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Solute contents in roots and root calli of NaCl-tolerant and NaCl-sensitive tissues of *Lycopersicon*

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Abstract

Biomass, relative growth rate (RGR), organic and inorganic solute contents in control and NaCl (50 - 100 mM) affected roots or calli of the wild tomato genotype *Lycopersicon pennellii* and the *Lycopersicon esculentum* wilty mutant *flacca* were compared. Under NaCl-stress, the RGR of calli from *L. pennellii* was higher than that of the mutant *flacca*, while the root biomass of the former was lower than that of the latter. Constant water contents were found in calli and roots, irrespective of the genotypes and NaCl concentrations. Taking into account the solute contents of the apoplasm, Na^+ accumulation was similar in the sensitive tissues (calli from *L. flacca*, roots of *L. pennellii*) and the tolerant ones (calli from *L. pennellii*, *flacca* roots). Decreased K^+ and Mg^{2+} and increased proline contents were found in both sensitive tissues. In comparison with sensitive *L. pennellii* roots, salt sensitive *flacca* calli showed increased total organic acid and amino acid contents.

Introduction

Comparing physiological responses between excised organs cultured *in vitro* and organs *in vivo*, Von Hedenstrom and Breckle (1978) and Tal *et al.* (1978) clearly demonstrated that salt tolerance may reside at the cellular level. However, in some species as in tomato cv. St. Pierre, the growth responses of calli and whole plants are not correlated (Bourgeais *et al.* 1987, Bourgeais-Chaillou and Guerrier 1992): thus, the root calli of this cultivar were more NaCl-tolerant than the roots, calli from stems and leaves being more NaCl-sensitive than the corresponding whole plant organs.

Thus, two main questions arise: (*1*) does the high NaCl-tolerance of root calli also appear in two tomato populations characterized by two different NaCl-response degrees, *e.g.* the wild tolerant species (*Lycopersicon pennellii*) and the sensitive wilty

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flacca mutant of *L. esculentum*; (*2*) if so, what are the differences between the relative growth rates of calli and plants of the both genotypes and the physiological references differentiating calli and plantlets *in vitro*.

The study of the reaction of callus to salt may explain the cellular difference in salt tolerance and the influence of the organizational level through which the control of salt tolerance operates. Therefore, we report in this paper the nutritional disturbances and proline accumulations, markers of NaCl-response in the *Lycopersicon* genus (Tal *et al.* 1978, Bourgeais-Chaillou and Guerrier 1992) in order to provide some answers as regards the mechanisms of tolerance operating at both cell and whole plant levels.

Materials and methods

Plant production: Seeds of *Lycopersicon pennellii* (Correll), D'Arcy, accession PE-47, and wilty mutant *flacca* of *L. esculentum* (L.) Mill. were surface sterilized for 10 min in a 8 % calcium hypochlorite solution, then rinsed three times for 5 min. with sterilized water. Lots of 12 - 15 seeds were transferred into glass jars (9 cm diameter, 10 cm high) containing mineral nutrients (germination medium - GM) according to Bourgeais *et al.* (1987).

After 12 d of germination under ambient conditions (16 h light at 20 °C and a 60 W m^{-2} irradiance, 8 h dark at 20 °C), explants of 1 cm length (*i.e.* the terminal part of the stem including the terminal bud and the last internode) were transferred aseptically into glass tubes containing multiplication medium (MM) corresponding to GM supplemented with saccharose, inositol, thiamin, nicotinic acid, pyridoxine and indoleacetic acid (Bourgeais-Chaillou and Guerrier 1992). The 28-d-old plants were routinely propagated (48 explants per treatments), then transferred on MM supplied with NaCl (from 0 up to 100 mM). After 28 d of culture the roots were excised and then analysed.

Callus initiation: Calli were initiated from 5 mm segments of tomato roots growing on control medium (without NaCl). Then, 15 - 20 segments were transferred per glass jar containing initiation medium (IM) including Murashige and Skoog (1962) mineral nutrients and Bourgeais-Chaillou and Guerrier (1992) plant growth regulators. Routinely, 40 - 50 calli per treatments were subcultured each 4 weeks (after 4 subcultures in order to stabilize calli) on GM supplied with NaCl (from 0 up to 100 mM) and saccharose, inositol, thiamin, nicotinic acid, pyridoxine, indole-acetic acid, 2,4-dichlorophenoxyacetic, kinetin and glycine (Bourgeais-Chaillou and Guerrier 1992).

Relative growth rate (RGR) of callus was expressed as the [(mf-mi)/mi] ratio where mi is the fresh mass of calli at the time of the transfer and mf the fresh mass after 28 d of culture.

Mineral analysis: After mineralisation of dry matter with HNO_3, K^+, Ca^{2+}, Na^+ and Mg^{2+} were determined by atomic absorption spectrophotometry (*Unicam Sp9, Pye Unicam,* Cambridge, UK) Cl^- was determined by colorimetry after extraction of fresh

matter with boiling water under agitation for 1 h (Bourgeais *et al.* 1987). Three replicates of 10 roots or 10 calli were performed.

Organic solute analysis: Soluble sugars, free amino acids and organic acids were extracted from fresh matter with boiling water: total soluble sugars were measured by colorimetry using glucose as standard (Morris 1948). Free amino acids were quantified at 554 nm (*Rank-Hilger Chromaspek Autoanalyser, Rank Hilger Co.*, Westwood-Margatg, UK) using a pH gradient on an ionic exchange column with a ninhydrin post-column reaction and norleucine as internal standard; free proline was determined colorimetrically (Bates *et al.* 1973). Analysis of organic acids was carried out on *Rezex* column (*Phenonenex*, Rancho Palos Verdes, USA) and isocratic *Kontron HPLC 420* pump (*Kontron AG*, Zürich, Switzerland), according to Bourgeais-Chaillou and Guerrier (1992).

Measurement of solute contents in callus free space: Solutes from apoplasm (A) of calli were released during 3 min in HCl 0.01 M or H_2O; remaining solutes from protoplasm (B) were measured after mineralisation with HNO_3 (K^+, Ca^{2+}, Mg^{2+}, Na^+) or extraction with boiling water (Cl^-, sugar, amino acids). Proportions of solutes in apoplasm were defined by the ratio A/(A+B) (Bourgeais-Chaillou and Guerrier 1992).

Results and discussion

Relative growth rate of calli and production of root biomass: The relative growth rates of calli were related to the natural habitat of the populations (Tal *et al.* 1978): RGR of calli from the wilty mutant and the wild species decreased by 95 % and 25 % respectively, on a 100 mM NaCl medium (Fig. 1). However, no relation was found between biomasses of roots and callus growth rates, the roots being more NaCl-sensitive than their corresponding calli: thus, surprisingly, *L. pennellii* was the most sensitive species at whole plant level.

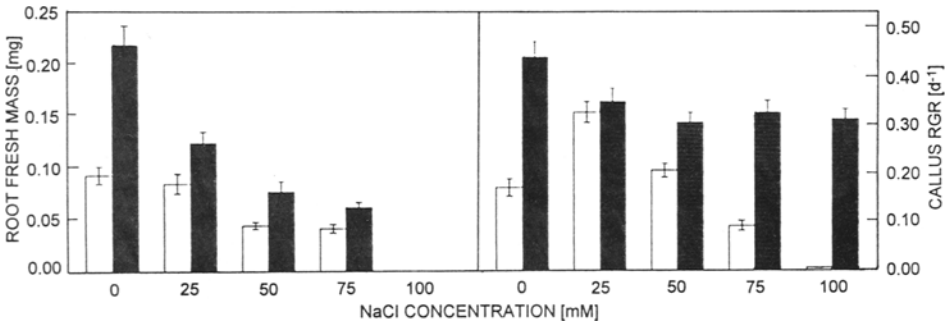

Fig. 1. Changes in root biomass and in relative growth rate of calli from roots of *L. esculentum flacca* (*open columns*) and *L. pennellii* (*closed columns*) as a function of NaCl concentrations (from 0 up to 100 mM). Vertical bars represent ± SD (n = 10).

Water content (93 % irrespective of calli and organs) did not change significantly with NaCl treatments. So, the NaCl responses of calli and plants were not linked to their unability to adjust their osmotic potential.

Solute contents of apoplasm: Comparisons between cell solute contents may take into account the bulk of apoplasm which was higher in calli than in meristematic cells of the roots (Gibbs *et al.* 1989); however, as the roots were thin and crisp, only the measurement of apoplasm content was carried out on calli (Table 1).

Table 1. Influence of NaCl concentrations on the inorganic solutes in the apoplasm [in % of total solutes, see Materials and methods] of calli of *Lycopersicon esculentum flacca* and *L. pennellii*.

NaCl [mM]	species	Ca^{2+}	Na^{+}	Mg^{2+}	K^{+}	Cl^{-}
0	both	67	67	48	47	45
50	*L.e. flacca*	63	64	41	43	47
100	*L.e. flacca*	56	54	43	44	40
50	*L. pennellii*	57	60	44	40	35
100	*L. pennellii*	49	48	40	42	33

Under control conditions, the proportions of apoplasmic inorganic solutes did not differ in the calli of the sensitive and the tolerant populations: high proportions of solutes (mainly Ca^{2+} and Na^{+}) were located within the cell walls. According to Gibbs *et al.* (1989), the apoplasmic contents decreased under saline conditions; however, these decreases (mainly in Na^{+}, Ca^{2+}, Cl^{-}) were higher in the tolerant tissues of the wild species, as it was previously found in tolerant tissues of *L. esculentum* cv. St. Pierre (Bourgeais-Chaillou and Guerrier 1992), than in those of the sensitive tomato. Thus, the quantity of protoplasmic solutes increased in salt-treated calli of the wild species.

Similar total sugar proportions (25 % of total soluble sugar contents) were found within the cell walls in both control calli; the apoplasmic soluble sugar proportions fell down to 15 - 20 % in 50 mM NaCl-treated calli and to 10-15 % in 100 mM NaCl-treated calli. On the other hand, amino acid proportions in cell walls were constant (7 - 12 % of total amino acid contents) in control and salt-treated calli of both species.

Biomass production and inorganic solute contents: Taking into account the water contents and the apoplasmic solute contents, the NaCl sensitivity of *flacca* calli, as well as that of *L. pennellii* plants, did not appear to result from an increase in Na^{+} and Cl^{-} levels (Fig. 2). So, the salt-tolerance cell mechanisms did not involve either Na^{+} or Cl^{-} exclusion capacities, as in *Sapindus* embryos (Unnikrishnan *et al.* 1991), or Na^{+} or Cl^{-} excess as in *Cicer* and *Brassica* calli (Pandey and Ganapathy 1984, Paek *et al.* 1988).

According to Tal *et al.* (1978), Sabbah and Tal (1990) and Bourgeais-Chaillou and Guerrier (1992), the deficiencies in mineral nutrient could be involved in the NaCl-sensitivities of tissues: indeed, K^{+}, Ca^{2+} and Mg^{2+} contents decreased in salinized

L. pennellii roots and in 100 mM NaCl-treated *flacca* calli (Fig. 3); conversely, K^+ contents remained constant in *flacca* roots, while Ca^{2+} and Mg^{2+} increased. Moreover, high $K^+/(K^+ + Na^+)$ ratios, which are linked to K^+ selectivity and NaCl-adaptation (Guerrier 1984), were found in *L. pennellii* calli and *flacca* roots.

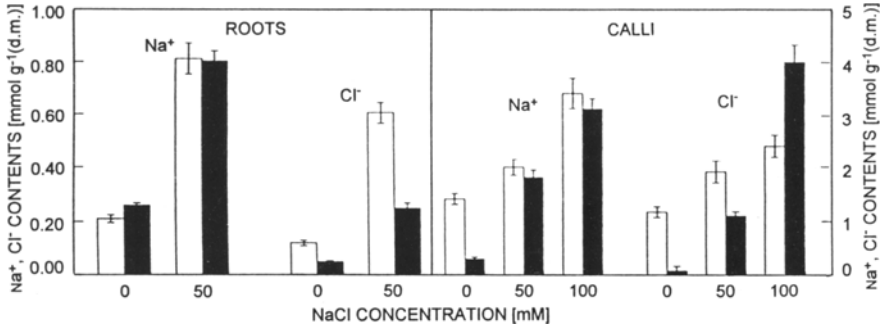

Fig. 2. Changes in Na^+ and Cl^- contents in roots and calli from roots of *L. esculentum flacca* (*open columns*) and *L. pennellii* (*closed columns*) as a function of NaCl concentrations (0, 50, 100 mM). Vertical bars represent ± SD (n = 10).

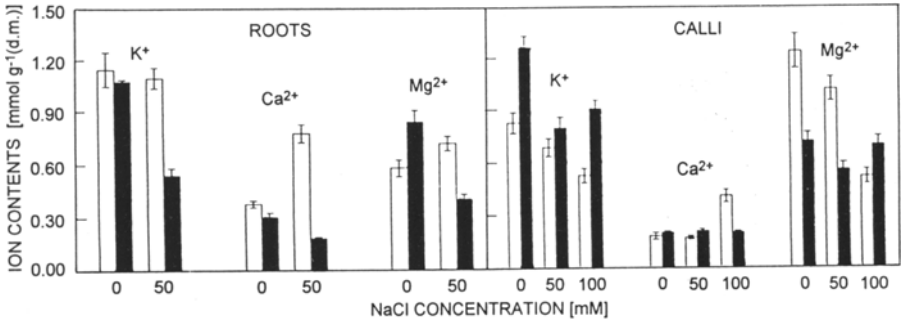

Fig. 3. Changes in K^+, Ca^{2+}, Mg^{2+} contents in roots and calli from roots of *L. esculentum flacca* (*open columns*) and *L. pennellii* (*closed columns*) as a function of NaCl concentrations (0, 50, 100 mM). Vertical bars represent ± SD (n = 10).

The halophytic character of *L. pennellii* (Tal *et al.* 1978) appeared only at the cell level: the 100 mM-treated calli of the wild species accumulated as many inorganic ions as and less soluble sugar than those of *flacca* (Figs. 2, 3; Table 2). This was in accordance with the capacity of halophytic species to osmotic adjustment by inorganic solute accumulation (Cram 1980). On the contrary, the loss of the inorganic solute accumulation capacity could be linked to the sensitivity of *L. pennellii* plants: thus, the roots of the wild species accumulated and translocated towards shoots fewer inorganic ions (*e.g.* 70 % of total Na^+ and Cl^-) than *flacca* (85 % of total Na^+ and Cl^-). Whether the abundances of sugars 6- to 10-fold higher

in the heterophically grown plant roots than in autotrophic plants (Rush and Epstein 1976) and nutrients in the culture media, contrary to the natural habitat, account for this peculiar character of *L. pennellii* is unclear.

Organic solute contents: The levels in organic acids linked to the oxidative respiration decreased in the salinized-roots of the both species (Table 2). On the other hand, organic acid levels remained practically constant in their calli. Therefore, the NaCl-sensitivities of the plants, compared with those of the calli, could result from a low energy supply while the energetic costs of NaCl-adaptation increased: K^+-retention in the cytoplasm, biosynthesis of organic solutes, Na^+ and Cl^- vacuolar compartmentations, specific Na^+ extrusion from cytoplasm against a concentration gradient, nutrient translocations.

Table 2. Total soluble sugar (TS, [mmol glucose g^{-1}(d.m.)]), total organic acid (TOA [mg g^{-1}(d.m.)]), total amino acids and proline (TAA, PRO [µmol g^{-1}(d.m.)]) contents in roots and calli from roots of *L. esculentum* cv. flaca and *L. pennellii* as a function of NaCl concentrations.

	L. esculentum wilty mutant *flacca*					*L. pennellii*				
	roots		calli			roots		calli		
NaCl [mM]	0	50	0	50	100	0	50	0	50	100
TS [mmol glucose g^{-1}(d.m.)]	1.20	2.95	1.01	1.19	2.80	2.01	1.46	1.35	2.08	1.33
TOA [mg g^{-1}(d.m.)]	50.4	0	101	124	130	50.5	18.1	45.3	32.2	86.3
TAA [µmol g^{-1}(d.m.)]	183	798	140	268	539	606	270	70.2	62.2	86.3
PRO [µmol g^{-1}(d.m.)]	3.09	1.16	8.27	40.1	174	5.73	35.4	2.88	3.84	9.5

Increasing free amino acid contents were found in salinized *flacca* roots/calli, while amino acid contents decreased in salinized *L. pennellii* roots or remained constant in their calli. Proline accumulation was independent from a photosynthetic component (in calli) and from the presence of ABA: high proline levels were found in calli of the ABA-deficient wilty mutant *flacca* (Stewart and Voetberg 1987), as in *L. pennellii* roots, both sensitive tissues (Stewart and Hanson 1980, Bourgeais-Chaillou and Guerrier 1992). In comparison with sugar and inorganic solute contents, the small pool sizes of proline did not play a key role in the osmotic adjustment of tomato (Tal *et al.* 1979, Perez-Alfocea *et al.* 1993). Proline contents could be involved in the H^+ removal ability and the cell pH neutralization (Bellinger and Larher 1987, Venekamp 1989): indeed, high proline contents were linked in the sensitive tissues to excess of cationic charge [1.60 mmol g^{-1}(d.m.) in 50 mM NaCl-treated *flacca* calli and 2.10 mmol in 100 mM NaCl-treated ones], while a low proline content in 100 mM-treated calli of *L. pennellii* accounted for a relative balance between anionic and cationic charges [0.10 mmol g^{-1} (d.m.) of cationic excess].

The comparisons between the tolerance mechanisms operating in calli and whole plants of wild and sensitive tomato populations were possible, since the experiments

were carried out (*1*) with the same nutritive medium for all the calli, thus avoiding interactions between Na^+-accumulation and hormone or solute concentrations, and (*2*) on 50 clones of calli and heterotrophically grown plants, to minimize the somaclonal variation due to *in vitro* culture (Bourgeais *et al.* 1987).

The cellular mechanisms (nutrient deficiencies) did not appear directly responsible for NaCl-stress, since the NaCl-tolerance of calli from roots of the both populations (*e.g.* the halophytic character of *L. pennellii*) may be functionaly limited to the expression at the cell level. The highest NaCl-sensitivity of plants would result from of nutrient deficiencies and biochemical processes: sugar utilization, paucity in organic acids linked to oxidative respiration (while increased the energetic costs), amino acid biosynthesis (*e.g.* salinized roots accumulated 2- to 10-fold more free amino acids than their calli). The mechanisms for salt-tolerance in *L. pennellii* cells/whole plants (K^+ selectivity, requirement of Ca^{2+} for the functioning of biological membranes, involvement of proline in the H^+ removal) are currently studied.

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