Vitis **38** (2), 55–60 (1999)

# Evaluation of salt tolerance of *in vitro*-grown grapevine rootstock varieties

by

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Summary: The response of 11 grapevine rootstock varieties to increasing salt concentrations (0, 50, 85, 120, 155 mM NaCl) was studied under *in vitro* and growth chamber conditions. The effect of salinity on the mortality of explants was compared with that of plantlets grown under growth chamber conditions and with data in literature on rootstock resistance under field conditions. In addition, *in vitro* stem elongation, bud number, and rooting ability were related to salinity. The rootstock varieties can be divided into sensitive (41 B, R.Lot, 110 R, 140 R and 161-49), moderately tolerant (13.5 and Ramsey) and tolerant (196-17, CH-1, CH-2 and Superior). Measurements of the water and nutrient contents of plantlets indicate that increasing salt concentrations decreased the hydration of aerial parts and roots of all plants; however, the decrease of hydration was smaller in salt tolerant varieties. Increasing salt concentrations significantly reduced the K content and, to a smaller extent, the P and Ca contents. With and without salt treatments the levels of K and P were lower in sensitive plants. Na and Cl accumulated to a higher extent in tolerant plants. The tolerance to NaCl of *in vitro*-grown rootstocks seems to be due to their capacity to accumulate salt, to increase K concentration in the tissue and to maintain a high water content. Our results indicate that salt tolerance of grapevine varieties may be tested under growth chamber conditions and using *in vitro* explants.

Key words: rootstock cultivars, in vitro culture, salt tolerance, sodium chloride, phosphorus, potassium.

## Introduction

Maas and Hoffman (1977) classified grapevine species to be moderately sensitive to salinity. Depending on concentration salt in the medium may lead to a reduction of the growth rate (Downton and Crompton 1979; Garcia *et al.* 1993) and a decrease of shoot, bunch and root number (François and Clark 1979; Al Saidi *et al.* 1988; Prior *et al.* 1992); high concentrations may even cause death of grapevines.

The mechanism of salt stress in plants can, in part, be related to osmotic stress (water deficit) and ionic imbalance (Cushman *et al.* 1990). Marschner (1995) reported that plants exposed to high salt concentrations during a short time, mainly suffer from water stress. After prolonged exposure ionic toxicity may become more relevant. Thus the ability of plants to maintain an adequate level of tissue hydration is closely related to salt tolerance (Downton and Millhouse 1985).

Arbabzadeh and Dutt (1987) related the salt-induced plant growth inhibition to the total sum of cations in leaves. In many experiments the cationic increase is mainly due to Na (Downton 1977; Bartolini *et al.* 1991; García and Charbaji 1993). It is well known that an excess of Na causes a decrease of other cations ("ionic antagonism"). Garcia and Charbaji (1989) showed a Na-K antagonism and Downton (1985) a Na-Ca antagonism in grapevines.

Some authors (Sauer 1968; Bernstein *et al.* 1969; Groot and Alexander 1973) attribute negative salinity effects to an accumulation of Cl. Higher Cl concentrations, however, may not necessarily cause saline stress in grapevine (Arbabzadeh and Dutt 1987; Walker 1994).

In spite of numerous reports in which the tolerance of grapevines to salinity has been studied, under both, field and greenhouse conditions, there are only few studies dealing with *in vitro* salt tolerance of grapevine. Barlass and Skene (1981) demonstrated that *in vitro* salt tolerance of grapevines is variety-dependent and considered *in vitro* culture a suitable method to select salt tolerance of grapevine. However, they also stressed the need to verify the results by field experiments (Skene and Barlass 1988).

The aim of the present study was to study salt tolerance (NaCl) of different rootstock varieties by comparing potted plants with *in vitro*-grown tissues and plants in order to develop a rapid and non-expensive test for salt tolerance of grape.

## Material and Methods

Cuttings from rootstock varieties Rupestris du Lot (R.Lot), 161-49 Couderc (161-49), 41-Berlandieri (41 B), 110-Richter (110 R), 196-17 Castel (196-17), 13.5 Evex (13.5), 140 Ruggeri (140 R), Ramsey, Superior, and two clones, CH-1 and CH-2 from a saline semi-arid zone in Arica-Chile, were treated with 0.2 % $CuSO_4$ and placed in a medium of granulated perlite in a greenhouse. The developing shoots were desinfected by the following steps: (a) washing with water and a detergent and gently rinsing with distilled water; (b) immersion in 70 % ethanol for 30 s; (c) immersion in 12 % sodium hypochlorite (3.5 % active chlorine) after addition of some drops of Tween-20 (20 min at 30 °C with stirring); (d) gently rinsing with distilled, sterilized water. After desinfection, the young shoots were cut into sections (1 cm long)

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with one bud. The explants were placed in sterile test tubes (21 x 150 mm), one per tube, with 10 ml of "VID" culture medium (TRONCOSO *et al.* 1990). The tubes were covered with plastic caps, sealed with parafilm and placed in a growth chamber at 23 ± 2 °C and a 16 h photoperiod (light intensity: 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$). After 60 d of *in vitro* culture, one part of the plantlets of each variety was used to prepare new explants which were subcultured in the presence of different concentrations of NaCl. Other plantlets of the rootstock varieties R.Lot, 140 R, 13.5, 161-49, Ramsey, 196-17, Superior, CH-1 and CH-2 were transplanted into pots with 250 ml of a perlite-peat mixture and placed for hardening in a growth chamber (CANTOS *et al.* 1993). After 45 d, the potted plants were grouped and treated as follows: 10 plants per variety were irrigated every 15 d with 150 ml per pot of distilled water (control) or with NaCl solutions (50, 85, 120 or 155 mM). At the 15th and 30th day of treatment the number of dead plants and the growth of the surviving ones were determined.

The *in vitro* explants were subcultured under the conditions indicated above. 24 explants per variety were cultured in the VID medium without (control) or with 50, 85, 120 or 155 mM NaCl. After 45 d of *in vitro* culture the number of dead plants, plant size, root development, degree of hydration and mineral composition of the plantlets were determined in relation to the saline treatment. The level of hydration (H) was calculated as follows: H(%)=[(fw-dw)/fw]100, where fw=fresh weight and dw=dry weight.

N, P, K, Ca, Mg, Na were determined according to PINTA *et al.* (1969) and Cl according to FLORENCE and FARRAR (1971). Student t, and Student-Newman-Keuls, Mann Whitney Rank Sum tests were used for statistical analysis.

## Results and Discussion

Experiments with potted plants: All plants on control medium survived except for R.Lot which had a survival rate of 90 % (Fig. 1). 15 d after the saline treatments a high mortality, proportional to the salt concentration, was observed in R.Lot, 140 R and 13.5, while the other varieties were not significantly affected (Fig.1). Thus, R.Lot, 140 R and 13.5 were classified as less tolerant than Superior, CH-1, CH-2, 196-17, Ramsey and 161-49.

After 30 d, Ramsey and 161-49 showed less salt tolerance than Superior, CH-1, CH-2, and 196-17 (Fig. 1). Thus, R.Lot, 140 R and 13.5 were classified as sensitive, 161-49 and Ramsey as moderately tolerant, and 196-17, CH-1, CH-2, and Superior as tolerant. This classification coincides well with the results of SOTÉS *et al.* (1989) and MARTINEZ *et al.* (1990). Superior is grown mainly in warm regions with frequent environmental stresses. CH-1 and CH-2 are clones selected for their adaptability in the saline-arid zone of Arica, Chile. Only 161-49 showed a somewhat higher tolerance in our experiment. On the other hand, 161-49 was found among the best performing roostocks in a study with both, table and wine grapes under dry semi-arid conditions (SPIEGEL-ROY *et al.* 1971, 1972).

Experiments with *in vitro*-grown plants: The salt-sensitive rootstocks 41 B and 110 R, widely used in Andalusia (Spain), were also used. After 45 d of *in vitro* culture, all plants grown in the control medium were still alive. Addition of 50 mM NaCl to the medium enabled us to differentiate between the varieties. 41 B, R.Lot, 110 R and 140 R (explant mortality of 40-63 %) were classified as highly sensitive to salt stress (Fig. 2). The behaviour of all 4 rootstocks coincides with the degree of sensitivity described by SOTÉS *et al.* (1989) and MARTÍNEZ *et al.* (1990). The roostock 13.5 showed intermediate sensitivity with 17 % of dead explants and the remaining varieties revealed a low sensitivity. The grouping was identical to that of the growth chamber experiment.

By adding 85 mM of NaCl to the medium we confirmed the low tolerance of 41 B, R.Lot, 110 R, and 140 R. At this concentration, 13.5, 161-49, and Ramsey also revealed a high number of dead explants, while 196-17, CH-1, CH-2 and Superior largely survived. At 120 and 155 mM, 196-17, CH-1, CH-2 and Superior proved to be more tolerant than the other varieties.

Together with the aforementioned results it was possible to establish three groups of varieties: (i) a sensitive group

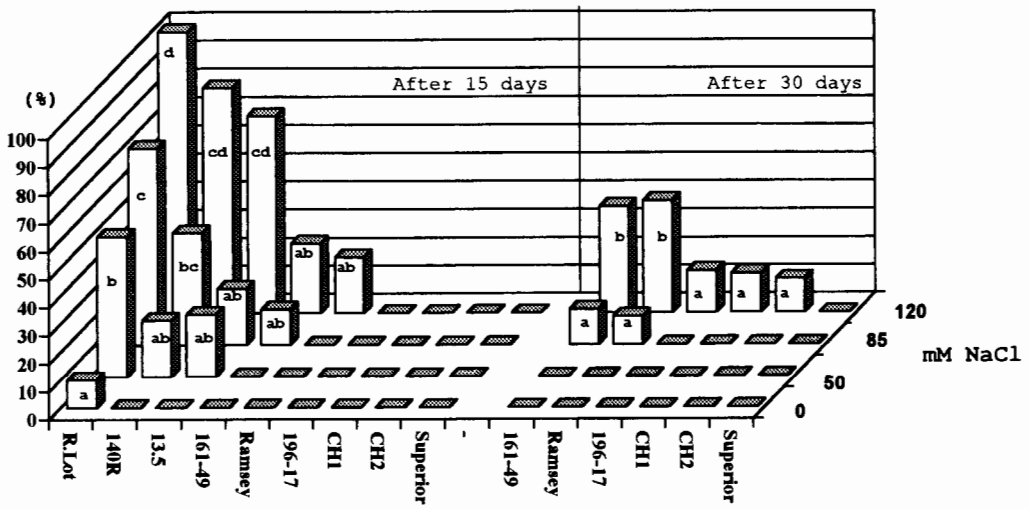


Fig. 1: The effect of salinity (NaCl) on mortality (%). Growth chamber-grown potted plants of various rootstock cvs 15 and 30 d after the treatment. Different letters indicate significance at the p=0.05 level.

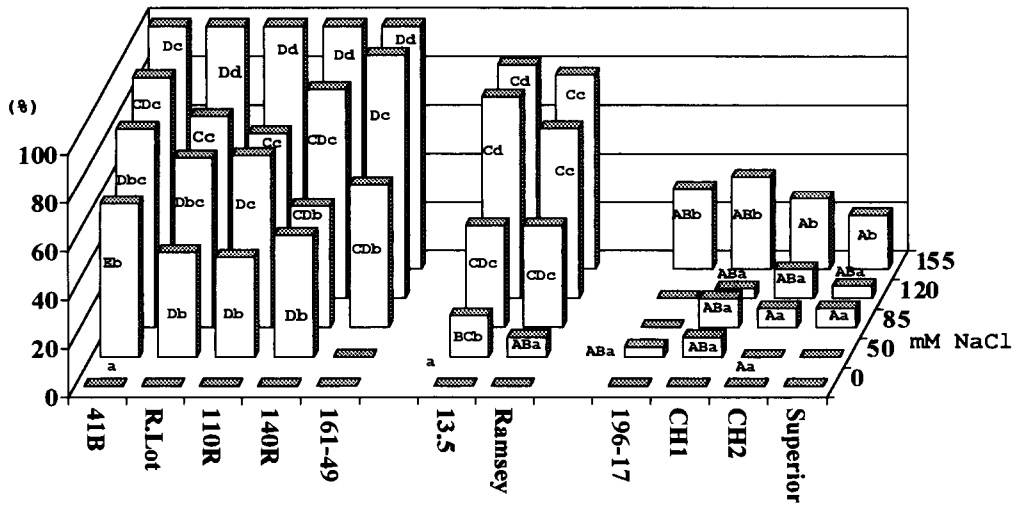


Fig. 2: The effect of salt concentration (NaCl) on plant mortality (%) of rootstock cvs grown *in vitro* for 45 d. Different letters indicate significance at the p=0.05 level; capital letters indicate differences among varieties, small letters among treatments.

including 41 B, R.Lot, 110 R, 140 R, and 161-49, (ii) a moderatly tolerant group including 13.5 and Ramsey, and (iii) a tolerant group including 196-17, CH-1, CH-2 and Superior.

This classification correlated well with that of the potted plants in the growth chamber experiment, except for 161-49, which appeared less tolerant *in vitro*. The close relationship between the results obtained from *in vitro* and pot experiments including results from field studies concerning salt tolerance suggests that salt tolerance of grapevine may rapidly and conveniently be tested *in vitro*, the level of salinity where mortality starts to increase significantly being a diagnostic parameter.

The presence of NaCl in the medium also affected the *in vitro* development of the surviving plants. Stem growth, number of buds, and rate of rooting were inversely related to the salt concentration. The magnitude of the effects differed between the varieties according to their classification as sensitive, moderatly tolerant and tolerant (Figs. 3, 4, 5).

Root formation and development was even more affected by salt than growth and development of aerial parts of the plantlets with NaCl concentrations >85 mM preventing rooting of the explants.

The water content of the aerial plant tissues decreased in all grapevine varieties with increasing salt levels, though less in the more tolerant varieties. Hydration was most strongly reduced in 41 B, R.Lot, and 110 R, followed by 140 R, 161-49 and 13.5. The varieties classified as tolerant were almost unaltered between 0 and 85 mM NaCl, and even maintained a high water content at 155 mM (Tab. 1).

An increase in salinity also caused a reduction of the water contents of the roots (Tab. 2) which was related to the tolerance level of thc varieties. Thus, the tissue water content seems to be another suitable parameter for testing salt tolerance *in vitro*.

Since nutritional imbalances and ionic toxicity are important factors of salt tolerance the contents of N, P, K, Ca, Mg, Na, and Cl of sensitive and tolerant grapevine varieties as influenced by NaCl concentrations in the media were determined (Tab. 3). The N content was hardly affected. P levels decreased in NaCl-treated plants but less so in tolerant varieties. The K content was strongly affected by NaCl, showing an inverse relation with NaCl concentrations. This Na-K antagonism is well known in field-grown plants. However, the salt tolerant varieties maintained higher K levels than

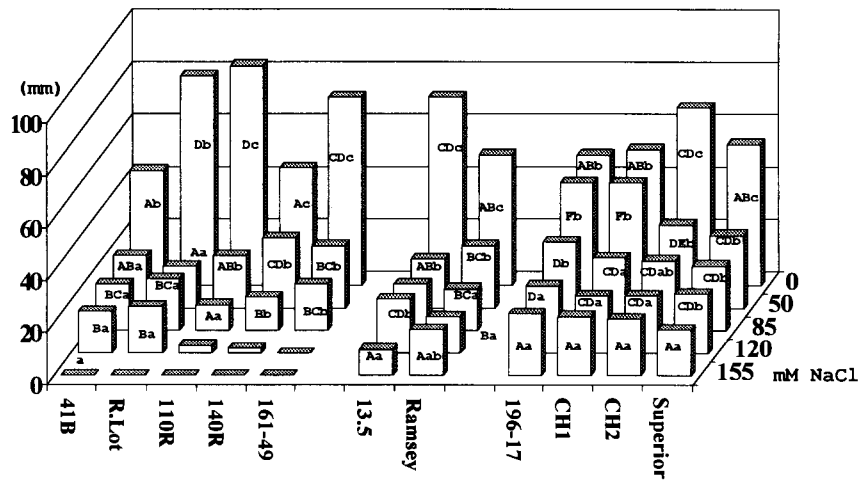


Fig. 3: The effect of NaCl concentration on shoot growth of rootstock cvs 45 d after *in vitro* culturing. For details see Fig. 2.

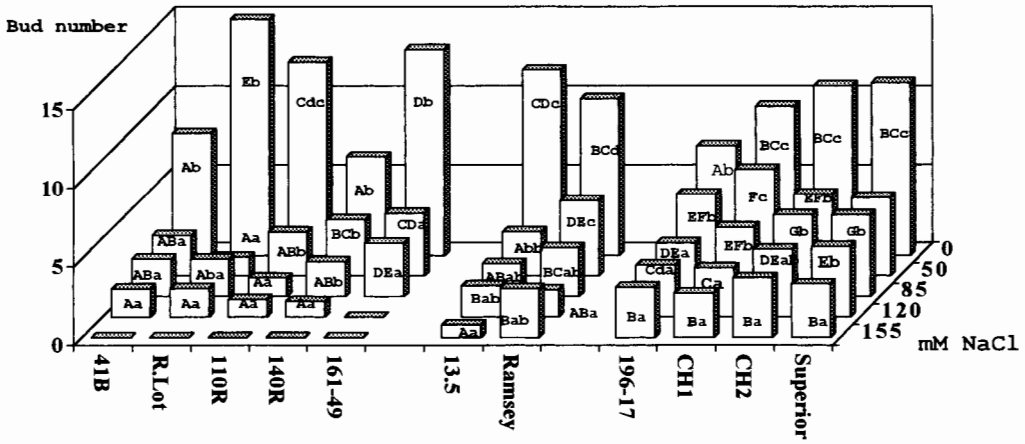


Fig. 4: The effect of NaCl concentration on bud formation of rootstock cvs, 45 d after *in vitro* culturing. For details see Fig. 2.

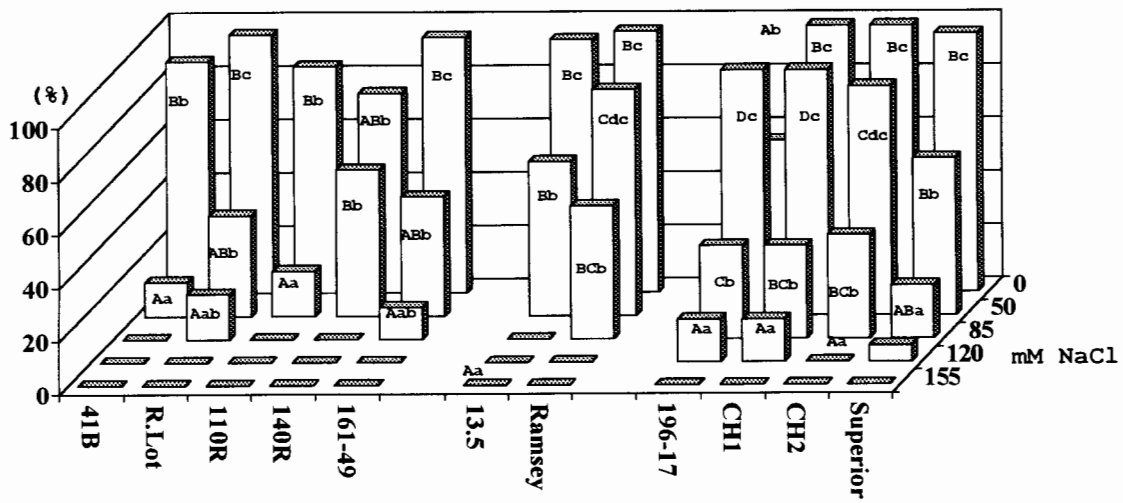


Fig. 5: The effect of salt concentration (NaCl) on explant rooting of *in vitro*-grown rootstock cvs 45 d after culturing. For details see Fig. 2.

Table 1

Water content (%) of the aerial tissues of grapevine plantlets 45 d after the onset of *in vitro* culture. Plantlets were exposed to various concentrations of NaCl in the medium

| Rootstock variety | NaCl (mM) 0 | 50 | 85 | 120 | 155 |
|---|---|---|---|---|---|
| 41 B | 87.8 | 82.8 | 80.0 | - | - |
| R. Lot | 91.9 | 83.7 | 80.0 | - | - |
| 110 R | 89.9 | 83.3 | 80.0 | - | - |
| 140 R | 90.0 | 84.0 | 83.7 | - | - |
| 161-49 | 90.2 | 87.5 | 82.5 | - | - |
| 13.5 | 89.7 | 84.0 | 83.0 | - | - |
| Ramsey | 91.7 | 86.7 | 87.1 | 86.0 | - |
| 196-17 | 89.6 | 90.5 | 90.9 | 87.0 | 88.7 |
| CH-1 | 91.7 | 90.6 | 88.6 | 87.5 | 86.0 |
| CH-2 | 91.7 | 90.7 | 89.2 | 89.3 | 89.2 |
| Superior | 91.1 | 85.8 | 86.7 | 85.5 | 86.7 |

Table 2

Water content (%) of the roots of grapevine plantlets 45 d after the onset of *in vitro* culture. For details see Tab. 1

| Rootstock variety | NaCl (mM) 0 | 50 | 85 | 120 | 155 |
|---|---|---|---|---|---|
| 41 B | 90.0 | 81.2 | - | - | - |
| R. Lot | 96.1 | 88.3 | 83.3 | - | - |
| 110 R | 94.9 | 84.4 | - | - | - |
| 140 R | 94.8 | 89.6 | - | - | - |
| 161-49 | 94.7 | 88.9 | 85.7 | - | - |
| 13.5 | 93.9 | 89.2 | - | - | - |
| Ramsey | 94.0 | 90.0 | 87.5 | - | - |
| 196-17 | 94.2 | 92.3 | 85.6 | - | - |
| CH-1 | 95.9 | 94.7 | 90.0 | - | - |
| CH-2 | 94.2 | 89.3 | 91.2 | - | - |
| Superior | 94.5 | 91.2 | 82.5 | - | - |

the sensitive ones. The Ca content also decreased slowly with increasing salt concentration in the medium, though, to a smaller extent and irrespective of their tolerance. This agrees with results of DOWNTON (1985).

Salt tolerant varieties accumulated significantly higher amounts of Na in salinity treatments. DOWNTON (1977) observed Na to increase in field-grown grapevines treated with NaCl. ARBABZADEH and DUTT (1987) and GARCÍA and CHARBAJI

Table 3

Influence of NaCl concentration of the medium on the mineral composition (% d.w.) of the plant tissue (groups of sensitive and tolerant varieties) after 45 d of the onset of *in vitro* culture

| | Group of variety | NaCl (mM) 0 | 50 | 85 | 120 | 155 |
|---|---|---|---|---|---|---|
| N | sensitive | 3.01 | 3.33 | 3.82 | 3.69 | - |
| | tolerant | 3.50 | 3.22 | 3.48 | 3.70 | 4.09 |
| P | sensitive | 0.44 | 0.37 | 0.31 | 0.33 | - |
| | tolerant | 0.67 | 0.59 | 0.43 | 0.47 | 0.48 |
| K | sensitive | 1.63 | 1.50 | 1.40 | 1.12 | - |
| | tolerant | 1.93 | 1.72 | 1.58 | 1.53 | 1.27 |
| Ca | sensitive | 0.59 | 0.48 | 0.40 | 0.46 | - |
| | tolerant | 0.63 | 0.57 | 0.43 | 0.42 | 0.41 |
| Mg | sensitive | 0.27 | 0.20 | 0.22 | 0.22 | - |
| | tolerant | 0.24 | 0.24 | 0.20 | 0.20 | 0.19 |
| Na | sensitive | 0.19 | 1.82 | 2.62 | 2.89 | - |
| | tolerant | 0.16 | 2.46 | 3.71 | 4.48 | 5.97 |
| Cl | sensitive | 0.14 | 2.76 | 4.08 | 4.31 | - |
| | tolerant | 0.16 | 4.09 | 5.44 | 6.73 | 8.80 |

(1993) related the salt content of grapevine leaves to the salt injuries of field-grown plants. They report lower Na contents in tolerant plants. REAM and FURR (1976) indicated that under field conditions tolerant plants accumulate less Na than sensitive ones. These observations contradict our results under *in vitro* conditions. It seems that under *in vitro* conditions the tolerant material is better adapted to high tissue Na concentrations. The Na/K ratio has been considered to be a good indicator for salt stress in field-grown plants (DEVITT *et al.* 1981; ASLAM *et al.* 1988). In our *in vitro* system we also found a direct relation between the salt concentration in the media and the Na/K ratio in tolerant and sensitive varieties. Due to the high levels of Na in the tissue of tolerant varieties their Na/K ratio was higher than that of sensitive varieties.

The results obtained for Na are also valid for Cl (Tab. 3). The accumulation of Cl observed in our experiments agrees with results of SAUER (1968), BERNSTEIN *et al.* (1969) and GROOT and ALEXANDER (1973). Here again, avoidance was apparently not the tolerance mechanism in our plant material; this is in contrast to reports of ARBABZADEH and DUTT (1987) and WALKER (1994).

In conclusion, we observed a clear relationship between plant survival, stem growth, number of new buds, and rooting and salt tolerance. Thus, the grapevine varieties of our experiments were grouped according to their salt tolerance under *in vitro* conditions. The classification coincided closely with that obtained by pot experiments and that reported in the literature. *In vitro* testing may be used for rapid selection of tolerant grapevine varieties. The *in vitro* tolerance of rootstock varieties seems not to be due to avoidance.

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*Received November 30, 1998*