

**A comparative study of Cl<sup>-</sup> transport across the roots  
of two grapevine rootstocks, K 51-40 and  
Paulsen, differing in salt tolerance**

**By**

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# Table of Content

<b>TABLE OF CONTENT</b> .....	<b>I</b>
<b>LIST OF FIGURES</b> .....	<b>V</b>
<b>LIST OF TABLES</b> .....	<b>VII</b>
<b>ACKNOWLEDGEMENT</b> .....	<b>VIII</b>
<b>PUBLICATION FROM THIS THESIS</b> .....	<b>X</b>
<b>DECLARATION</b> .....	<b>XI</b>
<b>ABSTRACT</b> .....	<b>XIII</b>
<b>ABBREVIATION</b> .....	<b>XVI</b>
<b>CHAPTER 1</b> .....	<b>1</b>
<b>INTRODUCTION</b> .....	<b>1</b>
<i>1-1 Salinity stress</i> .....	<i>1</i>
1-1-1 Definition of salinity.....	2
1-1-2 Components of salt stress .....	2
1-1-3 Salinity tolerance .....	4
1-1-4 Mechanisms of salt tolerance.....	5
1-1-5 Salt tolerance in plants that show Cl <sup>-</sup> sensitivity.....	6
<i>1-2 Mechanisms of entry of salt (Na<sup>+</sup> and Cl<sup>-</sup>) into plant roots</i> .....	<i>7</i>
1-2-1 Transport across membrane in cells.....	7
1-2-2 Na <sup>+</sup> transport.....	9
1-2-3 Cl <sup>-</sup> Transport.....	11
1-2-4 NO <sub>3</sub> <sup>-</sup> Transport .....	13
1-2-5 NO <sub>3</sub> <sup>-</sup> and Cl <sup>-</sup> interactions .....	14
1-2-6 Different affinities of NO <sub>3</sub> <sup>-</sup> uptake [LATS, HATS (iHATS and cHATS)] .....	15
<i>1-3 Compartmentation of Na<sup>+</sup> and Cl<sup>-</sup> in plant parts</i> .....	<i>16</i>
1-3-1 Compartmental flux analysis .....	17
1-3-2 X-ray microanalysis.....	20
<i>1-4 Pathways of Na<sup>+</sup> and Cl<sup>-</sup> transport across roots to the xylem</i> .....	<i>21</i>
1-4-1 Apoplast pathway .....	22
1-4-2 Release of salt into the xylem from the symplast .....	23
<i>1-5 Water relations and salinity</i> .....	<i>24</i>
1-5-1 The pressure probe (PP) technique .....	26
1-5-2 Using the root pressure probe to assess root permeability to ions .....	26
1-5-3 Measurement of the reflexion coefficient .....	30
<i>1-6 Grapevines and salinity</i> .....	<i>31</i>
1-6-1 Origin of rootstocks and grapevine varieties .....	31

1-6-2 Vitis rootstocks and salinity.....	32
1-6-3 Na <sup>+</sup> and Cl <sup>-</sup> accumulation .....	33
1-6-4 Salt tolerance in grapevines .....	35
1-6-5 Rootstocks contrasted.....	37
1-7 Aims of the project.....	37
<b>CHAPTER 2 .....</b>	<b>39</b>
CL <sup>-</sup> /NO <sub>3</sub> <sup>-</sup> SELECTIVITY IN GRAPEVINE ROOTS .....	39
2-1 Introduction .....	39
2-2 Materials and Methods.....	41
2-2-1 Plant material.....	41
2-2-2 Experimental design .....	41
2-2-3 Determination of NO <sub>3</sub> , Cl and <sup>15</sup> N.....	42
2-3 Results.....	43
2-4 Discussion.....	47
<b>CHAPTER 3 .....</b>	<b>50</b>
WATER AND SOLUTE RELATIONS OF GRAPEVINE ROOT UNDER SALINITY.....	50
3-1 Introduction .....	50
3-2 Materials and methods .....	52
3-2-1 Plant material.....	52
3-2-2 Measurement of root hydraulic conductivity ( $L_{pr}$ ) and reflection coefficient ( $\sigma_s$ ).....	53
3-2-3 Anatomy .....	54
3-3 Results.....	55
3-3-1 Root hydraulic conductivity and reflection coefficient.....	55
3-3-2 Root anatomy .....	59
3-4 Discussion.....	61
3-4-1 $L_{pr}$ and $\sigma_s$ .....	61
3-4-2 Root anatomy .....	63
<b>CHAPTER 4 .....</b>	<b>64</b>
APOPLASTIC FLUX (FLUORESCENT DYE APPROACH) .....	64
4-1 Introduction .....	64
4-2 Materials and Methods.....	65
4-2-1 Plant material.....	65
4-2-2 Experimental design .....	65
4-2-3 Determination of Cl and PTS .....	66
4-3 Results.....	66
4-3-1 Chloride.....	66
4-4 Discussion.....	71
<b>CHAPTER 5 .....</b>	<b>73</b>
<sup>36</sup> CL <sup>-</sup> COMPARTMENTATION AND FLUX CHARACTERISTICS .....	73

IN GRAPEVINE .....	73
5-1 Introduction .....	73
5-2 Materials and methods .....	75
5-2-1 Plant material.....	75
5-2-2 Measurement of <sup>36</sup> Cl <sup>-</sup> fluxes.....	76
5-2-2-1 Experiment 1 (Initial influx) :.....	76
5-2-2-2 Experiment 2 (short period influx): .....	76
5-2-2-3 Experiment 3 (Concentration Kinetics of <sup>36</sup> Cl <sup>-</sup> influx):.....	77
5-2-2-4. Experiment 4 ( <sup>36</sup> Cl <sup>-</sup> uptake by main and lateral roots):.....	77
5-2-2-5 Experiment 5 (uptake of <sup>36</sup> Cl <sup>-</sup> to the shoot):.....	77
5-2-2-6 Experiment 6 (efflux of <sup>36</sup> Cl <sup>-</sup> ):.....	78
5-2-2-7 Analysis of efflux experiments:.....	79
5-3 Results.....	80
5-3-1 Experiment 1: .....	80
5-3-2 Experiment 2: .....	80
5-3-3 Experiment 3: .....	81
5-3-4 Experiment 4: .....	83
5-3-5 Experiment 5: .....	83
5-3-6 Experiment 6: .....	84
5-4 Discussion.....	89
5-5 Appendix.....	93
<b>CHAPTER 6 .....</b>	<b>96</b>
CL <sup>-</sup> , NA <sup>+</sup> AND K <sup>+</sup> DISTRIBUTION IN GRAPEVINE ROOT .....	96
PRETREATED WITH NA <sup>+</sup> CL (X-RAY MICROANALYSIS).....	96
6-1 Introduction .....	96
6-2 Materials and methods .....	98
6-2-1 Plant material.....	98
6-2-2 X-ray microanalysis.....	98
6-3 Results.....	100
6-4 Discussion.....	104
<b>CHAPTER 7 .....</b>	<b>107</b>
MEMBRANE POTENTIALS OF GRAPEVINE ROOT CORTICAL CELLS .....	107
AND ROOT SURFACE-POTENTIAL UNDER HIGH SALINITY .....	107
7-1 Introduction .....	107
7-1-1 The root surface potential. ....	109
7-2 Materials and methods .....	110
7-2-1 Plant material.....	110
7-2-2 The cortical cell membrane potential.....	110
7-2-3 Measurement of the root surface potential.....	111
7-3 Results.....	111
7-3-1 The cortical cell membrane potential.....	111

7-3-2 The root surface potential.....	115
7-4 Discussion.....	119
<b>CHAPTER 8 .....</b>	<b>124</b>
GENERAL DISCUSSION AND FUTURE PERSPECTIVES.....	124
8-1 Discussion.....	124
8-2 Future perspectives.....	130
<b>REFERENCES:.....</b>	<b>133</b>

# List of Figures

Figure 1-1.....	17
Figure 1-2.....	22
Figure 1-3.....	27
Figure 1-4.....	29
Figure 2- 1.....	40
Figure 2- 2.....	44
Figure 2- 3.....	44
Figure 2- 4.....	45
Figure 2- 5.....	45
Figure 2- 6.....	46
Figure 2- 7.....	46
Figure 2- 8.....	47
Figure 3- 1.....	56
Figure 3- 2.....	57
Figure 3- 3.....	57
Figure 3- 4.....	58
Figure 3- 5.....	59
Figure 3- 6.....	60
Figure 4- 1.....	66
Figure 4- 2.....	67
Figure 4- 3.....	68
Figure 4- 4.....	68
Figure 4- 5.....	69
Figure 5- 1.....	78
Figure 5- 2.....	79
Figure 5- 3.....	81
Figure 5- 4.....	82
Figure 5- 5.....	83
Figure 5- 6.....	84
Figure 5- 7.....	85
Figure 5- 8.....	85

Figure 5- 9.....	86
Figure 5-10.....	87
Figure 6- 1.....	99
Figure 6- 2.....	101
Figure 6- 3.....	102
Figure 6- 4.....	103
Figure 7- 1.....	112
Figure 7- 2.....	113
Figure 7- 3.....	114
Figure 7- 4.....	116
Figure 7- 5.....	117
Figure 7- 6.....	118
Figure 8- 1.....	130

## List of Tables

Table 1-1 .....	33
Table 1-2 .....	34
Table 1-3 .....	35
Table 3- 1 .....	66
Table 4- 1 .....	70
Table 4- 2 .....	70
Table 5- 1 .....	81
Table 5- 2 .....	88
Table 5- 3 .....	93
Table 7- 1 .....	113
Table 7- 2 .....	115
Table 7- 3 .....	118



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## **Publication from this thesis**

**Nasser Abbaspour, Brent Kaiser and Stephen D. Tyerman (2005)**  $\text{Cl}^- / \text{NO}_3^-$  selectivity in grapevine roots and relationship to salt tolerance. Combio2005, Adelaide, Australia (Poster).

## **Declaration**

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Signed:

Date:

*To:*

*my wife, Irandokht*

*my sons, Ali and Hesam*

*and*

*my father and mother*

## Abstract

Soil salinity is one of the major abiotic stresses that decreases agricultural crop production through imposition of both ionic and osmotic stresses. The accumulation of  $\text{Na}^+$  and  $\text{Cl}^-$  in the cytosol to toxic levels inhibits metabolism. Unlike  $\text{Na}^+$ , less is known about  $\text{Cl}^-$  uptake and transport in plants. Grapevine is moderately sensitive to salinity and accumulation of toxic levels of  $\text{Cl}^-$  in leaves is the major reason for salt-induced symptoms. In this study  $\text{Cl}^-$  uptake and transport mechanism(s) were investigated in two grapevine (*Vitis* sp.) rootstock hybrids differing in salt tolerance: 1103 Paulsen (salt tolerant) and K 51-40 (salt sensitive).

Increased external salinity caused high  $\text{Cl}^-$  accumulation in shoots of the salt sensitive K 51-40 in comparison to Paulsen. Measurement of  $^{15}\text{NO}_3^-$  net fluxes under high salinity showed that by increasing external  $\text{Cl}^-$  concentrations K 51-40 roots showed reduced  $\text{NO}_3^-$  accumulation. This was associated with increased accumulation of  $\text{Cl}^-$ . In comparison to Paulsen, K 51-40 showed reduced  $\text{NO}_3^- / \text{Cl}^-$  root selectivity with increased salinity, but Paulsen had lower selectivity over the whole salinity range (0-45 mM).

In order to examine if root hydraulic and permeability characterisations accounted for differences between varieties, the root pressure probe was used on excised roots. This showed that the osmotic  $Lp_r$  was significantly smaller than hydrostatic  $Lp_r$ , but no obvious difference was observed between the rootstocks. The reflection coefficient ( $\sigma$ ) values (0.48-0.59) were the same for both rootstocks, and root anatomical studies showed no obvious difference in apoplastic barriers of the main and lateral roots. Comparing the uptake of  $\text{Cl}^-$  with an apoplastic tracer, PTS (3-hydroxy-5, 8, 10-pyrenylsulphonic acid), showed that there was no correlation between  $\text{Cl}^-$  and PTS transport. These results indicated that by-pass flow of salts to the xylem is the same for both rootstocks ( $10.01 \pm 3.03$  % and  $12.1 \pm 1.21$  %) and hence pointed to differences in membrane transport to explain difference in  $\text{Cl}^-$  transport to the shoot.

$^{36}\text{Cl}^-$  fluxes across plasma membrane and tonoplast of K 51-40 and Paulsen roots showed that  $^{36}\text{Cl}^-$  influx in root segments of Paulsen was greater than K 51-40 over the first 10 minutes. Unidirectional influx within 10 min loading time showed increases with increases in the external concentrations in both rootstocks but Paulsen had higher influx rate when compared to K 51-40. This appeared to be due to a greater  $V_{\max}$ . There was no significant difference in  $K_m$ .

It was shown that  $^{36}\text{Cl}^-$  accumulation and transport rate to the shoot of K 51-40 was higher than that of Paulsen. Compartmental analysis of  $^{36}\text{Cl}^-$  efflux from intact roots confirmed that the difference in influx observed between the rootstocks was consistent with the results obtained for excised roots, although the values were not exactly the same. It was also shown that the main root of Paulsen had greater contribution to  $^{36}\text{Cl}^-$  uptake than lateral roots.  $^{36}\text{Cl}^-$  fluxes by lateral roots were not significantly different between the rootstocks.

$\text{Cl}^-$  and  $\text{Na}^+$  distribution patterns in different root cell types were determined using the X-ray microanalysis technique. It was shown that  $\text{Cl}^-$  content in the hypodermis and cortical cells was higher than the other cell types in both rootstocks, but overall  $\text{Cl}^-$  content in the root of Paulsen was higher than K 51-40. The pericycle of the main root of Paulsen accumulated more  $\text{Cl}^-$  than K 51-40. It was concluded that  $\text{Cl}^-$  loading to the xylem was different in the rootstocks and Paulsen tended to prevent the xylem  $\text{Cl}^-$  loading process. Lateral roots also displayed opposite behaviour consistent with flux analysis.

Membrane potential difference (PD) of the cortical cells showed a rapid and transient depolarization by adding 30 mM NaCl in both rootstocks that was followed by a gradual hyperpolarization. Depolarizations caused by 30 mM Choline-Cl, Na-MES and NaCl measured by the root surface potential method showed that Choline-Cl in K 51-40 and Na-MES in Paulsen caused greater depolarization than that of Na-MES in K 51-40 and Choline-Cl in Paulsen respectively. Assuming that PD measured in this method was the trans-root potential (TRP), it was concluded that the higher depolarization by Choline-Cl in K 51-40 can be due to higher  $\text{Cl}^-$  efflux rate to the xylem. Two different mechanisms were also detected for  $\text{Cl}^-$  transport: HATS which was observed in the range of 0.5-5 mM and a LATS in the range of 10-30 mM of the

external NaCl concentration. This was consistent with the concentration dependence of Cl<sup>-</sup> influx.

In conclusion, evidence obtained from different experiments of this study indicated that in the grapevine rootstocks (Paulsen and K 51-40) Cl<sup>-</sup> was mostly transported through the symplastic pathway. From  $E_{Cl}$  values determined for the rootstocks by the Nernst equation, a proton-driven transport system was responsible for Cl<sup>-</sup> transport in both the HATS and LATS range of external NaCl concentrations. The rate of Cl<sup>-</sup> transport from the root to shoot (xylem loading) was the major difference in Cl<sup>-</sup> transport between the rootstocks in terms of salinity tolerance.



## Abbreviation

<b>ABA</b>	Abscisic acid
<b>AMTS</b>	Ammonium transport system
<b>ANOVA</b>	Analysis of variance
<b>cpm</b>	Counts per minute
<b>CW</b>	Cell water
<b>DW</b>	Dry weight
<b><math>E_{Cl}</math></b>	Nernst potential of $Cl^-$
<b>EDTA</b>	Ethylene diamine tetra-acetic acid
<b>FW</b>	Fresh weight
<b>HKT</b>	High affinity potassium transporter
<b>iHATS</b>	Substrate induced high affinity transport system
<b>cHATS</b>	Constitutively active high affinity transport system
<b>IBA</b>	Indole-3- butyric acid
<b><math>J_{BF}</math></b>	Bypass flow of water
<b><math>Lp_r</math></b>	Root hydraulic conductivity
<b>MBq</b>	Megabecquerel
<b>MIFE</b>	Microelectrode ion-flux estimation
<b>MIPs</b>	Major intrinsic proteins
<b>NAXT</b>	Nitrate excretion transporter
<b>NRT</b>	Nitrate transporter
<b><math>\mu Ci</math></b>	Microcuri
<b>PD</b>	Potential difference
<b>PP</b>	Pressure probe
<b>PTR</b>	Peptide transporter
<b>PTS</b>	8-hydroxy-1,3,6- pyrenetrisulfonic acid
<b><math>S_o</math></b>	Specific activity
<b>S</b>	Selectivity
<b>SDS</b>	Sodium dodecyl sulphate
<b>SEM</b>	Scanning electron microscope
<b>SE</b>	Standard error
<b>S.P.Q</b>	6-methoxy-N-(3-sulfopropyl) quinolinium

<b>TEA</b>	Tetraethyl ammonium chloride, $K^+$ channel blocker
<b>TTX</b>	Tetrodotoxin, $Na^+$ channel blocker
<b>USL</b>	Unstirred layer
$\sigma$	Reflection coefficient
$\Phi$	Ion flux