Early Detection of Salt Stress Tolerance of *Prunus* Rootstocks by Excised Root Culture

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Abstract. Salt tolerance varies between species and genotypes of plants, but evaluation of these differences is cumbersome, because whole plants that are highly complex systems show a variety of responses depending on the applied methodology. However, focusing on plant roots, which are in direct contact with the soil, could offer a simpler and more efficient model for analyzing salt stress tolerance in different species. This study explores whether root growth under salt stress is associated with genotypic differences in *Prunus* species with different degrees of salt tolerance. Excised root cultures were grown in vitro under increasing salt concentrations (0, 20, 60, and 180 mM NaCl). Root tips taken from in vitro-rooted shoots of *Prunus* species with different salt tolerance were measured after 3 weeks of culture in a shaker, and changes in their anatomy were examined. Both growth and starch content of in vitro root cultures were affected by salt concentration. Root length increments were related to salt stress tolerance at 60 mM NaCl, in which significant differences were also found between species. A significant inverse correlation was found between salt tolerance and starch accumulation in the maturation zone of root tips. Genotypic differences were observed in agreement with species' salt stress tolerance in vivo. These results suggest the use of excised root cultures for rapid, early detection of salt stress tolerance in plants. Chemical names: sodium chloride (NaCl).

Salinity is a problem increasing facing agriculture, especially in irrigated lands located in semiarid zones. These agricultural zones account for 100 to 110 million hectares, of which ≈ 20 to 30 million hectares are seriously damaged by salt accumulation and an estimated 0.25 to 0.5 million hectares are lost from production every year as a result of salt accumulation (FAO, 2002). Only 1% of the world's flora is considered halophyte, and the most important agricultural crops are not among the most tolerant species, making salinity a serious human as well as ecological concern (Byrt and Munns, 2008). Hence, efforts to increase salt tolerance of crop plants could potentially improve crop yield and support agriculture on marginal lands (Türkan and Demiral, 2009).

The response of plants to saline environments has generally been determined by measuring emergence, survival, growth, phenology, maturity, aboveground biomass, and commodity yields. However, the responses of crops to salinity often vary with different

plant growth stage, across varieties, and for each measure of plant growth, development, and product yield (Steppuhn and Wall, 1999). Discrepancies arise from the fact that whole plants, as highly complex systems, show a variety of responses depending on the methodologies used in evaluation: different growth stage, evaluation criteria, or salt stress application. Focusing on plant roots, which are in direct contact with the soil, could offer a simpler, more direct, and more efficient model for analyzing salt stress tolerance in different species.

Fruit tree rootstock selection would benefit from research strategies that shorten the long times required for growing woody plants. Selection studies with fruit trees have been accelerated in recent years with the use of potting and hydroponic techniques, thus reducing the duration needed for field studies. However, generating a sufficient number of clonal plants and measuring the effects of applied stresses still require a great deal of time and effort. In vitro shoot cultures of cherry (Ertürk et al., 2007), peach, and the almond $\times$ peach hybrid GF677 (Biricolti and Pucci, 1995) have proven to be applicable for the study of salt stress on plant tissues. In vitro studies represent a practicable strategy for selection studies, because promising results have been achieved in the screening of different genotypes of grapevine (Troncoso et al., 1999) and mulberry rooted shoots (Vijayan et al., 2003), in which the salinity tolerance of selected genotypes in vitro were correlated to those ex vitro. Recently, an in vitro approach was used for the selection of mung bean and

tomato plantlets regenerated from cotyledons under salt stress (Hassan et al., 2008).

It is clear that different genetically determined mechanisms for salt tolerance exist, but these remain largely unknown. Comparisons between species that are genetically closely related but vary in terms of salt tolerance would speed the progress of understanding the mechanisms involved in resistance of salinity, as suggested by Byrt and Munns (2008). This is an objective of the present study, which examines different degrees of salt stress tolerance in the related species of the genus *Prunus*.

Abiotic stress tolerance is an important trait for fruit tree rootstock selection. Although resistant to important abiotic stresses such as drought or alkalinity, many *Prunus* species are included in the group of salt-sensitive species (Day, 1953; Kotuby-Amacher et al., 2000). Nevertheless, *Prunus* species display different degrees of tolerance to salt stress. Past studies of different *Prunus* species have allowed us to estimate their relative salt tolerance with plum type species being among the more tolerant and cherry type species among the least tolerant. Specifically, local selections of *P. insititia* ("Pollizo") in the southeast of Spain grew better than apricot seedlings or Myrobalan (*P. cerasifera*) in heavy and saline soils (Egea, 1970). Mexican plum (*P. mexicana*) and the Titan almond $\times$ Nemaguard hybrid showed more relative tolerance than Nemaguard or Nemared, and these were more tolerant than Myrobalan or bitter almond when cultured in sand cultures watered with saline solutions (Ottman and Byrne, 1988). The almond $\times$ peach hybrid GF677 showed an intermediate salt tolerance compared with the relatively more tolerant GF655/2 (*P. insititia*) and the less tolerant Myrobalan hybrid MrS. 2/5 or to peach seedlings (Massai and Gucci, 1998). These studies roughly agree with that of Kotuby-Amacher et al. (2000), which ranked different species according to the salinity (expressed as conductivity) needed to reduce yield by 50%. This work positioned cherry species (sweet and tart) at the more sensitive end with only 3.1 dS·m⁻¹, whereas plum needed 4.3 dS·m⁻¹. Rootstocks can play an important role in salt tolerance; thus, the use of the adequate rootstock allowed Japanese plums to withstand low salt levels when grafted onto Marianna 2624 (*P. cerasifera* $\times$ *munsoniana*) (Ziska et al., 1991) and loquat (*Eriobotrya japonica*) was unaffected under salt stress when grafted on Angers rootstock (*Cydonia oblonga*) (López-Gómez et al., 2007).

Here, we propose a novel approach using excised roots as a simplified experimental model (Marín and Marín, 1998), because roots are the plant organs directly in contact with soil. Instead of studying the effect of salt stress in whole plants or in plant parts distant from the origin of salt source, we examined the responses of isolated roots to salt stress in vitro in controlled culture conditions without the influence of substrates and microorganisms. Excised roots have been used in the past in short-duration (few hours) experiments for the study of ion uptake mechanisms of

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woody plants from saline solutions (Altman and Mendel 1973). Besides, the use of long-duration (3 weeks) aseptic cultures allowed us to avoid the possible effects of the rhizosphere (Yang et al., 2009). A simple model, instead of studying whole plants, can yield results more quickly, which would shorten the selection processes. In vitro root cultures either attached to or detached from plants have previously shown a certain correlation to whole organism salt stress tolerance (Prakash and Widholm, 1993; Vijayan et al., 2003), in addition, root cultures showed an accumulation of proline as a response to salt stress similar to that of shoot cultures in vitro (Marín et al., 2009; Sotiropoulos, 2007). However, more in-depth studies of the root response to salt stress are important, mainly in fruit tree species, where the plant characteristics make these studies difficult.

Besides root growth, we studied the starch content of the root tissues, because starch formation and accumulation in vitro were related in tobacco to the metabolic activity of the callus tissue (Thorpe et al., 1986), and the metabolic activity could be affected by salt, as recently reported in tomato, in which salinity stress enhanced carbohydrate accumulation as starch during the early development stages of tomato fruits (Yin et al., 2010). Both root growth and starch content are parameters relatively easy and quick to determine, allowing a quick test of genotypes.

In this work, the effect of increasing salt concentrations on excised root cultures of different *Prunus* rootstocks is studied to determine the validity of the hypothesis that root responses to salinity are related to those of whole plants and then to confirm this technique as an early selection method. A patent application has been filed to protect the procedure (Spanish application P200803727).

Materials and Methods

Plant material. Following already published data on salt stress tolerance in *Prunus* (see previously for references), we have chosen different *Prunus* genotypes from groups that cover a relatively broad range: 'Adesoto 101' (*P. insititia* L.) and 'Marianna 2624' (*P. cerasifera* Ehrh. × *munsoniana* Wight & Hedr.) from the plum-type group that was more tolerant; 'Masto de Montañana' and CAB 6P (*P. cerasus* L.) cherry rootstocks that were less tolerant; and the almond × peach hybrid GF 677 [*P. dulcis* (Miller) D.A. Webb × *persica* (L.) Batsch] that showed an intermediate tolerance.

Root culture and NaCl treatments. Roots were obtained from in vitro shoots micro-propagated for more than 1 year following previously described methods (Andreu and Marín, 2005) using modified Murashige and Skoog (MS) medium (0.4 mg·L⁻¹ thiamine-HCl, 5 µM 6-benzylaminopurine (BAP), 0.5 µM indole 3-butyric acid (IBA), and 3% sucrose, pH 5.5) and gelled with 0.7% Difco-Bacto agar. Shoots were rooted in the same medium but with half-strength macronutrients without BAP and with 5 µM IBA at

24 °C and a 16-h photoperiod. Rooting medium was dispensed (100 mL each) in unsealed Sigma polypropylene jars (700 mL; Sigma Chemical Co., St. Louis, MO) and autoclaved 20 min at 121 °C.

Root tips, 10 mm in length, were taken from roots washed in sterile distilled water and trimmed on a petri dish placed over graph paper. Ten root tips per treatment (salt concentration) of each rootstock were cultured in 30 mL of liquid MS medium (Murashige and Skoog, 1962) with 3% sucrose, but without growth regulators, in glass culture vessels (Sigma V8630) with Magenta polypropylene caps (Sigma B8648) and placed in the dark in an orbital shaker (90 rpm) in a culture room at 24 °C. Salt stress was applied by adding NaCl at four different concentrations to the culture medium: 0 (control), 20, 60, and 180 mM. Total electric conductivity (EC) of the culture media at 25 °C was 6.0, 8.2, 12.5, and 23.6 dS·m⁻¹, respectively. Whole experiments were repeated in triplicate using in total 120 (40 × 3) roots per rootstock.

Root cultures were evaluated after 3 weeks and root length was measured with the aid of graph paper. For anatomical studies, 'Adesoto 101' from the more tolerant rootstock group was compared with 'Masto de Montañana' from the less tolerant rootstock group, as described previously. At least 20 roots per treatments per rootstock were microscopically analyzed. Handmade root sections using a razor blade were observed fresh or after fixation in a 2.5% glutaraldehyde in phosphate buffer (0.03 M, pH = 7) (Sabatini et al., 1963) after staining with I₂KI (Jensen, 1962) for starch routine observations. Alternatively, roots were fixed in 2.5% glutaraldehyde in 0.03 M phosphate buffer, dehydrated in an ethanol series, and embedded in JB4 plastic resin (Polysciences Inc., Warrington, PA). Embedded roots were sectioned using a Leica EM UC6 ultramicrotome (Leica Microsystems, Wetzlar, Germany) at 5 µm and stained with periodic acid-Schiff's reagent for insoluble carbohydrates (Feder and O'Brien, 1968). Sections were examined for starch using a Leica DM 2500 microscope and images were recorded with a digital camera (Leica DFC 320). Sections were ranked in one of three classes for convenience depending on the starch content: absent, abundant, and scarce (one to five starch grains).

Data analysis. Relative root length increment was calculated for each root in each experiment as a percentage of the average root length increment for control roots. Mean values of the relative root length increment at 60 mM NaCl were compared by the least significant difference test after analysis of variance (ANOVA). Regression analyses of relative root length increments versus salt concentration were performed. Correlation between starch content and different parameters (root length, rootstock, NaCl concentration) were determined using the non-parametric Kendall's τ coefficient, which is based on counting the number of concordant and discordant pairs (Dalggaard, 2002). R statistical package (R Development Core Team, 2008) was used.

Results

Root growth. The response of excised roots to increasing salt concentrations followed a consistent pattern. Although roots grew well at 0 mM NaCl, no growth was observed at 180 mM NaCl, and intermediate responses were recorded within this range. Importantly, differences in response to salt stress by genotype were found.

Roots grew at different rates in liquid culture media depending on genotype and salt concentration. At 0 mM NaCl (control treatment), the cherry rootstock 'Masto de Montañana' showed the greatest average root growth (47.3 mm root length) followed by 'CAB 6P' (36.4 mm), 'GF677' (35.2 mm), 'Adesoto 101' (30.7 mm), and 'Marianna 2624' (24.4 mm).

Root length decreased as salt concentration increased in all rootstocks, but not homogeneously (Fig. 1). Rootstocks followed two different patterns of growth at increasing salt concentrations. At 60 mM of NaCl, plum-type rootstocks ('Adesoto 101' and 'Marianna 2624') showed root lengths similar to those of controls, whereas the rest of the rootstocks (the cherry type 'Masto de Montañana' and CAB 6P and the peach × almond hybrid GF677) showed only 40% of control root length.

Differences in relative root length increment at 60 mM NaCl were statistically significant ($P \leq 0.001$) following ANOVA (Table 1). There were differences between the more tolerant (plum-type rootstocks) and the less tolerant (cherry and peach × almond hybrid rootstocks) plants, but no differences were found between rootstocks within each group (Fig. 2). Interestingly, root length varied considerably in roots from each genotype; despite originating from the same clonal stocks, a wide variability in growth was recorded for each salt concentration (except at 180 mM). This was most apparent in the more tolerant genotypes, in which a population of roots showed little growth, similar to the less tolerant species, but a proportion of the roots within each genotype was able to overcome the stress and grow to considerable lengths (Fig. 2).

In addition, a regression analysis of the relative root length increments of the rootstocks, grouped according to their degree of tolerance in vitro, showed that NaCl concentration had a highly significant effect on root growth ($P \leq 0.001$). The presence of a significant interaction ($P \leq 0.001$) between the rootstock group and the square of NaCl concentration (Table 2) indicated that salt stress differently affected each group. A square term for concentration should be included, because there was a significant deviation from linearity. For this reason, data were fitted to second-degree polynomials but with different shapes depending on the group as revealed by the coefficient signs (Fig. 3). Thus, the more tolerant group showed a concave curve, whereas the less tolerant group curve was convex.

The interaction term became not significant when each group was analyzed separately, indicating that rootstocks kept the same curve

Fig. 1. Average relative root length increment of different rootstocks at increasing NaCl concentrations. Plum type rootstocks (open symbols) showed more relative root length increment at 60 mM than the cherry and peach × almond hybrid rootstocks (black symbols) ($n = 10$, three replicates).

Table 1. Analysis of variance of the relative root length increments of the different rootstocks at 60 mM NaCl.

	df	SS	F	Pr(>F)
Rootstock	4	65,937	11.818	2.45·10 ^{-8***}
Residuals	145	202,254		

***Significant difference at $P \leq 0.001$.

type within the same group. In addition, no significant differences were found between rootstocks within the same group, and separate regression analyses for each rootstock showed similar curve fits to those of the group in which they were included (data not shown).

Starch accumulation in root cells at 10 mm from the root tip (maturation zone). Starch grains accumulated in root cells of the cortex at high salt concentrations, but there were clear differences between 'Adesoto 101' and 'Masto de Montañana' rootstocks (Fig. 4). 'Adesoto 101' did not show starch grains at 0 and 20 mM NaCl concentration, but starch was present in some roots at different amounts when the salt concentration was increased to 60 mM (Fig. 4). On the other hand, 'Masto de Montañana' showed starch grains even in the absence of NaCl, and the starch content was more abundant at all NaCl concentrations than in 'Adesoto 101'. In both genotypes, starch content reached the highest values at 180 mM. Starch grains showed variable size, being larger at higher NaCl concentrations (Fig. 5).

We have found a positive correlation between NaCl concentration and starch content in both rootstocks, but the strength of the correlation varied with genotype. The Kendall's τ rank correlation coefficient was higher in 'Adesoto 101' ($\tau = 0.51$; $P \leq 0.001$) than in 'Masto de Montañana' ($\tau = 0.41$; $P \leq 0.01$), being lowest when data were pooled ($\tau = 0.25$; $P \leq 0.05$).

In contrast, we have found a negative correlation between root length and starch content, but the situation is not general. In 'Adesoto 101', there was not starch accumulation in the maturation zone at 20 mM, but it accumulates at different degrees at 60 mM. Thus, although the more actively growing (longer) roots at 60 mM did not have starch grains, the less active (shorter) roots showed a range from scarce to abundant starch (Fig. 4), and the negative correlation between root length and starch accumulation at 60 mM NaCl was high and statistically very significant ($\tau = -0.79$; $P \leq 0.001$). However, in 'Masto de Montañana', no significant correlation was found between root length and starch content both at 20 mM and at 60 mM NaCl.

There was a significant correlation between starch content and rootstock type, but this differed with NaCl concentration. The correlation coefficient between rootstock type and starch content was higher at 20 mM NaCl ($\tau = 0.83$; $P \leq 0.001$) than at 60 mM ($\tau = 0.35$; $P \leq 0.05$) or when data from both concentrations were pooled ($\tau = 0.68$; $P \leq 0.001$). Thus, the highest difference between rootstock starch content was displayed at 20 mM NaCl.

Discussion

In this study, we have shown that excised root culture could be a useful model to study the degree of tolerance to salt stress in *Prunus* species, because we found that the degree of tolerance to salt stress shown in root cultures was consistent with previous findings in related genotypes in whole plant studies. The method possesses the advantages of simplicity, reproducibility, and speed.

Root culture allowed us to rank *Prunus* rootstocks of different species according to

their capability to grow in a concentration of salt (60 mM NaCl) that would inhibit the growth of whole fruit tree plants (Kotuby-Amacher et al., 2000). Roots have grown in MS culture medium that contains a relatively high salt concentration as well as sucrose and other organic components. The EC of the culture medium without NaCl was much higher than Hoagland's solution, commonly used with whole plants (6.0 dS·m⁻¹ versus 1.5 dS·m⁻¹ at 25 °C). This represents an important difference between the root culture model and the hydroponic culture that limits further comparisons. The growth of isolated roots, even when directly exposed to relatively high NaCl concentrations, could be related to the fact that roots are surprisingly robust and that root growth is less affected than leaf growth by salinity in whole plants (Munns, 2002). Several reports have stressed the effect of certain ions as Ca²⁺ or B³⁺ on the modulation of the effect of NaCl in different related genotypes (Bolat et al., 2006; Cramer and Läuchli, 1986; El-Motaium et al., 1994; Lucchesini and Vitagliano, 1993; Nasr et al., 1977; Sotiropoulos, 2007; Sotiropoulos et al., 2006; Tattini and Traversi, 2009; Ziska et al., 1991). This fact can cause some trouble when comparing experiments on the effect of NaCl made with different substrates or media that can be avoided with the use of experimental sets with a defined culture medium, like in our root culture model. On the other hand, the effect of the rhizosphere, which has an important effect on abiotic stress tolerance (Yang et al., 2009), is avoided when using axenic cultures.

The in vitro method described here is based on the measurement of the root length after a period of culture under different NaCl concentrations. Despite its simplicity, this method yielded a satisfactory measure of the tolerance of the different species tested. Similarly, a good correlation was found between the in vitro root length and the ex vitro growth of mulberry plants under different salt concentrations (Vijayan et al., 2003) and between the growth of root cultures of potato cultivars in solid media with that of whole plants (Prakash and Widholm, 1993). The results reported here indicate that our simplified method can provide detailed information on the root response to stress.

Our results are in agreement with those of Munns and Tester (2008), in which genetic differences in wheat and barley were not reflected in responses to salt treatment in the short term (few days). Only after several weeks of salt treatment, when the osmotic effects of salt gave way to the toxic ionic effects, could genotype-related differences be observed. In this study, we found root growth differences among different rootstocks after 3 weeks of culture, and these differences were related to salt stress tolerance. With our model we found differences between groups of species belonging to different sub-genera: *Prunus* ('Adesoto 101' and 'Marianna 2624'); *Cerasus* ('Masto de Montañana' and CAB 6P); and *Amygdalus* (the almond × peach hybrid GF 677). The possible differentiation of

Fig. 2. Relative root length increment of different rootstocks at 60 mM NaCl. Open symbols show the actual data, whereas black symbols show the average root length increments ($\pm$ SEM). Vertical bar represents least significant difference (LSD) at $P \leq 0.05$ ($n = 30$)

Table 2. Regression analysis of variance of relative root length increments, grouped according to the rootstock degree of tolerance, versus NaCl concentration and its square (Concentration^2).

	df	SS	F	Pr (>F)
Concentration	1	839,852	567.26	$2.2 \cdot 10^{-16}***$
Concentration ²	1	8,250	5.57	0.0186 *
Tolerance group (T. Group)	1	23,125	15.62	$8.68 \cdot 10^{-5}***$
Concentration: T. Group	1	975	0.66	0.4174 NS
Concentration ² : T. Group	1	41,748	28.20	$1.55 \cdot 10^{-7}***$
Residuals	594	879,437		

*Significant difference at $P \leq 0.05$; ***significant difference at $P \leq 0.001$; NS = non-significant.

Fig. 3. Fitted curves (solid lines) of relative root length increment data grouped according to the tolerance degree of the rootstocks in vitro. The less tolerant group (A) included the cherries ‘Masto de Montañana’ and CAB 6P and the almond $\times$ peach hybrid GF 677, and the more tolerant group (B) included the plums ‘Adesoto 101’ and ‘Marianna 2624’ rootstocks. Prediction bands (dashed lines), associated with 95% confidence limits of data, and confidence bands (dotted lines), associated with SEM, are included.

rootstocks belonging to other taxonomic groups using root culture requires further study.

Despite the fact that differences between genotypes were statistically significant, roots showed a certain degree of variability. Even in the more tolerant rootstocks, some roots did not grow or grew only a small amount. Thus, only some roots possessed competent tissues that could withstand salt stress and continue growing. Although this further in-

vestigation is necessary to explain this phenomenon, it should be noted that the cellular response to salt stress in *Arabidopsis* roots was found to be non-uniform and was related to the transcriptional state of the cells (Dinneny et al., 2008).

In addition to root growth, we found differences in the accumulation of starch in the maturation zone of the root between both rootstocks with different degrees of tolerance

and among roots from the same rootstock at different salt concentrations. The comparative study of starch content between roots was however difficult because the starch grain distribution was variable along the root becoming more abundant in the basal part. Roots are dynamic structures that maintain their structures while growing continuously, and new cells/tissues replace older ones when they mature. Studying the maturation zone allowed us to compare different roots with different growth rates, because this zone keeps a similar developmental state in every root. In the maturation zone, the new tissues formed by the root tip acquire a functional state, which can be easily observed by anatomical changes such as the cell wall thickening of xylem vessels. The maturation zone is located ≈ 10 mm from the root tip, and similar measurement areas have been used in other studies (Eticha et al., 2005; Meyer et al., 2009).

Starch is directly synthesized in vitro from the sucrose in the culture medium throughout the culture period, as reported in tobacco callus cultures in which starch formation and accumulation in vitro were shown to be related to the presence of sucrose in the culture medium and with the metabolic activity of the callus tissue (Thorpe et al., 1986). Here, starch formation and accumulation in cortical tissues might not be directly the result of salt stress, but the decrease in root growth induced by the stress. Thus, in ‘Adesoto 101’ roots cultured at 60 mM NaCl, starch accumulation in the maturation zone was inversely correlated to root length and, therefore, to the use of starch by root tissues as the energy source, as reported by Thorpe et al. (1986). On the other hand, recent studies have shown that salinity stress enhanced carbohydrate accumulation as starch during the early development stages of tomato fruits and that the ADP-glucose pyrophosphorylase encoding genes, *AgpL1* and *AgpS1*, involved in the promotion of starch biosynthesis, were up-regulated under salinity stress (Yin et al., 2010). Thus, if a similar mechanism applies here, in another sink organ like the root, the starch accumulation could reflect the degree of salinity stress of root tissues. Starch content in ‘Masto de Montañana’ was higher than in Adesoto both at 20 mM and at 60 mM NaCl, and although there was still a good correlation between starch content and salt concentration, there was no such correlation between starch content and root length. This lack of correlation in ‘Masto de Montañana’ could be the result of the stronger effect of salt stress and the smaller variability in root length and starch content. Similar to root growth, in which there was a NaCl concentration (60 mM) at which we were able to differentiate genotypes according to the salt stress tolerance of their taxonomic group, starch content also differentiates the more tolerant from the less tolerant genotypes, but at a smaller concentration of NaCl (20 mM). This can indicate that the salt stress needed to induce differences between rootstocks in starch content is lower than the stress needed to differentiate rootstock in their root growth.

Fig. 4. Root length and starch content of root sections taken at 10 mm from root tips of 'Adesoto 101' and 'Masto de Montañana' rootstocks roots grown at 20 and 60 mM NaCl.

Fig. 5. Cross-sections of the maturation zone of the roots showing starch grains (arrows) after acid-Schiff's reagent staining. (A–B) 'Adesoto 101' at 0 mM NaCl (A) or 180 mM NaCl (B); (C–D) 'Masto de Montañana' at 0 mM NaCl (C) or 180 mM NaCl (D). Scale bar = 50 µm.

Root culture of excised root tips is a good model for the study of salt stress tolerance. Root growth and the starch content of excised roots vary according to different concentrations of salt. Genotypic differences affect these responses, and these differences are consistent with the reported findings with these genotypes in vivo. It will be worthwhile to evaluate whether this approach can be used in other genera, because root growth and salinity response are highly conserved processes.

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