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Argentinean cultivars of *Vitis vinifera* grow better than european ones when cultured *in vitro* under salinity

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Key words: *Vitis vinifera*, cultivars, salt tolerance, *in vitro,* growth.

ABSTRACT: Argentinean *Vitis vinifera* cultivars although originated from Europe, have clear ampelographic and genotypic differences as compared with the European cultivars currently used in wine making. *In vitro* evaluation of salt tolerance has been used in many species. Our hypothesis was that Argentinean cultivars are more tolerant to salinity than European ones. Three European cultivars, Malbec, Cabernet Sauvignon and Chardonnay and four Argentinean cultivars, Cereza, Criolla Chica, Pedro Gimenez and Torrontes Riojano were tested by *in vitro* culture. Treatments included: 1) Control, 2) 60 mEq/L of a mixture of three parts of NaCl and one part of $CaCl_2$ and 3) 90 mEq/L of the salt mixture. Results from two experiments (I and II) are reported. No differences were found in plant survival, expressed as % of the respective control, among cultivars. Leaf area, leaf, stem and total dry matter (DM) in Experiment I and leaf area, leaf number and leaf, stem, root and total DM in Experiment II, were higher in Argentinean cultivars than in European ones. We conclude that Argentinean cultivars show better performance in growing under salinity, especially in the highest salt concentration. Differences among cultivars, inside each group, were found for most of the measured variables.

Introduction

Soil salinity is one the biggest problems for crop production in many areas of the world (Zhu, 2000; Munns, 2002). Salinity affects plant growth by lowering water potential and by ionic imbalance (Greenway and Munns, 1980; Volkmar *et al*., 1998). In addition to salt concentration and ions composition of the saline solution, salt injury also depends on the period of time over which plants are exposed to saline conditions (Munns, 2002). Depending on the concentration, salts can cause growth restriction or even lead to plant death (Greenway and Munns, 1980; Munns, 2002; Volkmar *et al*., 1998).

The ionic imbalance effect varies with the ions present, although the most important damages in grapevine have been attributed to Cl^- and Na^+ (Alexander and Groot Obbink, 1971; Antcliff *et al*., 1983; Bernstein *et al*., 1969; Greenway and Munns, 1980; Volkmar *et al*., 1998).

Maas and Hoffman (1997) reviewing data for many crops classified grapevine species as moderately sensitive to salinity. Despite generalizations, wide variability in salt tolerance has been demonstrated among grape rootstocks and varieties in pot and field experiments. Broadly speaking, *Vitis vinifera* cultivars are less tolerant to salt stress than other *Vitis* species frequently used as rootstocks in saline soils (Alexander and Woodham,

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1968; Alexander and Groot Obbink, 1971; Antcliff *et al*., 1983; Barlass and Skene, 1981; Bernstein *et al*., 1969; Pandey and Divate, 1976; Sauer, 1968; Walker, 1994; Walker *et al*., 2002).

Effects of salinity on grape plants include reduced rates of CO_2 fixation, lower dry matter accumulation in organs, decrease in bunch number, smaller berry size, poorer yield and an overall reduced growth. Salinity also affects berry composition by increasing Cl^- and Na^+ concentration, and reducing sugars and acidity (Alexander and Woodham, 1968; Downton, 1977; Walker, 1994).

In several papers it has been shown that the use of *in vitro* culture is a reliable method for testing salt tolerance in many crops, since in most cases the results agree with those obtained under greenhouse or field

TABLE 1.

***Experiment I.* Plant survival (expressed as % of the respective control) under three salt treatments, in Argentinean (CER, CRI, PGI, TOR) and European (MAL, CHA, CAB) cultivars.**

Treatments	MAL	CHA	CAB	CER	CRI	PGI	TOR	Mean	Statistical Significance
Control	100.0	100.0	100.0	100.0	100.0	100.0	100.0	**100.0**	**A**
Salt 60	82.8	91.7	76.7	81.1	63.3	96.0	55.5	**78.1**	**B**
Salt 90	75.7	72.3	75.2	86.7	83.3	70.0	79.2	**77.5**	**B**
Mean	**86.1**	**88.0**	**84.0**	**89.2**	**82.7**	**88.7**	**78.2**		
Statistical significance	**A**	**A**	**A**	**A**	**A**	**A**	**A**		

Different capital letters in the last row indicate significant differences among cultivars at $p<0.05$. Different letters in the last column indicate significant differences among salt treatments at $p<0.05$ (n= 6)

TABLE 2.

***Experiment II.* Plant survival (expressed as % of the respective control) under three salt treatments, in Argentinean (CER, CRI, PGI and TOR) and European (MAL and CHA) cultivars.**

Treatments	MAL	CHA	CER	CRI	PGI	TOR	Mean	Statistical Significance
Control	100.0	100.0	100.0	100.0	100.0	100.0	**100.0**	**A**
Salt 60	72.0	78.0	62.5	85.0	88.0	96.0	**80.2**	**B**
Salt 90	52.0	80.0	68.4	68.4	88.0	86.0	**73.8**	**B**
Mean	**74.7**	**86.0**	**77.0**	**84.4**	**92.0**	**94.0**		
Statistical significance	**A**	**A**	**A**	**A**	**A**	**A**		

Different capital letters in the last row indicate significant differences among cultivars at $p<0.05$. Different letters in the last column indicate significant differences among salt treatments at $p<0.05$ (n=5).

conditions (Basu *et al*., 2002; Mercado *et al*., 2000; Naik and Widholm, 1993; Watanabe *et al*., 2000). The method has the advantages that the results are obtained relatively fast and also that the plant environment is fully controlled. Several authors have reported good agreements between field and *in vitro* results in grapevine (Barlass and Skene, 1981; Bavaresco *et al*., 1993; Singh *et al*., 2000; Sivritepe and Eris, 1997, 1999; Troncoso *et al*., 1999).

In Argentina, along with the traditional European varieties that are cultivated because of their enological value, there is a group of cultivars with unique morphological and enological features that are different from Europeans. Such cultivars are locally called "Criollas" or "Argentinean" despite the fact that some of them are also cultivated in other South American countries. These *Vitis vinifera* cultivars were originated from European ones, but nowadays they present clear ampelographic (Alcalde, 1989) and genotypic (Martinez *et al*., 2003) differences as compared with the current European cultivars. They were probably selected by ancient viticulturists from cuttings or seeds brought by Spaniard colonizers 3-4 centuries ago (Alcalde, 1989). The Argentinean cultivars represent almost 50% of the whole area cultivated with grape in Argentina and they are: Criolla Grande, Criolla Chica, Cereza, Pedro Gimenez Argentino, Moscatel Rosado, Torrontes Riojano, Torrontes Sanjuanino and Torrontes Mendocino. Based just on field observations (i.e. not proven experimentally), most viticulturist believe that Argentinean cultivars are more tolerant to salinity than European ones. In fact some nurseries recommend them as salt tolerant rootstocks. However, no scientific or technical information about an improved salt tolerance in Argentinean cultivars can be found in the literature.

Based on the hypothesis that Argentinean cultivars are more tolerant to salinity than European ones, the objective of this work was to compare *in vitro* the performance of some European and Argentinean cultivars of *Vitis vinifera*, under saline conditions.

TABLE 3.

Probability values obtained from statistical analysis for different factors (group, salt treatment and cultivars) and variables (leaf area, leaf number, stem length, leaf DM, stem DM, Root DM and Total DM) in *Experiment I* (n=6) and *Experiment II* (n=5) ($p<0.05$).

Experiment I							
	Leaf Area	**Leaf number**	**Stem length**	**Leaf DM**	**Stem DM**	**Root DM**	**Total DM**
Argentinean vs. European	0.0000	0.7399	0.7810	0.0001	0.0203	0.9297	0.0025
Cultivar	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Salt Treat.	0.0000	0.0000	0.0000	0.1299	0.3775	0.7280	0.8410
Cult. x Salt	0.0356	0.0200	0.0001	0.0154	0.0617	0,0258	0.0566
Experiment II							
	Leaf Area	**Leaf number**	**Stem length**	**Leaf DM**	**Stem DM**	**Root DM**	**Total DM**
Argentinean vs. European	0.0000	0.0112	0.0000	0.0000	0.0003	0.0389	0.0000
Cultivar	0.0000	0.0003	0.0000	0.0000	0.0000	0.0481	0.0000
Salt Treat.	0.0000	0.0001	0.0000	0.0000	0.0002	0.0150	0.0000
Cult. x Salt	0.2682	0.1462	0.0006	0.4649	0.1987	0.6755	0.6572

Material and methods

Two experiments were performed, namely Experiment I and II. For both experiments explants were obtained from plants of different cultivars cultured *in vitro*. Microcuttings of 1 cm long including one node each were used. Cuttings were cultured in the basal medium of Galzy (1964) modified by adding NH_4NO_3 320 mg/L, Na_2MoO_4. $2H_2O$ 0.025 mg/L and 1/10 of the vitamins used in this paper. The medium was supplemented with sucrose 30 g/L, regional agar 7 g/L and naphthaleneacetic acid (NAA) 0.005 mg/L. The pH was adjusted to 6 before autoclaved for 20 min at 120ºC. Explants were grown in 250 mL flask containing 40 mL of medium.

Salt treatments were performed by adding different concentrations of a salt solution to the basal medium. Salt solutions were made with 3 parts of NaCl + 1 part of $CaCl_2$. Salts were aseptically added to the medium two weeks after explants have formed 1-2 roots per plants. The final volume per flask was 45 mL. In both Experiments salt treatments added to basal medium included 60 and 90 mEq/L. A mixture of NaCl and $CaCl_2$ was used because the role of calcium in maintaining membrane functionality under saline conditions (Volkmar *et al*., 1998).

The number of explants per flask, salt treatments and particular characteristics of each experiment is described below.

Experiment I:

Three European cultivars, Malbec (MAL), Cabernet Sauvignon (CAB), Chardonnay (CHA) and four Argentinean, Cereza (CER), Criolla Chica (CRI), Pedro Gimenez (PGI), Torrontes Riojano (TOR) were tested. Salt treatments were: A) Control (basal medium without salts); B) Salt 60 (medium + 60 mEq/L NaCl+$CaCl_2$) and C) Salt 90 (medium + 90 mEq/L NaCl+$CaCl_2$). Six replications, each consisting in six plants per flask, were used.

Experiment II:

Two European, (MAL and CHA) and four Argentinean (CER, CRI, PGI, and TOR) cultivars were used. In this experiment treatments were: A) Control (basal medium without salts); B) Salt 60 (medium + 60 mEq/L NaCl+$CaCl_2$) and C) Salt 90 (medium + 90 mEq/L NaCl+$CaCl_2$). Five replications, each consisting in five plants per flask, were used.

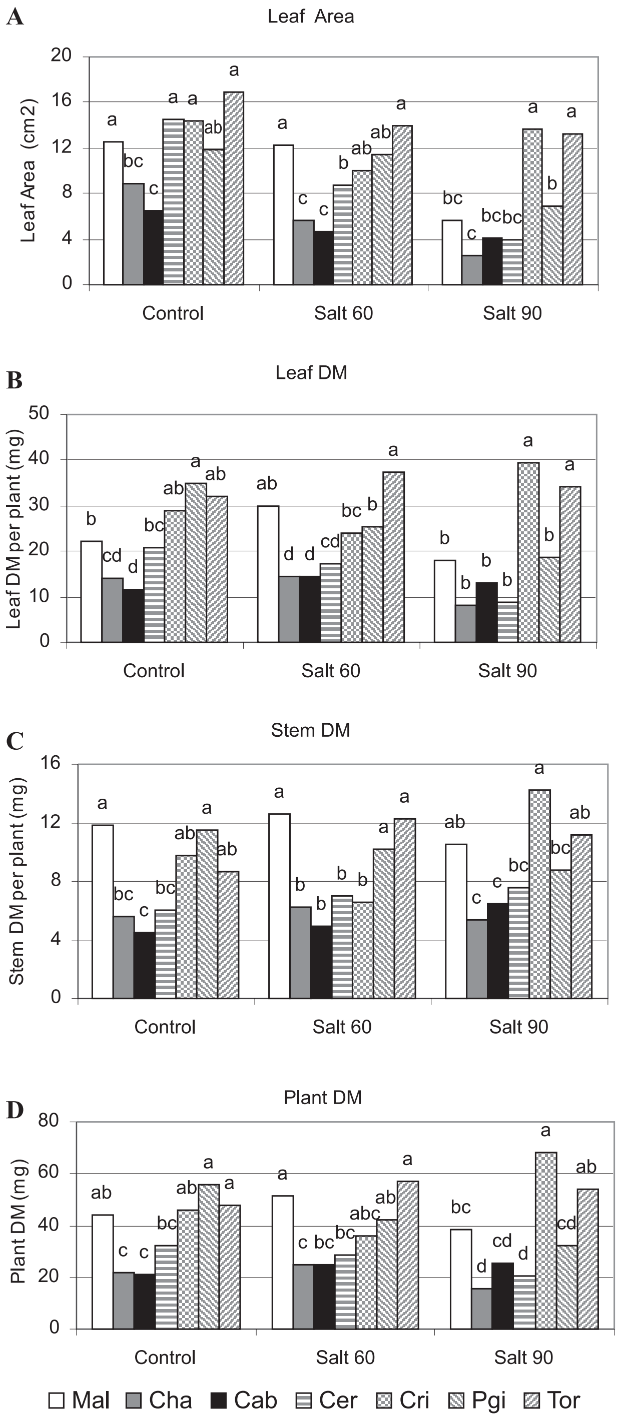

FIGURE 1. Effect of increasing salt concentration on leaf area (A), leaf dry matter (B), stem dry matter (C) and plant dry matter (D) of seven *Vitis vinifera* L. varieties cultured *in vitro*, after four weeks of treatment in Experiment I. Different letters indicate significant differences among cultivars under each treatment (Fisher test LSD, $p \leq 0.05$).

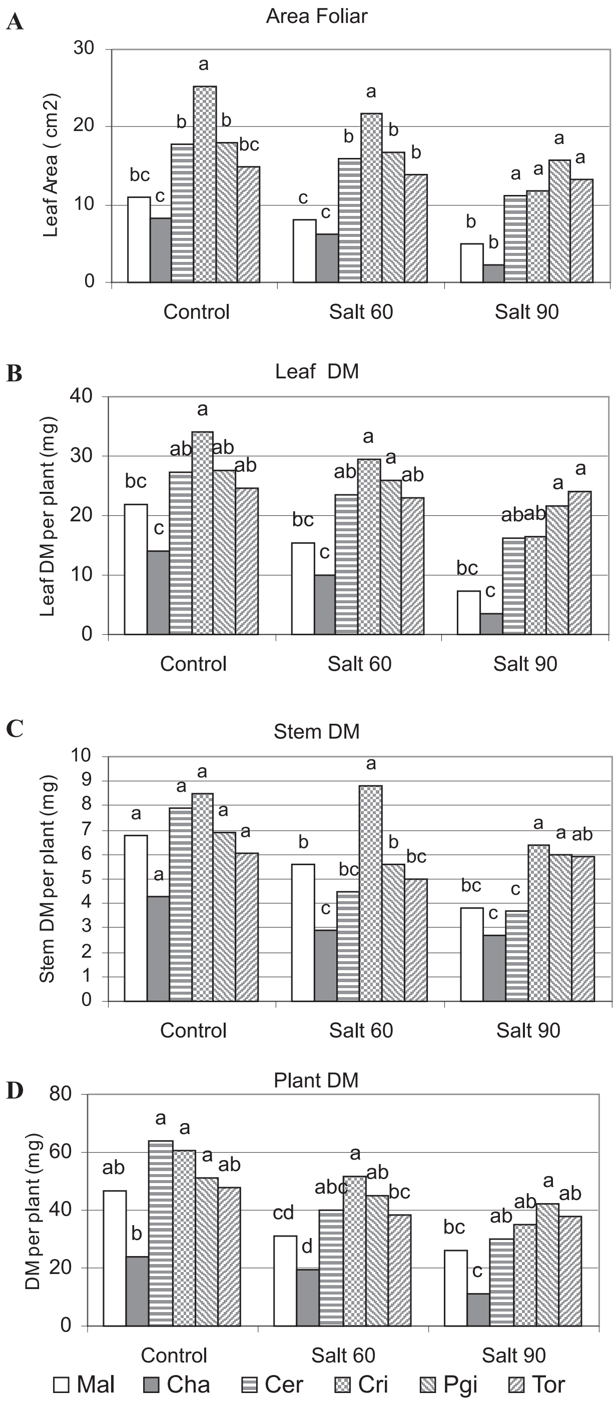

FIGURE 2. Effect of increasing salt concentration on leaf area (A), leaf dry matter (B), stem dry matter (C) and plant dry matter (D) of six *Vitis vinifera* L. varieties cultured *in vitro*, after four weeks of treatment in Experiment II. Different letters indicate significant differences among cultivars under each treatment (Fisher test LSD, $p \leq 0.05$).

In both experiments plants were grown under 16 h photoperiod (150 µmol m^{-2} s^{-1} photosynthetic active radiation, PAR) provided by fluorescent lamps, at 24 ± 2°C. After 4 weeks of salt treatment plant survival, leaf area, stem length, leaf, stem and root dry matter per plant were determined. A factorial experimental design including cultivars and salt treatments was used. Data were statistically analyzed by ANOVA and the Fisher test, LSD, (Sokal and Rohlf, 1971) was used for mean comparisons.

For every cultivars, leaf area of all control plants and the corresponding leaf dry matter were used to obtain a regression equation between these variables. Leaf area of plants belonging to other treatments was calculated from leaf DM of each plant and the corresponding equation. Leaf area of plants corresponding to control, were measured using a leaf area meter (LiCor model LI 3000A). Dry matter determinations were performed in a ventilated oven at 70ºC.

Results and discussion

Tables 1 and 2 (experiments I and II, respectively) show plant survival after four weeks of salt treatments, expressed as percentages of the respective controls. Since some cultivars showed dead plants in their control treatments, we expressed values as a percentage of controls, to allow comparisons among genotypes. This cultivar differential adaptability for this media is in agreement with the results obtained by Martinez and Tizio (1990). Using the same substrate, they observed that plant survival and growth rate varied, in a cultivar dependent fashion. New research is necessary to develop an optimum medium for *in vitro* growth of grapevines, regardless of the cultivar used.

Significant differences among salt treatments were found for plant survival but not among cultivars. The number of living plants, scored at the end of the experiment, decreased as salt concentration increased (Tables 1 and 2), but no significant differences were found between salt 60 and salt 90. These results are in agreement with those obtained by Alexander and Woodham (1968) using other *V. vinifera* cultivars under glasshouse conditions and by Troncoso *et al.* (1999) working in growth-chamber and *in vitro* culture. However, in the latter case they found great differences in mortality among 11 grapevine species and cultivars proposed as rootstocks, grown under salt concentrations ranging from 0 to 150 mM NaCl. Foliar symptoms including partial or total browning and necrosis of blades were observed in plants that eventually died.

We did not find differences in plant survival among cultivars, expressed as % of the respective controls, and therefore we should reject the previous hypothesis that Argentinean varieties are more tolerant to salinity than European ones.

Survival is one of the criteria mentioned by Munns (2002) to assess salt tolerance in plants. However, this author pointed out that salt tolerance could also be assessed in terms of biomass production over a prolonged period of time. In this respect, it is interesting to note that Argentinean cultivars, in both experiments, showed better performance for most of the plant-growth variables measured (Table 3, Figs. 1 and 2).

Table 3 resumes probability values (p-values) obtained in the statistical analysis of growth variables from experiments I and II. Considered as a group, Argentinean varieties performed better than Europeans for leaf area and DM of leaves, stems and whole plants in experiment I and II. In the latter, stem length and roots DM followed the same trend (Table 3). Differences between results of both experiments can be attributed to a larger variability among plants in experiment I.

Leaf area (LA) of plants was affected by salt concentration in both experiments. Argentinean cultivars showed higher LA than European ones. However, in experiment I, cultivars performance under salt treatments 60 and 90 meq/L did not always show a simple additive effect, indicating a significant Genotype x Environment interaction (Table 3). Figure 1A show cultivars LA under salt treatments from experiment I. Plants of CHA and CAB were the most affected under Salt 60 treatment whereas for Salt 90 CRI and TOR were the less affected. In experiment II (Fig. 2A) Argentinean cultivars (CER, CRI, PGI, TOR) presented significant higher LA than European in both, Salt 60 and Salt 90 saline treatments.

For leaf DM results were similar to LA, in both experiments. In experiment I (Fig. 1B) CRI and TOR were the most tolerant for Salt 90 conditions, MAL showed the highest values among European cultivars, especially in the Control and in Salt 60 treatments. In experiment II (Fig. 2B) CRI was the cultivar with the highest leaf DM for Salt 60 treatment while PGI and TOR were the most productive under Salt 90. Considered as separate groups, Argentinean varieties were significantly superior than European, for both salt treatments.

In experiment I, MAL, PGI, TOR and CRI, MAL, TOR, showed the highest stem DM under Salt 60 and Salt 90, respectively (Fig. 1C). In experiment II, CRI was the most productive at the lowest salt concentration whereas for Salt 90, CRI, PGI and TOR performed best (Fig. 2C).

For the variable total plant DM (the sum of leaf DM + stem DM + root DM) results showed a similar trend as for leaf DM. This is probably due to the fact that leaf DM accounts for 50-60% of the total DM, reflecting the relative weight of this organ in the resulting variable. In experiment I, under Salt 60, plants from MAL and TOR had the highest DM but under Salt 90, plants from CRI and TOR were the outstanding cultivars (Fig. 1D). Experiment II (Fig. 2D) showed Argentinean cultivars as more productive than Europeans for both, control and saline conditions.

Figure 2 clearly shows that Argentinean cultivars CER, CRI, PGI and TOR presented a better performance for all the growth variables evaluated under both salt conditions. MAL was the less affected of Europeans. CRI, PGI and TOR among the Argentinean cultivars and the European MAL performed best for most variables under Salt 60 (Fig. 1). Under Salt 90 CRI and TOR were the outstanding cultivars. Further research is required to elucidate what compounds, if any, of those proposed by Ashraf and Harris (2004), are related to the salt tolerance observed in Argentinean cultivars.

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