 17). Values for all parameters for Shiraz on own roots and the other 3 rootstocks were all within 10% of the median value for own roots and the 3 rootstocks (Table 17).

For the sensory data, there was no significant difference between wines from own roots and the various rootstocks in the *Salty* taste attribute, indicating that the highest concentrations of Cl^- (274.1 mg/L) and Na^+ (130.5 mg/L) were below the sensory detection threshold for *Salty* taste for a red wine, even though K^+ concentrations in these wines were in the range 969 to 1174 mg/L.

There was however, a significant effect of rootstock (rootstock/s with highest scores in brackets) for the attributes *Opacity* (140 Ruggeri), *Dark Fruit* aroma (140 Ruggeri, followed by K 51-40 and 1202C), *Cooked Vegetable* aroma (Kober 5BB and own roots, followed by 1202C), *Viscosity* and *Hotness* (K51-40 and 1202C).

Table 17: Colour density, colour hue, total and ionised anthocyanins and total phenolics in Shiraz wines in 2011; n = 2.

Rootstock	Colour Density au	Colour Hue au	Total Antho. mg/L	Ionised Antho. mg/L	Total Phenolics au
Own Roots	8.45^{bc}	0.52^{a}	494^{c}	74.9^{b}	39.4^{b}
140 Ruggeri	8.82^{c}	0.55^{a}	429^{b}	68.7^{b}	40.6^{b}
K51-40	7.89^{bc}	0.53^{a}	434^{b}	67.2^{b}	38.7^{b}
Kober 5BB	5.80^{a}	0.63^{b}	263^{a}	43.1^{a}	28.5^{a}
1202C	7.46^{b}	0.54^{a}	487^{bc}	62.2^{b}	41.0^{b}
Significance	***	***	***	**	***
5%LSD	1.23	0.03	59	13.9	5.2

** = $P< 0.05$; *** = $P< 0.01$; ns = not significant; different superscript letters indicate significant differences between means.

As well as significant rootstock effects for *Opacity* and *Cooked Vegetable* attributes, there were significant winemaking replicate effects. For *Cooked Vegetable* aroma, replicate 1 of Kober 5BB and replicate 2 of 1202C were rated highest of all wines,

and significantly higher than the respective second replicate (Figure 4). This attribute may be related to sulphide aroma compounds such as hydrogen sulphide or methane thiol, produced during the winemaking process, or alternatively may arise from the grape berry. The compound dimethyl sulphide (DMS), largely grape derived, is often described as *Cooked Vegetable* at high concentrations, while at lower concentrations can give a blackcurrant-like aroma.

These relationships are also evident in the PCA sensory biplot with compositional variables included (Figure 4). For K 51-40, at least one replicate shows an association with high wine Cl^-, Na^+ and K^+ concentrations, with alcohol concentrations and with *Viscosity*. For 140 Ruggeri, it shows the association with *Dark Fruits* and with *Opacity*. For Kober 5BB, it shows the association with *Cooked Vegetable* attribute (Figure 4).

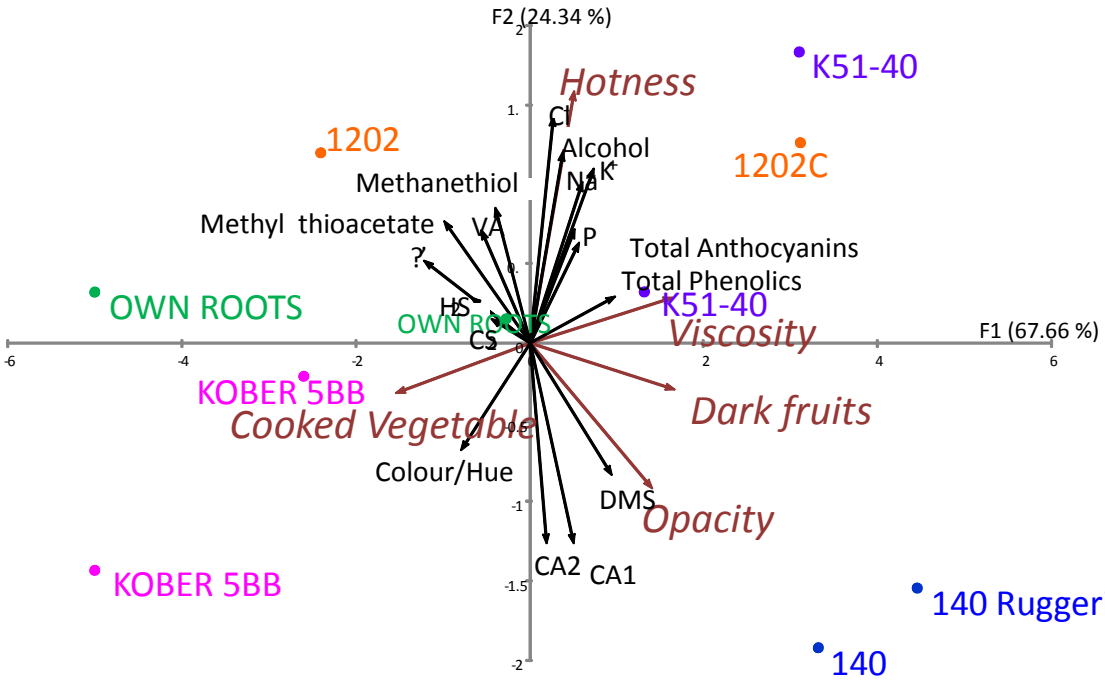


Figure 4: PCA biplot (PC1 and 2) for the winemaking replicates of the Shiraz wines from the five rootstocks: sensory attributes with compositional measures included as supplementary variables.

6.4 Discussion

Salty taste has been investigated in a number of studies, from those using aqueous solutions of standard salts for assessment by panels of tasters (Schiffman 2000), through to studies using wines containing various concentrations of salts, achieved naturally by growing vines in a salt affected region (Walker et al. 2003), or by addition of salts to wines to achieve desired concentrations for sensory assessment.

Wines from Mexico have been reported to have an average Na^+ concentration of around 158 mg/L, but Na^+ concentrations as high as 757 mg/L have been reported for a white wine and 1152 mg/L for a red wine (Cabello-Pasini et al. 2013). It was concluded that the *Salty* character described in some Mexican wines is, in part, the result of high concentrations of ions, especially Na^+. Wines from Brazil have also been described as having weak *Salty* characteristics (Miele and Rizzon 2011), but concentrations of ions in the wines were not provided.

The study of de Loryn et al. (2014) evaluated sensory thresholds and perception of NaCl in grape juice and wine. The NaCl taste detection threshold was determined to be the lowest concentration at which the subject could perceive a taste sensation, while the taste recognition threshold was the lowest concentration at which the subject could correctly identify the taste quality as *Salty*. For white and red wines, the median NaCl detection thresholds were found to be the same, with a mean of 0.305 g/L, respectively, which translates to 0.185 g/L or 185 mg/L Cl^-. The median NaCl recognition threshold values were higher than the median NaCl detection threshold values, and higher for a white wine (2.05 g/L NaCl or 1,240 mg/L Cl^-) than for a red wine (1.77 g/L NaCl or 1,074 mg/L Cl^-).

In the study of Scacco et al. (2010), wines containing 0.5 to 1.0 g/L NaCl were considered more *Salty, Soapy*, *Drying* and with *Lower Fruit Expression*. They also found that the increase in soil salinity enhanced colour intensity, purple reflexes, *Salty*, *Citrus*, and *Fruit* aroma. Furthermore, wines from the medium salinity (EC_e of 1.2 dS/m 0-55 cm depth and 2.1 dS/m for 56-105 cm depth) and high salinity (EC_e of 1.0 dS/m for 0-55 cm depth and 7.6 dS/m for 56 to 105 cm depth) soils were

preferred by wine experts, while those from negligible salinity (EC_e of 0.7 dS/m for 0-105 cm depth) were defined as flat and dull (Scacco et al. 2010).

6.4.1 Chardonnay

There were clear differences between the wines made from different rootstocks in our study, including differences in the *Salty* and *Viscous* attributes which are consistent with differences in Cl^- and Na^+ concentrations in wines from the various rootstocks. This in turn indicates differences in the capacity of certain rootstocks to restrict the root to shoot transport of Cl^- and its accumulation in the juice of developing grape berries. Rootstock K 51-40, for example, has poor capacity for Cl^- exclusion, while other rootstocks, such as 140 Ruggeri, have good capacity for Cl^- exclusion (Walker et al. 2010).

The higher rating for *Salty* and perhaps also *Viscous* in K 51-40 wines is consistent with earlier data in which *Salty* has been linked with *Soapy* palate attribute (Walker et al. 2003; Scacco et al. 2010). Furthermore, it links with data on Best Estimate Threshold (BET) values for NaCl and KCl in white wine, and shows that a Cl^- concentration of 407 mg/L exceeds the BET for white wine of 270 mg/L, expressed as the Cl^- concentration in wine. This is a little higher than the NaCl taste detection threshold determined as 185 mg/L for a white wine by de Loryn et al. (2014). The Na^+ concentration in K 51-40 wines was 374 mg/L, which was significantly higher than for wines from the other rootstocks, and may have also contributed to the *Salty* attribute in K 51-40 wines. *Salty* and *Viscosity* were both approximately equally correlated with Cl^- and Na^+ concentration ($r=0.823$, $P=0.001$, $n=12$).

The K 51-40 rootstock gave a wine that had relatively high *Overall Fruit* aroma and was lower in perceived *Acidity*. The low perceived *Acidity* may relate to the *Salty* taste attribute of the K 51-40 wine which may have had a masking effect on *Acidity*. There were further differences in fruit attributes such as *Overall Fruit* aroma and *Overall Fruit* flavour and *Citrus* among the rootstocks, with the wines from Schwarzmann rootstock rated particularly highly for these attributes.

6.4.2 Shiraz

There were no differences between wines of Shiraz on own roots and Shiraz on rootstocks in the *Salty* scores for these wines. The highest concentration of Cl^- in wine was 274 mg/L and the highest concentration of Na^+ was 114 mg/L, both recorded for Shiraz wine from K 51-40 rootstock, and both well below the Best Estimate Threshold (BET) level for red wine (700 mg/L) determined in earlier work at the AWRI. However, the wine Cl^- concentration of 274 mg/L exceeded the median NaCl detection threshold determined as 185 mg/L Cl^- for a red wine by de Loryn et al. (2014).

There were significant differences in sensory properties among Shiraz wines made from different rootstocks, with 140 Ruggeri wines having high scores for *Opacity*, *Dark Fruit* aroma and *Viscocity*. Wines from this rootstock were among the highest with regard to colour density, total and ionised anthocyanins and total phenolics. Kober 5BB produced wines that could be considered least desirable, having lower *Opacity* which reflected its lower wine colour density, lower *Dark Fruit* aroma and V*iscocity* scores, and higher C*ooked Vegetable* aroma. Although the perception of *Viscosity* is one of the parameters which may relate to higher salt concentrations in wines, there was no relationship between ion concentrations for Cl^-, Na^+ or K^+ and the *Viscosity* scores for these wines. As expected, *Hotness* was related to alcohol concentration, and correlated with K^+ in the wines, with alcohol and K^+ being positively closely related ($r=0.95$, $P<0.001$).

7. Establish test site for best CSIRO salt tolerant rootstocks

7.1 Objective

- Propagate the best CSIRO bred rootstocks from long term (14 year) salinity trials at Merbein (seasons 1995-96 to 2006-2007, EC_{iw} = 2.1 dS/m and total seasonal irrigation application = 7.15 ML/ha; seasons 2007-08 and 2008-09, EC_{iw} = 1.65 dS/m and total seasonal irrigation application = 3.04 ML/ha) and establish a test site with industry input for comparison with standard rootstocks. Final evaluation (2014-2017) of these rootstocks will carry forward into GWRDC project CSP 1304.

7.2 Materials and Methods

Two trials were established on a commercial vineyard at Padthaway, S. Australia.
1. Shiraz on own roots and grafted to 140 Ruggeri, and CSIRO rootstocks A5 and B7. This trial was planted in 2010, with replacements planted in 2011.
2. Pinot Gris on own roots and grafted to 140 Ruggeri, Ramsey and CSIRO rootstock B7. This trial was planted in 2012, with replacements planted in 2013.

Both trials were trained to develop bilateral cordons on a single fruiting wire at 100 cm from the ground with provision for vertical shoot positioning. Row and vine spacing was 3.5 m and 1.8 m, respectively. GroGuards aided establishment.

Vines were drip irrigated with drippers spaced 50 cm apart and flow rates of 2.67L/h. The drip line was situated 25 cm above ground level.

7.3 Results and Discussion

Earlier work had shown that CSIRO rootstocks A5 and B7 had demonstrated good capacity to limit the accumulation of Cl^- and Na^+ in grape juice at harvest. Rootstock trials were established in a different environment to the original trial site in order to

test their yield performance and Cl^- and Na^+ exclusion capacity. Yield analysis was not possible in 2013, but a preliminary analysis of Cl^-, Na^+, K^+, Ca^{2+} and Mg^{2+} concentrations in grape juice at harvest was made. Berry samples (100 per vine) were taken from fruiting vines on 5 March 2013 (140 Ruggeri) and on 11 March 2013 (B7 and Shiraz on own roots). Insufficient fruit of A5 were available in 2013, so data are only available for Shiraz on own roots and Shiraz on 140 Ruggeri and B7 rootstocks.

Table 18: Rootstock effects on Shiraz grape juice Cl^-, Na^+, K^+, Ca^{2+} and Mg^{2+} concentrations at harvest in 2013; n = 12.

Rootstock	Parameter					
	TSS (ºBrix)	Cl^-	Na^+	K^+ (mg/L)	Ca^{2+}	Mg^{2+}
Own roots	25.8[b]	97.2[b]	101.6[b]	1307[a]	130.8[b]	99.6
140 Ruggeri	24.9[a]	21.8[a]	47.2[a]	1560[b]	89.5[a]	108.6
B7	23.7[a]	33.9[a]	61.3[a]	1305[a]	78.4[a]	94.0
$LSD_{Rootstock}$	1.2 **	21.1 ***	18.9 ***	136 ***	18.3 **	ns

** = $P< 0.01$; *** = $P< 0.001$; different superscript letters indicate significant differences between means.

Similar concentrations of Cl^- and Na^+ were accumulated by 140 Ruggeri and B7, and both were significantly lower than Shiraz on own roots (Table 18). This suggests similar capacity of 140 Ruggeri and B7 rootstocks with regard to Cl^- and Na^+ exclusion from grape juice. Shiraz on own roots and on B7 rootstock has significantly lower grape juice K^+ concentrations than Shiraz on 140 Ruggeri. Grape juice Ca^{2+} and Na^+ were higher in own-rooted than in the grafted vines (Table 18).

8. Marker for chloride exclusion

8.1 Objective

- Using characterised rootstocks, advanced rootstock hybrids and a population of progeny segregating for salt tolerance, develop a marker for chloride exclusion for use in rapid screening for salt tolerance.

8.2 Materials and Methods

8.2.1 Rooted leaf propagation and establishment

The source of leaves for use in the experiments were 'stock plants' or container-grown grapevines of K 51-40 and 140 Ruggeri, established from cuttings and maintained as rooted vines in a glasshouse. Cabernet Sauvignon was included as a third cultivar in some experiments. The 'stock plants' were watered daily, initially with tap water, but later changed to rain water, after it was found that the tap water contained 3.2 mM Cl^-. The experiments involving rooted leaves for microarray and q RT-PCR analysis of candidate genes involved use of tap water for daily watering of stock plants, whereas the experiments to investigate cation accumulation by rooted leaves, Cl^- versus NO_3^- uptake and transport and the impacts on ion uptake of the Cl^- channel inhibitor, DIDS, involved use of rainwater for daily watering of stock plants. The plants were also given fortnightly applications via fertigation of Megamix Plus (Rutec Pty. Ltd., Tamworth, New South Wales) at a rate of 70 mLs and Iron Sodium EDTA (concentration 42.6 g/L) 25 mLs per 250 L. Typical analysis of this solution was macro elements in mM: K^+, Na^+, Ca^{2+}, Mg^{2+} and Cl^- respectively 1.3, 2.4, 1.5, 1.1 and 2.7 and micro elements in µM: Fe, B, respectively 25.2 and 3.4.

Mature leaves were excised from the 'stock plants' and the proximal ends of petioles were dipped in a hormone gel containing 1.5 g/L indole-3-butryic acid (Clonex Growth Technology, Taunton, Somerset, UK) before planting in propagation boxes (v:v) 50% vermiculite:50% perlite (Australian Vermiculite and Perlite Pty Ltd. Campbellfield, Victoria, Australia).

The propagation boxes (33 x 28 cm) were placed upon heat beds (30ºC) in a mini-shadehouse within the glasshouse. The mini-shadehouse was covered with 90% shadecloth and the leaves were misted with fine sprays of tap water (later changed to rain water) for 1 min every hour during the day between 7 am and 7 pm. About 3 weeks later, rooted leaves were transferred to aerated modified Hoagland #1 solution culture (Hoagland and Arnon 1938) prepared with Millipore water and containing the following nutrients (mM) for a 2-week pre-treatment period: KNO_3, 1.0: $Ca(NO_3)_2.4H_2O$, 1.0; $MgSO_4.7H_2O$, 0.4; KH_2PO_4, 0.2; H_3BO_3, 4.6 x 10^{-2}; $MnCl_2.4H_2O$, 9.1 x 10^{-3}; $ZnSO_4.7H^2O$, 7.6 x 10^{-4}; $CuSO_4.5H_2O$, 3.2 x 10^{-4}; $Na_2MoO_4.2H_2O$, 2.4 x 10^{-4}; EDTA-Fe-Na, 7.1 x 10^{-2}.

8.2.2 Cation accumulation by rooted leaves

This experiment was undertaken by Dr Haijun Gong as part of a previous GWRDC project (CSP0605). Details of the experiment were described in the final report for that project. In brief, following successful establishment of rooted leaves, including the two week pre-treatment as described above, the rooted leaves were subjected to control (nutrient solution only) or 25 mM Cl^- (Na^+:Ca^{2+}: Mg^{2+} = 6:1:1) in nutrient solution for up to 14 days. At each harvest (0, 7 or 14 days), seven uniform rooted leaves of K51-40 and 140 Ruggeri were separated into lamina, petiole and roots, dried, then stored for cation analysis.

8.2.3 Chloride versus nitrate uptake and transport

Following pre-treatment, the rooted leaves were subjected to control (nutrient solution only), 47 mM Cl^- (Na^+:Ca^{2+}: Mg^{2+} =5:1:1) in nutrient solution with standard NO_3^- concentration of 3 mM, and 47 mM Cl^- (Na^+:Ca^{2+}: Mg^{2+} =3.5:1:1) in nutrient solution with additional NO_3^- applied as $NaNO_3$ to give a final NO_3^- concentration of 12 mM. There were four biological replicates per genotype and treatment, each comprising two rooted leaves. All salt treated plants received 47 mM Cl^- on day 1 (no gradational increase in salinity). The treatments were applied for 4 days (31 January - 3 February). Laminae, petiole and roots were sampled for ion analysis.

8.2.4 Impact on ion uptake of the Cl^- channel inhibitor, DIDS

DIDS (4,4"-Diisothiocyanatostilbene-2,2'-disulfonic acid) is a non-specific inhibitor of Cl^- ion channels. Following pre-treatment, the rooted leaves were subjected to control (nutrient solution only), 50 mM Cl^- (Na^+:Ca^{2+}: Mg^{2+} =6:1:1) in nutrient solution without DIDS, and 50 mM Cl^- plus 70 µM DIDS for 4 days (25 January - 29 January). There were 3 biological replicates per genotype and treatment, each comprising a pooled sample of 5 rooted leaves. Salt treated plants received 50 mM Cl^- on day 1 (no gradational increase in salinity). Laminae, petiole and roots were sampled for ion analysis.

8.2.5 Rooted leaf experiments for microarray and q RT-PCR analysis of candidate genes

Two experiments were conducted using rooted leaves of 140 Ruggeri, K 51-40 and Cabernet Sauvignon. The first experiment involved a 'no salt' control treatment of nutrient solution only and a salt treatment of 25 mM Cl^- (Na^+:Ca^{2+}: Mg^{2+} =6:1:1) in nutrient solution. The second experiment involved a 'no salt' control treatment of nutrient solution only and a salt treatment of 50 mM Cl^- (Na^+:Ca^{2+}: Mg^{2+} =6:1:1) in nutrient solution.

All salt treated plants received 25 or 50 mM Cl^- on day 1 (no gradational increase in salinity). Experiment duration was 3 days (19 - 22 November for Experiment 1, and 21 to 24 January for Experiment 2). Each experiment involved 3 biological replicates per genotype and treatment, each comprising a pooled sample of 4 rooted leaves. Roots were sampled for RNA extraction. Laminae, petiole and roots were sampled for ion analysis

8.2.6 Plant harvest and sampling

Rooted leaves were washed, then blotted dry with paper towel. Total fresh weight of each individual whole rooted leaf was weighed and recorded. They were then dissected into roots, petiole and lamina, with each part weighed. The number of rooted leaves pooled in a replicate varied according to the experiment (minimum of 2 rooted leaves and maximum of 5 rooted leaves). The fresh weight of the pooled dissected component was weighed and recorded for each tissue component. The

root sample that was taken for RNA extraction was immediately frozen in liquid nitrogen. Lamina, petiole and root samples for ion analysis were placed in bags for later oven drying. The samples were dried in an oven at 60°C for at least 72 h then retained for Cl^- and cation analyses.

8.2.7 RNA extraction

Frozen whole roots of pooled rooted leaf material were ground to fine powder using liquid nitrogen and a mortar and pestle. A 0.1 g sample of frozen, ground roots was used to extract total RNA using the Spectrum Plant Total RNA Kit (Sigma) following manufacturer's procedures. Contaminating genomic DNA was removed from RNA samples by digestion with DNase I (Turbo DNA-free, Life Technologies) for 1 hour at 37 ^{o}C. RNA was then purified by precipitation at minus 80 ^{o}C in 5 volumes of 100 % ethanol (v/v) and 1/10 volumes of 3 M NaOAC for 16 hours. Following ethanol precipitation, RNA was pelleted, washed with 80 % ethanol and air dried. Dried RNA was re-suspended in nuclease free water and analysed on a NanoDrop 1000 spectrophotometer for quality and quantity. RNA integrity was measured on a Bioanalyzer 2100 (Agilent Technologies) at Adelaide Microarray Centre.

8.2.8 Quantitative real time PCR (q RT-PCR)

1 µg of purified, total RNA was reverse transcribed in a 20 µl reaction using iScript cDNA Synthesis Kit (Bio-Rad). For qRT-PCR reactions, cDNA was diluted 1:5. qRT-PCR was performed on a Bio-Rad iCycler. Reactions consisted of 250 nM forward and reverse primer, 1x KAPA SYBR FAST qPCR Master Mix (KAPA Biosystems), and 2 µl of diluted cDNA. Reactions were performed in triplicate following a two-step or three-step protocol. The three-step protocol consisted of 40 cycles of the following: 95 ^{o}C 15 sec, 56 ^{o}C 20 sec, 72 ^{o}C 10 sec (plus data acquisition). The two-step protocol was the same except the 72 ^{o}C step was excluded and data acquisition occurred at 56 ^{o}C. Melt curve analysis was performed by heating PCR products from 52 ^{o}C to 92 ^{o}C for 20 seconds increasing at 0.5 ^{o}C per cycle with continuous fluorescence detection. Relative expression was determined using the primer pair efficiency ($E = 10^{-1/slope}$) and the formula described by Pfaffl (2001), with the geometric mean of Actin, Ubiqutin and Ef1-α used as the reference for normalisation.

8.2.9 Microarray hybridisation

Custom 8 x 60K gene expression microarrays were designed using eArray (Release 7.6) (Agilent Technologies). 60-mer oligonucleotide probes were designed against 26,346 annotated *V. vinifera* transcripts from the 12x Genoscope build available from http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/. A total of 22 microarray hybridisations were performed. This consisted of 4 biological replicates for Cabernet Sauvignon (± 50 mM Cl^-), 4 biological replicates of K51-40 (± 50 mM Cl^-) and 3 biological replicates of 140 Ruggeri (± 50 mM Cl^-). Single colour labelling and hybridisations were performed at the Ramaciotti Centre for Gene Function Analysis.

8.2.10 Ion concentrations in plant tissues

Chloride concentrations: Dried samples were examined in duplicate for Cl^- concentrations by silver ion titration with a chloridometer (model 442-5150, Digital Chloridometer, Lenexa, Kansas, USA). Samples (50 mg for laminae, 25 mg for petioles and roots) were weighed into 4 mL glass vials and 3 mL of acid reagent (100 mL glacial acetic acid and 6.4 mL of concentrated nitric acid per litre) added and allowed to extract for 30 minutes, then four drops of gelatin reagent were added just prior to analysis.

Cations: Dried samples were examined in duplicate for cation (K^+, Na^+, Ca^{2+} and Mg^{2+}) concentrations. Extracts of approximately 100 mg powdered samples were weighed into 15 mL polypropylene digestion vessels (SC415) (Environmental Express, Mt Pleasant, SC, USA) and 2 mL of 15 M nitric acid was added. They were held in a fumehood overnight at room temperature. A disposable polypropylene (SC417) watch glass was added to each tube. The tubes were sequentially heated on a 96 well HotBlock Pro Digestion System (SC189-240) with a Pro Controller-Touch Screen (SC180-240) (Environmental Express, Mt Pleasant, SC, USA) increasing the temperature at 3ºC/min initially to 60ºC, with a holding period at 60ºC for 20 minutes, then at 85ºC for 60 minutes and subsequently at 85ºC for 210 minutes. Ninety six vessels were heated per batch. The tubes were allowed to cool, then made up to 15 mL with MilliporeR reverse osmosis water and then inverted to mix thoroughly. The solutions were then filtered through Whatman No 42 filter paper 125 mm. Aliquots of approximately 10 mL were transferred to polypropylene tubes

for cation analyses. Cation concentrations were measured by inductively coupled plasma-atomic emission spectrometry using appropriate standards for calibration and recalibration to correct for any drift that may occur during analytical runs.

8.2.11 Statistical methods

Means are presented with standard errors or ± standard errors in most Figures. In other cases, one way ANOVA was applied to the data. Genstat 10 Release 12.1-13.1 (VSN International, Hemel Hempstead, UK) (Payne et al. 2009) was used.

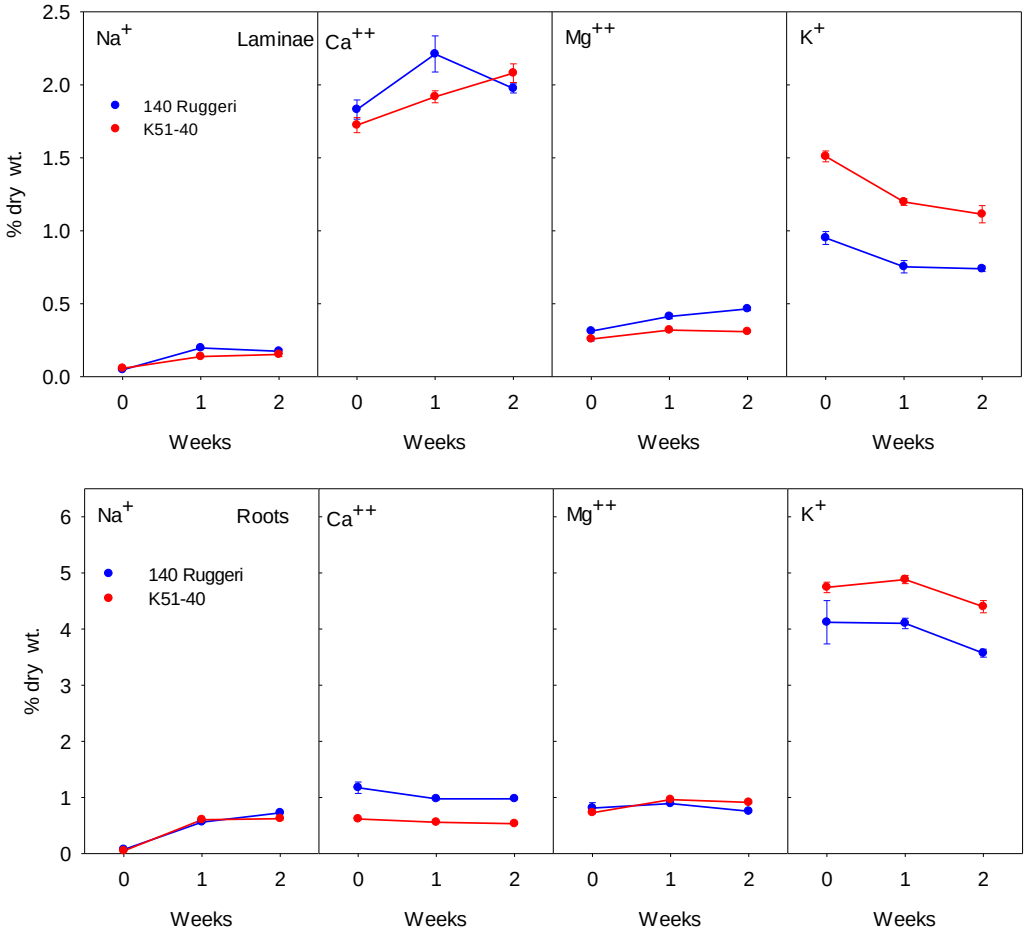
8.3 Results

8.3.1 Cation accumulation by rooted leaves

Laminae of K 51-40 rooted leaves accumulated higher concentrations of K^+ and lower concentrations of Ca^{2+} (at 0 and 1 week only) and Mg^{2+} (at 0, 1 and 2 weeks) relative to 140 Ruggeri (Figure 5). In a parallel study, laminae of K 51-40 accumulated higher concentrations of Cl^- than 140 Ruggeri, similar to previous studies (Tregeagle et al. 2010; Gong et al. 2011) (data not shown). In this study, roots of K 51-40 accumulated higher concentrations of K^+ and lower concentrations of Ca^{2+} (Figure 5). However in other studies (R. R. Walker et al., unpublished data), root K^+ concentrations were similar between the genotypes or higher in 140 Ruggeri, indicating that a range of factors can influence K^+ accumulation in roots of the genotypes. There was no difference between the genotypes in concentrations of Mg^{2+} and Na^+ in roots of the rooted leaves (Figure 5).

Figure 6 shows that in all three genotypes NO_3^- accumulation was similar (mean values within 5% of each other) in laminae of control and 47 mM Cl^- treated plants where NO_3^- concentration in the nutrient solution was similar (3 mM) between the treatments, but higher by 11.4 to 17.7% in 47 mM Cl^- treated plants with 12 mM nitrate in the nutrient solution.

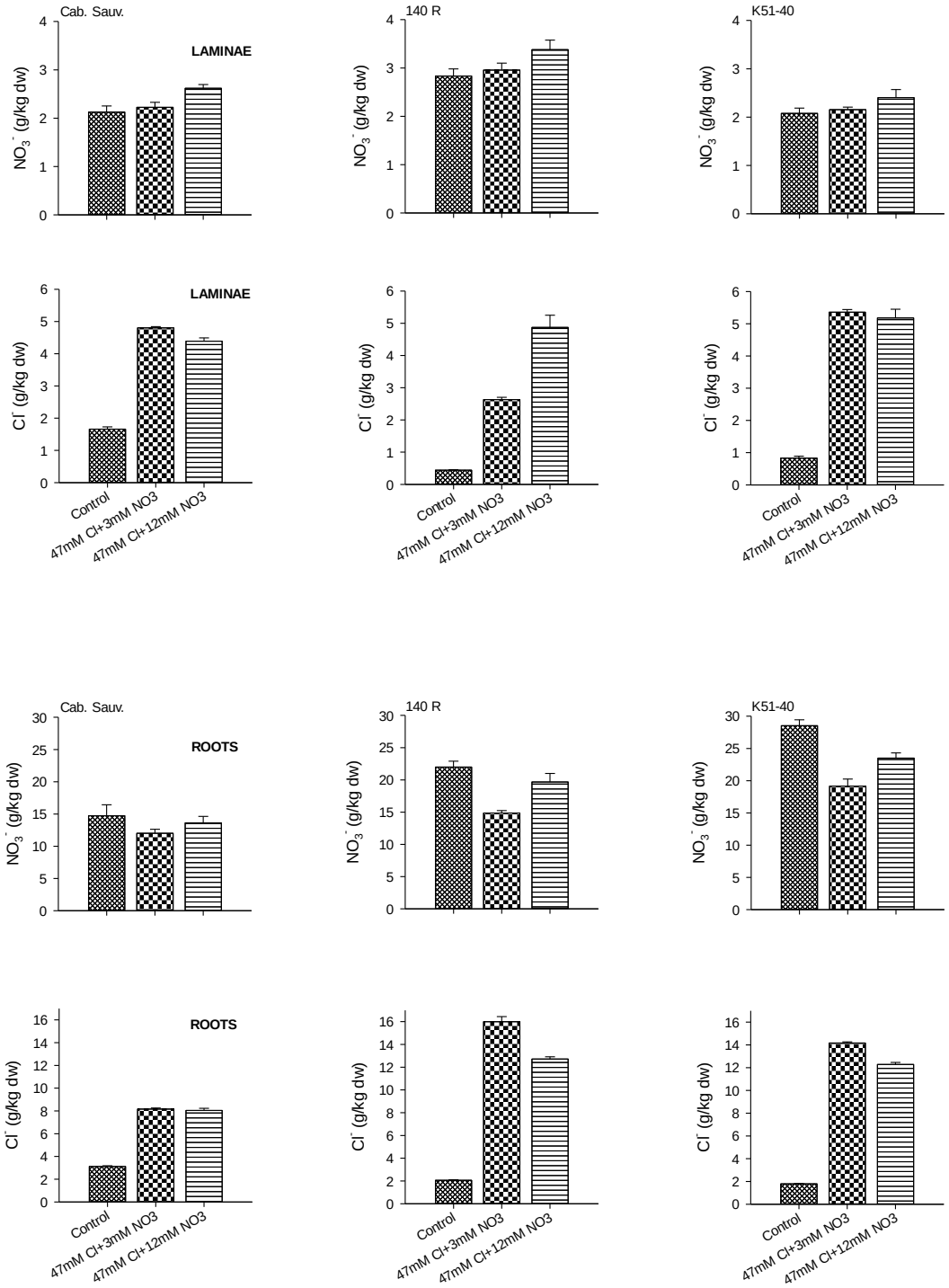
Figure 5: Na^+, Ca^{2+}, Mg^{2+}, K^+ and Cl^- concentrations in rooted leaves of K51-40 and 140 Ruggeri at 0, 7 and 14 days in nutrient solution (control) or nutrient solution plus 25 mM Cl^-. Each value is a mean ± standard error of 7 rooted leaves.



The situation was different for Cl^-, where genotype differences were apparent when comparing Cl^- concentrations in laminae between the 47 mM Cl^- treatments containing 3 and 12 mM nitrate (Figure 6). Cabernet Sauvignon showed a 8.5% reduction Cl^- accumulation in laminae in the presence of 12 mM NO_3^-, K 51-40 showed no significant change, but 140 Ruggeri surprisingly showed a significant (85%) increase in laminae Cl^- in the presence of 12 mM NO_3^- (Figure 6). In the roots, NO_3^- accumulation was significantly reduced (18.5 to 32.9%) by 47 mM Cl^- treatment in all three genotypes (when the NO_3^- concentration in the nutrient solution was the same at 3 mM), but increased (by 13.5 to 32.4%) at the 47 mM Cl^- treatment in the presence of 12 mM NO_3^- (Figure 6).

8.3.2 Chloride versus nitrate uptake and transport

Figure 6: Cl^- and NO_3^- concentrations in laminae and roots of K 51-40, 140 Ruggeri and Cabernet Sauvignon treated with nutrient solution (control, containing 3 mM NO_3^-), nutrient solution with 47 mM Cl^- and with 47 mM Cl^- plus extra NO_3^- (12 mM total). Means ±SE.



For plants treated with 47 mM Cl^-, root Cl^- concentration was not significantly different between the low and high NO_3^- treatments for Cabernet Sauvignon, whereas for K 51-40 and 140 Ruggeri, root Cl^- concentrations were reduced in the presence of high NO_3^-, by 20.5% for 140 Ruggeri and 13.3% for K 51-40 (Figure 6), indicating NO_3^- / Cl^- antagonism. In this study, in the 47 mM Cl^- with normal nutrient solution treatment, the Cl^- concentration in the roots of 140 Ruggeri was significantly higher (by 12.9%) than in the roots of K 51-40, as previously demonstrated by Tregeagle et al. (2010) and Gong et al. (2011).

This higher Cl^- concentration in roots of 140 Ruggeri (than in K 51-40) treated with 47 mM Cl^- implies higher Cl^- concentration in vacuoles of 140 Ruggeri roots than in K 51-40 roots, and that in the presence of high NO_3^- in the solution culture, the capacity of 140 Ruggeri roots to store Cl^- in vacuoles may have been reduced due to competition with NO_3^-, leading to more Cl^- being transported through to laminae.

These results may support involvement of a NO_3^- / Cl^- - type transporter at root cell vacuole level in storage of greater concentrations of Cl^- in 140 Ruggeri relative to K 51-40 roots and hence provides justification for investigating the CLC family of tonoplast localised Cl^- / NO_{3-} channels in grapevine for their potential as markers for Cl^- exclusion.

8.3.3 Impact on ion uptake of the Cl^- channel inhibitor, DIDS

It is assumed that DIDS entered roots in this experiment, but we were not able to measure the extent to which that occurred. The typical phenotype observed in the 50 mM Cl^- minus DIDS treatment was significantly less Cl^- accumulated in laminae and petiole and more Cl^- accumulated in roots of 140 Ruggeri compared to K 51-40 (Table 19). DIDS had no effect on Cl^- accumulation in laminae and petiole for any of the 3 genotypes. This suggests that DIDS did not significantly disrupt the processes of xylem Cl^- loading and / or Cl^- retrieval from xylem during root to shoot transport. However, DIDS significantly reduced Cl^- accumulation in 140 Ruggeri roots from 1.95 % dw to 1.25 % dw, and increased Cl^- accumulation in K51-40 roots from 1.40 % dw to 1.72 % dw (Table 19).

We can only speculate on the reasons for this result. When whole rooted leaf Cl^- concentrations were calculated (Table 19), it was evident that DIDS reduced Cl^- accumulation into the whole rooted leaves of 140 Ruggeri, but increased Cl^- accumulation into whole rooted leaves of K 51-40 and with no change in Cabernet Sauvignon, and that this effect was driven largely by the effect of DIDS on root Cl^- concentrations. If the principal effect of DIDS was inhibition of Cl^- loading and release into/ from root cortical cell vacuoles, reduced loading may explain lower root Cl^- concentrations in 140 Ruggeri and increased retention may explain higher root Cl^- concentrations in K 51-40. However, the actual situation is clearly more complex than this, given the results of the Cl^- / NO_3^- study as reported above.

Table 19: Concentrations of Cl^- in laminae, petiole,roots and whole rooted leaves of 140 Ruggeri, Cabernet Sauvignon and K 51-40 grown in nutrient solution only (control), nutrient solution plus 50 mM Cl^- and nutrient solution containing 50 mM Cl^- and 70 µM DIDS. Each of the 3 biological replicates for each treatment consisted of 5 rooted leaves.

Variety	Treatment	Reps	Laminae % dry wt	Petiole % dry wt	Roots % dry wt	Whole plant % dry wt
140 Ruggeri	Control	3	0.07^{a}	0.20^{a}	0.31^{a}	0.13^{a}
140 Ruggeri	Salt 50mM	3	0.29^{b}	0.43^{d}	1.95^{c}	0.76^{c}
140 Ruggeri	DIDS + Salt 50mM	3	0.35^{b}	0.41^{cd}	1.25^{b}	0.57^{b}
Cab. Sauv.	Control	3	0.19^{ab}	0.36^{c}	0.43^{a}	0.29^{a}
Cab. Sauv.	Salt 50mM	3	0.48^{c}	0.70^{f}	1.27^{b}	0.78^{c}
Cab. Sauv.	DIDS + Salt 50mM	3	0.53^{cd}	0.71^{f}	1.41^{b}	0.78^{c}
K51-40	Control	3	0.10^{a}	0.29^{b}	0.34^{a}	0.19^{a}
K51-40	Salt 50mM	3	0.50^{cd}	0.55^{e}	1.40^{b}	0.75^{bc}
K51-40	DIDS + Salt 50mM	3	0.59^{d}	0.58^{e}	1.72^{c}	1.00^{d}
		LSD_T	0.05	0.05	0.13	0.10
		LSD_V	0.05	0.05	0.13	0.10
		LSD_{T*V}	0.09	0.09	0.22	0.18

8.3.4 Chloride accumulation in plants used for microarray and q RT PCR

Chloride accumulation data for laminae, petioles and roots for the two experiments that were conducted are shown in Table 20. The relatively high values for Cl^- in control rooted leaves reflects the fact that stock plants were watered daily with tap water that contained 3.2 mM Cl^-. It also shows that the Cl^- exclusion phenotype is constitutive, evident under low Cl^-, absence of stress and not necessarily induced by high salt levels in the root medium. The phenotype was typical of past experiments that show significantly greater accumulation of Cl^- in laminae and petioles of K 51-40 relative to 140 Ruggeri, and significantly higher concentrations of Cl^- in roots of 140 Ruggeri than in K 51-40 (Table 20). Chloride accumulation in Cabernet Sauvignon was generally intermediate between 140 Ruggeri and K 51-40 (Table 20).

8.3.5 Microarray

Microarray screening of candidates was undertaken following isolation of high quality RNA from the material generated in the rooted leaf experiments. Relative to the non-salt treated control plants, 140 Ruggeri had 121 genes up-regulated and 107 down-regulated, K 51-40 had 539 genes up-regulated and 827 down-regulated, while for Cabernet Sauvignon, 335 genes were up-regulated and 379 were down-regulated. This correlates with the extent of Cl^- exclusion in each variety with 140 Ruggeri, the best salt excluder, being the least responsive, K 51-40, the least effective Cl^- excluder, being the most responsive, and Cabernet Sauvignon, intermediate with respect to Cl^- exclusion capacity, giving an intermediate response. These results may relate to the extent of stress induced within the plant as the internal salt load increases.

Table 20: Cl^- concentrations in laminae, petioles and roots of K 51-40, 140 Ruggeri and Cabernet Sauvignon rooted leaves treated with nutrient solution only (control), 25 mM Cl^- in Experiment 1, and 50 mM Cl^- in Experiment 2. LSD = least significant difference; T = treatment; V = variety; T x V = treatment x variety; ns = not significant; * = $P< 0.05$; ** = $P< 0.01$; *** = $P< 0.001$.

		Cl^- concentration (% dw)					
		Lamina		Petiole		Roots	
Experiment number		1	2	1	2	1	2
Variety	Treatment						
140 Ruggeri							
	Control	0.092	0.089	0.280	0.24	0.190	0.233
	25mM Cl^-	0.162		0.390		1.740	
	50mM Cl^-		0.268		0.52		1.732
Cab. Sauvignon							
	Control	0.340	0.272	0.510	0.59	0.360	0.326
	25mM Cl^-	0.415		0.620		1.100	
	50mM Cl^-		0.692		0.91		1.144
K51-40							
	Control	0.389	0.814	0.620	0.90	0.160	0.209
	25mM Cl^-	0.643		0.710		1.520	
	50mM Cl^-		1.237		1.11		1.156
ANOVA	LSD_T	0.077^{**}	0.072^{***}	0.087^{*}	0.034^{***}	0.171^{***}	0.113^{***}
	LSD_v	0.094^{***}	0.088^{***}	0.107^{***}	0.042^{***}	ns	0.138^{***}
	LSD_{T*V}	ns	0.125^{*}	ns	ns	0.296^{**}	0.195^{***}

The largest differences in the array were those between varieties in non-salt treated conditions, again indicating that the greater Cl^- exclusion capacity of 140 Ruggeri relative to K 51-40 is constitutive. A number of candidate genes with potential to be involved in the greater Cl^- exclusion capacity of 140 Ruggeri were identified by sorting the genes represented by these probe intensities in control conditions in the order of the most significantly differentially expressed between 140 Ruggeri and K 51-40. A similar statistical approach was taken to identify genes that were functionally annotated as anion transporters or anion channels within the *V. vinifera* genome, and that were significantly differentially expressed between 140 Ruggeri and K 51-40 under control conditions. This provided further gene candidates for Cl^- exclusion.

8.3.6 Q RT-PCR of candidate genes

Quantitative RT-PCR using RNA extracted from root samples of rooted leaves of 140 Ruggeri, K 51-40 and Cabernet Sauvignon treated with nutrient solution only or nutrient solution plus 25 or 50 mM Cl^- was completed for a range of candidate genes.

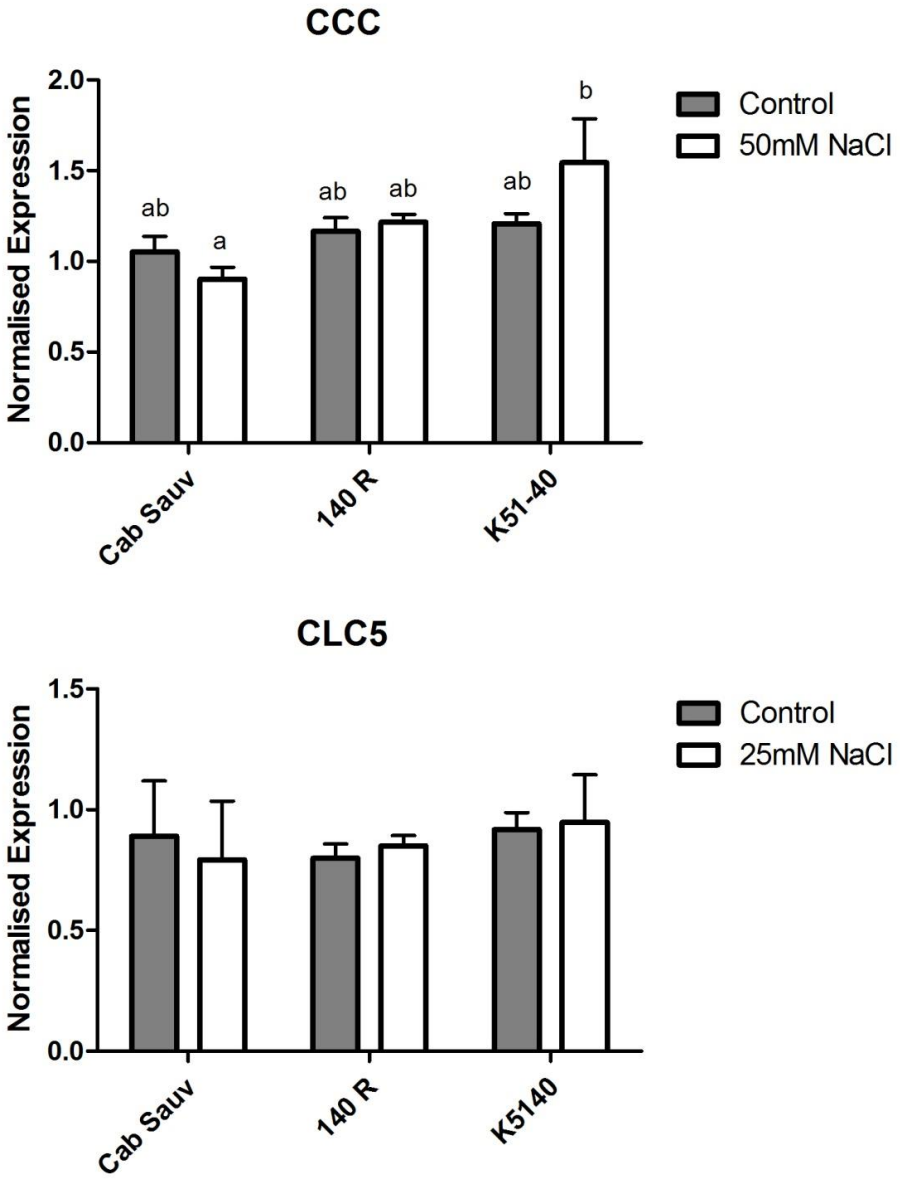
8.3.6.1 - CCC:

CCC is a cation coupled Cl^- transporter. There are three types of CCC. The KCC group are K^+:Cl^- co-transporters. The NCC group are Na^+:Cl^- co-transporters. The NKCC group are Na^+:K^+:Cl^- co-transporters, in the latter case transporting two Cl^- for every one Na^+ and one K^+ in the same direction across a membrane (Teakle and Tyerman 2010). Transport is electroneutral. While in Arabidopsis, CCC expression has been found in xylem parenchyma cells, further work is needed to determine the localisation of CCCs in plants (Teakle and Tyermman 2010). Expression was higher in 50 mM Cl^- treated K 51-40 than in Cabernet Sauvignon, but overall there was no obvious association with the Cl^- exclusion phenotype (Figure 7).

8.3.6.2 - CLC:

There are 7 known members of this family in Arabidopsis and rice, generally expressed in all tissues in various endomembranes, e.g. tonoplast, golgi and chloroplast thylakoid, with no evidence as yet for a plasma membrane location (Teakle and Tyerman 2010). There is evidence that members of the family are involved in Cl^- transport (Teakle and Tyerman 2010). Further, it has been hypothesised that under non-saline conditions, this transporter transports NO_3^- into the vacuole (Teakle and Tyerman 2010). In citrus, it was found that there was no differential expression of an ortholog of Arabidopsis CLCd in leaves of a Cl^- accumulating and Cl^- excluding genotype under saline conditions (Brumos et al. 2009).

Figure 7: Normalised q RT-PCR expression levels of *VvCCC* and *VvCLC5* from roots of control and 25 or 50 mM Cl^- treated rooted leaves of 140 Ruggeri, K 51-40 and Cabernet Sauvignon. Bars are SEM of three biological replicates. Different lettering above bars indicates significant differences between means (ANOVA, $P < 0.05$). Absence of lettering indicates no significant differences.



Two members of the CLC family were assessed in this study, *VvCLC5* and *VvCLC3*. There were no significant differences in expression level of *VvCLC5* between 140 Ruggeri, K 51-40 and Cabernet Sauvignon, nor between control and 25 mM Cl^- treated plants (Figure 7). Similarly, there were no significant differences in expression level of *VvCLC3* between 140 Ruggeri, K 51-40 and Cabernet

Sauvignon, nor between control and 25 mM Cl^- treated plants (Figure 8), or between control and 50 mM Cl^- treated plants (Figure 8).

8.3.6.3 - NRT:

NRT transporters form a large gene family in higher plants (Teakle and Tyerman 2010). They are suggested to be involved in Cl^- and NO_3^- transport. Nitrate transport is suggested to be the predominant role, with Cl^- transport about 20% of that for NO_3^- (Teakle and Tyerman 2010). Two members of the family were assessed in this study, *VvNRT1.5* and *VvNRT1.8*. At both 25 and 50 mM Cl^-, expression levels of *VvNRT1.5* in the control treatment were higher in Cabernet Sauvignon than in 140 Ruggeri and K 51-40 (Figure 9). Treatment with 25 mM Cl^- reduced expression of *VvNRT1.5* in Cabernet Sauvignon, but not in the other genotypes (Figure 9). Treatment with 50 mM Cl^- did not reduce expression relative to the control (Figure 9).

For *VvNRT1.8*, expression level of 140 Ruggeri from the 25 mM Cl^- treatment was higher than the Cabernet Sauvignon control, and expression level of K 51-40 from the 50 mM Cl^- treatment was also higher than the Cabernet Sauvignon control (Figure 10). For each genotype, there was no difference in expression level between control and Cl^- treated (25 or 50 mM) plants (Figure 10).

8.3.6.4 - POT:

Promising differences in expression level between all three genotypes were obtained irrespective of control or 50 mM Cl^- treatment (Figure 11). Highest expression was obtained with Cabernet Sauvignon, and least with K 51-40. The expression level for 140 Ruggeri was intermediate (Figure 11). This did not correspond with the Cl^- exclusion phenotype across the three genotypes.

Figure 8: Normalised q RT-PCR expression levels for *VvCLC3* from roots of control and (a) 25 mM Cl^- and (b) 50 mM Cl^- treated rooted leaves of 140 Ruggeri, K 51-40 and Cabernet Sauvignon. Bars are SEM of three biological replicates. Absence of lettering above bars indicates no significant differences between means (ANOVA, $P < 0.05$).

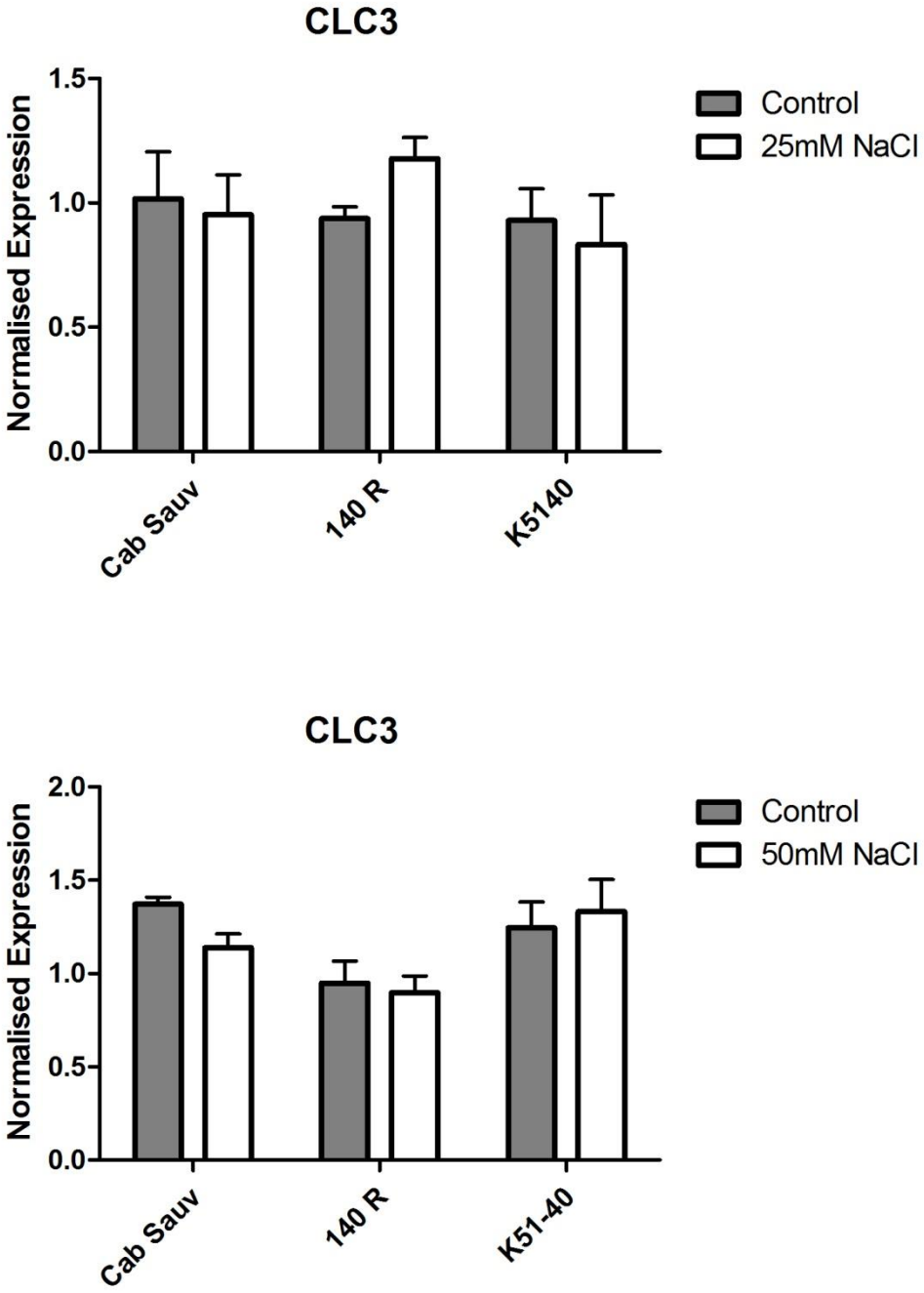


Figure 9: Normalised q RT-PCR expression levels for *VvNRT1.5* from roots of control and (a) 25 mM Cl treated and (b) 50 mM Cl^- treated rooted leaves of 140 Ruggeri, K 51-40 and Cabernet Sauvignon. Bars are SEM of three biological replicates. Different lettering above bars indicates significant differences between means (ANOVA, $P < 0.05$).

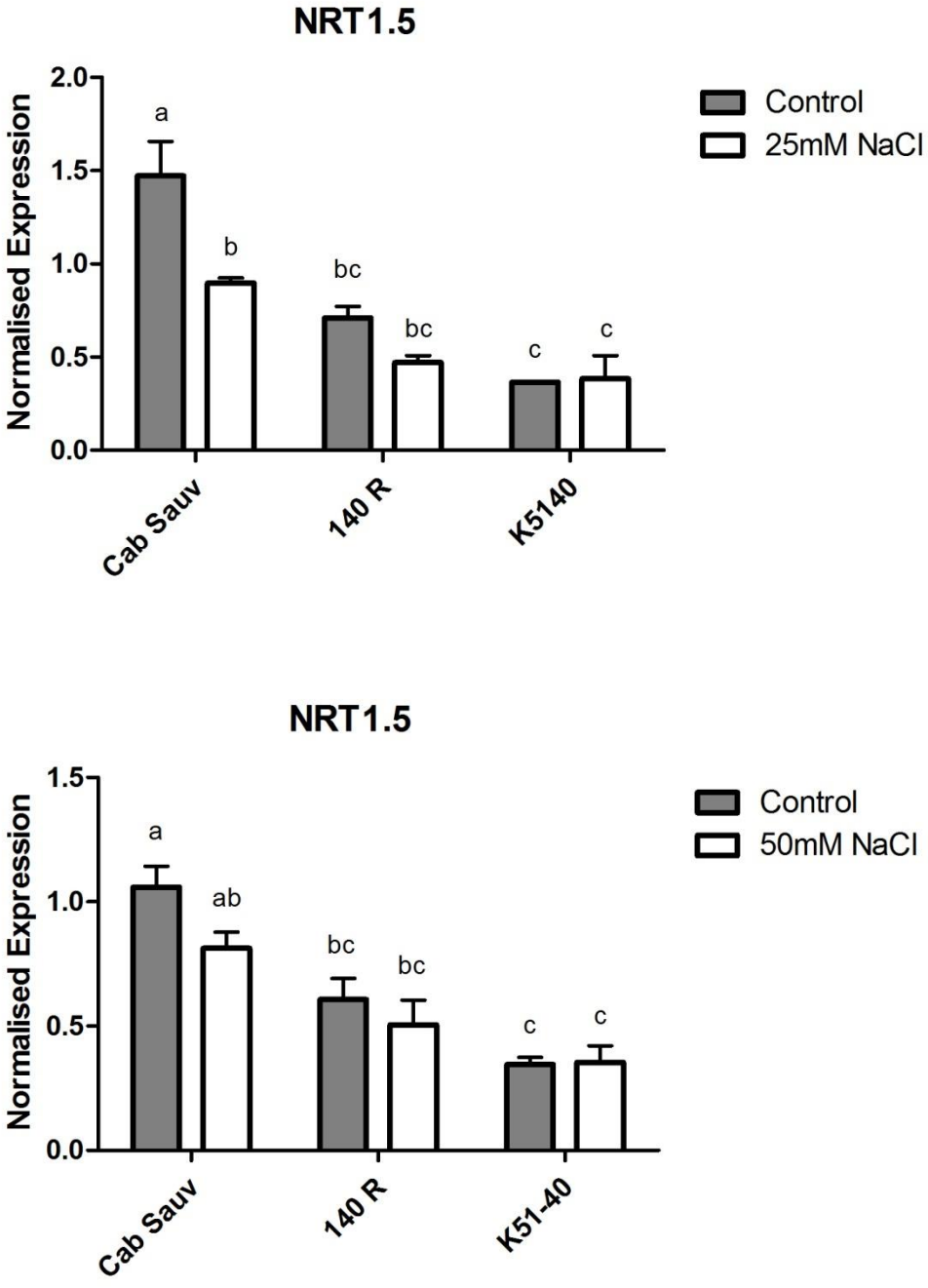
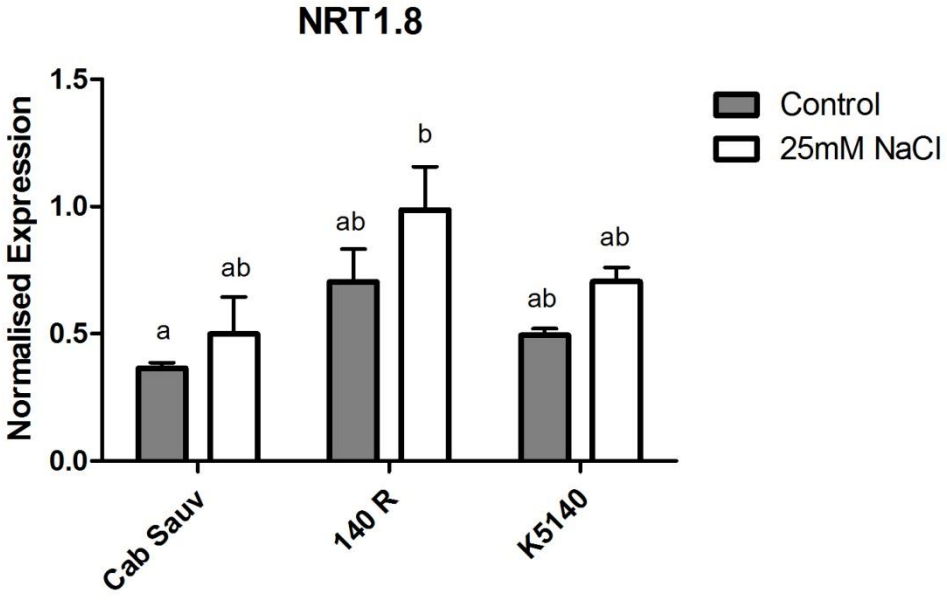
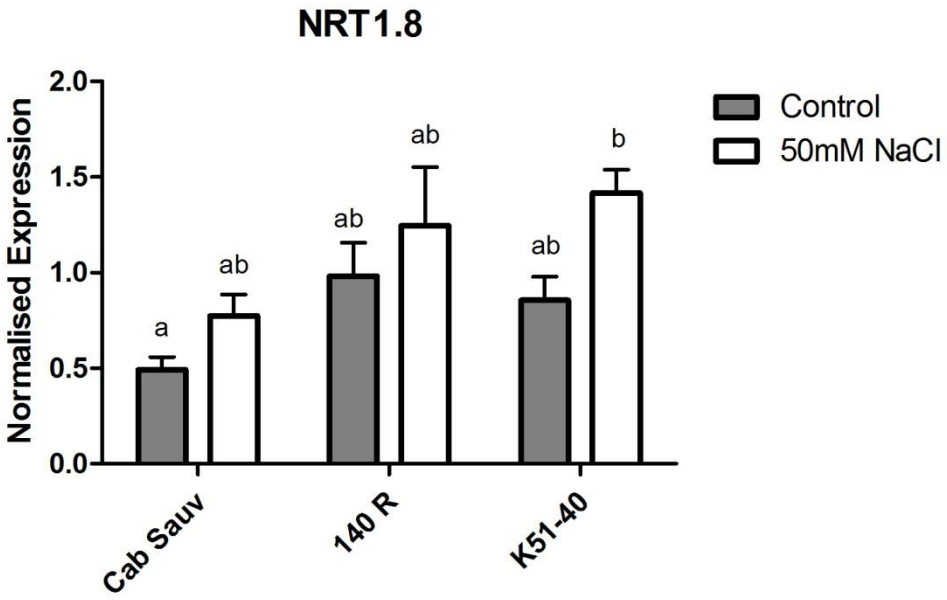


Figure 10: Normalised q RT-PCR expression levels for *VvNRT1.8* from roots of control and (a) 25 mM Cl treated and (b) 50 mM Cl^- treated rooted leaves of 140 Ruggeri, K 51-40 and Cabernet Sauvignon. Bars are SEM of three biological replicates. Different lettering above bars indicates significant differences between means (ANOVA, $P < 0.05$).





8.3.6.5 - SLAH:

SLAH are a relatively new family of possible anion transporters (Teakle and Tyerman 2010). SLAH 1, 2 and 3 are known to be expressed in roots, with SLAH1 showing strong expression in the root vascular cylinder (Teakle and Tyerman 2010). Two members of the family were assessed in this study, *VvSLAH1.2* and *VvSLAH1.3*. *VvSLAH1.2* was neither differentially expressed between the 3 genotypes nor between the control and Cl^- (25 or 50 mM) treatments (Figure 12). The same applied for *VvSLAH1.3* at the control and 25 mM Cl^- treatments for the 3 genotypes (Figure 13a). However, in the experiment comparing control and 50 mM Cl^- treatment, expression level for control Cabernet Sauvignon was higher than for control 140 Ruggeri and K 51-40 treatments, and for Cabernet Sauvignon (but not the other genotypes), expression level at 50 mM Cl^- was reduced relative to the control treatment (Figure 13b).

Figure 11: Normalised q RT-PCR expression levels of *VvPOT1* from roots of control and 50 mM Cl^- treated rooted leaves of 140 Ruggeri, K 51-40 and Cabernet Sauvignon. Bars are SEM of three biological replicates. Different lettering above bars indicates significant differences between means (ANOVA, $P < 0.05$).

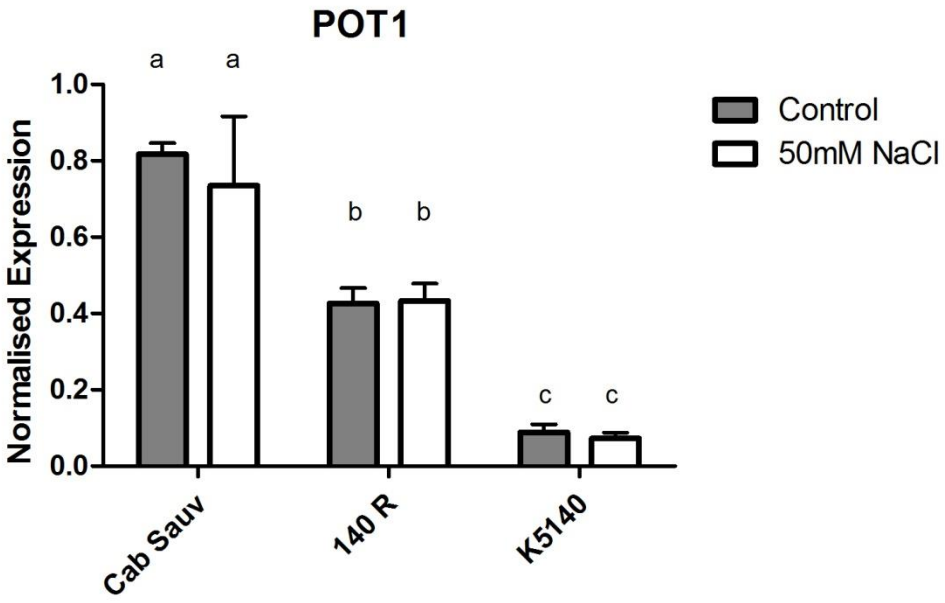
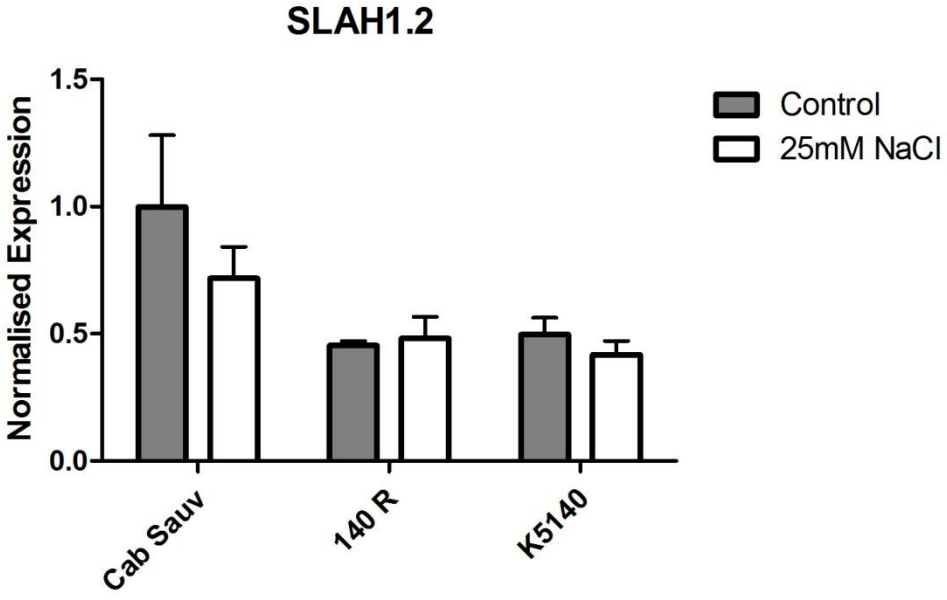


Figure 12: Normalised q RT-PCR expression levels for *VvSLAH1.2* from roots of control and (a) 25 mM Cl^- treated and (b) 50 mM Cl^- treated rooted leaves of 140 Ruggeri, K 51-40 and Cabernet Sauvignon. Bars are SEM of three biological replicates. Absence of lettering above bars indicates no significant differences between means (ANOVA, $P < 0.05$).



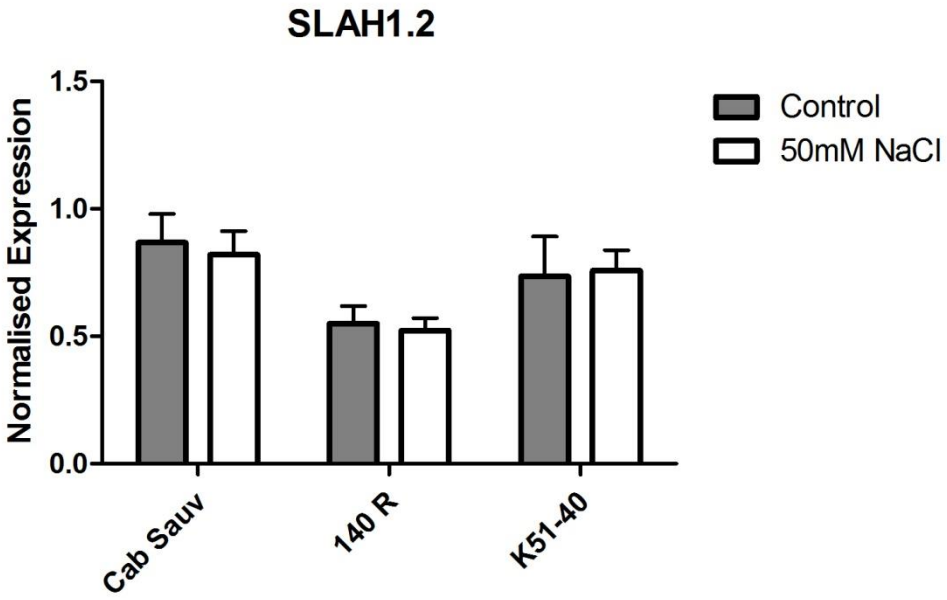
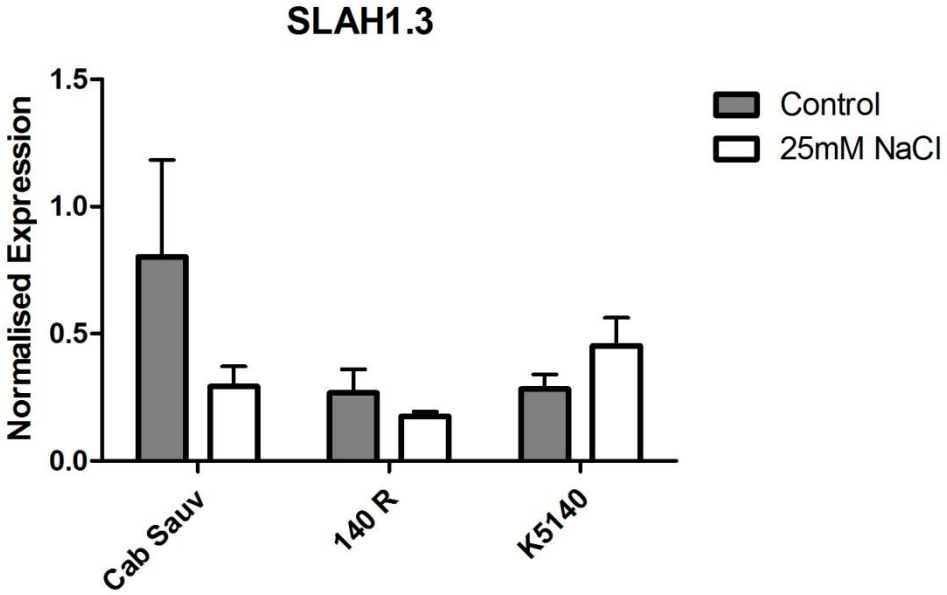
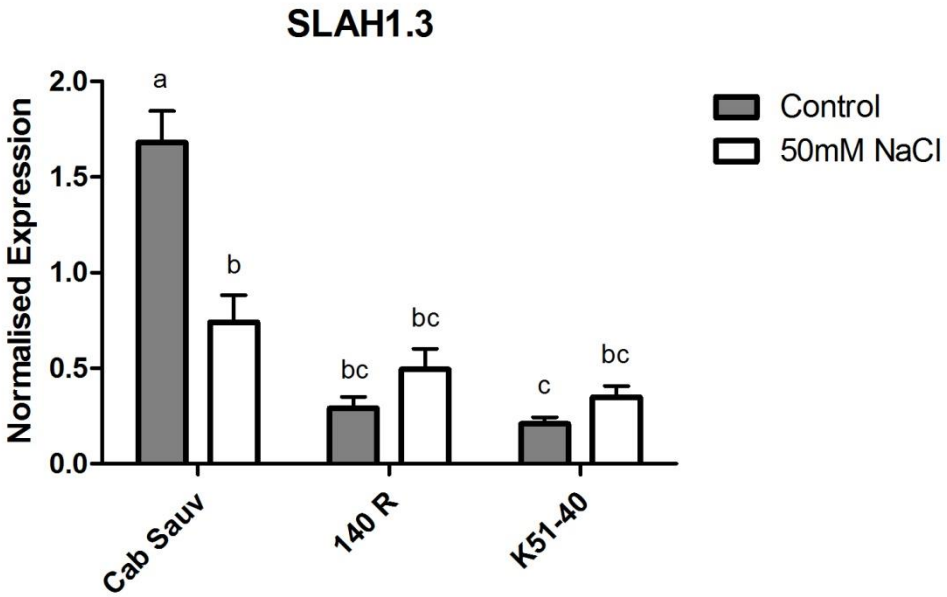


Figure 13: Normalised q RT-PCR expression levels for *VvSLAH1.3* from roots of control and (a) 25 mM Cl^- treated and (b) 50 mM Cl^- treated rooted leaves of 140 Ruggeri, K 51-40 and Cabernet Sauvignon. Bars are SEM of three biological replicates. Different lettering above bars indicates significant differences between means (ANOVA, $P < 0.05$). Absence of lettering indicates no significant differences.





8.3.7 Sequencing

The aim was to design PCR based markers that correlate with Cl^- exclusion by designing a specific product with DNA from 140 Ruggeri that is different to that for non-excluding varieties. Once achieved, these could then be screened across a population of hybrid vines (developed from a cross between 140 Ruggeri and K 51-40) that had been pre-phenotyped for Cl^- exclusion capacity.

Sequencing and some re-sequencing of all 7 candidate genes has been undertaken. Direct sequencing has proved to be a complicated approach as it gives multiple sequences in one single point of the sequence. This has proved to be confusing because the amplified sequences were from genomic DNA, not cDNA, so there are a lot of differences in the sequence. It was decided that the best way get a single copy sequence was to clone the amplified sequence into a vector for sequencing as every single colony only carries one copy of the gene. Markers have been discovered for two of the seven candidates, but sequencing is ongoing and has been carried through into the follow-up GWRDC project CSP1302.

8.4 Discussion

Studies with rooted leaves confirmed observations made in previous studies (Tregeagle et al. 2010; Gong et al. 2011) that Cl^- accumulation is higher in roots and lower in the shoot (lamina and petiole) of the good Cl^- excluder (140 Ruggeri) relative to the poor Cl^- excluder (K 51-40). When concentrations of Cl^- on a whole rooted leaf basis are compared, there is no difference between the three genotypes, indicating that the exclusion phenotype, at least for rooted leaves, involves partitioning of Cl^- within the plant. The lower concentrations of Cl^- in petiole and lamina of rooted leaves of 140 Ruggeri relative to K 51-40 is associated with lower concentrations of Cl^- in xylem of 140 Ruggeri (Tregeagle et al. 2010) and higher flux rates of Cl^- to the shoot in K 51-40 relative to 140 Ruggeri (Tregeagle et al. 2010; Gong et al. 2011). The studies of Tregeagle et al. (2010) and Gong et al. (2011) also demonstrated that uni-directional influx of Cl^- (flux of Cl^- across root cell plasma

membrane) was similar between the genotypes, suggesting that rates of uptake into roots may also be similar. The higher Cl^- concentrations in roots of 140 Ruggeri may therefore be associated with similar rates of Cl^- uptake into roots, reduced loading of Cl^- into xylem and therefore higher root Cl^- concentrations.

Higher concentrations of Cl^- in roots of 140 Ruggeri imply higher concentrations of Cl^- in the vacuoles of root cortical cells. It has already been demonstrated, using a population of hybrids generated from a cross between 140 Ruggeri and K 51-40, that variation of Cl^- accumulation is continuous across the hybrid family suggesting involvement of more than one gene. Chloride transporters on root cell membranes are implicated. Gong et al. (2011) and Abbaspour et al. (2014) provided evidence that apoplastic transport was not different between the genotypes, suggesting that differences between the genotypes are more likely to be associated with the symplastic pathway and implicating a role for membrane Cl^- transporters. The apparent higher loading of Cl^- into root cell vacuoles may involve a Cl^- transporter at the root cell tonoplast. Furthermore, it has previously been hypothesised that lower concentrations of Cl^- in xylem of 140 Ruggeri are regulated at root level. This may be the case, but further x-ray microanalysis of roots will be required to demonstrate that xylem Cl^- concentrations are indeed lower in roots of 140 Ruggeri relative to K 51-40. Storey et al. (2003) demonstrated higher concentrations of Cl^- in the pericycle cells of an excluding genotype relative to a poor excluder, but xylem concentrations were not measured.

There are anion transporters on plant cell membranes that have a role in transport of both Cl^- and NO_3^- (Teakle and Tyerman 2010). Using rooted leaves and a high and low NO_3^- treatment in the presence of 47 mM Cl^- in the solution culture, we were able to show that high NO_3^- (12 mM versus 3 mM) reduced Cl^- accumulation in roots, to a greater extent in 140 Ruggeri (20.5%) than in K 51-40 (13.3%) and significantly increased Cl^- concentration in laminae of 140 Ruggeri by 85% relative to a 3% decrease in K 51-40. This is evidence for the presence of anion transporters able to transport both Cl^- and NO_3^- in roots, and potentially on the root cortical cell tonoplast. Reasons for the increase in Cl^- concentrations in laminae of the high NO_3^- treatment are unclear, but a possible explanation is that because more NO_3^- was being

transported into root cell vacuoles, more Cl^- in roots would be available for transport from root to shoot.

The non-specific blocker of Cl^- transport, DIDS, resulted in a reversal of the Cl^- exclusion phenotype in roots, where in the absence of DIDS, 140 Ruggeri had higher concentrations of Cl^- in roots, whereas in the presence of DIDS, K 51-40 had higher concentrations of Cl^- in roots. Reasons for the change are not clear, but it suggests a role for anion transporters in the Cl^- exclusion phenotype.

Initially, 23 candidate marker probes were selected from a microarray screen on the basis of their differential expression between K 51-40 and 140 Ruggeri. The 23 candidate marker probes were re-checked against their targets in both genomic DNA and predicted complementary DNA. Because multiple probes were present in the same genes, it was possible to decrease the list of 23 probes to 5 target genes plus an additional two genes that were selected based on literature review and prior knowledge in the initial part of the project.

9. Outcomes and Conclusions

9.1 Rootstocks for use in moderately saline situations

- There was a strong link between lamina Cl^- and grape juice Cl^- concentration at harvest, as shown by the consistently higher concentrations of Cl^- in laminae and grape juice of Shiraz on M 6262 in both seasons.

- Rootstocks 101-14 and 1103 Paulsen resulted in earlier ripening (by 5 to 12 days) of Shiraz than rootstocks M 5489 and M 5512 in both years of the trial.

- The increase in Cl^- concentration in progressing from grape juice to wine of Shiraz on M 6262 ranged from 1.65 in 2012 to 2.72 in 2013. There was a decrease in Na^+ in progressing from juice to wine in 2012 and a slight increase (1.3 fold) in 2013. The greater increase in Cl^- than Na^+ in progressing from juice to wine reflects higher concentrations of Cl^- in grape skins relative to Na^+ (Gong et al 2010).

- Riper Shiraz fruit from rootstocks 101-14 and 1103 Paulsen in both years generally had higher colour density, *Dark Fruit*, *Hotness*, *Overall Fruit* and *Fruity Aftertaste* attributes. In 2013, when adjusted for alcohol content, many of the significant rootstock effects on sensory attributes of the Shiraz wines were no longer significant, leaving only *Astringency*, *Stalky*, *Dark Fruits* aroma and *Red Fruits* aroma as significant rootstock effects.

- A salt tolerance index (STI) was calculated taking into consideration Shiraz yield, leaf area index, grape juice Cl^- and Na^+ and wine colour density. Best STI values (6.06 to 7.60) were obtained with rootstocks M 5489, 110 Richter and 140 Ruggeri, intermediate for 1103 Paulsen, Ramsey, 101-14 and M 5512 (4.83 to 5.65), while M 6262 had a very low STI (0.54).

- On the basis of these data, rootstock M 6262 is not recommended for saline situations.

9.2 Thresholds for salty taste detection in wine

- Wine Cl^- concentration of 482 mg/L was sufficient for detection of *Salty* taste in Shiraz wines in 2012, whereas wine Cl^- concentration of 384 mg/L was insufficient for detection of *Salty* taste in Shiraz wine in 2013. Hence, based on the method used in this work, the detection threshold for Shiraz is between 384 and 482 mg/L. These concentrations were well below the Best Estimate Threshold (BET) for *Salty* taste in red wines, 701 mg/L (Leigh Francis et al. Australian Wine Research Institute), where a different procedure was used relative to the procedure described in this work.

- Wine K^+ concentrations in the range 1074 to 1425 mg/L K^+, in the presence of Cl^- concentrations in the range 63-92 mg/L and Na^+ concentrations in the range 25 to 35 mg/L, were not sufficient to result in *Salty* taste.

- Cl^- and Na^+ concentrations of 274 and 114 mg/L respectively in Shiraz were not sufficient were detection of *Salty* taste.

- Cl^- and Na^+ concentrations of 407 and 374 mg/L respectively in Chardonnay wine were sufficient for *Salty* taste detection. The Cl^- concentration of 407 mg/L was above the Best Estimate Threshold (BET) for *Salty* taste in white wines, 270 mg/L (Leigh Francis et al., Australian Wine Research Institute).

- The *Salty* taste attribute in both Shiraz and Chardonnay wines was linked with the *Viscosity* attribute.

9.3 Evaluation of CSIRO salt tolerant rootstocks

- Trials with Shiraz and Pinot Gris as scions have been successfully established.

- There was no significant difference in grape juice Cl^- and Na^+ concentrations for Shiraz on 140 Ruggeri and Shiraz on B7 rootstocks and both were

significantly lower than concentrations of Cl^- and Na^+ in grape juice from Shiraz on own roots.

- Shiraz on own roots and B7 rootstocks accumulated similar concentrations of K^+ in grape juice, which were significantly less than the K^+ concentration accumulated in grape juice of Shiraz on 140 Ruggeri rootstock. Data from additional seasons are required to confirm this finding.

9.4 Mechanism for chloride exclusion

- The Cl^- exclusion phenotype in rooted leaves of 140 Ruggeri was shown to be constitutive i.e. present under both high and low salt conditions, and not salt-inducable.

- The Cl^- exclusion phenotype in rooted leaves of 140 Ruggeri involves partitioning of higher Cl^- concentrations in roots, lower Cl^- concentrations in xylem and lower concentrations of Cl^- in petiole and lamina relative to that in K 51-40.

- Rooted leaves of 140 Ruggeri and K 51-40 have similar concentrations of Cl^- when expressed on a whole plant basis, again indicating that the (rooted leaf) Cl^- exclusion phenotype in 140 Ruggeri is based on partitioning within the plant rather than on differences in whole plant ion uptake and accumulation.

- High NO_3^- in solution culture (root zone) (a) reduced Cl^- accumulation in roots of both genotypes, but to a greater extent in 140 Ruggeri rooted leaves, and (b) significantly increased Cl^- accumulation in laminae of 140 Ruggeri. This provides evidence for a role of a Cl^-/NO_3^- transporter or transporters in the roots of both genotypes, and a possible role in altering the Cl^- exclusion phenotype between the genotypes in response to high root zone NO_3^-.

- The non-specific inhibitor of Cl^- transporter activity, DIDS, eliminated the Cl^- exclusion phenotype in 140 Ruggeri, providing further evidence that the Cl^- exclusion phenotype in 140 Ruggeri is regulated by Cl^- transporters.

9.5 Marker for chloride exclusion

- Background literature searching and microarray on high quality RNA from roots of 140 Ruggeri, Cabernet Sauvignon and K 51-40 identified 23 candidate genes with potential as markers for Cl^- exclusion. This list was reduced to 7 candidates. From this list at least two putative markers have been identified.

- The number of genes either up regulated or down regulated by salt was highest in K 51-40, least in 140 Ruggeri and mid-range in Cabernet Sauvignon, reflecting the Cl^- exclusion rankings of the 3 genotypes.

10. Recommendations

10.1 Rootstocks for use in moderately saline situations

- The salt tolerance index (STI) shows promise as an alternative way to rank rootstocks for tolerance of salinity. This should be further tested over a range of environments. Tests should utilise data from trials involving a range of wine grape scions and rootstocks in regions affected by salinity.

- Examine season to season variability in trunk wood Cl^- and Na^+ concentrations and test the relationship between grapevine trunk wood Cl^- and Na^+ and grape berry / grape juice Cl^- and Na^+ concentrations as a means for early (within season) prediction of high grape juice Cl^- and Na^+.

- Undertake further work to examine the genotype by environment interaction on the low grape juice K^+ phenotype. This work should examine why rootstocks that express the low grape juice K^+ phenotype in low salinity environments, do not express the low grape juice K^+ phenotype in high salinity environments.

- Use data and wines from the rootstock trial in extension activities that assist growers in making choices on what rootstock to use for their vineyard.

10.2 Thresholds for salty taste detection in wine

- Utilising wines that are either high in Cl^- and low in Na^+, or high in Na^+ and low in Cl^-, but similar in K^+, better define the role of Na^+ and Cl^- in determining *Salty* taste in wines.

- Assess the consumer preference response to *Salty* taste in wines of different styles.

10.3 Evaluation of CSIRO salt tolerant rootstocks

- Continue evaluation of B7 rootstock with Shiraz and Pinot Gris as scions in a moderately saline environment, and extend to other saline environments, and evaluate also in non-saline environments.

10.4 Marker for chloride exclusion

- Continue development of putative markers and test for efficacy using populations that have been characterised for Cl^- exclusion.

- Commence work to develop a marker or markers for Na^+ exclusion,

- Further characterise the Cl^- exclusion phenotype in grapevines. In rooted leaves, the phenotype is associated with genotype differences in within-plant Cl^- partitioning. It is not associated with genotype differences in total plant Cl^- accumulation. Rooted leaves are a non-growing model system. The phenotype should be examined using growing plants of good and poor Cl^- excluding genotypes.

11. Appendix 1: Communication

11.1 Presentations

Walker, R. (2010) Rootstock regulation of salt uptake by grapevines. La Trobe University Seminar Series. Bundoora, Victoria, September 2010.

Walker, R. (2010) Sustainable salt tolerance of salt excluding rootstocks. Fosters Wine Group, South East Salinity Information Day. Padthaway, October, 2010

Dry, N., McCarthy, M. and Walker, R. (2010) Rootstocks for salt affected soils. Barossa Wine Grape Growers Salinity Seminar. Nuriootpa, November, 2010.

Walker, R. (2011) Grapevine salt exclusion and salt tolerance. Crush Symposium, Adelaide, September 2011.

Walker, R. (2011) Physiology of salt tolerance in grapevines. Lecture to School of Agriculture, Food and Wine Students. Adelaide, October, 2011.

Holt, H., Francis, L., Pearson, W., Blackmore, D., Clingeleffer, P and Walker, R. (2012) Sensory properties of Chardonnay and Shiraz wines made from rootstocks differing in capacity for chloride and sodium exclusion. Crush Symposium, Adelaide, November, 2012.

Walker, R. (2012) Physiology of salt tolerance in grapevines. Lecture to School of Agriculture, Food and Wine Students. Adelaide, October, 2012.

Walker, R. (2013) Grapevine rootstocks and salinity. McLaren Vale Wine Grape Growers Seminar. McClaren Vale, April 2013.

Walker, R., Clingeleffer, P. and (the late) Brady Smith (2013) Rootstock use and major needs – Australia. American Society of Enology and Viticulture, Rootstock Symposium and Annual Meeting, Monterey, June 2013.

Walker, R., Holt, H., Francis, L., Pearson, W., Blackmore, D. and Clingeleffer, P. (2013) Yield and wine sensory properties of Shiraz and Chardonnay from rootstocks differing in capacity for salt exclusion. American Society of Enology and Viticulture, Annual Meeting, Monterey, June 2013.

Walker, R. (2013) Grapevine rootstocks and salinity. Rootstocks Workshop. Australian Wine Industry Technical Conference. Sydney, July, 2013.

Walker, R. (2013) Physiology of salt tolerance in grapevines. Lecture to School of Agriculture, Food and Wine Students. Adelaide, October, 2013.

Walker, R., Clingeleffer, P. and (the late) Brady Smith (2013) Rootstocks for Australian conditions. AWRI Webinar, Adelaide, October, 2013

Walker, R, Clingeleffer, P., Walker, M, Gilliham, M, Francis, L. Holt, H and Pearson, W. (2013) Chloride and sodium excluding rootstocks for quality wine production. GWRDC Rootstock Reference Group Meeting, November 2013.

Walker, R., Clingeleffer, P., Blackmore, D., Holt, H., Pearson, W. and Francis, L. (2013) Rootstock responses to salinity, SE of SA. Treasury Wine Estates South East Information Day. Padthaway, November 2013.

Walker, R., Clingeleffer, P., Blackmore, D., Holt, H., Pearson, W. and Francis, L. (2013) Rootstock responses to salinity. Treasury Wine Estates Barossa meeting. Nuriootpa, November 2013.

11.2 Conference Posters

Walker, R.R., Blackmore, D.H. and Clingeleffer, P.R. (2013) Grapevine response to long term saline irrigation. Australian Wine Industry Technical Conference, Sydney, July 2013.

Henderson, S.W., Blackmore, D, Walker, M, Walker, R, Baumann, U, Tyerman, S, and Gilliham, M. (2012) Identification of anion transporters that confer chloride exclusion and salt tolerance to grapevine rootstocks. Gordon Research Conference on Salt and Water Stress in Plants, Chinese University of Hong Kong, Hong Kong, China.

Henderson, S.W., Blackmore, D.H., Baumann, U., Walker, A.R., Tyerman, S.D., Walker, R.R. and Gilliham, M. (2013) Membrane transporters differentially expressed between salt tolerant and salt sensitive Vitis rootstocks and their role in chloride exclusion. 16th International Workshop on Plant Membrane Biology, Kurashiki, Japan.

11.3 Peer Reviewed Journal Papers

Gong, H.J., Blackmore, D.H., Clingeleffer, P.R., Sykes, S.R. and Walker, R.R. (2014) Variation for potassium and sodium accumulation in a family from a cross between grapevine rootstocks K 51-40 and 140 Ruggeri. *Vitis*, in press.

Walker, R.R., Blackmore, D.H., Clingeleffer, P.R. and Emanuelli, D. (2014) Rootstock type determines tolerance of Chardonnay and Shiraz to long term saline irrigation. *Australian Journal of Grape and Wine Research*, in press.

11.4 General Communications

Anon. (2012) Water use in vineyards. Drinks Business (UK)

Anon. (2012) Salty skin: reds most at risk. R&D @ Work

12. Appendix 2: Intellectual Property and/or valuable information arising from the Research

Data obtained in the project and presented in this report are in the public domain and are either in the process of being published or intended to be published in international peer reviewed journals.

Much of the public domain material has been presented at industry meetings and symposia and will be continue to be extended as opportunities arise.

13. Appendix 3: References cited in the Report

13.1 References

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14. Appendix 4: Staff (A list of all staff that have been engaged on the Project)

Dr Rob Walker, CSIRO Plant Industry (Project Leader)
Mr Peter Clingeleffer, CSIRO Plant Industry (Research Scientist)
Dr Mandy Walker, CSIRO Plant Industry (Research Scientist)
Ms Deidre Blackmore, CSIRO Plant Industry (Research Support Scientist)
Mr Ruwan Lenora, CSIRO Plant Industry (Casual Assistant, 2013)
Ms Tania Ferreirinho, CSIRO Plant Industry (Technical Officer, 2012-13)
Ms Pooja Vashist, CSIRO Plant Industry (Technical Officer, 2011-12)
Ms Corrinne Pruess, CSIRO Plant Industry (Technical Officer, 2010-11)

Dr Matthew Gilliham, University of Adelaide
Mr Sam Henderson, University of Adelaide, PhD Student
Mr Weng Wa Ng, University of Adelaide (Research Assistant)

Dr Leigh Francis, The Australian Wine Research Institute (Research Manager)
Dr Helen Holt, The Australian Wine Research Institute (Research Scientist)
Mr Wes Pearson, The Australian Wine Research Institute (Scientist)

15. Appendix 5: Budget reconciliation

Submitted separately to GWRDC.