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Responses to NaCl stress of cultivated and wild tomato species and their hybrids in callus cultures

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Summary. If *in vitro* culture is to be used for evaluating the salt tolerance of tomato hybrids and segregant populations in a breeding programme, it is previously necessary to get quick and reliable traits. In this work, growth and physiological responses to salinity of two interspecific hybrids between the cultivated tomato (*Lycopersicon esculentum* Mill) and its wild salt-tolerant species *L. pennellii* are compared to those of their parents. The leaf callus of the first subculture was grown on media amended with 0, 35, 70, 105, 140, 175 and 210 mM NaCl for 40 days. Relative fresh weight growth of callus in response to increased salinity in the culture medium was much greater in *L. pennellii* than in the tomato cultivars, and greater in the hybrids than in the wild species. Moreover, the different salt tolerance degree of hybrids was related to that of female parents. At high salt levels, only Cl^- accumulation was higher in *L. pennellii* than in tomato cultivars, whereas in the hybrids both Cl^-, and Na^+ accumulation were higher than in their parents. Proline increased with salinity in the callus of all genotypes; these increases were much higher in the tomato cultivars than in *L. pennellii*, and the hybrids showed a similar response to that of the wild species. Salt-treated callus of the tomato cultivars showed significant increases in valine, isoleucine and leucine contents compared to control callus tissue. In contrast, these amino acids in callus tissues of the wild species and hybrids showed a tendency to decrease with increasing salinity.

Key words: Amino acids - *L. esculentum* - *L. pennellii* - NaCl tolerance - Saline ions - Tomato interspecific hybrids

Introduction

Salinity is a major factor limiting the crop productivity in the semi-arid areas of the world. In the last years, *in vitro* selection has seemed to be the methodological solution to this problem. However, the results have not been as satisfactory as expected, since it is very difficult to regenerate plants from cell culture, and when this is possible, usually the trait is not expressed in the plant (Tal 1993). In some cultivated species such as tomato, despite the existence tolerance sources from the wild species, practical results have not been obtained either by means of traditional breeding programmes or by protoplast fusion, which is in part due to the difficulty of screening thousands of plants without having available rapid and reliable traits. Although different growth and physiological characteristics have been selected at the whole plant level for evaluating the salt tolerance of the wild tomato species (Cruz *et al.* 1990), it should be very interesting to be able to evaluate the salt tolerance by *in vitro* culture, because of the relatively little space and the lower time required for the selection, as well as a controlled environment. However, it is previously necessary to obtain a clear relationship between *in vitro* and *in vivo* responses. We reported earlier that growth and physiological responses were similar at both organizational levels in a tomato cultivar and an accession of the wild species *L. pennellii*, whereas unclear results were obtained in the interspecific hybrid (Perez-Alfocea *et al.* 1994a).

At the cell level, the response can be affected by different factors, such as the culture medium and the explant source from which callus was derived (Garcia-Reina *et al.* 1988a). A factor which has not been taken in account is the large variation generated during culture within genotypes, known as somaclonal variation (Van den Bulk *et al.* 1990), which could contribute to a modification of the degree of salt tolerance. Moreover, different authors have shown that plant cells have a high adaptation capacity (Binzel *et al.* 1985; Sabbah *et al.* 1995), which will increase with the subculture number. Some authors have indicated that the identification of the the individual physiological processes that confer tolerance could be more useful to select salt tolerant plants. Thus, Dracup (1993) and Tal (1993) indicated that more understanding of the mechanisms of salt tolerance in cultured cells is necessary, as without knowledge of the limiting

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process at high NaCl it is difficult to know what is being selected through *in vitro* culture.

In this work, the salt tolerances of two tomato cultivars, one accession of the wild tomato species *L. pennellii*, and their interspecific hybrids are determined in the callus of the first subculture with the objective of trying to avoid as far as possible the variation generated by the *in vitro* culture. This paper also examines the effects of a range of NaCl concentrations (0-210 mM) on some previously selected physiological characteristics (Perez-Alfocea *et al.* 1994a,b), Na^+ and Cl^- accumulation and free amino acids, in the callus of different genotypes and hybrids.

Materials and methods.

Two tomato cultivars (*Lycopersicon esculentum* Mill), cv Pera (relatively salt-tolerant) and cv Muchamiel (relatively salt-sensitive), a salt-tolerant accession of the wild species *L. pennellii* (PE-47), and the two F_1 hybrids between *L. esculentum* and *L. pennellii* were used in this experiment. Callus was induced as previously described (Cano *et al.* 1990). The explant source used was leaf, as the most similar responses between callus and whole plants were found in callus from leaf (Perez-Alfocea *et al.* 1994a). Forty days after initiation, approximately 50 mg of callus tissue were transferred to fresh media amended with different NaCl levels (0, 35, 70, 105, 140, 175 and 210 mM). The callus was grown at 25°C, with a photoperiod of 16 h (76 µmol/m^2/s supplied by fluorescent lights) and a 8 h dark period. After 40 days of salt treatment, the cultures were harvested, weighed, and stored at -20°C for subsequent analyses of ions and free amino acids. The callus growth was measured as biomass in six replicates (six calluses per replicate) per treatment.

The Na^+ and Cl^- contents and the free amino acid analyses were determined according to Perez-Alfocea et al. (1994a,b). Briefly, Cl^- was determined by potentiometric titration with 0.01 N $AgNO_3$, and Na^+ by atomic absorption spectrophotometry. Amino acids were determined in a Rank Hilger Autoanalyzer using the ninhydrin post-column reaction and a pH gradient for ionic exchange separation of individual amino acids. Concentrations were calculated by the internal standard method (norleucine 0.1 mM). Four replicates (six calluses per replicate) were used for analysis.

All the data were subjected to a one way analysis of variance, and significance was determined at 95% confidence limits.

Results

Growth

The callus fresh weights of the two tomato cultivars were reduced by salinity (Fig. 1). The growth reduction was greater in cv Muchamiel than in cv Pera from 35 to 105 mM NaCl, whereas both cultivars showed similar growth reduction at high salt levels. Growth of *L. pennellii* callus increased slightly with salinity, except at 210 mM NaCl, where a 50% reduction was found in relation to the control. The callus growth of both hybrids increased at the different salt levels in relation to the controls, with these increases being higher in the hybrid between cv Pera and *L. pennellii*.

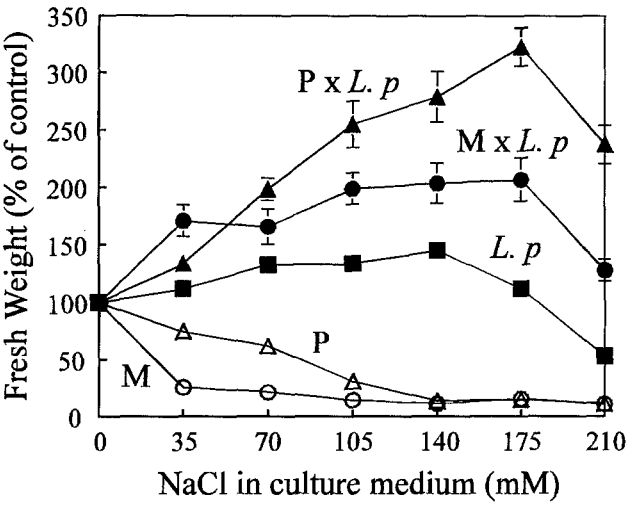

Fig.1. Callus relative growth of two tomato cultivars (Pera, P; Muchamiel, M), the wild species *L. pennellii* (*L. p*), and their interspecific hybrids as a function of external NaCl concentration. Vertical bars represent ± standard error.

Accumulation of Na^+ and Cl^-

The cv Pera accumulated more Na^+ and Cl^- than the cv Muchamiel at low and moderate salinities (35-105 mM) (Fig. 2).

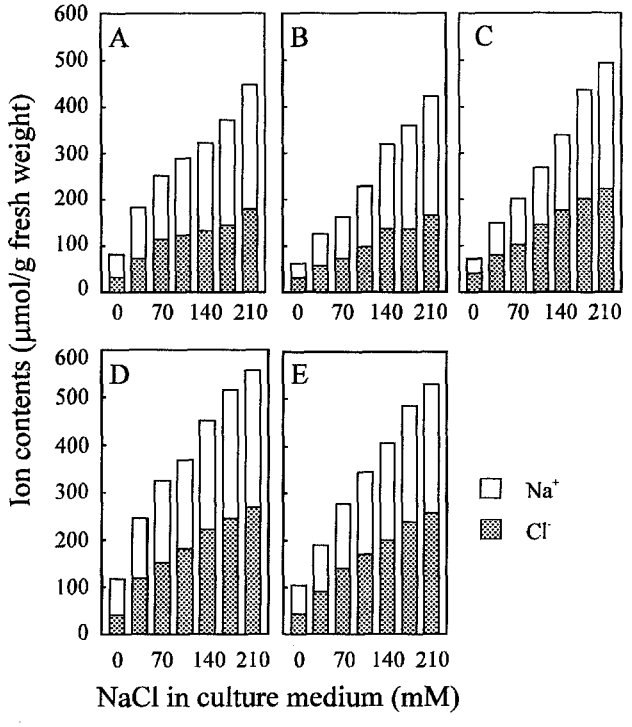

Fig.2. Sodium and Cl^- concentrations in callus of cvs Pera (A) and Muchamiel (B), the wild species *L. pennellii* (C), and their interspecific hybrids, cv Pera x *L. pennellii* (D) and cv Muchamiel x *L. pennellii*, as a function of external NaCl concentration. Within each genotype, Na^+ + Cl^- values for each salt level are significantly different at P = 0.05.

The accumulation of saline ions was greater in *L. pennellii* than in the tomato cultivars provided the salt levels were high, and this was due to Cl^- accumulation. Even at low and moderate salt levels, the Na^+ accumulation was lower in the wild species than in cv Pera. Na^+ and Cl^- accumulation in the hybrids was higher than in the parents. Moreover, the cv Pera x *L. pennellii* hybrid showed more Na^+ and Cl^- accumulation than the cv Muchamiel x *L. pennellii*, according to the tomato cultivar.

Amino acids

Proline was the amino acid that increased most with increasing salt levels in the callus of all genotypes (Fig. 3), but these increases were much higher in the most salt-sensitive calluses, from cvs Muchamiel and Pera, than in *L. pennellii* and the hybrids, especially at high salt levels. The two interspecific hybrids showed a similar response to that of the wild species. As in the case of proline, large differences between the tomato cultivars and the wild species and their hybrids were found in other amino acids, such as valine, isoleucine, and leucine (Fig. 3). Thus, the accumulation of these amino acids only occurred in callus of the tomato cultivars, while a tendency to decrease with increasing salt levels was found in callus of *L. pennellii* and hybrids.

Discussion

Based on the differences in callus growth reduction, the cv Pera was more salt-tolerant than cv Muchamiel at low and moderate salt levels, as was observed in whole plant studies on the basis of fruit yield (Caro *et al.* 1991), whereas the salt tolerances of both cultivars were similar at high salt levels. Thus, a wide range of salt levels must be used for evaluating the salt tolerance of tomato cultivars, otherwise inaccurate conclusions can be obtained. The wild species was much more salt tolerant than the tomato cultivars, as the callus growth was not reduced except for at 210 mM NaCl in *L. pennellii*, whereas in both tomato cultivars growth was reduced from the first salt level.

The callus of the two interspecific hybrids were the most salt tolerant. Both hybrids showed a tendency similar to the wild parent, in that their growth was significantly decreased between 175 and 210 mM NaCl. However, their salt tolerances were related to those of the tomato parents. These differences in callus tolerance support the hypothesis for a mechanism of salt tolerance operating at the cellular level (Smith and McComb 1981). In a previous work (Perez-Alfocea *et al.* 1994a), however, the salt tolerance of an interspecific hybrid (*L. esculentun* cv P-73 x *L. pennellii*) was lower than that of the wild species. This could be due to the fact that the culture medium used in that experiment did not allow for the optimum growth of the hybrid. In our experiment, different growth regulators have been used in the culture medium. The different growth responses could also be due to alterations within the genotypes (Blits *et al.* 1993), which might be more likely, since in the previous work callus from the fourth subculture were used.

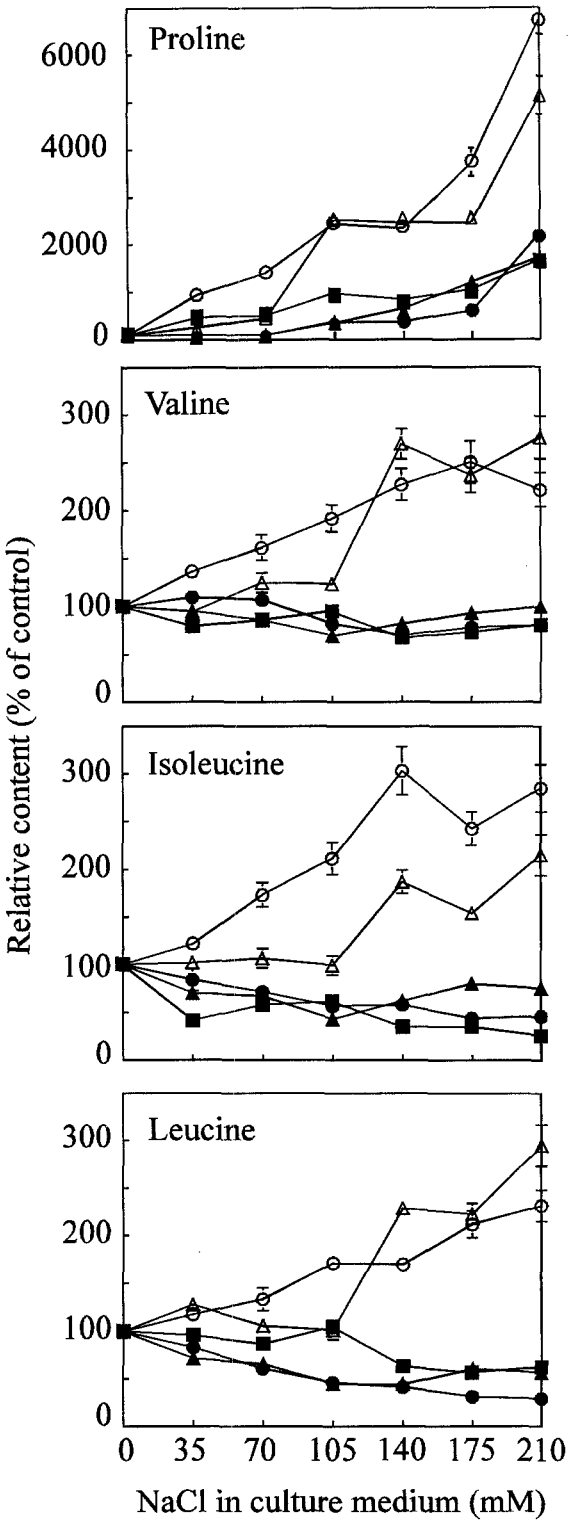

Fig.3. Proline, valine, isoleucine, and leucine concentrations in callus of two tomato cultivars, the wild species *L. pennellii*, and their interspecific hybrids as a function of external NaCl concentration. The symbols are as in Fig. 1.

The greater Na^+ and Cl^- accumulation found in callus of cv Pera than in callus of cv Muchamiel is in agreement with the results obtained in whole plants (Perez-Alfocea *et al.* 1993). However, the differences between the Na^+ concentrations in callus of both cultivars are lower than those found in leaves of whole plants, which may be due to the fact that the ion exclusion operative in whole plants is not shown in cell culture, as was reported by Garcia-Reina *et al.* (1988b) and Yang *et al.* (1990). Thus, we previously found (Perez-Alfocea *et al.* 1994a) that leaf callus tissues accumulated more Na^+ than leaves only in the tomato cultivar (where the exclusion mechanism is predominant), whereas in the wild species (where the inclusion mechanism is predominant) no differences were found.

The role of proline appears to differ from species to species and its increase has been explained in different ways (Joice *et al.* 1992). In callus of tomato cultivars, proline accumulation seems to be related to inhibition of callus growth, such as was previously found (Perez-Alfocea *et al.* 1994b), and consequently it can be used as a salt-sensitive trait. According to the results obtained in this work, the valine, isoleucine and leucine contents could be also used as salt-sensitive traits. In conclusion, the high salt tolerances shown by the hybrids may result from either a hybrid vigour character, which has not been indicated previously in callus culture, or from physiological traits of the wild species (Tal and Shannon 1983). According to the similar physiological responses obtained between the wild species and their hybrids, it seems to be suitable to use physiological traits to evaluate the salt tolerance of tomato interspecific hybrids.

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