A dynamic model for sodium intoxication unravels salt tolerance in grapevine (*Vitis vinifera* L.) rootstocks

Un modelo dinámico de intoxicación por sodio permite comprender la tolerancia a salinidad en portainjertos de vid (*Vitis vinifera* L.)

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Abstract

A correct selection of salt-tolerant plants should consider the relative effects of the various existing tolerance mechanisms. When toxic ions, like Na^+, reach the leaves, they affect the photosynthetic apparatus, reducing plant growth and performance. Leaf concentration of toxic ions depends on exclusion efficiencies at root level, or compartmentation in organs other than leaves. On the other hand, flow within the plant depends on leaf area, transpiration rate, and soil ion concentrations. From this perspective, in a feedback process, leaf area may be, simultaneously, cause and consequence of salt toxicity. To unravel how this feedback process influences salinity damage in grapevines, a dynamic model of Na^+ toxicity was developed. The theoretical model proposed a way to estimate plant exclusion and compartmentation efficiencies. Parametrization was based on a 60-days trial with potted cv. Malbec vines (*Vitis vinifera* L.), own-rooted and grafted onto 101-14Mgt, 1103P and Cereza, under three soil NaCl levels (0, 50 and 100 mM). The model simulated different grapevine rootstock responses to different salinity levels. These simulations evidenced the key role of Na^+ exclusion in long-term tolerance. Stomatal adjustment, compartmentation and rootstock conferred vigor showed relatively minor effects.

Keywords
salinity • ion exclusion • compartmentation • innate vigor • *Vitis vinifera* L.

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Resumen

Una correcta selección de plantas tolerantes a la sal requiere una evaluación del peso relativo de los diferentes mecanismos de tolerancia. Cuando los iones tóxicos, como el Na^+, alcanzan el tejido foliar, causan lesiones en el aparato fotosintético, lo que afecta el crecimiento y el rendimiento de la planta. La concentración foliar de iones tóxicos depende de la eficiencia de la planta para excluir el flujo de iones tóxicos desde el suelo a nivel de la raíz, o para compartimentar estos iones en órganos distintos a las hojas. Por otro lado, el flujo de sustancias tóxicas dentro de la planta también depende, entre otros factores, del área foliar, la velocidad de transpiración y la concentración de los iones tóxicos en la solución del suelo. Desde esta perspectiva, en un proceso de retroalimentación, el área foliar puede ser simultáneamente una causa y consecuencia de la toxicidad de la sal. Para dilucidar cómo este proceso de retroalimentación influye en el daño por salinidad en las vides, se desarrolló un modelo dinámico de toxicidad para Na^+. El modelo tuvo en cuenta este marco teórico y propuso una forma de calcular las eficiencias de exclusión y compartimentación de tóxicos de la planta. Para parametrizar el modelo, se llevó a cabo un ensayo de 60 días de cv. Malbec (*Vitis vinifera* L.) a pie franco e injertadas en 101-14Mgt, 1103P y Cereza, bajo tres niveles de NaCl (0, 50 y 100 mM). El modelo se usó para simular el comportamiento de distintos portainjertos de vid bajo diferentes niveles de salinidad. Estas simulaciones nos permitieron comprender el papel clave de la exclusión de Na + en la tolerancia a la salinidad a largo plazo. El ajuste estomático, la compartimentación y el vigor conferido por el portainjerto mostraron menores efectos.

Palabras clave
salinidad • exclusión de iones • compartimentación • vigor innato • *Vitis vinifera* L.

Introduction

Salinity is one major crop stressor (4, 25). In irrigated arid zones, affected areas can reach up to 25%, threatening food supply in many countries (9). Genetic selection of tolerant varieties is an effective way to face this problem (2). However, a particularly difficult issue arises with perennial fruit crops, where damages caused by salinity may accumulate over time, affecting plant productivity and longevity (36). In grapevines and citrus, excluder rootstocks have been widely proposed. These rootstocks avoid toxic ions like Na^+ and Cl^- from reaching the leaves, avoiding their harmful effect in leaf tissues (3, 22, 39). When Na^+ and Cl^- accumulate in the leaves, they induce membrane denaturation and affect photosynthesis and vegetative growth (6, 10, 23, 40). Ultimately, toxic ions cause leaf marginal necrosis and early defoliation (13, 24, 29). The accumulation of toxic ions in leaves depends, primarily, on their flow from soil to root, and secondly, on root exclusion efficiency. Salinity also influences plant transpiration by affecting leaf growth and stomatal conductance (6, 10, 23, 39). This may constitute an indirect mechanism for ion flow limitation, as it depends on ion concentration in the soil solution and on water flow into the root (which, in turn, depends on transpiration). For instance, many tolerant species grow in winter (*e.g.*, barley), when the evaporative demand is lower (17). Regarding the exclusion mechanism, roots are responsible for ion selective entrance to the plant. For example, in wheat, epidermal and subepidermal root cells are responsible for excluding most soil Na^+ and Cl^- (19). Nonetheless, the ability of a rootstock to confer salt tolerance to a plant cannot be only attributed to their root selective power. Indeed, roots and organs like stems and leaf petioles can also compartmentalize toxic ions. Grapevines can compartmentalize Na^+ in cell vacuoles of the root pericycle (34), while influencing the ability of stems to compartmentalize Cl^- and Na^+ (10) when used as rootstocks.

A correct selection of salt-tolerant genotypes should consider the relative effects of the various tolerance mechanisms. This requires being able to separate the *direct* effects of exclusion and compartmentation, from the *indirect* effect of limiting soil ion flow. With respect to *direct* effects, ion flow reaching the leaves results from subtracting, from the soil solution, those ions deviated to other destinations. These deviations depend on

plant excluding and compartmentation efficiencies. A theoretical scheme of how these efficiencies affect ion flow reaching leaf blades can be represented by typical equations, in which inflow is limited by entry efficiencies. For example, in the case of Na^+, its flow into the plant, to the canopy, reaching the leaves and leaf blades, can be represented by equations i to iv, respectively.

$$J_{Na^+ Plant} = J_{Na^+ Soil} \cdot (1 - Exc_{Roots}) \quad \text{(i)}$$

$$J_{Na^+ Canopy} = J_{Na^+ Plant} \cdot (1 - Comp_{Roots}) \quad \text{(ii)}$$

$$J_{Na^+ Leaves} = J_{Na^+ Canopy} \cdot (1 - Comp_{Stems}) \quad \text{(iii)}$$

$$J_{Na^+ Leaf\ blades} = J_{Na^+ Leaves} \cdot (1 - Comp_{Petiols}) = J_{Na^+ Soil} \cdot (1 - Exc_{Roots}) \cdot (1 - Comp_{Roots}) \cdot (1 - Comp_{Stems}) \cdot (1 - Comp_{Petiols}) \quad \text{(iv)}$$

In these equations, different J_{Na^+} represent the different net ion flows (*e.g.*, in mg day^{-1}) moving from the soil to different plant organs, independently from the route followed to reach a certain organ. This is considered because, for several species, some of the Cl^- and Na^+ reaching the stem via xylem, can be redistributed via phloem (15, 41). So, J_{Na^+} would be equal to organ inflow minus organ outflow. Exc_{Roots} represents the root excluding efficiency, *i.e.*, clearing eq. i, $Exc_{Roots} = 1 - \frac{J_{Na^+ Plant}}{J_{Na^+ Soil}}$ (v).

Comp represents compartmentation efficiencies , *i.e.*, clearing eqs. ii to iv, $Comp_{Roots} = 1 - \frac{J_{Na^+ Canopy}}{J_{Na^+ Plant}}$ (vi); $Comp_{Stems} = 1 - \frac{J_{Na^+ Leaves}}{J_{Na^+ Canopy}}$ (vii); and $Comp_{Petiols} = 1 - \frac{J_{Na^+ Leaf\ blades}}{J_{Na^+ Leaves}}$ (viii).

On the other hand, $J_{Na^+ Soil}$ depends on leaf area (LA), transpiration rate (E), and ion concentration in the soil solution. This can be formaliz $J_{Na^+ Soil} = \frac{E}{2} \cdot t \cdot LA \cdot [Na^+]_{Soil}$ (ix);

where, in a simplified manner, E/2 is the average daily sap flow (*e.g.*, L h^{-1} m^{-2}), considering that daily maximum E almost doubles the average daily rate (26); t is the number of daylight hours; LA is plant leaf area (*e.g.*, in m^2); and $[Na^+]_{Soil}$ is sodium concentration in the edaphic solution (*e.g.*, in g L^{-1}). The plant controls transpiration through stomatal adjustment, an "indirect" mechanism limiting ionic inflow.

By calculating the different ion flows and efficiencies through the proposed equations, and separating the direct and indirect factors involved, their relative weight can be assessed. Thus, evaluating scion-rootstock combinations in relation to toxicity avoidance, would be possible. Factors such as root exclusion and compartmentation would obviously be exerted by the rootstock. Other factors, such as stem compartmentation, limiting sap flow or LA expansion could also be influenced by the rootstock (10).

For the proposed analysis, leaf growth was chosen as model output. As indicated, salinity affects carbon assimilation, leaf growth and, in severe cases, causes defoliation. Above a certain threshold, increasing concentrations of a toxic ion such as Na^+ cause linearly proportional leaf growth decreases (38). Under this framework, foliar ion concentration depends on net ion flow, multiplied by the time lapsed under the saline condition. This concentration will also depend on leaf biomass (as ionic concentration is expressed per unit of dry weight), closely associated with cell and leaf expansion. In addition, according to equation ix, soil ion flow also depends on LA, meaning that under a certain saline condition, LA results from the dynamic changes that occur in a feedback process (*i.e.*, LA is dependent on salt concentration at a time t, but in turn, it influences salt concentration at a time t+1). In grapevines, some rootstocks conferring higher vigor, result more tolerant to salinity (38, 40). Therefore, it might be possible that ion dilution in a higher LA may act as an indirect tolerance mechanism, related to a feedback process, as proposed above.

This study presents the construction of a dynamic model based on the explained feedback processes. Our model aimed to unravel how salinity influences leaf growth and maintenance in grafted grapevines. The model was parameterized with experimental data and used to simulate the behavior of four grapevine rootstocks under saline stress. The model also evaluated the relative of various direct and indirect mechanisms for salt tolerance.

Materials and methods

A 60-day trial was carried out with 1-year old potted cv. Malbec vines (clone 18 INTA), grafted onto 101-14Mgt (*V. riparia x V. rupestris*), 1103P (*V. berlandieri x V. rupestris*) and Cereza (*Vitis vinifera* L.; locally recommended as a salt-tolerant cultivar or rootstock), and a control (own-rooted Malbec); in the Agricultural Experiment Station of INTA Mendoza, Argentina. The grapevines were grafted by omega technique in the previous season and planted one month before the onset of the trial, in cylindrical pots (60 cm long by 20 cm in diameter). The substrate was composed of two parts of perlite and one part of sand. Grapevines pruned to one shoot, were grown in a greenhouse (average temperature 25°C, PAR 800 µmol m^{-2} s^{-1}). Initial LA showed no differences among the vines (0.14 m^2 on average, $p<0.05$). Irrigation was performed with saline solutions at three levels: 0, 50 and 100 mM NaCl. The solutions were prepared with regular water (EC, 1.04 dS m^{-1}, 70 mg L^{-1} Na^+; 88.75 mg L^{-1} Cl^-), and supplemented with nutrient solution (172.5 N, 15 P, 30 K, 90 S, 0.76 Fe, 0.38 Mn, 0.74 Zn, mg L^{-1}). The treatments, 0, 50 and 100 mM NaCl, reached an EC of 2.10, 7.32 and 12.23 dS m^{-1}, respectively. Automatic irrigation was achieved with a closed-loop system keeping constant field capacity (8 L day^{-1} $plant^1$, distributed in 8 irrigations). Salinity was gradually imposed, taking 15 and 30 days for the 50 and 100 mM NaCl treatments to reach their stress levels, respectively. The plants remained subjected to stress for 45 and 30 extra days, respectively. The experimental design consisted of a complete randomized design with two factors: salinity level and rootstock genotype, for a total of 12 treatments replicated three times (36 vines). Electric conductivity of the solutions was daily monitored with a conductivity meter (HI 9033 Multi-Range; Hanna Instruments, USA). At the end of the trial, the following variables were determined. Leaf area (LA) was estimated from measurements of leaf length and width using the following equation: $LA= 2.98 + 0.66\ (l \cdot w)$, where l and w represent maximum leaf length and width, in m (R^2 0.98; 38). Stomatal conductance (g_s) and transpiration rate (E) were measured in fully expanded leaves at midday with an infrared gas analyzer (CIRAS-2 PP Systems, Hertfordshire, UK). Water use (WU) was calculated by multiplying E by LA, considering number of days under stress. Dry weights (leaf blades, petioles, stems and roots) were obtained with an electric oven, at 60°C. Na^+ concentration was determined by a flame photometer (Crudo Caamaño; Argentina) from hydrochloric extracts of leaf blades, petioles, stems and root samples, (1:100; 27) and from pot drainage water (*i.e.,* $[Na^+]_{Soil}$). Total ion content in each organ was obtained by multiplying sample ion concentration by tissue dry weight. Net average daily flows of Na^+ ($J_{Na}+$) were calculated as total Na^+ in each organ, divided by the number of days under saline irrigation. Soil Na^+ flow ($J_{Na}+_{Soil}$) was calculated using eq. vi, from measurements of $[Na^+]_{Soil}$, E, and LA. With these flows, exclusion efficiencies (Exc) and root, stem and petiole compartmentations (Comp), were calculated for each rootstock and saline condition, using equations v to viii.

Rootstocks and salinity interactions, for each variable, were analyzed by ANOVA, after verifying normality and homoscedasticity. Means were compared by Fisher's LSD test, with a confidence level of 95%. After linear regression analysis, dummy variables were used to compare the regression lines obtained for the studied rootstocks. Statistical analyses were performed using the software StatGraphics Plus 4 for Windows (Statistical Graphics Corp., USA). The dynamic simulation model was designed using the system dynamics methodology developed by Forrester (1994). Simulations were resolved with the spreadsheet program Microsoft Office Excel (Microsoft Corp., USA), using the option of iterative calculation.

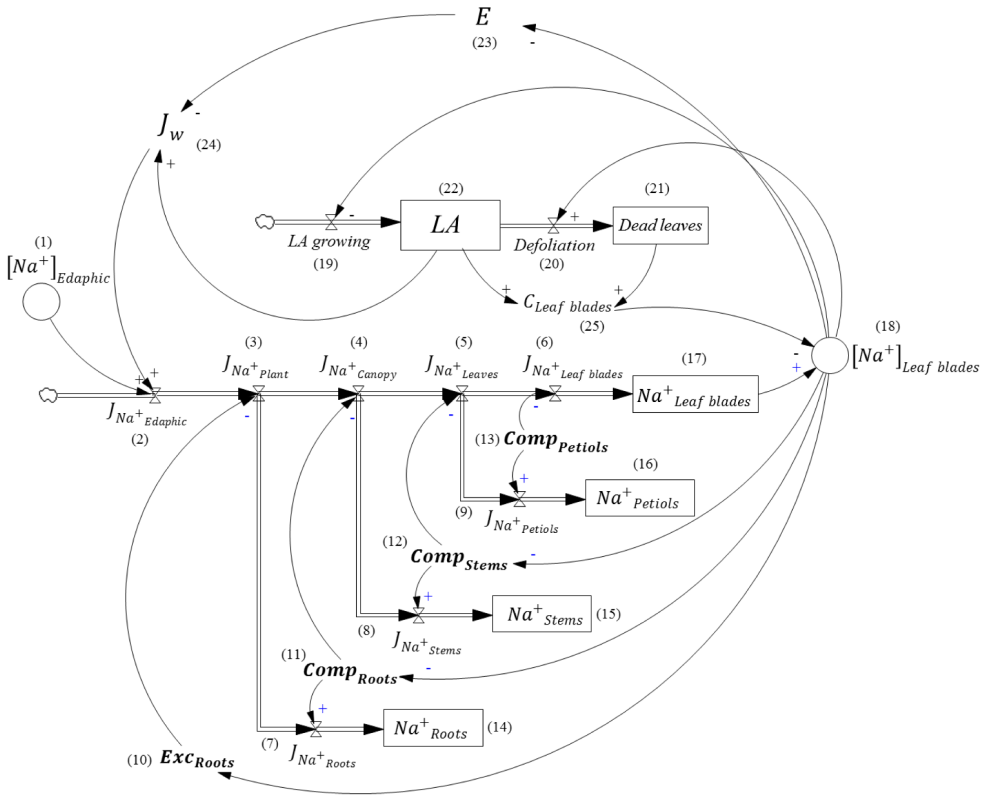
Results

Construction of the dynamic model of Na^+ flow

Based on the theoretical framework presented by equations i to ix, a model to estimate net sodium flow ($J_{Na}+$) dynamics from soil to leaves, was constructed. The model considered soil Na^+ concentration as input variable. It also considered empirical observations of water flow and exclusion, and organs compartmentation efficiencies (*i.e.*, Exc_{Roots}, $Comp_{Roots}$, $Comp_{Stems}$ y $Comp_{Petiols}$). Temporarily integrating daily Na^+ flow to the leaves, the model estimated the amount of Na^+ accumulated in leaves during the growing season. Na^+ concentration in leaf

blades (*i.e.*, $[Na^+]_{Leaf\ blades}$), the main output of the model, depended on the amount of Na^+ accumulated in this organ, and on dry matter ($C_{Leaf\ blades}$). The latter is an empirical variable closely correlated with LA. Furthermore, both LA growth and defoliation (and therefore, net biomass) were calculated based on their empirical functional dependence on $[Na^+]_{Leaf\ blades}$. At this point, the first feedback loop, was established. Plant LA depended on toxicity level, but was also partially responsible for this toxicity, by promoting transpiration and ionic flow, considering that plant transpiration partly depends on LA. The model also considered that salt toxicity negatively influenced E, via stomatal adjustment, so an empirical relationship between E and $[Na^+]_{Leaf\ blades}$, was used.

A scheme of the model and its multiple functional relationships is presented in figure 1. This follows the typical outline given by Forrester (1994), separating mass and information flows. As mass flows, the first variables are the different J_{Na}+from soil to the various organs. Net LA growth, promoted by salt toxicity (*i.e.*, LA minus defoliation) also represents a type of mass flow. As information signals, the model uses the functional relationships among variables in the plant. It considers that, increasing toxicity could end up affecting plant ability to exclude or compartmentalize Na^+. Given this, exclusion and compartmentation efficiencies depend on $[Na^+]_{Leaf\ blades}$. These last functional relationships defined other possible feedback loops, given that $[Na^+]_{Leaf\ blades}$ also depended on these efficiencies.



Simple arrows indicate functional relationships between variables; double-lined arrows indicate flow of matter; clouds are sources and sinks; text sentences correspond to variables (texts in boxes or close to circles correspond to levels); numbers in brackets are codes of parameters and equations included in table 1 (page 94-95).

Las flechas simples indican las relaciones funcionales entre las variables; las flechas dobles indican el flujo de materia; las nubes son fuente y sumideros; los textos corresponden a variables (los textos en recuadros o cerca de los círculos corresponden a niveles); los números entre paréntesis son códigos de parámetros y ecuaciones incluidos en la tabla 1 (pág. 94-95).

Figure 1. Diagram of the dynamic model proposed to explain how soil Na^+ affects growth and leaf area maintenance in grapevines.

Figura 1. Diagrama del modelo dinámico propuesto para explicar cómo el Na^+ del suelo afecta el crecimiento y el mantenimiento del área foliar en vides.

Experimental observations and model parameterization

To reveal differences among rootstocks, linear regressions, with auxiliary variables, were performed. Physiological and vegetative variables were related to foliar toxicity, considering all cases together (*i.e.*, 0, 50 and 100 mM NaCl). Only those empirical relationships with high levels of determination (R^2 > 70%) and statistical significance ($p < 0.01$) were used to parameterize the model. A list of the functional relationships empirically found, and then used in the model, is shown in table 1 (page 94-95). In this table, the same numerical codes (#) assigned to the functions are those indicated on the model, showing the different functional relationships among variables (figure 1, page 92). Increasing LA, defoliation, E, Exc_{Roots}, $Comp_{Roots}$, and $Comp_{Stems}$ depended on foliar toxicity –*i.e.*, they depended on $[Na^+]_{Leaf\ blades}$. Three of these variables (*i.e.*, Exc_{Roots}, $Comp_{Stems}$ and LA) also showed differences between rootstocks and were, therefore, chosen to simulate different behaviors. Increasing LA depended on $[Na^+]_{Leaf\ blades}$ in a logarithmic pattern (R^2 82%), but Cereza was more affected by Na^+ (*i.e.*, LA=-0.0031. $In[Na^+]_{Leaf\ blades}$+0.0037) than the other rootstocks (*i.e.*, LA=-0.0060. $In\ [Na^+]_{Leaf\ blades}$+0.0037; table 1, page 94-95). For all rootstocks, a close linear association between leaf blade $[Na^+]$ and $[Cl^-]$, was observed (R^2 91%). Interestingly, Cl^-, a well-known toxic ion, nearly doubled Na^+. Results also showed that regardless of salinity level, leaf blades dry weight ($C_{Leaf\ blades}$) was linearly related with LA (R^2 96%); but in a rootstock-dependent manner. At equal biomass, own-rooted Malbec resulted to have less LA than the other rootstocks (*i.e.*, for own-rooted vines, $C_{Leaf\ blades}$ = 9.18+41.81.LA; while for the others, $C_{Leaf\ blades}$ = 1.55+41.81.LA; table 1, page 94-95). This could have meant a minor advantage for Malbec, given that Na^+ flow was linked to E, thus, indirectly dependent on LA.

In addition to the regression analysis (*i.e.*, $[Na^+]_{Leaf\ blades}$ *vs.* plant variables), an evaluation of the impact of soil saline condition on plant variables, confirmed marked differences between rootstocks when subjected to salinity. Under saline conditions, own-rooted Malbec retained 34% more LA than 1103P, while 1103P retained 96% more than 101-14Mgt and Cereza (table 2, page 96; average LA augmented 52% less at 100 mM NaCl than at 50 mM, and 45% less at 50 mM NaCl than control -data not shown). A lower LA in 101-14Mgt and Cereza at 100 mM NaCl, was due to decreased growth but also to defoliation (101-14Mgt and Cereza lost 0.37 m^2 and 0.18 m^2 of LA per plant, respectively). No differences were observed in g_s between 50 and 100 mM NaCl treatments. Saline treatments had 53% lower g_s than the control (data not shown). However, under saline conditions, own-rooted Malbec maintained a higher g_s than 101-14Mgt (92 *vs.* 30 mmol m^{-2} s^{-1}); whereas 1103P did not differ from own-rooted vines, and Cereza did not significantly differ from 101-14Mgt. No significant differences were observed in E between 50 and 100 mM NaCl, but own-rooted Malbec also maintained a higher E than 101-14Mgt (1.33 *vs.* 0.46 mmol m^{-2} s^{-1}). Cereza and 1103P showed intermediate behaviors for this variable.

Regarding $J_{Na^+\ Leaf\ blades}$, own-rooted Malbec and 1103P had a 63% lower $J_{Na^+\ Leaf\ blade}$ than Cereza and 101-14Mgt (table 3, page 96). Rootstocks with a lower $J_{Na^+\ Leaf\ blade}$ presented higher LA increments and lower defoliation. The Exc_{Roots} showed differences among rootstocks, but not between saline treatments. Own-rooted Malbec maintained a slightly higher Exc_{Roots} than 101-14Mgt and Cereza (99.997% against 99.988%), whereas 1103P did not differ from both groups. $Comp_{Roots}$ was also high in all rootstocks and saline conditions, except for 101-14Mgt at 100 mM NaCl (70% against 20%). $Comp_{Stems}$ was high at 50 mM NaCl (61% on average for all rootstocks) but declined at 100 mM NaCl (43% on average). Differences among rootstocks were also observed for this variable. Own-rooted Malbec and 1103P showed 32% more $Comp_{Stems}$ than 101-14Mgt and Cereza. In short, the higher LA loss of 101-14 Mgt and Cereza was due to their lower exclusion and stem compartmentation efficiencies. However, these rootstocks had a 57% lower $J_{Na^+\ Soil}$ than other more tolerant rootstocks (*i.e.*, own-rooted vines and 1103P), alleviating the stress.

Table 1. Theoretical and empirical equations used by the dynamic model of Na^+plant toxicity.
Tabla 1. Ecuaciones teóricas y empíricas utilizadas por el modelo dinámico de toxicidad por Na^+.

Variable	Equation or parameter	#	Units	R^2	*p-value*
Concentration of Na^+ in the edaphic solution (entry variable):					
$[Na^+]_{Soil}$	Entry data	1	mg L^{-1}	-	-
Ionic flows (J_{Na^+}; calculation variables):					
$J_{Na^+_{Soil}}$	$[Na^+]_{Edaphic} \cdot J_w$	2	mg day^{-1}	-	-
$J_{Na^+_{Plant}}$	$J_{Na^+_{Edaphic}} \cdot (1 - Exc_{Roots}) \cdot 1000$	3	µg day^{-1}	-	-
$J_{Na^+_{Canopy}}$	$J_{Na^+_{Plant}} \cdot (1 - Comp_{Roots})$	4	µg day^{-1}	-	-
$J_{Na^+_{Leaves}}$	$J_{Na^+_{Canopy}} \cdot (1 - Comp_{Stems})$	5	µg day^{-1}	-	-
$J_{Na^+_{Leaf\ blades}}$	$J_{Na^+_{Leaves}} \cdot (1 - Comp_{Petiols})$	6	µg day^{-1}	-	-
$J_{Na^+_{Roots}}$	$J_{Na^+_{Plant}} \cdot Comp_{Roots}$	7	µg day^{-1}	-	-
$J_{Na^+_{Stems}}$	$J_{Na^+_{Canopy}} \cdot Comp_{Stems}$	8	µg day^{-1}	-	-
$J_{Na^+_{Petiols}}$	$J_{Na^+_{Leaves}} \cdot Comp_{Petiols}$	9	µg day^{-1}	-	-
Exclusion (Exc) and compartmentation (Comp) efficiencies (empirical data and variables):					
Exc_{Roots}	In own-rooted Malbec: 0.99997; In 101-14Mgt: 0.99987 In 1103P: $If\{1 - 0.000290729 \cdot [Na^+]_{Leaf\ blades} > 0.99999992;\ 0.99999992;\ 1 - 0.000290729 \cdot [Na^+]_{Leaf\ blades}\}$ In Cereza: $If\{1 - 0.000143754 \cdot [Na^+]_{Leaf\ blades} > 0.9999496;\ 0.9999496;\ 1 - 0.000143754 \cdot [Na^+]_{Leaf\ blades}\}$	10	-	0.79	0.0007
$Comp_{Roots}$	In 101-14Mgt: $exp(-0.207 - 0.819 \cdot [Na^+]_{Leaf\ blades})$ In 1103P, Cereza, and own-rooted Malbec: 0.7	11	-	0.87	0.0002
$Comp_{Stems}$	$If\{0.379 - 0.114 \cdot ln[Na^+]_{Leaf\ blades} > 0.8;\ 0.8;\ 0.379 - 0.114 \cdot ln[Na^+]_{Leaf\ blades}\}$	12	-	0.89	0.0000
$Comp_{Petiols}$	0,17	13	-	-	-

Na⁺ content in organs (calculation variables):					
Variable	**Equation or parameter**	**#**	**Units**	**R^2**	***p-value***
Na^+_{Roots}	$\int_{t=1}^{n} J_{Na^+_{Roots}} \cdot dt$	14	µg	-	-
Na^+_{Stems}	$\int_{t=1}^{n} J_{Na^+_{Stems}} \cdot dt$	15	µg	-	-
$Na^+_{Petiols}$	$\int_{t=1}^{n} J_{Na^+_{Petiols}} \cdot dt$	16	µg	-	-
$Na^+_{Leaf\ blades}$	$\int_{t=1}^{n} J_{Na^+_{Leaf\ blades}} \cdot dt$	17	µg	-	-
Na⁺ concentration in leaf blades (calculation variable):					
$[Na^+]_{Leaf\ blades}$	$Na^+_{Leaf\ blades} / (C_{Leaf\ blades} \cdot 10)$	18	mg % mg	-	-
Physiological traits (calculation and empirical variables):					
LA growing	In 101-14Mgt, 1103P, and own-rooted Malbec: $-0.0060 \cdot ln[Na^+]_{Leaf\ blades} + 0.0037$ In Cereza: $-0.0031 \cdot ln[Na^+]_{Leaf\ blades} + 0.0037$	19	m^2 day^{-1}	0.82	0.0000
Defoliation	$0.0035 \cdot [Na^+]^2_{Leaf\ blades} - 0.0013 \cdot [Na^+]_{Leaf\ blades} + 0.00004$	20	m^2 day^{-1}	0.97	0.0000
Dead leaves	$\int_{t=1}^{n} Defoliation \cdot dt$	21	m^2	-	-
Leaf area (LA)	$\int_{t=1}^{n} (LA\ growing - Defoliation) \cdot dt$	22	m^2	-	-
Transpiration (E)	$-0.134 \cdot ln[Na^+]_{Leaf\ blades} + 0.172$	23	L m^{-2} day^{-1}	0.72	0.0000
Sap flow (J_w)	$E \cdot SF$	24	L day^{-1}	-	-
Leaf blades biomass $(C_{Leaf\ blades})$	In 101-14Mgt, 1103P, y Cereza: $1.55 + 41.81 \cdot (LA + Dead\ leaves)$ In own-rooted Malbec: $9.18 + 41.81 \cdot (LA + Dead\ leaves)$	25	mg	0.96	0.0000

In empirical relationships, R^2 and p-values are indicated.
En las relaciones empíricas se indican los valores R^2 y valor p de las regresiones.

Table 2. Leaf area (LA), stomatal conductance (g_s), midday leaf transpiration rate (E) and plant water use (WU) of own-rooted and grafted Malbec onto different rootstocks, after 60 days under two salinity treatments.

Tabla 2. Área foliar (LA), conductancia estomática (g_s), tasa de transpiración de la hoja del mediodía (E) y uso de agua de la planta (WU) de Malbec a pie franco e injertado en diferentes portainjertos, después de 60 días bajo dos tratamientos de salinidad.

	LA (m^2 $plant^{-1}$)	g_s (mmol H_2O m^{-2} s^{-1})	E (mmol H_2O m^{-2} s^{-1})	WU (L $plant^{-1}$)
NaCl 50 mM	0.56 a	64	0.99	17.85 a
NaCl 100 mM	0.27 b	52	0.74	7.84 b
Salinity p-value	0.0001	ns	ns	0.0086
Own-rooted Malbec	0.66 a	92 a	1.33 a	26.12 a
1103P	0.49 b	64 ab	0.97 ab	13.72 ab
Cereza	0.30 c	45 bc	0.71 bc	8.27 bc
101-1Mgt	0.20 c	30 c	0.46 c	3.48 c
Rootstock p-value	0.0001	0.019	0.02	0.0016
Rootstock x salinity p-value	*ns*	*ns*	*ns*	*ns*

Different letters indicate differences for the LSD test for a *p-value* < 0.05.

Letras diferentes indican diferencias para la prueba LSD, valor de *p* < 0,05.

Table 3. Sodium flow reaching leaf blades ($J_{Na}+_{Leaf\ blades}$); edaphic sodium flow drawn into the sap-stream ($J_{Na}+_{Soil}$); roots sodium exclusion efficiency (Na^+ Exc_{Roots}); and sodium compartmentation efficiency (Na^+ *Comp*) of roots, stems and petioles, of Malbec, own-rooted and grafted over different rootstocks, after 60 days under two salinity treatments.

Tabla 3. Flujo de sodio que llega a la lámina foliar ($J_{Na}+_{Leaf\ blades}$); flujo de sodio edáfico atraído hacia la corriente de savia ($J_{Na}+_{Soil}$); eficiencia de exclusión de sodio por las raíces (Na^+ Exc_{Roots}); y eficiencia de compartimentación para sodio (Na^+ *Comp*) en raíces, tallos y pecíolos, de Malbec a pie franco e injertado en diferentes portainjertos, después de 60 días bajo dos tratamientos de salinidad.

	$J_{Na}+_{leaf\ blades}$ (µg day^{-1} $plant^{-1}$)	$J_{Na}+_{Soil}$ (µg day^{-1} $plant^{-1}$)	Na^+ $_{Exc\ Roots}$ (%)	*Na⁺ Comp*		
				Roots **(%)**	***Stems*** **(%)**	***Petiols*** **(%)**
NaCl 50 mM	1.16 b	325	99.990	68.79	60.83 a	26.48
NaCl 100 mM	3.25 a	321	99.992	55.35	42.76 b	21.94
Salinity p-value	0.0004	*ns*	*ns*	0.0246	0.0001	ns
Own-rooted Malbec	1.12 b	540 a	99.997 a	74.85	58.74 a	23.99
1103P	1.24 b	361 ab	99.993 ab	61.93	59.23 a	28.28
Cereza	2.81 a	253 b	99.989 b	62.19	46.88 b	27.45
101-14Mgt	3.65 a	138 b	99.987 b	49.32	42.44 b	17.11
Rootstock p-value	0.0025	0.016	0.029	*ns*	0.009	*ns*
Rootstock x salinity p-value	*ns*	*ns*	*ns*	*0.01*	*ns*	*ns*

Different letters indicate differences for the LSD test for a *p-value* < 0.05. / Letras diferentes indican diferencias para la prueba LSD, valor de *p* < 0,05.

Simulations with the model

The model was firstly evaluated by simulating the studied soil salt concentrations. Predicted values of LA for each plant under these conditions at day 60, were compared with the observed values. Correlation between observed *vs.* predicted values was accurate and significant (R= 0.87 p < 0.0000; figure 2, page 97).

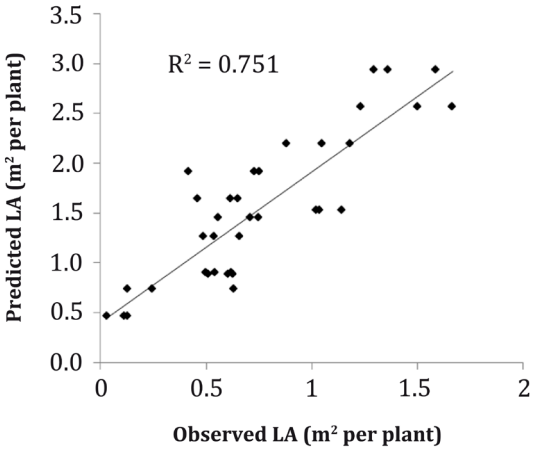


Figure 2. Observed *vs.* predicted by the model leaf areas (LA) of Malbec own-rooted and grafted onto different rootstocks, after 60 days under two edaphic Na^+ concentrations (50 and 100 mM NaCl).

Figura 2. Áreas foliares (LA) observadas *vs.* predichas por el modelo para Malbec a pie franco e injertado en diferentes portainjertos, después de 60 días bajo dos concentraciones edáficas de Na^+ (50 y 100 mM NaCl).

Simulations also showed that, under 50 mM NaCl at day 100, 1103P would grow 33% more than own-rooted Malbec, while own-rooted Malbec would grow 87% more than 101-14Mgt and Cereza (figure 3). At this salinity level, 101-14Mgt would accumulate significantly more Na^+ than the other rootstocks (1.29 *vs.* 0.13% dw at day 100), mainly due to its lower exclusion capacity, causing growth arrest from day 60. Simulations also showed that 100 mM *NaCl* would be catastrophic for 101-14Mgt, but not for the others. This concentration would cause growth arrest at day 40, and complete defoliation at day 100, after a massive entry of Na^+ into this rootstock´s leaves. At 100 mM NaCl, 1103P would show a decline in its performance from day 60, given a marked increase of Na^+ in leaf blades.

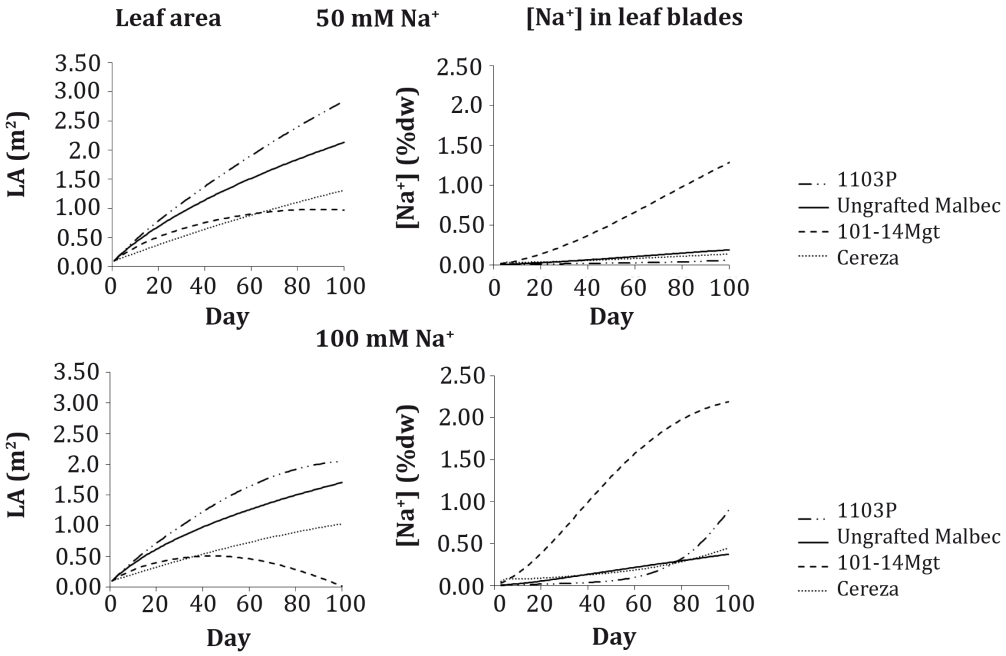


Figure 3. Modeled evolution of leaf area (LA) and *Na*$^+$ concentration in leaf blades of Malbec own-rooted and grafted onto 101-14Mgt, 1103P and Cereza, under two edaphic Na^+ concentrations (50 and 100 mM *NaCl*).

Figura 3. Evolución modelada del área foliar y la concentración de Na^+ en láminas foliares de Malbec a pie franco e injertadas en 101-14Mgt, 1103P y Cereza, en dos concentraciones edáficas de Na^+ (50 y 100 mM *NaCl*).

Further simulations altering exclusion and compartmentation efficiencies, one at a time, allowed to assess the relative contribution of these tolerance mechanisms. First, a virtual "enhanced 101-14Mgt" was generated by assigning 101-14Mgt (*i.e.*, a "bad sodium excluder"), with the Exc_{Roots} of the relatively "good excluder" 1103P. Considering a concentration of soil Na^+ of 100 mM, an increasing exclusion capacity of only 0.009%, decreased leaf Na^+ concentration on 97% while increasing LA on 296%, at day 60 (figure 4). The same procedure was performed when simulating an improvement of 101-14 Mgt, by adding a higher $Comps_{Stems}$ (similar to that of the other rootstocks). In this case, an increased compartmentation efficiency of 174%, only decreased leaf Na^+ concentration by 41%, and increased LA 28%. This indicated that, when conferring salt tolerance, the compartmentation mechanism resulted to be weaker than the exclusion mechanism.

Finally, the effect of conferred vigor on salt tolerance was evaluated. For this, a virtual "enhanced Cereza" was generated by matching its innate vigor to that of the other rootstocks. In simulations without salt factor (*i.e.*, 1 mM Na^+) "enhanced Cereza" grew 76% more than regular Cereza, almost reaching LA values of 1103P, at day 60. Under high salinity (*i.e.*, 100 mM Na^+), "enhanced Cereza" could grow 58% more than its regular counterpart, but its LA was in between 1103P and Cereza, at day 60. Altering innate vigor (*i.e.*, equation 19 in table 3, page 96) did not substantially change leaf Na^+ concentration. In this sense, under high salinity, at day 60, "enhanced Cereza" showed a subtle 11% decrease in leaf Na^+.

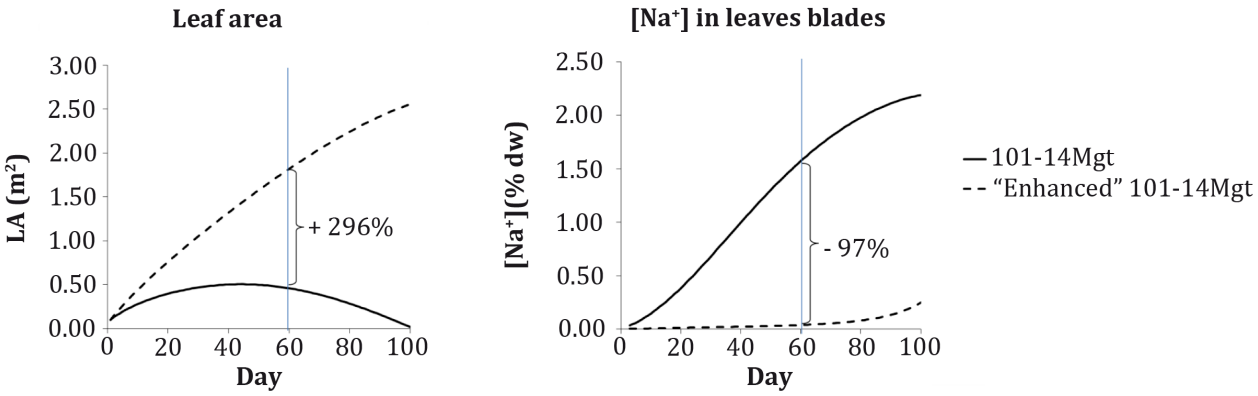


Figure 4. Modeled evolution of leaf area (LA) and *Na*⁺ concentration in leaf blades of Malbec grafted onto the "bad excluder" 101-14Mgt, and onto "enhanced 101-14Mgt" (by adding to 101-14Mgt an exclusion efficiency similar to 1103P), under 100 mM *NaCl*.

Figura 4. Evolución modelada del área foliar y la concentración de *Na*⁺ en láminas foliares de Malbec injertado en el "excluidor malo" 101-14Mgt, y en "101-14Mgt mejorado" (al agregar a 101-14Mgt una eficiencia de exclusión similar a 1103P), bajo 100 mM *NaCl*.

Discussion

Even though others have already modeled the effects of salinity on plants (5, 7, 14, 16, 30, 31, 32), the model herein developed represents, to the best of our knowledge, the first attempt to include, in a theoretical framework, the mechanisms of root exclusion and organ compartmentation that allow leaf sodium toxicity avoidance in glycophytes. Previous models based on a more mechanistic basis, predict important aspects such as water absorption, transpiration, carbon uptake (14, 16), vegetative growth and productivity (5, 32). These models considered climatic conditions like radiation and vapor pressure deficit, soil salinity, and other plant factors such as the modification of stomatal and hydraulic conductances. Our model, in contrast, confronted soil saline supply with exclusion and compartmentation efficiencies, predicting salt toxicity in leaf tissue and its impact on vegetative biomass. It should be noted that our model has a more empirical basis, especially regarding the impact of salinity on transpiration and carbon assimilation.

Our model intended to evaluate salt toxicity in different rootstocks with the intention of improving future grapevine rootstock screenings. For genotype evaluation, mechanisms such as ion exclusion or root compartmentation, appear as essential. The high correlation between predicted and observed responses indicated that this dynamic model presented a fairly accurate representation of reality. This also proved that under salinity, vegetative biomass is the result of dynamic changes that occur in a feedback process (where LA is dependent on tissue salt concentration, but that, in turn, also influences this concentration) as theoretically proposed. Regardless, ours is a semi-mechanistic model, and a good fit to reality requires accurate observations of the effects of foliar toxicity on several physiological traits (*e.g.*, tissue damage reflected in the model by variables as LA increments, defoliation, and exclusion efficiency loss). Comparing linear regression models with dummy variables resulted in an interesting approach to contrast different rootstocks, framed within previous proposals (27).

Despite its semi-mechanistic character, the model allowed to weight the effect of different avoidance mechanisms, in a process as complicated as the observed. In this regard, the simulations confirmed ion exclusion as the most relevant mechanism. Very small variations in exclusion produced large changes in rootstock performance. This genotype-dependent mechanism has been previously observed (3, 8, 10, 12, 40). Our modeling approach permitted to compare its influence to that of compartmentation or innate vigor. Regarding conferred or innate vigor, an association between high vigor and increased salt tolerance has already been found (40). These investigations suggested that saline dilution could represent an avoidance mechanism. Simulations with our model confirmed a real effect of innate vigor on salt tolerance, and the implicit dilution effect. The higher vigor of "enhanced Cereza" caused a higher inflow of toxic ions, however, compensated by their dilution in its higher LA. For this reason, leaf sodium concentration of more vigorous "enhanced Cereza" did not increase in comparison with that of less vigorous (*i.e.* regular) Cereza.

This study proved that root compartmentation contributes to salt tolerance. In several species including grapevine, Na^+ compartmentation in cell vacuoles of different tissues, depends on the combined activity of a Na^+/ H^+ tonoplast antiporter and an H^+ pump (1, 18). It has also been observed that the expression of genes encoding this antiporter varies in different grapevine rootstocks when grown under saline conditions (37). This could explain the differences found between 101-14 Mgt and the other rootstocks, in terms of their root compartmentation efficiency.

A partial drawback of our model is the fact that it only considers Na^+ toxicity, while Cl^- may have been partly responsible for the saline effects as well. Several studies have confirmed the severe toxicity of Cl^- and the relative ease with which it enters the plants (10, 21, 39). Despite this, two things may indicate that these facts do not invalidate our model. First, the possible additive effect of Na^+ and Cl^- that has been observed by some researchers in glycophytes (31, 35) and secondly, the high correlation between foliar Na^+ and Cl^- concentrations. Thus, given that Na^+ and Cl^- concentrations proved to be highly collinear variables, their combined use in multiple regressions, such as those used to parameterize the model, would result in serious redundancy. This type of redundancy can lead to numerical problems in the estimation of the regression parameters (*i.e.*, parameters of incorrect magnitude or sign, with large standard errors) (33).

Another problem limiting model scope is that it describes what happens in a short period of time, when grapevines grow at steady rate. This ignores that, considering the whole vegetative cycle, grapevine canopies follow a sigmoid pattern (20). The model is also unable to predict long term effects, considering grapevines are perennial plants. It has been observed that salinity has additive effects overtime on root exclusion efficiency (31, 36). This limits the model to the understanding of the dynamics of salt toxicity, and the comparison of different genotypes. Given that the model is semi-mechanistic, these aspects do not necessarily imply serious limitations. It could be parameterized by experimental observations considering longer growth periods. The same occurs when realizing that the model only describes plants growing at field capacity. In the future, a clearer understanding of the interaction between salinity and water deficit (frequent situation in the field) should be achieved.

Conclusion

Our model simulated different grapevine rootstock responses to different salinity levels. These simulations evidenced the key role of Na^+ exclusion in salinity tolerance. Stomatal adjustment, compartmentation and rootstock conferred vigor showed relatively minor effects. Considering that the semi-mechanistic model presented has an accurate explanatory and predictive power, we highlight its potential use for rootstock evaluation regarding salt tolerance.

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