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BRIEF COMMUNICATION

Proline accumulation in leaves of NaCl-sensitive and NaCl-tolerant tomatoes

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Abstract

Proline (Pro) accumulation in leaf discs of the NaCl-treated salt sensitive *Lycopersicon esculentum* was higher than in the salt tolerant *L. pennellii.* The magnitude of Pro accumulation differed when leaf discs of both species were floated in the light or under darkness, and in various incubation media: buffer solutions (pH from 3.9 to 7.8), abscisic acid, isobutyric acid, NH_4Cl, malate, citrate, and mixtures of NaCl and KCl, NaCl and $CaCl_2$, and NaCl and mannitol. Under darkness, Pro accumulation in *L. esculentum* was not regulated by salts. Conversely, the light-independent NaCl-induced Pro accumulation observed in *L. pennellii* became light-dependent when $CaCl_2$ was added. The different expression patterns of Pro accumulation reinforce the proposal of two Pro metabolic pathways in the domestic and the wild tomatoes.

Additional key words: excised tissues, *Lycopersicon esculentum, L. pennellii,* proline, salt stress.

The physiological significance and the mechanisms leading to proline (Pro) accumulation in the *Lycopersicon* genus are poorly understood. The NaCl-tolerant wild tomato species (*Lycopersicon pennellii, L. pimpinellifolium*) exhibit stress-induced Pro accumulation, while in the NaCl-sensitive domestic one (*L. esculentum*) Pro content does not exert any osmotic or protective functions (Perez-Alfocea *et al.* 1993, Guerrier 1996, Renard and Guerrier 1997). Considering that, wild plants are adapted to stresses (Bohnert *et al.* 1995), the causes of decreasing stress tolerance

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Abbreviations: ABA - abscisic acid; BAP - benzylaminopurine; IAA - indole-3-acetic acid; IBA - isobutyric acid; Kin - kinetin.

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along the evolution from the wild relatives of tomato to the domestic species need to be studied.

One approach to elucidate the mechanism of adaptation to stress was to establish the differences in Pro metabolic pathways in both plant species. Studies on calli and excised organs (Guerrier 1995, unpublished data) have allowed to characterize in the salt-treated sensitive species a restriction of Pro catabolism and the use of specific Pro precursors (arginine, glutamate and ornithine *vs.* arginine and glutamine) in the wild tolerant species. Another approach was to consider the involvement of the intracellular cascade of events (regulation of specific genes, modification of cellular activity) induced by NaCl-stress (Skriver and Mundy 1990, Bostock and Quatrano 1992). The possible link between Pro accumulation and cell pH was already described in various plants incubated for short-term in abscisic acid (ABA), isobutyric acid (IBA), vanadate and NH_4Cl (all treatments resulting in decreased cell sap pH) (Pesci and Beffagna 1984, Göring and Flescher 1986, Chou *et al.* 1990). In salt-treated *L. esculentum* (Bourgeais-Chaillou and Guerrier 1994), the decrease in phosphoenolpyruvate carboxylase activity, a sensor of cell pH (Kurkdjian and Guern 1989) suggested a similar decrease of the cell pH. Here, we present Pro accumulation in leaf discs of the NaCl-sensitive *L. esculentum* and the NaCl-tolerant *L. pennellii* after short-term incubation in various buffers and acidifying solutions, and exposed either to darkness or to light. Light is known to affect the distribution of ABA inside the cell, to activate the electrogenic H^+ extrusion and to enhance the stromal pH (Kaiser *et al.* 1985, Marre *et al.* 1989).

Seeds of the domestic NaCl-sensitive tomato (*Lycopersicon esculentum* Mill. cv. P 73) and its wild NaCl-tolerant relative [*L. pennellii* Correll (D'Arcy) accession PE 47] were germinated and then grown in the same 30 dm^3- trays (60 seeds per tray) filled with washed silica sand. After germination in the dark at 28°C and 90 % relative humidity, growth took place under day/night temperatures 30/17 °C, 16-h photoperiod (*Gro-lux* and *Lux-line Sylvania* lamp); irradiance of 245/81 $\mu mol\ m^{-2}\ s^{-1}$, and a relative humidity of 60 - 70 %. A full strength Hoagland's solution was daily applied after emergence of the first leaf, *i.e.* 7 d after soaking. When plants were 45 d-old, the mature leaves were used for the experiments.

Groups of 30 - 40 leaf-discs (diameter 1.2 cm, 0.5 g of fresh matter) were excised at the end of the dark period and then floated for 8 h in a Petri dish containing 20 cm^3 of one of the following incubation solution: distilled water, pH 5.7 (control), barbital/citrate/phosphate buffers allowing a range of pH from 3.9 to 7.8, 0.1 mM ABA, 10 mM IBA, 5 mM malate, 5 mM citrate, 5 mM NH_4Cl; and mixtures of 100 mM NaCl with 15 mM KCl, 15 mM $CaCl_2$ or 15 mM mannitol.

After incubation carried out at 20 °C under dark or under light (irradiance of 150 $\mu mol\ m^{-2}\ s^{-1}$ at the disc level), a sample of leaf dics was rinsed, homogenized with 5 cm^3 boiling distilled water, and then centrifuged (10 000 *g* for 10 min). Free Pro was determined in the supernatant with a *UVIKON 810* spectrophotometer (*Kontron*, Zurich, Switzerland) at a wavelength of 520 nm, according to Bates *et al.* (1973). Six replicates were performed per treatment; means were statistically compared at $P = 0.05$ using either confidence intervals or the Mann-Whitney test.

Both species studied exhibited three major differences in Pro accumulation pathways. Firstly, different magnitudes of Pro accumulation were induced by the individual treatments (Table 1, Figs. 1,2): *e.g.* in *L. esculentum*, the magnitude of Pro accumulation induced by ABA was twice higher than that induced by IBA, while the reverse was observed in *L. pennellii*. These differences suggested that different mechanisms may result in ABA-, IBA- and salt-induced Pro accumulation (Pesci and Beffagna 1984, Stewart and Voetberg 1985). Comparing the effects of ABA and IBA on Pro levels of barley, Pesci (1987, 1992) had already observed that only the ABA-induced Pro accumulation involved a protein which was activated by K^+ and Na^+ and inhibited by NH_4^+.

Table 1. Proline content [μmol g^{-1}(d.m.)] in leaf discs of *Lycopersicon esculentum* and *L. pennellii* when incubated for 8 h in the light and under darkness in distilled water and in various acidifying solutions: 10 mM isobutyric acid (IBA), 0.1 mM abscisic acid (ABA), 5 mM malate, 5 mM citrate and 5 mM NH_4Cl. Means of 6 replicates ± confidence intervals at $P = 0.05$.

	L. esculentum		*L. pennellii*	
	light	dark	light	dark
H_2O	0.65 ± 0.05	0.65 ± 0.05	0.78 ± 0.05	0.78 ± 0.05
ABA	1.14 ± 0.13	1.01 ± 0.11	0.95 ± 0.08	0.92 ± 0.08
IBA	0.88 ± 0.07	0.55 ± 0.04	1.48 ± 0.12	0.68 ± 0.07
Citrate	0.73 ± 0.08	0.55 ± 0.06	0.66 ± 0.05	0.67 ± 0.06
Malate	0.98 ± 0.11	0.55 ± 0.05	0.69 ± 0.07	0.65 ± 0.06
NH_4Cl	0.72 ± 0.07	0.51 ± 0.04	0.30 ± 0.04	0.33 ± 0.04

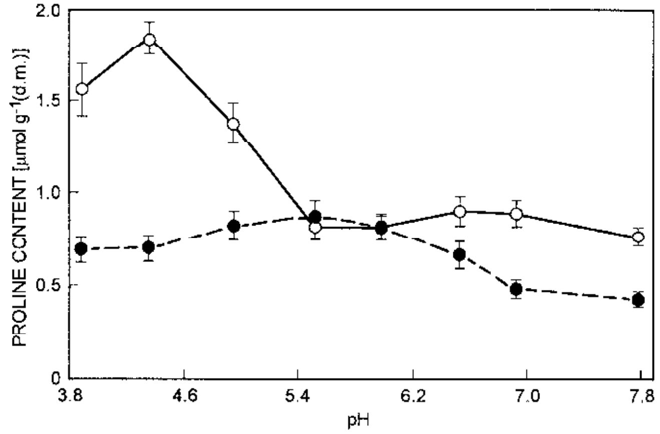

Fig. 1. Proline content in leaf discs of *L. esculentum* (*open circles*) and *L. pennellii* (*closed circles*) when incubated for 8 h in the light or under darkness in barbital/citrate/phosphate buffers (range of pH from 3.9 to 7.8). Means of 6 replicates ± confidence intervals at $P = 0.05$ level.

Secondly, under light, ABA, IBA, malate and citrate dramatically increased Pro content in *L. esculentum*, while only IBA and ABA enhanced Pro content in

L. pennellii (Fig. 1, Table 1). This suggests the possible involvement of cell acidification as a part of a signal transduction chain initiating a cascade of stress-induced responses that could resulted in injury damage in *L. esculentum*. Therefore, this link between Pro accumulation and cell acidity should not be consistent with the previously observed protective effect of Pro on peroxidases at alkaline pH (Renard and Guerrier 1997). It remains then to elucidate why Pro level was strongly enhanced in *L. esculentum* incubated in ABA, 0.1 mM IAA, BAP and Kin, all protecting tomato against water deficit and deleterious effect of NaCl (Tal and Imber 1971, Bourgeais *et al.* 1990).

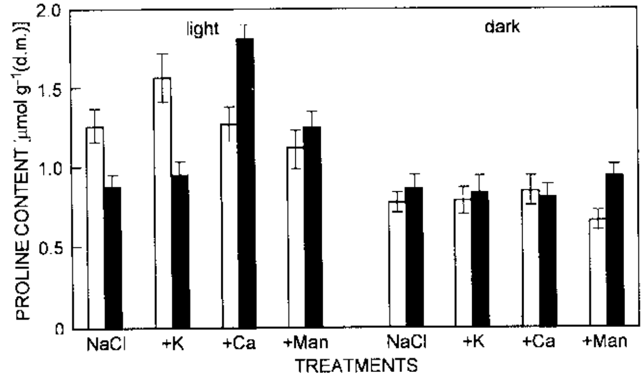

Fig. 2. Proline content in leaf discs of *L. esculentum* (*empty columns*) and *L. pennellii* (*full columns*) when incubated for 8 h in the light and under darkness in 100 mM NaCl (NaCl), or in 100 mM NaCl + 15 mM KCl (+K), in 100 mM NaCl + 15 mM $CaCl_2$ (+Ca) or in 100 mM NaCl + 15 mM mannitol (+Man). Means of 6 replicates ± confidence intervals at $P = 0.05$ level.

Thirdly, when *L. esculentum* was incubated in IBA, citrate, malate, NH_4Cl and NaCl, Pro accumulation differed according to whether leaves were exposed to light or darkness (Table 1, Fig. 2); under darkness, the NaCl-induced Pro response was not regulated by salts (Fig. 2). It was also worthnoting that the light-independent NaCl-induced Pro accumulation observed in *L. pennellii* became light-dependent when $CaCl_2$ and mannitol were added to the NaCl medium (Table 1, Fig. 2). These high Pro levels are not directly related to membrane permeability, taking into account the opposite effects of mannitol and $CaCl_2$. They could involve Ca^{2+}-dependent transduction pathways (Ward *et al.* 1995) and some regulatory aspects of light through , *e.g.*, the enhancement of the stromal pH from pH 7 in the dark to pH 8 in the light (Delauney and Verma 1993), modification of ABA distribution inside the cell (Kaiser *et al.* 1985), and activation of the electrogenic H^+ extrusion (Marre *et al.* 1989). This range of pH precisely corresponds to the activation of *1*) Pro biosynthesis enzyme NAD(P)H-pyrroline-5-carboxylate reductase (Rayapati *et al.* 1989) and of *2*) Pro catabolism enzymes proline dehydrogenase, proline oxidase (Huang and Cavalieri 1979).

Studies on the enzyme activity of Pro metabolic pathway (Guerrier 1995) and on the here reported regulatory aspects of Pro accumulation reinforce the proposal of two different Pro metabolic pathways in the domestic tomato and its wild relative. Studies on the simplified model of excised organs did not allow to observe the magnitude of Pro accumulation occurring in organs of intact plants (Perez-Alfocea *et al.* 1993, Guerrier 1996); in particular Pro accumulation was not found in excised root fragments and was not modulated by the here tested solutions (data not shown). Additional experiments are then required to elucidate the events leading to the lack of the adaptive Pro accumulation along the evolution in the *Lycopersicon* genus.

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