A comparative study of Cl^- transport across the roots of two grapevine rootstocks, K 51-40 and Paulsen, differing in salt tolerance

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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 Na^+ and Cl^- in the cytosol to toxic levels inhibits metabolism. Unlike Na^+, less is known about Cl^- uptake and transport in plants. Grapevine is moderately sensitive to salinity and accumulation of toxic levels of Cl^- in leaves is the major reason for salt-induced symptoms. In this study 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 Cl^- accumulation in shoots of the salt sensitive K 51-40 in comparison to Paulsen. Measurement of $^{15}NO_3^-$ net fluxes under high salinity showed that by increasing external Cl^- concentrations K 51-40 roots showed reduced NO_3^- accumulation. This was associated with increased accumulation of Cl^-. In comparison to Paulsen, K 51-40 showed reduced NO_3^- / 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 (σ) 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 Cl^- with an apoplastic tracer, PTS (3-hydroxy-5, 8, 10-pyrentrisulphonic acid), showed that there was no correlation between Cl^- and PTS transport. These results indicated that by-pass flow of salts to the xylem is the same for both rootstocks (10.01±3.03 % and 12.1±1.21 %) and hence pointed to differences in membrane transport to explain difference in Cl^- transport to the shoot.

$^{36}Cl^-$ fluxes across plasma membrane and tonoplast of K 51-40 and Paulsen roots showed that $^{36}Cl^-$ 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 $^{36}Cl^-$ accumulation and transport rate to the shoot of K 51-40 was higher than that of Paulsen. Compartmental analysis of $^{36}Cl^-$ 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 $^{36}Cl^-$ uptake than lateral roots. $^{36}Cl^-$ fluxes by lateral roots were not significantly different between the rootstocks.

Cl^- and Na^+ distribution patterns in different root cell types were determined using the X-ray microanalysis technique. It was shown that Cl^- content in the hypodermis and cortical cells was higher than the other cell types in both rootstocks, but overall Cl^- content in the root of Paulsen was higher than K 51-40. The pericycle of the main root of Paulsen accumulated more Cl^- than K 51-40. It was concluded that Cl^- loading to the xylem was different in the rootstocks and Paulsen tended to prevent the xylem 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 Cl^- efflux rate to the xylem. Two different mechanisms were also detected for 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^- influx.

In conclusion, evidence obtained from different experiments of this study indicated that in the grapevine rootstocks (Paulsen and K 51-40) Cl^- 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^- transport in both the HATS and LATS range of external NaCl concentrations. The rate of Cl^- transport from the root to shoot (xylem loading) was the major difference in Cl^- transport between the rootstocks in terms of salinity tolerance.

Abbreviation

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

TEA	Tetraethyl ammonium chloride, K^+ channel blocker
TTX	Tetrodotoxin, Na^+ channel blocker
USL	Unstirred layer
σ	Reflection coefficient
Φ	Ion flux