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Response of eight *Cucumis melo* cultivars to salinity during germination and early vegetative growth

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Abstract – In order to determine salt tolerance in eight varieties of *Cucumis melo*, two controlled environment experiments were carried out with seeds and seedlings. The tolerance study was carried out using the Van Genuchten equation which was found to have a very high level of fitting for melon. During the germination process, the most tolerant varieties were ‘amarello’ and ‘amarillo oro’ and during the early vegetative growth it was ‘tendral terreno’. In general, there was no relationship between salt tolerance at the two stages of development. Salinity induced a decrease in the concentrations of Ca^{2+} , K^+ and Mg^{2+} in the shoots, but only of K^+ in the roots of all varieties. Phosphate concentration showed no significant changes either in the shoots or in the roots. Salt tolerance mechanism in *Cucumis melo* seems to be associated with a compartmentation process, since a correlation was found between Na^+ or Cl^- uptake and tolerance. (© Inra/Elsevier, Paris.)

mineral composition / muskmelon / salinity / tolerance

Résumé – Réaction de huit cultivars de *Cucumis melo* à la salinité durant la germination et le début de la croissance végétative. Afin de déterminer la tolérance au sel de huit variétés de melon, deux expériences ont été faites en milieu contrôlé sur des graines et des plants. La tolérance au sel a été étudiée à l’aide de l’équation de Van Genuchten qui s’est trouvée convenir très bien pour le melon. Au cours du processus de germination les variétés les plus tolérantes au sel ont été « amarello » et « amarillo oro » et, pendant le début de la croissance végétative, la variété « tendral terreno ». La tolérance au sel n’est liée à aucun des deux stades de développement étudiés. La salinité a provoqué une diminution de la concentration en Ca^{2+} , K^+ et Mg^{2+} dans les pousses mais, dans les racines de toutes les variétés, seule la concentration en K^+ a diminué. La

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concentration en phosphate n'a montré de variation significative ni dans les pousses, ni dans les racines. Le mécanisme de la tolérance au sel chez *Cucumis melo* semble être associé à un processus de cloisonnement, puisqu'aucune relation n'a été trouvée entre l'absorption de Na^+ ou de Cl^- et la tolérance. (© Inra/Elsevier, Paris.)

melon / composition minérale / salinité / tolérance

1. INTRODUCTION

Melon is an important crop which is increasingly being cultivated using low-quality saline waters in semiarid regions. It has been widely studied how different cultivars vary from salt sensitive to moderately tolerant [19, 22, 23]. However, the sensitivity or tolerance can differ according to the culture medium, the type of salinity and the plant growth stage [19, 26]. Thus, relative tolerance during seed germination, seedling emergence and later stages of the plant may be different. It has been reported that melon is more tolerant to salinity during germination and emergence than during vegetative growth [22]. In any case, the evaluation of salt tolerance is complicated by the fact that growth rates are inherently different among cultivars, and seed viability differs depending upon seed age and the source of nutrient [31]. The response during germination has been reported to be more complex than during plant growth because it depends on the availability of storage compounds [7]. Some plants that have been classified as salt sensitive can germinate under high concentrations of NaCl [13, 18]. However, other tolerant species are more sensitive during germination [2, 12]. And, some halophyte species, which have their growth stimulated by salt, have even been reported to be salt sensitive during germination [11]. Consequently, the separate study of salt tolerance during germination or early and late growth stages is important for determining the limits of each variety in every development phase.

Exposure to NaCl salinity affects transport processes in the plant, the result of which can be an alteration of the nutritional status and tissue ion balance [14]. Salt stress inhibits the uptake and transport of K^+ [15], Ca^{2+} [16] and P [21] which influence plant growth. The nutritional imbalance has a

greater or lesser degree of importance depending on the ambient conditions, the species and, within the same species, on the cultivars.

In this paper we studied eight common varieties of melon and compared their levels of tolerance to NaCl during the phases of germination and early growth. We also measured the concentrations of Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Cl^- and P in shoots and roots and tried to correlate them with salt tolerance in order to explain the differences among cultivars.

2. MATERIALS AND METHODS

2.1. Germination assay

Seeds of eight varieties of *Cucumis melo* L. (Galia, amarello, tendral terreno, piel de sapo híbrido, cantaloup americano, piel de sapo piñonet, amarillo oro and temprano rochet) were surface-sterilized in a 5 % sodium-hypochlorite solution for 10 min, washed with deionized water and disposed in Petri dishes (100 seeds per Petri dish) on a wet filter paper. The solutions used to moisten the filter paper contained 0.5 mM CaSO_4 with either 0, 30, 60, 90 or 120 mM NaCl. Seeds were germinated in an incubator at 29 °C and a relative humidity of 90 %. Every day the germinated seeds were counted. Each treatment was replicated four times.

2.2. Vegetative growth assay

The seeds were germinated in vermiculite with 0.5 mM CaSO_4 . The seedlings were disposed in containers of 15 L (30 plants per container) with aerated Hoagland nutrient solution (mmol L^{-1}): 6 KNO_3 ; 4 $\text{Ca}(\text{NO}_3)_2$; 1 Mg SO_4 ; 1 $\text{NH}_4\text{H}_2\text{PO}_4$, micronutrients ($\mu\text{mol L}^{-1}$) were applied in the form of 20 Fe-EDTA, 25 H_3BO_3 , 2 MnSO_4 , 2 ZnSO_4 , 0.5 CuSO_4 and 0.5 H_2MoO_4 . The con-

ainers were transferred to a controlled environment chamber with a cycle of 16 h light and 8 h darkness at 27 and 18 °C, respectively. The relative humidity (RH) was 60 % (day) and 80 % (night) and the photosynthetically active radiation (PAR) was set to 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Ten days after plantation, treatments of 0 (control), 30, 60, 90 and 120 mM NaCl (corresponding to 1.93, 5.34, 11.59, 14.44 and 20.31 dS m^{-1} , respectively) were applied to plants. Nutrient solution was renewed every 5 d. At 16 and 29 d after sowing, samples of roots and shoots were taken in order to determine fresh and dry weights and mineral contents. Three plants constituted one sample and three samples per treatment were taken.

2.3. Analysis of salt tolerance

The growth data were analysed by the equation proposed by Van Genuchten and Hoffman [35]. They suggested a non-linear least-squares inversion method for evaluating the salt tolerance response of plants. The expression of the formulation considered was:

$$Y_r = Y_m / [1 + (c/c_{50})^p]$$

where Y_m is the relative growth rate under non-saline conditions (0 mM NaCl treatment), Y_r is the relative growth rate (g f.w. $\text{g}^{-1} \text{d}^{-1}$) at a given electric conductivity (c), c is the root zone electric conductivity (dS m^{-1}), c_{50} is the electric conductivity at which growth is reduced by 50 %, and p is an empirical constant (generally $p = 3$ [34]).

2.4. Mineral analysis

Dried plant tissues (0.1 g) were digested in a concentrated nitric/perchloric acid (2:1, v/v) mixture. Phosphorus was measured by the molybdenum-blue method described by Dickman and Bray [5]. Sodium and potassium concentrations were determined directly by atomic emission spectrometry and calcium and magnesium by atomic absorption spectrometry (Perkin Elmer-500).

Chloride was extracted from 0.1 g ground material with 50 mL deionized water and measured by electro-metric titration [9].

2.5. Data analysis

All the experiment were repeated at least three times and data were analysed calculating least significant differences (LSD).

2.5. Cl^- and Na^+ uptake

Cl^- and Na^+ uptake was calculated as specific absorption rate (SAR) since the experiment was carried out during the exponential growth phase, using the following equation [36]:

$$\text{SAR } (\mu\text{mol mg}^{-1} \text{day}^{-1}) = 1/\text{RDW } \partial M / \partial T$$

where M is the ion content (μmol), t is the time (days) and R the root dry weight (mg), at two different periods.

3. RESULTS

Variations in the relative germination percentage (RGP) with time are represented in *figure 1*. It can be observed that there was a high variation in the dynamics of germination of the untreated (control) seeds of the different varieties. However, 100 % of germination was reached by all varieties within 7 d. The first variety to produce 100 % germination was 'amarillo oro' (3 d) followed by 'tendral terreno' and 'amarello' (4 d), and the last was 'cantaloup americano' (7 d). In general, NaCl reduced RGP, but 'amarello' and 'amarillo oro' were the least affected varieties. For the remaining varieties, the decrease in RGP became larger with increasing NaCl concentration. RGP values lower than 20 % were observed for the 120 mM NaCl treatment. The variety 'cantaloup americano' was the most affected, showing minimal values at 90 and 120 mM NaCl.

An objective estimation of the salt tolerance of each variety studied was obtained by the Van Genuchten equation (see Material and methods). For each variety, the fit of the equation is represented in *figure 2* (shoots) and *figure 3* (roots). In all varieties, there was a high degree of significance in the fitting of shoot and root relative biomass data: the determination coefficients were between 0.59 (shoots of 'amarello') and 0.93 (shoots of 'piel de sapo piñonet' and roots of 'tendral terreno'). When the varieties were analysed individually, large differences in the C_{50} of shoots and roots were observed. The varieties with the highest and lowest C_{50} in shoots (*figure 2*) were 'tendral terreno'

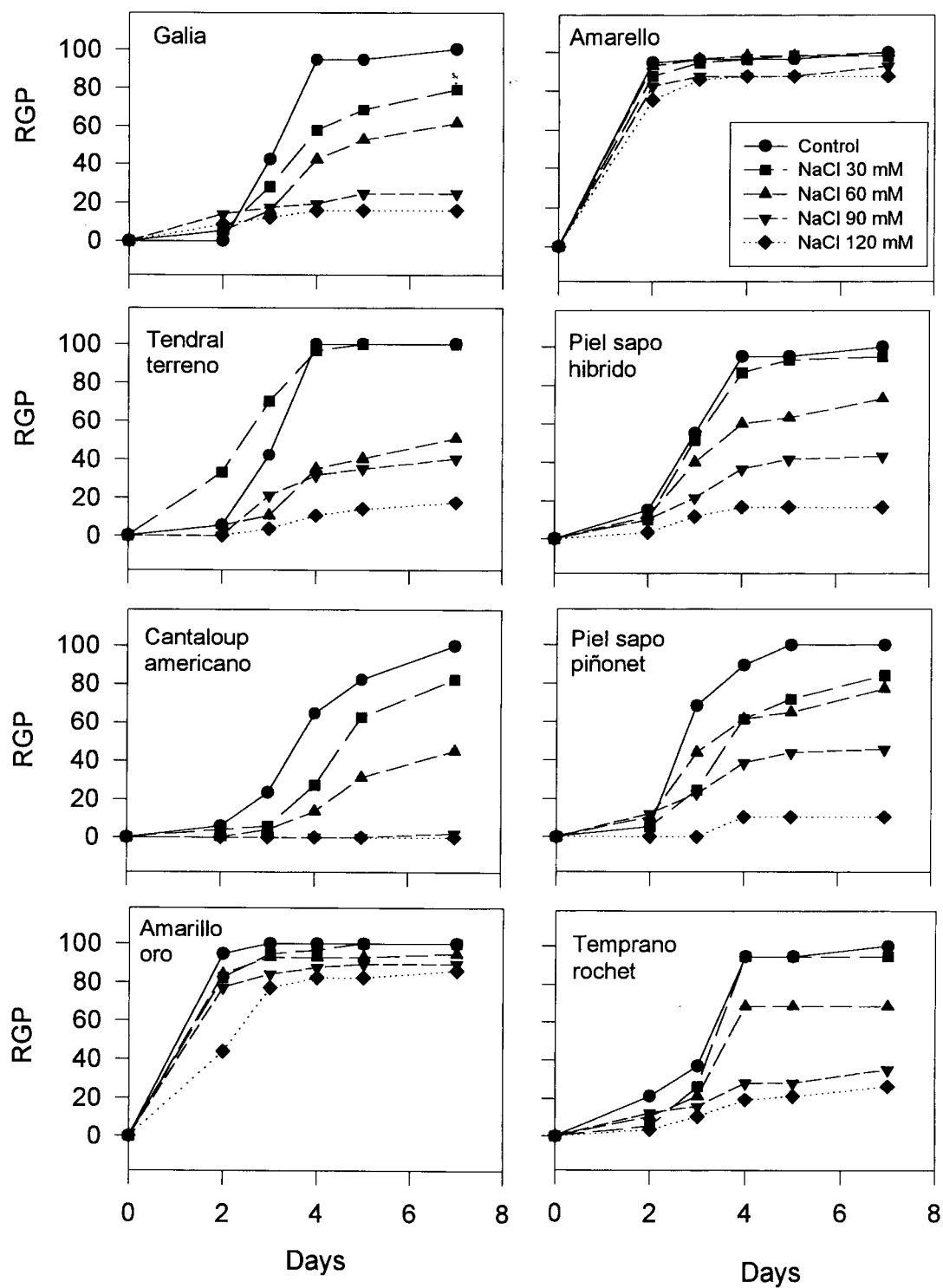


Figure 1. Relative germination percentage (RGP) of eight varieties of melon over 7 d. The seeds were hydrated with 0.5 mM CaSO_4 (control) and different concentrations of NaCl (30, 60, 90 and 120 mM); ($n = 4$).

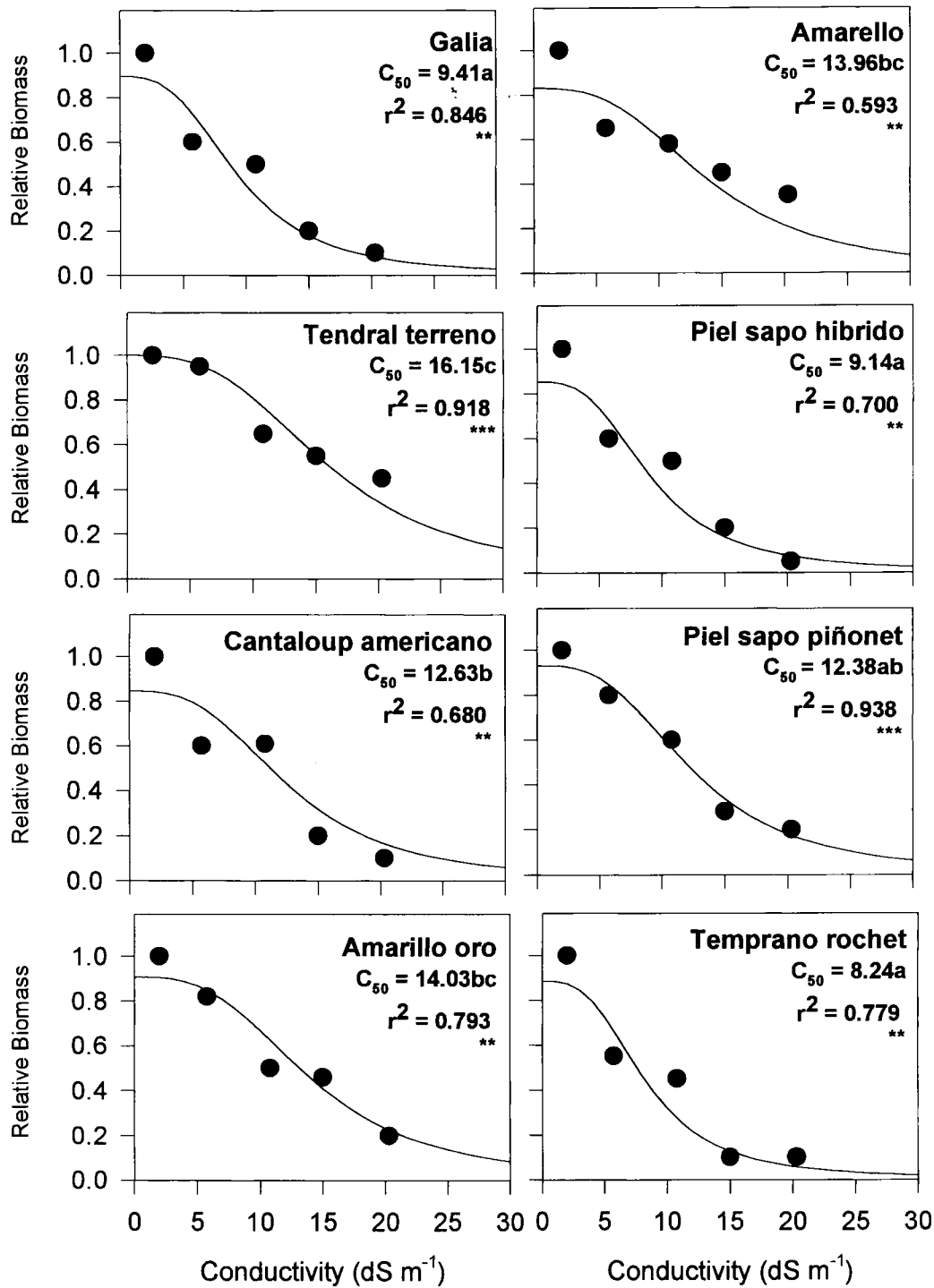


Figure 2. Effect of NaCl treatments on the shoot dry weight. The data are expressed as relative to control plants (0 mM NaCl added). The solid line represents the best fit obtained by the Van Genuchten equation. * Significance level < 0.1, ** < 0.05 and *** < 0.01. C₅₀ followed by different letter indicates significant differences at the 95 % level of confidence or better.

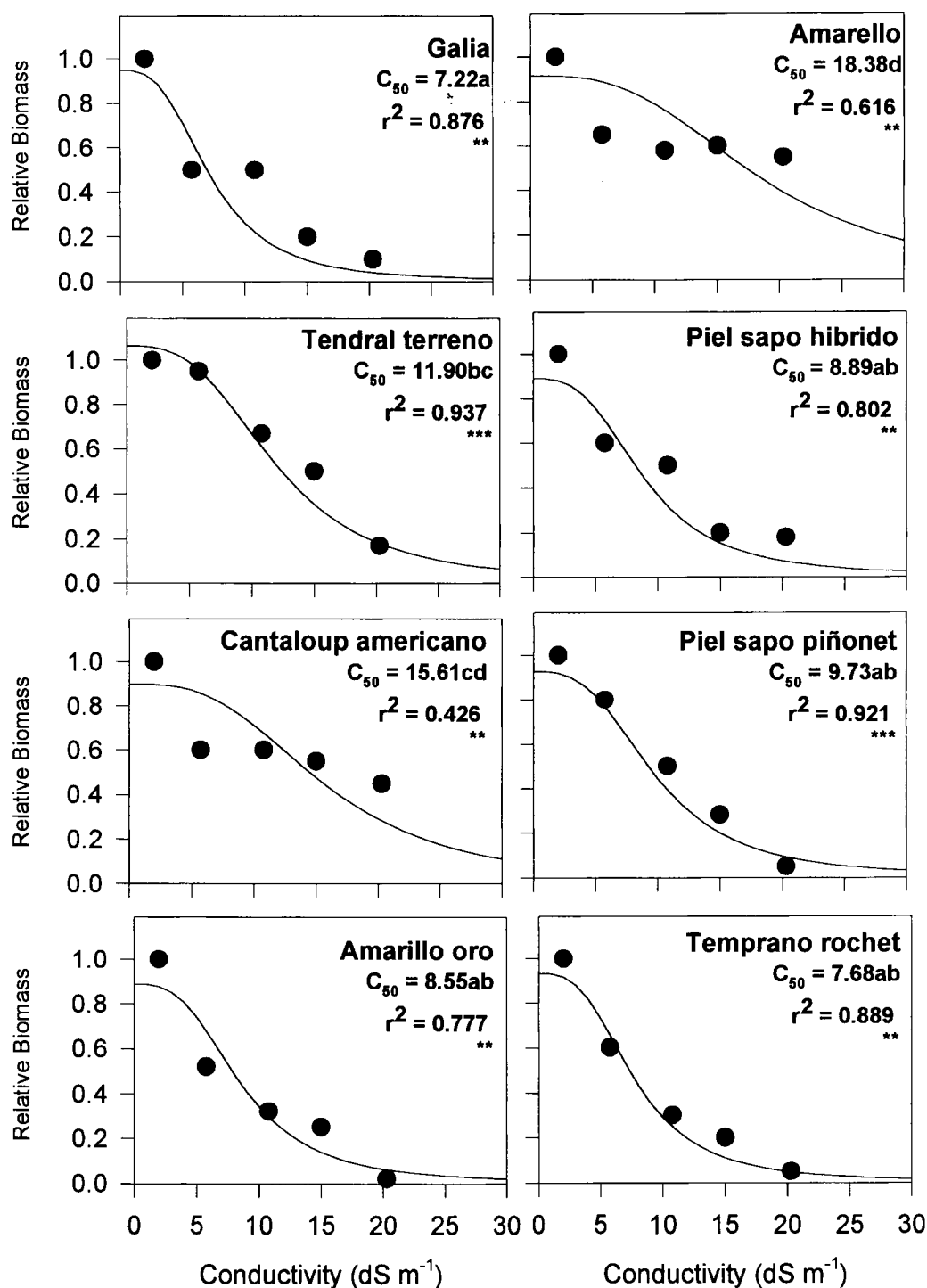


Figure 3. Effect of NaCl treatments on the root dry weight. The data are expressed as relative to control plants (0 mM NaCl added). The solid line represents the best fit obtained by the Van Genuchten equation. * Significance level < 0.1, ** < 0.05 and *** < 0.01. C_{50} followed by different letter indicates significant differences at the 95 % level of confidence or better.

(16.15) and 'temprano rochet' (8.24), respectively, whereas in roots (*figure 3*) they were 'amarrello' (18.38) and 'galia' (7.22). The range of variation of C_{50} in the roots was greater than in the shoots.

The changes in shoot and root ion concentrations in response to salinity are shown in *figure 4*. Only average data are presented since ion concentration varied little among varieties at each NaCl concentration. The concentrations of Na^+ or Cl^- in the shoots were significantly increased when higher NaCl concentrations were applied in the nutrient solution. However, in the roots, only a slight increase was observed after the 30 mM NaCl treatment but the concentration remained constant afterwards when higher NaCl concentrations in the nutrient solution were applied. Calcium concentration in shoots was significantly decreased with all NaCl treatments. However, no difference was found between the roots of treated and control plants (0 mM NaCl). The concentration of K^+ similarly decreased in shoots and roots after applying the NaCl treatments, but no significant differences were observed among the NaCl treatments. The concentration of Mg^{2+} in shoots decreased progressively at 30 mM and 60 mM NaCl, and remained constant at higher NaCl concentration, but no changes were observed in roots. No significant difference was found in P concentration either in shoots and roots or among treated plants.

The SAR of Na^+ and Cl^- was calculated between 16 and 29 d and is shown in *figure 5*. A progressive increase in SAR of both ions was observed in all varieties studied when NaCl in the medium was increased from 30 to 90 mM. However, from 90 to 120 mM, SAR decreased reaching in most cases values similar to those obtained with 60 mM.

For each variety, the shoot C_{50} calculated by the Van Genuchten equation was linearly related to Cl^- or Na^+ SAR measured at 90 mM NaCl (*figure 6*). The correlation between Cl^- SAR and C_{50} was highly significant ($P < 0.05$) as shown in *figure 6b*. Nevertheless, this correlation was lower in the case of Na^+ ($P < 0.1$; *figure 6a*). No correlation was found for the other analysed ions (data not shown).

4. DISCUSSION

The germination process depends on the capacity of the seeds to absorb water [1]. This capacity can be altered if the osmotic potential of the external solution increases and if the enzyme and hormone levels in the seeds change as a consequence of the toxic effect produced by high concentrations of Na^+ or Cl^- . Salinity may affect germination in two different ways, by either reducing the percentage of germination owing to inhibition or simply causing a delay immediately after applying the treatments. These two effects were observed in our experiments. Some treatments such as 30 and 60 mM NaCl produced a delay in RGP of almost all the varieties, the 100 % germination being reached some days later than that of the control. However, 90 and 120 mM NaCl usually caused an inhibition of RGP either total (cantaloup americano), or partial from 20 to 80 % (the rest of the varieties). This inhibition can be reversible [31] which implies that there is no damage to the structures of the seeds and the normal process may occur when the control treatment is added. This protection mechanism is very common in wild-type species, where seeds can remain in a latent stage under saline conditions [33].

In an attempt to fit a general response function to all salt tolerance data, Maas and Hoffman [17] published a comprehensive analysis based upon an extensive review of the literature. They found that most crops tolerate soil salinity up to a threshold level, above which yields show an approximately linear decrease. In some cases, subjective judgement was required to include or exclude data from the analysis. The method we have used in this paper allows a convenient analysis of the data by coupling a salt tolerance model with a least squares optimization procedure. In our experiments, this model proposed by Van Genuchten and Hoffman produces highly significant fittings for the early growth values of all varieties studied ($P < 0.05$) which implies that it could be a good method for determining the tolerance of melon plants to salinity. Several studies demonstrated that, in general, there is no relationship between the tolerance during germination and

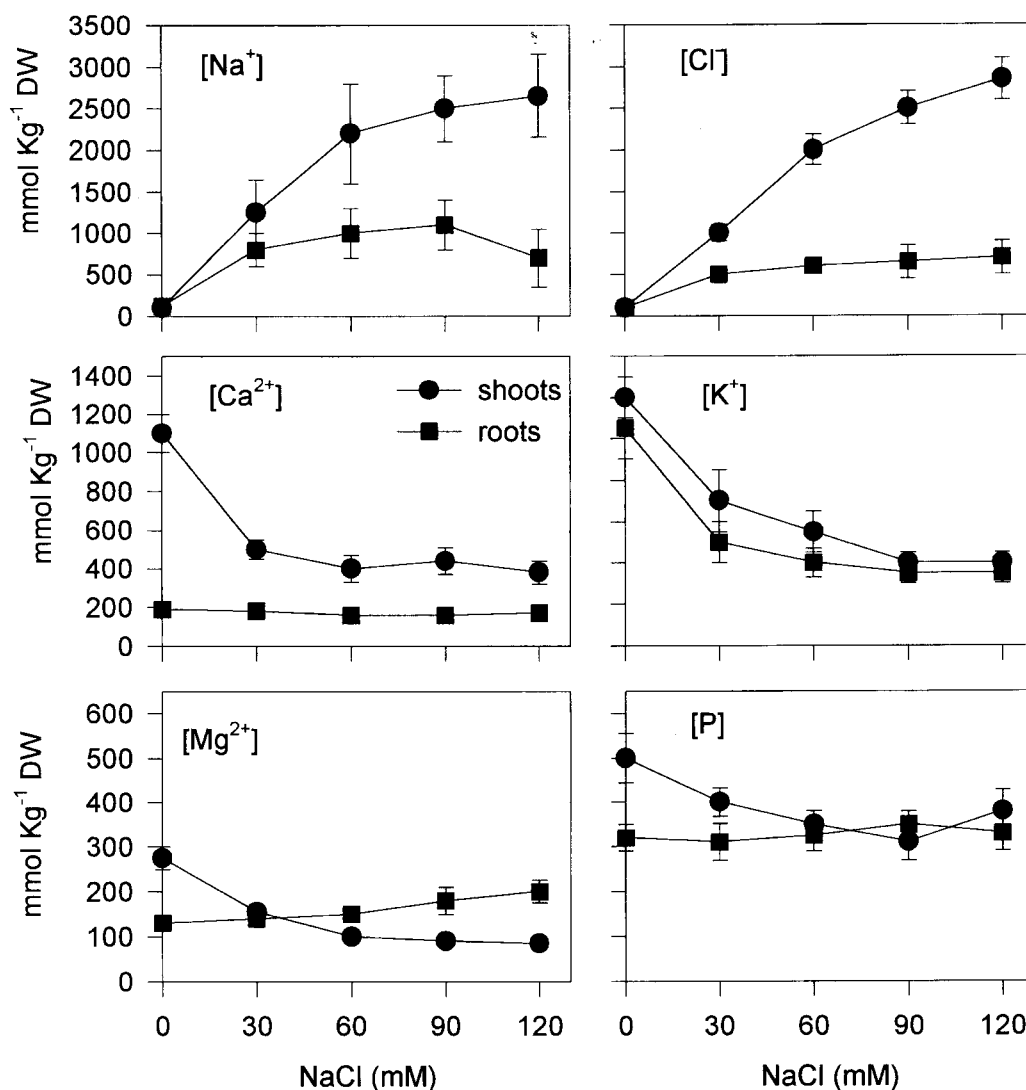


Figure 4. Effect of NaCl treatments on the nutrient concentration in the shoots and the roots of eight varieties of melon plants. Each point is the mean of results $\pm$ SE obtained from all varieties and three replicates per variety.

growth period of melon [6, 26]. However, the study of tolerance during germination and emergence is important for establishing the limit for salt concentrations and the germination delays for each variety. From our results, the most tolerant seeds were 'amarrello' and 'amarillito oro'. For shoot growth of seedlings, the variety showing the highest C_{50} was 'tendral terreno' and for roots it was 'amarrello'. The differences observed between tolerance of

shoots and roots of each variety could be due to water availability after the osmotic adjustment since no relationship between tolerance and nutrient concentration appeared in our study. The separate study of the shoot and root tolerance is interesting because of the different behaviour of both parts under salt conditions. Although 'amarrello' and 'tendral terreno' can be classified amongst the most tolerant cultivars, a factor which has to be taken

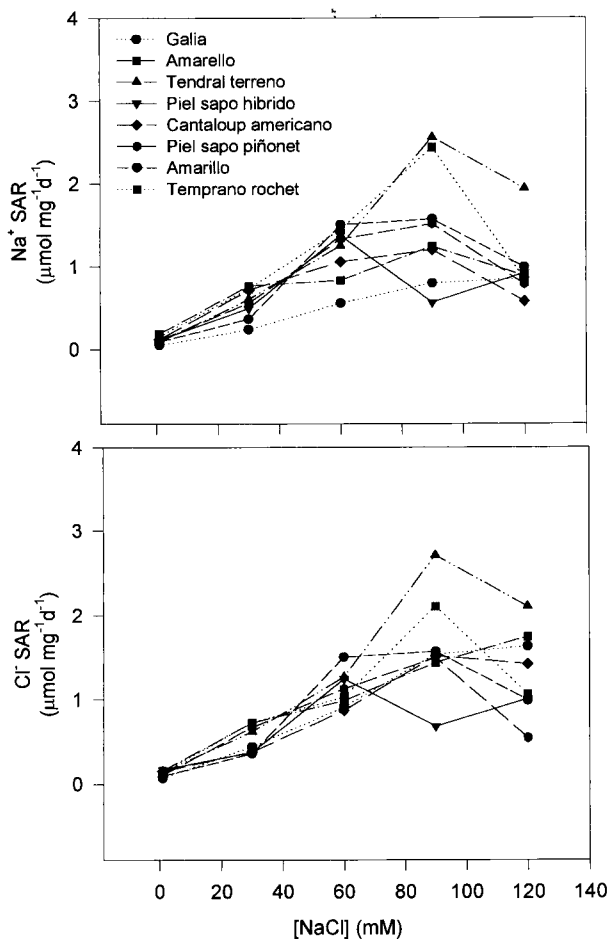


Figure 5. Effect of NaCl treatments on Na⁺ and Cl⁻ specific absorption rates (SAR) of the eight varieties of melon. SARs are the mean values calculated between 16 and 29 d of growth.

into account is the growth conditions. In our study, the germination and growth data were obtained from experiments under controlled environments. Some changes in tolerance are expected to occur when the experiments are carried out in field conditions since plants show different degrees of resistance to changes in climate. The results of this paper, however, could be very interesting for breeding experiments with *Cucumis melo* in order to choose the most tolerant varieties, because a good correlation between early and late growth has been established for this crop [24].

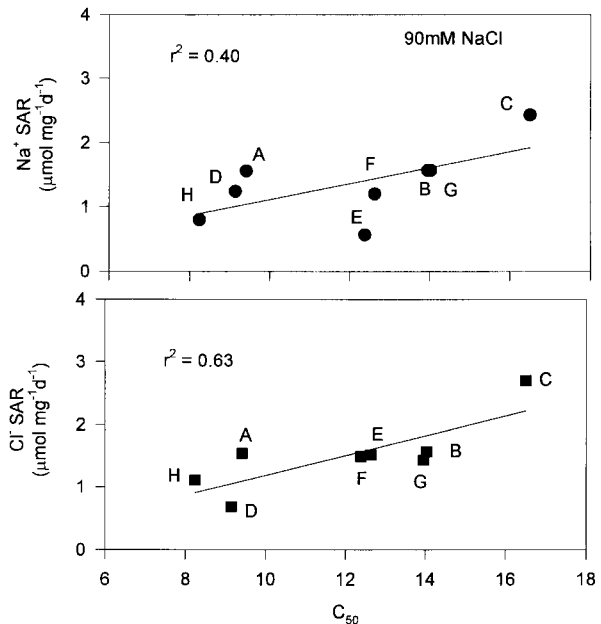


Figure 6. Correlations between Na⁺ or Cl⁻ SARs and NaCl tolerance (expressed as C₅₀) of the eight varieties of melon plants (A: Galia; B: amarello; C: tendral terreno; D: piel de sapo híbrido; E: cantaloup americano; F: piel de sapo piñonet; G: amarillo oro; and H: temprano rochet) grown under 90 mM NaCl.

The decrease in K⁺ concentration found in *Cucumis melo* plants grown under saline conditions has been previously described and explained in terms of the competitive uptake between Na⁺ and K⁺ [20]. In our experiments, the saline stress reduced the Ca²⁺ and Mg²⁺ concentrations only in the shoots, while in the roots they remained constant. This effect has been reported in other species such as *Helianthus annuus* [28] or *Solanum melongena* [29]. Lynch and Läuchli [16] proposed that the decrease in the concentration of these cations in the shoots may be related to the reduced Ca²⁺ release into the root xylem, possibly by affecting the cation active loading in the xylem vessels. Cramer et al. [4] also found a significant interaction between Na⁺ and Ca²⁺. However, Savvas and Lenz [29] suggested that a higher proportion of lignified root mass indicates less protoplasm and more cell walls and therefore more sites of indiffusible anions which

are saturated by Ca^{2+} and Mg^{2+} . That could explain the fact that in our melon plants the concentration of both cations was not altered after increasing salinity from 30 to 120 mM.

It is usually predicted that salt sensitivity is associated with poor control of Na^+ or Cl^- transport from root to shoot [10]. But the most common evidence is the correlation found between the exclusion of Na^+ or Cl^- and the salt tolerance [27, 30, 32]. In our plants, the fact that there is no inverse relationship between Na^+ and Cl^- SAR and salt tolerance, has led us to think that there is no exclusion mechanism for salt tolerance in this crop [8]. Furthermore, this significant correlation found between shoot C_{50} and Cl^- or Na^+ SAR suggests that the implication of these ions in any aspect of the tolerance of melon could be related to compartmentation. Little is known about the compartmentation of Cl^- [3]. However, some approaches give Cl^- a role in the salt tolerance processes [25]. Recent findings (V. Martínez, unpublished results) in which Cl^- is accumulated in stems rather than in leaves of tolerant varieties of melon suggest that Cl^- might have a role in the tolerance process of melon plants and that a compartmentation at organ level is likely. High tolerance is based mainly on the inclusion of salts and their use in turgor maintenance. These inclusions and the partitioning of Na^+ and Cl^- into various organs and tissues are of major importance. This holds true in the case of partitioning between old and young leaves, leaf sheath and leaf blades, and vegetative and reproductive organs. However, this should be investigated further in melon in order to interpret its mechanism of relative salt tolerance.

Therefore, the overall conclusion of this study is that during the germination process, the most tolerant varieties were 'amarello' and 'amarillo oro' and during the early vegetative growth it was 'tendral terreno'. We believe that salt tolerance has no relation to the concentrations of Ca^{2+} , K^+ , Mg^{2+} , K^+ and P in the shoots or in the roots. However, the mechanism of salt tolerance in *Cucumis melo* seems to be associated with a compartmentation process.

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