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Increasing plant vigour and tomato fruit yield under salinity by inducing plant adaptation at the earliest seedling stage

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Abstract

In order to reduce the negative effect of salinity on fruit yield, 5-day-old tomato seedlings (*Lycopersicon esculentum*) were haloconditioned by complete immersion in osmotic/saline solutions composed of PEG (−0.5, −0.75, −1 MPa), with or without 10 mM NaCl, for 1, 3, 5 and 8 days. Under moderate salinity (7.5 dS m^{-1}), the pre-adapted plants produced 23% more shoot biomass and fruit yield than the non-adapted plants. In addition to the induced vigour, the improved tolerance in most pre-treatments was related to lower Na^+ and Cl^- concentrations in the leaves and increases in leaf K^+ contents and K^+/Na^+ ratio, but the contrary was also observed. Overall, the most effective haloconditioning treatment seems to be the application of −0.75 MPa for 3 days. During the experiment in greenhouse, some vigorous haloconditioned plants were propagated through adventitious apex culture and evaluated under salinity in a short-term experiment. The results suggested that the induced salt tolerance was not horizontally transmitted, indicating that (i) the individuals chosen were not genetically more vigorous, but (ii) it is likely that they responded better to the induced adaptation, and (iii) this adaptation is probably mediated by epigenetic changes taking place in the roots.

Keywords: Adaptation to salinity; Haloconditioning; Horizontal transmission; Ionic regulation; *Lycopersicon esculentum*; NaCl; Plant vigour; Polyethylene glycol; Potassium selectivity

1. Introduction

Phenotypic plasticity in plants is a phenomenon that involves adaptive developmental and physiological changes in response to internal and external environments in order to enhance the success in suboptimal conditions (Sultan, 2000). While evolution has developed a wide range of plant adaptations to different environmental conditions, in general, crop plants have evolved towards a high productivity in detriment to a low capacity to adapt to changes in their growth conditions. Shannon (1997) reported that the highly productive genotypes canalise all the energy in benefit of yield but in detriment to agronomical stability under variable conditions. The genotypes with low productive capacity, however, adapt better to the changes in the environment presumably because they retain some energy for stress adaptation. This situation is well described in the case of the salt tolerance of different tomato species and cultivars: according to the yield–salinity response curves, the most productive genotypes under non-saline conditions are also the most affected by increases in the salinity level (Caro et al., 1991). However, the well-adapted local ecotypes and the wild-relative salt-tolerant species are, in general, low-yielding plants. This phenomenon, together with the physiological complexity of salt tolerance, is one of the causes of the difficulty in improving simultaneously for salt tolerance and productivity by using conventional breeding programs (Flowers, 2004). From a physiological point of view, and considering an agronomical range of salinity (low to moderate salinity), the mechanisms involved in the defence against the stress are basically the same: regulation of the toxic ions in the leaves (exclusion or inclusion in vacuoles), nutrient selectivity, osmotic adjustment, synthesis of solutes such as proline or *myo*-inositol and antioxidative systems. However, despite promising reports of salt-tolerant transgenic crops by using genes involved in these mechanisms, none of these have

Abbreviations: DST, days of salt treatment; H_0-plants, haloconditioned plants selected for vegetative multiplication; H_1-plants, clones obtained from H_0-plants; NSC, non-saline control, non-adapted plants cultivated without salt; PEG-6000, polyethylene glycol M_W 6000; SC, saline control, non-adapted plants cultivated under salinity

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seen commercial use so far (Flowers, 2004; Munns, 2005). The differences between salt-tolerant and salt sensitive genotypes are more quantitative than qualitative, and it seems to be the ability to regulate these processes, which leads to a higher physiological stability under salinity. It has been hypothesised that slight differences in the regulation of similar salt-tolerance effectors and regulatory pathways account for large variations in tolerance between halophytes and glycophytes (Zhu, 2001; Munns, 2005). For example, the higher tolerance to the salt-dependent oxidative stress of the chloroplasts from *Lycopersicon pennellii* as compared to those from the cultivated *Lycopersicon esculentum* has been related to the ability to up-regulate a common set of antioxidative enzymes (Mittova et al., 2002). Moreover, both species have shown the ability to adapt to high salinity at the cell level (Rus et al., 2000). Although the salt tolerance of tomato has been linked mainly to the capacity to exclude the toxic ions from the shoot (Sacher et al., 1982, Pérez-Alfocea et al., 1993a, 1996; Estañ et al., 2005; Juan et al., 2005), some well-adapted ecotypes and wild species are able to include greater amounts of these ions while taking longer to reach the toxic effect than the more salt-sensitive genotypes (Pérez-Alfocea et al., 1993a,b; Bolarín et al., 1995). These species or ecotypes seem to have a more efficient system to retranslocate the toxic ions through the phloem and to increase the root/shoot ratio (Balibrea et al., 2000; Pérez-Alfocea et al., 2000). If a more efficient regulation of these pre-existing mechanisms could be induced, crop salt tolerance could be increased to a reasonable extent by inducing individual plant adaptation (Amzallag, 1999, 2002). The method of haloconditioning consisted of inducing adaptation mechanisms of salt tolerance by the immersion of whole seedlings in stressful solutions containing PEG, NaCl or a mixture of both, in a similar way to that of the highly successful glycophytic cell cultures reported during the eighties. This method has been proven to be efficient for increasing salt tolerance in short-term experiments carried out with tomato and lettuce through quantitative induction of pre-existing adaptive responses like root/shoot ratio and ionic homeostasis (Balibrea et al., 1999; Pérez-Alfocea et al., 2002). The objectives of this long-term experiment were to study (i) the effect of different parameters of the method (osmotic potential, ionic component and incubation time) on tomato fruit yield, and (ii) the horizontal transmission of the induced tolerance.

2. Material and methods

2.1. Germination and haloconditioning treatments

Germination and seedling haloconditioning were carried out as described by Balibrea et al. (1999). Five days after sowing, young tomato seedlings (*L. esculentum* L. Mill, cv. Durinta F1 from Western Seeds S.A.) were haloconditioned (30 per treatment) by complete immersion for 1, 3, 5 and 8 days in 300 mL of the following osmotic/saline solutions generated by PEG-6000 (−0.5, −0.75, −1 MPa), with or without 10 mM NaCl and under forced aeration to prevent anoxia. The different adaptive treatments were denoted by the capital letters A–B, C–D, E–F for −1, −0.75 and −0.5 MPa, respectively, without NaCl (A, C, E) or with 10 mM NaCl (B, D, F), followed by the number indicating the incubation time (A1, A8, . . ., F1, F8). The incubations were carried out in the dark and at 25 °C. The darkness condition was selected to avoid additional photochemical damage and destruction of light-sensitive hormones (not included in this study). In this case, no controls were made in the dark (to avoid ethyolation) or in deionized water (to avoid hyposmotic stress), although they have been tested in other short-term experiments (unpublished data). After completing each incubation time, the seedlings were rinsed for 3 min with deionized water and placed in jiffy pots containing a mixture of peat, perlite and siliceous sand soaked with Hoagland's solution to rehydrate the tissues and restore growth. Thirty control plants germinated simultaneously but without incubation were also placed in jiffy pots at the same time that the 1-day incubated plants.

2.2. Greenhouse evaluation under salinity

Forty-five days after sowing the plants were transferred for evaluation under salinity in a greenhouse using siliceous sand as substrate. A standard fertilisation for tomato was applied through drip irrigation (control). Fifteen days after the transfer, the saline treatment was applied by adding 50 mM NaCl to the nutrient solution. The treatment continued until the end of the harvest period. The mean electrical conductivities of the irrigation solutions were 2.01 (control) and 7.5 dS m^{-1} (moderate salinity). The viable pre-adapted plants of each treatment (with a minimum of three plants and a maximum of 13, see Table 1) were randomly distributed in three blocks under the saline regime. Fifteen non-adapted control plants were evaluated under saline (SC, randomly placed in front of pre-adapted plants) and non-saline (NSC) conditions. A total of 190 plants were individually evaluated. Fruit yield was recorded for 3 months. Sixty days after the beginning of salt treatment, young leaf material was collected in order to determine Na^+, Cl^-, K^+ concentrations, water content and osmotic potential (Balibrea et al., 1999). Vegetative shoot biomass was registered at the end of the experiment.

2.3. Vegetative multiplication and evaluation under salinity of the H_1 plants

About 50 days after the beginning of the salt treatment in the greenhouse, 1.5–2 cm shoot axilary buds were collected from 18 plants corresponding to the haloconditioning treatments B5 (−1 MPa + 10 mM NaCl × 5 days), D5 (−0.75 MPa + 10 mM NaCl × 5 days), F5 (−0.5 MPa + 10 mM NaCl × 5 days), C8 (−0.75 MPa × 8 days), E8 (−0.5 MPa × 8 days) (H_0) and to the SC population (SC_0). The apices were surface sterilized and rooted *in vitro* as described by Cano et al. (1998). The rooting medium contained 1/2 MS mineral salts, 100 mg L^{-1} *myo*-inositol, 1 mg L^{-1} thiamine–HCl, 0.1 mg L^{-1} IAA, 10 g L^{-1} sucrose and 8 g L^{-1} Difco-Bacto agar. After 17 days in the rooting medium, the H_1 and SC_1 plants were transferred to vermiculite for 20 days in a controlled culture chamber, and then to a hydroponic system for saline evaluation with 100 mM

Table 1
Number of plants evaluated, shoot biomass (kg plant^{-1}), fruit yield (kg plant^{-1}) and harvest index of the pre-adapted plants cultivated under salinity

Treatment	Number of plants	Shoot biomass	Fruit yield	Harvest index
NSC (non-saline control)	15	4.81 a (79)	5.48 ab (31)	0.53 b
SC (saline control)	15	2.69 bc	4.17 c	0.61 ab
A1 (−1 MPa)	6	3.11 abc (16)	5.07 abc (22)	0.62 ab
B1 (−1 MPa + NaCl)	7	3.72 ab (38)	5.20 abc (25)	0.58 ab
C1 (−0.75 MPa)	13	3.44 abc (28)	4.93 abc (18)	0.59 ab
D1 (−0.75 MPa + NaCl)	12	3.25 abc (21)	5.22 abc (25)	0.62 ab
E1 (−0.5 MPa)	6	3.26 abc (21)	4.74 abc (14)	0.59 ab
F1 (−0.5 MPa + NaCl)	9	3.26 abc (21)	5.09 abc (22)	0.61 ab
A3 (−1 MPa)	7	3.12 abc (16)	5.09 abc (22)	0.62 ab
B3 (−1 MPa + NaCl)	6	2.93 abc (9)	5.21 abc (25)	0.64 ab
C3 (−0.75 MPa)	8	4.04 a (50)	6.37 a (53)	0.68 a
D3 (−0.75 MPa + NaCl)	5	3.24 abc (20)	5.16 abc (24)	0.61 ab
E3 (−0.5 MPa)	12	3.38 abc (26)	5.15 abc (24)	0.60 ab
F3 (−0.5 MPa + NaCl)	13	2.96 abc (10)	5.16 abc (24)	0.64 ab
A5 (−1 MPa)	8	4.06 a (51)	4.85 abc (16)	0.54 b
B5 (−1 MPa + NaCl)	5	3.29 abc (22)	4.06 c (0)	0.55 ab
C5 (−0.75 MPa)	8	3.30 abc (23)	5.25 abc (26)	0.61 ab
D5 (−0.75 MPa + NaCl)	3	3.35 abc (25)	5.62 abc (35)	0.63 ab
E5 (−0.5 MPa)	8	2.94 abc (9)	4.84 abc (16)	0.62 ab
F5 (−0.5 MPa + NaCl)	6	3.30 abc (23)	4.96 abc (19)	0.60 ab
A8 (−1 MPa)	0	–	–	–
B8 (−1 MPa + NaCl)	3	2.50 abc (0)	4.02 c (0)	0.62 ab
C8 (−0.75 MPa)	6	4.09 a (52)	6.00 ab (44)	0.59 ab
D8 (−0.75 MPa + NaCl)	0	–	–	–
E8 (−0.5 MPa)	10	3.09 abc (15)	4.57 abc (10)	0.60 ab
F8 (−0.5 MPa + NaCl)	0	–	–	–

NSC, SC = non-adapted control plants cultivated without and with salt stress, respectively. (Numbers in brackets indicate the percentage of increase respect to SC plants; different letters indicate significant differences between groups, according to the Duncan test, $P \leq 0.05$.)

NaCl. A total of 39 H_1 (3–13 plants per treatment, see Table 4) and six SC_1 plants were obtained and individually evaluated under salinity for 25 days, as previously described (Balibrea et al., 1999). A 3-week evaluation period under 100 mM NaCl conditions has proved to be sufficient in order to discriminate between pre-adapted and non pre-adapted plants (Balibrea et al., 1999; Parra, 2002; and other unpublished data). Initial and final fresh weights, root/shoot ratio, relative growth rate and the same physiological parameters described above were determined in young leaves and roots of the H_1 and SC_1 plants.

2.4. Statistics

Analysis of variance was performed according to SPSS standard methods. Means of different treatments were compared using the Duncan's test at the 0.05 confidence level.

3. Results

3.1. Shoot biomass, fruit yield and harvest index

Although some of the severe pre-treatments decreased the seedling viability at the post-immersion stage by acting as a selection pressure, all the viable plants were individually evaluated because of their potential high interest. At the end of the harvest period, the saline control plants (SC) had a shoot biomass 45% lower than the non-salinized control plants (NSC) (Table 1). Most of the pre-adapted plants produced 2–52% more shoot biomass than the SC plants. It was 23% more in the average of the total population of these plants. The most vigorous plants were those from the A5 (−1 MPa × 5 days), C3 (−0.75 MPa × 3 days) and C8 (−0.75 MPa × 8 days) treatments. The treatments of −1 (A) and −0.75 MPa (C) seem to be the most effective (+25–27% in shoot biomass), with decreasing in the presence of 10 mM NaCl in the medium (B, D) (+17%). Salinity also decreased fruit yield by 25% in the SC plants (Table 1, Fig. 1). With the exception of the B5 and B8 plants (the most severe pre-treatments: −1 MPa + 10 mM NaCl × 5 and 8 days, respectively), different haloconditioning treatments yielded between 10 and 53% more than the SC plants (0.4–1.2 kg per plant). It was a 22% more in the average population of these plants. The most productive plants were those from the C3 (−0.75 MPa × 3 days) and C8 (−0.75 MPa × 8 days) treatments, yielding more than the plants cultivated without salt. On looking at the cumulative fruit yield curves (Fig. 1), it can be observed that most of pre-adapted populations yielded more than the non pre-adapted plants from the beginning of the harvest period. Interestingly, these differences increased in the −0.75 MPa-treated populations from the second half of this period, coinciding with the separation of the curves corresponding to the non-saline and saline control plants (Fig. 1). Indeed, when considered by groups, the most effective parameters seem to be −0.75 MPa (C and D) applied for 3 days. The presence of 10 mM NaCl in the medium had no clear effect

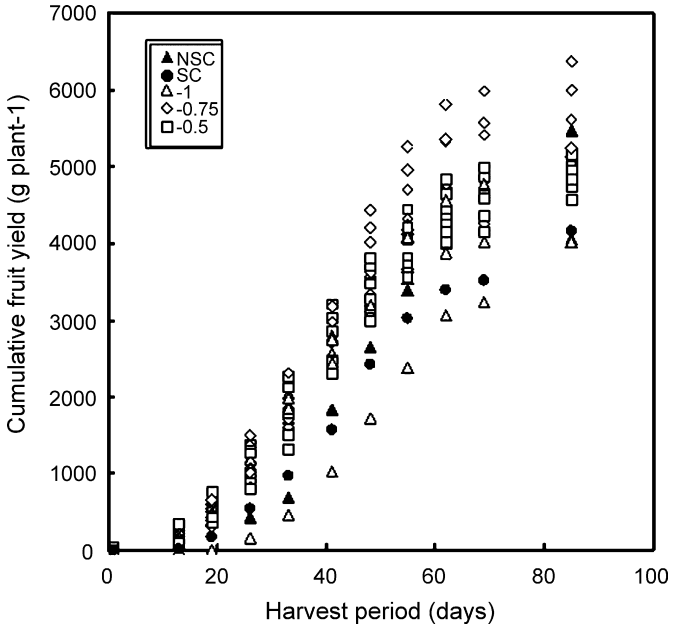

Fig. 1. Cumulative fruit yield along the harvest period in pre-adapted (grouped by identical osmotic potentials during pre-treatment: −0.5, open squares; −0.75, open diamonds; −1 MPa, open triangles) and non-pre-adapted salinized (SC, closed circles) and non-salinized (NSC, closed triangles) control plants. Each point represents the mean value of the corresponding population at each harvest.

on this parameter (Table 1). The harvest index increased with salinity from 0.53 (NSC) to 0.60–0.64 in most salinized plants, although the highest value was found in the most productive C3 plants (0.68) (Table 1).

3.2. Na^+, Cl^- and K^+ contents

After 60 days of salinization, the young leaves of the pre-adapted plants accumulated 10–12% less Na^+ and Cl^- than the SC plants (Table 2, Fig. 2a and b). The A3 plants (−1 MPa × 3 days) showed the lowest concentrations of these toxic ions (50% less than the SC plants). However, the plants from the treatments B5 (−1MPa + 10 mM NaCl × 5 days) and D5 (−0.75 MPa + 10 mM NaCl × 5 days) registered 20–30% increases in Na^+ and Cl^- compared to the SC plants, but with a very different effect on fruit yield. In general, the most vigorous and productive pre-adapted plants also presented the lowest leaf Na^+ and Cl^- concentrations, although the opposite was also found. For example, the highly productive C3 (−0.75 MPa × 3 days) plants (+50% in fruit yield) registered 20–35% less Na^+ and Cl^-, while those from treatment D5 (−0.75 MPa + 10 mM NaCl × 5 days) (+35% in fruit yield) showed a 20% increase in these toxic ions, when compared to the SC plants. Interestingly, the treatments inducing salt-inclusion included 10 mM NaCl in the composition of the incubation medium, in combination with −1 or −0.75 MPa and applied for 5 days (Table 2).

Salinity reduced the leaf K^+ concentration by 65% in the SC plants, but the haloconditioned population showed a significant 43% more K^+ in the leaves than the non-adapted plants (Table 2, Fig. 2c). The most effective treatments on this parameter were the same that on reducing the toxic ions, and so the K^+/Na^+ ratio increases in most pre-adapted plants and especially in the those from the treatments B1, F1, C3 and A3 (two to four times). The most productive C3 and C8 plants showed 2- and 1.6-fold more

Table 2
Na^+, Cl^-, K^+ contents (mM), K^+/Na^+ ratio, leaf water content (g g^{-1} DW) and osmotic potential (Ψ_π, −MPa) in leaves of the pre-adapted plants cultivated under salinity

Treatment	Na^+	Cl^-	K^+	K^+/Na^+	LWC	$\Psi\pi$
NSC (non-saline control)	28.2 e	28.8 e	99.5 a	3.53 a	5.34 ab	1.02 b
SC (saline control)	118.1 abcd	192.8 abc	35.5 ef	0.32 d	7.40 ab	1.29 ab
A1 (−1 MPa)	107.3 abcd	172.8 abcd	58.4 bcdef	0.65 bcd	6.54 ab	1.29 ab
B1 (−1 MPa + NaCl)	83.4 cd	141.0 bc	63.1 bcde	0.85 bcd	5.61 ab	1.10 b
C1 (−0.75 MPa)	127.3 abc	177.8 abcd	45.7 bcdef	0.36 d	6.74 ab	1.30 ab
D1 (−0.75 MPa + NaCl)	113.1 abcd	159.6 bc	38.2 def	0.44 d	6.67 ab	1.20 ab
E1 (−0.5 MPa)	86.2 cd	139.1 bc	38.9 def	0.45 d	6.32 ab	1.26 ab
F1 (−0.5 MPa + NaCl)	108.3 abcd	161.8 bcd	64.3 bcd	0.74 bc	5.99 ab	1.32 ab
A3 (−1 MPa)	63.7 d	104.4 d	75.6 b	1.19 b	5.01 b	1.27 ab
B3 (−1 MPa + NaCl)	82.2 cd	148.9 bc	55.1 bcdef	0.71 bcd	4.48 ab	1.24 ab
C3 (−0.75 MPa)	96.1 bcd	126.4 bc	63.3 bcde	0.73 bcd	4.89 b	1.19 ab
D3 (−0.75 MPa + NaCl)	115.8 abcd	189.9 abc	46.6 bcdef	0.45 d	6.93 ab	1.31 ab
E3 (−0.5 MPa)	107.8 abcd	151.7 bc	54.2 bcdef	0.59 bcd	5.78 ab	1.27 ab
F3 (−0.5 MPa + NaCl)	102.2 abcd	160.6 abcd	52.1 bcdef	0.59 bcd	5.65 ab	1.37 ab
A5 (−1 MPa)	123.7 abcd	178.7 abcd	49.3 bcdef	0.43 d	8.30 a	1.28 ab
B5 (−1 MPa + NaCl)	153.1 a	237.3 a	40.0 bcdef	0.35 d	6.11 ab	1.44 a
C5 (−0.75 MPa)	107.4 abcd	151.8 bc	44.5 bcdef	0.46 d	5.58 ab	1.25 ab
D5 (−0.75 MPa + NaCl)	141.3 ab	234.1 ab	41.7 cdef	0.29 d	6.66 ab	1.41 a
E5 (−0.5 MPa)	116.3 abcd	169.6 abcd	47.4 bcdef	0.49 d	6.31 ab	1.36 ab
F5 (−0.5 MPa + NaCl)	93.9 bcd	170.8 abcd	51.2 bcdef	0.55 bcd	4.89 b	1.42 a
B8 (−1 MPa + NaCl)	108.0 abcd	171.7 abcd	31.3 f	0.29 d	5.67 ab	1.14 ab
C8 (−0.75 MPa)	112.8 abcd	179.9 abcd	55.7 bcdef	0.54 bcd	6.35 ab	1.43 a
E8 (−0.5 MPa)	105.0 abcd	197.3 abc	47.5 bcdef	0.50 cd	6.15 ab	1.29 ab

NSC, SC = non-adapted control plants cultivated without and with salt stress, respectively. (Different letters indicate significant differences between groups, according to the Duncan test, $P \leq 0.05$.)

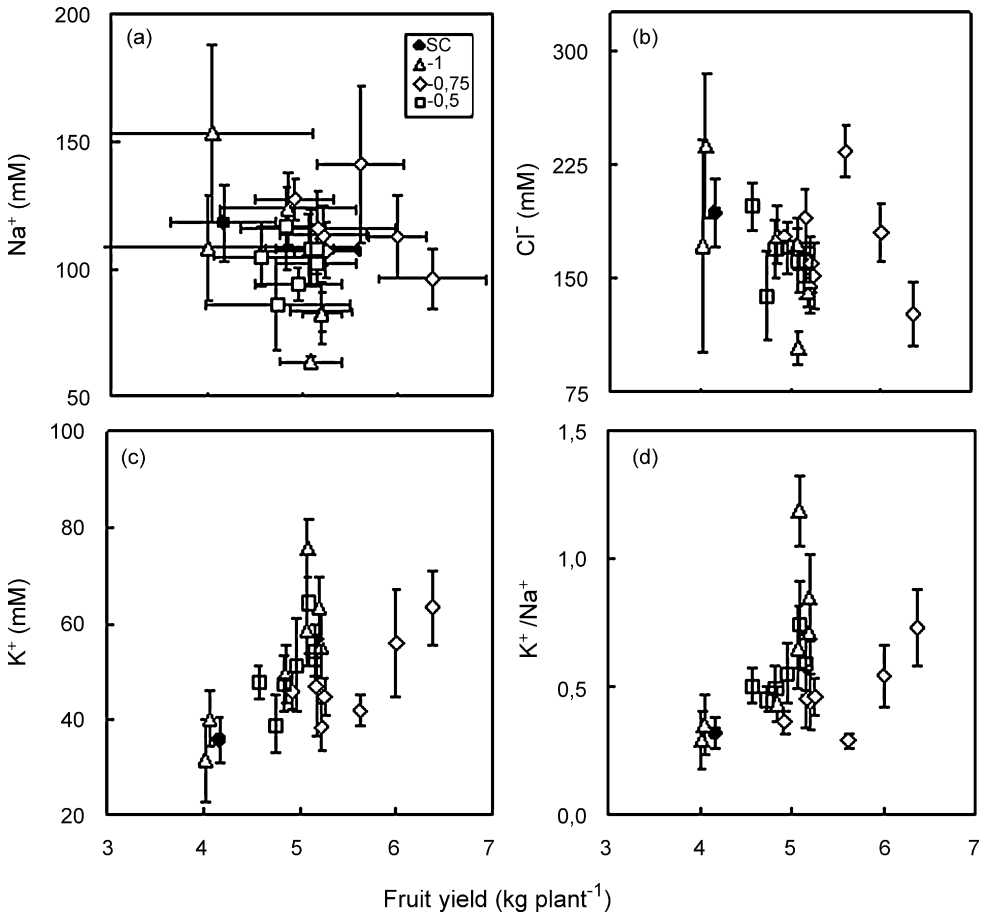

Fig. 2. Na^+ (a), Cl^- (b), K^+ (c) contents and K^+/Na^+ ratio (d) in young leaves vs. fruit yield under moderate salinity in non-adapted (SC) and pre-adapted (grouped by identical osmotic potentials during pre-treatment: −0.5, −0.75 and −1 MPa) tomato plants. Each point represents the mean value of the corresponding population of plants ± S.E.

K^+ and K^+/Na^+ ratio than the SC plants, respectively (Table 2, Fig. 2c and d).

3.3. Leaf water content and Ψ_π

The leaf water content of the SC plants after 60 days of salinization was 25% higher than in the non-salinized NSC plants (Table 2). The pre-adapted population showed 16% less water content than the SC plants (7.4 g g^{-1} DW) and values more similar to the NSC plants (5.34). Indeed, the most productive C3 plants also registered the lowest leaf water content (4.89). The leaf Ψ_π decreased by 20% under salinity with respect to the NSC plants (−1.02 MPa). Although no differences were found between most of pre-adapted and SC plants (−1.3 MPa), some relationship seems to exist between leaf Ψ_π and the Na^+ and Cl^- contents among different pre-adapted populations (Table 2).

3.4. Behaviour of the mother plants (H_0) under greenhouse conditions

When collecting the axilary buds, the mother plants (H_0) used for vegetative multiplication were selected for their vigorous aspect. This vigour was reflected in the better behaviour of these plants when compared to the mean of their respective populations in parameters such as fruit yield and shoot biomass (Table 3). The selected H_0-plants yielded 28–53% more fruit and up to 68% more shoot biomass than the SC plants. These improvements were 10–40% when compared to the mean of each treatment. Only the F5 and B8 H_0-plants did not show any improvement in shoot biomass with respect to both the SC and their respective populations. The leaf water content of the H_0-plants was similar to their respective treatments, and 10–25% lower than the SC plants (Table 3). The leaf Na^+ and Cl^- concentrations varied by up to 50% from the SC plants, with 15–20% increases in the B5 and D5 and 15–50% decreases in B8 and C8 mother plants. The leaf K^+ contents in the D5, F5, B8 and C8 mother plants were between 10 and 41% higher than in the SC plants, showing a similar tendency that all the pre-adapted population of plants.

3.5. Behaviour of the H_1 plants under controlled growth conditions

The initial fresh weight of the H_1 plants from the treatments D5, F5 and C8 at the beginning of salt treatment was about

Table 3
Behaviour of the H_0-plants (haloconditioned plants selected for vegetative propagation) and their corresponding pre-adapted population (mean) under salinity in the greenhouse experiment with respect to the following parameters (in percentage of the SC population): fruit yield, shoot biomass, Na^+, Cl^-, K^+ and water (LWC) contents in the leaves

H_0-plants	Fruit yield	Shoot biomass	Na^+	Cl^-	K^+	LWC
B5-H_0 (−1 MPa + NaCl)	137	158	115	123	103	74
B5-mean	97	122	130	123	96	85
D5-H_0 (−0.75 MPa + NaCl)	135	124	120	121	117	91
D5-mean	135	125	120	121	117	91
F5-H_0 (−0.5 MPa + NaCl)	130	105	83	99	111	62
F5-mean	119	123	80	89	144	70
B8-H_0 (−1 MPa + NaCl)	128	93	74	53	113	72
B8-mean	96	93	91	89	88	79
C8-H_0 (−0.75 MPa)	153	168	90	87	141	90
C8-mean	144	152	96	93	157	88
E8-H_0 (−0.5 MPa)	135	158	99	107	98	113
E8-mean	110	115	89	102	134	85
H_0-plants	136	134	97	98	114	84

Table 4
Behaviour of the SC_1 and H_1 plants (obtained by vegetative propagation from SC_0 and H_0-plants, respectively), after evaluation under salinity, with respect to the following parameters: initial (FWi) and final (FWf) fresh weights (g plant^{-1}), root/shoot ratio (R/S), relative growth rate (RGR, g g^{-1} day^{-1}), Na^+, Cl^-, K^+ (mM) and water (LWC, g g^{-1} DW) contents in the leaves

Treatment	FWi	FWf	R/S	RGR	Na^+	Cl^-	K^+	LWC
SC_1 (6)	20.8 ns	124.7 ns	0.38 ns	0.22 ns	95.5 ns	195.1 ns	77.9 ns	9.7 ns
B5-H_1 (13)	15.3	116.3	0.33	0.23	105.0	195.2	83.4	9.6
D5-H_1 (3)	10.4	75.4	0.29	0.24	77.3	181.4	88.2	10.7
F5-H_1 (5)	11.9	69.6	0.37	0.16	105.5	176.8	90.6	10.5
B8-H_1 (6)	18.5	109.4	0.43	0.18	92.8	179.1	94.6	9.9
C8-H_1 (8)	12.9	84.3	0.44	0.22	99.7	180.8	82.1	10.3
E8-H_1 (3)	18.2	88.9	0.39	0.19	80.2	173.9	65.6	10.6
H_1-plants	15.4	95.5	0.38	0.21	93.7	183.2	83.2	10.2

The number of plants evaluated in each group is indicated in brackets; ns indicates that no significant differences were found between groups.

50% lower than in the SC_1 plants, and similar in the rest of treatments (Table 4). At the end of the evaluation period under salinity, the SC_1 plants produced 30–55% more biomass than those from the treatments D5, F5, C8 and E8, and a similar biomass to the H_1 plants from B5 and B8. Although some differences were found in those parameters, they were not statistically significant because of the high variability observed in the initial biomass. Despite the reported differences in the initial weight, the root/shoot ratio and the relative growth rate were, nevertheless, very similar between all the evaluated H_1 and SC_1 plants (Table 4). Concerning the leaf ionic contents, 20% decreases (Na^+, Cl^-) or increases (K^+) were found with respect to the SC_1 plants in some treatments. Similar values were registered for LWC (Table 4). No significant differences in the physiological parameters evaluated were found when comparing the mean values of the H_1-population and the SC_1 plants, and no relationship was observed in the evolution of parameters such as Na^+, Cl^- and LWC with respect to the mother populations (Tables 3 and 4). However, the increase in K^+ content showed a similar trend when comparing both populations. These results indicate that both the biomass partitioning between roots and shoots and the RGR were independent of the initial parameters and of the adaptive treatment of the mother plants.

4. Discussion

4.1. Improving tomato crop salt tolerance by inducing plant adaptation

Tomato is a moderate salt-tolerant crop that reduces yield by 50% under a saline regime of 8 dS m^{-1} (Subbarao and Johansen, 1994). In our experimental conditions, the modern cultivar Durinta F1 was only affected by 25% (7.5 dS m^{-1}). However, with independence of the specific characteristics of the different treatments applied, the haloconditioning of young seedlings by immersion in low osmotic potentials for 1–8 days induced agronomic salt tolerance in this cultivar, reflected in a more than 20% improvement in both shoot biomass and fruit yield under the moderate salinity regime (Table 1, Fig. 1). Increased plant vigour seems to be responsible for these improvements since the harvest index was practically not affected with respect to the non-adapted plants and increases in the relative growth rates were observed during the vegetative stage in similar experiments (unpublished results). Taking into account the major physiological parameters linked to the tolerance to salinity, the pre-adapted plants registered 12% less Na^+ and Cl^- concentration and more than 40% more K^+ and K^+/Na^+ ratio in the leaves. The differences in Na^+ and Cl^- concentrations would be larger if expressed

on a dry weight basis since the leaf water content was higher in the SC plants than in most of pre-adapted plants (Table 2). Na^+ exclusion and K^+ selectivity are considered major targets to increase salt tolerance in crop plants (Shannon, 1997; Yeo, 1998; Zhu, 2001; Flowers, 2004; Munns, 2005). Indeed, the ability to uptake K^+ and to exclude Na^+ has been related to the agronomical salt tolerance in tomato (Cano et al., 1991; Pérez-Alfocea et al., 1993a, 1996; Estañ et al., 2005; Juan et al., 2005) and some improvements have been obtained by acting directly or indirectly on the K^+/Na^+ homeostasis: (i) overexpression of the *HAL1* gene in calli and plants (Gisbert et al., 2000; Rus et al., 2001b); (ii) by using grafting systems (Santa-Cruz et al., 2002; Estañ et al., 2005); (iii) by applying NaCl-pre-treatments (Cayuela et al., 2001). In most of these cases the salt tolerance was observed on fruit yield (Cayuela et al., 2001; Rus et al., 2001b; Santa-Cruz et al., 2002; Estañ et al., 2005). Positive effects on vegetative growth and K^+ selectivity were also observed in tomato plants pre-adapted with PEG −0.75 MPa for 12 h (Balibrea et al., 1999). In that case, the induced salt tolerance was also related to an enhanced Na^+ accumulation in the leaves, such as occurred in the D5-treated plants (−0.75 MPa + 10 mM NaCl × 5days). It is also likely that a more efficient inclusion mechanism into vacuoles is operating in the pre-adapted plants (unpublished results) such as seems to occur in some para-halophytic tomato ecotypes (Pérez-Alfocea et al., 1993a,b; Shannon, 1997). The dilution by the higher growth vigour of the haloconditioned plants could help to explain the lowered contents of toxic ions in the leaves. However, the increases registered in K^+ also indicate that a better regulation of the toxic and nutrient ions is probably happening in the roots. Indeed, the improvement in K^+ uptake under salinity is one of the most common responses to haloconditioned plants in different experiments, suggesting that these treatments affect the systems for K^+ uptake. Although this aspect will be further investigated, it has been reported that osmotic pre-treatments facilitates ion stress adaptation and K^+/Na^+ selectivity in yeast through increasing the affinity of K^+ transporters and inducing Na^+ exclusion (Matsumoto et al., 2002). Moreover, the lower leaf water content with a similar leaf Ψ_π to the SC plants may also indicate that the pre-adapted plants present a better osmotic adjustment, as has been observed in short-term experiments (Balibrea et al., 1999; Parra, 2002).

As stated above, the average improvement obtained in salt tolerance in the whole pre-adapted population was about 20–25%, but one of the objectives of this study was to define the best parameters of the method in terms of the nature of the pre-treatment (osmotic/saline), osmotic potential and incubation time. Taking into account the plant vigour reflected in both shoot biomass and fruit yield, the C3 (−0.75 MPa × 3 days) and C8 (−0.75 MPa × 8 days) were the best treatments, producing 50% more than the SC plants. The physiological data (Na^+, Cl^- and K^+ contents) do not support this advantage with respect to the other treatments, which only yielded about 25% more than the SC plants. Moreover, different behaviours concerning Na^+ and Cl^- homeostasis were found among the highly productive populations (i.e. C3, C5, D5 and C8) (Table 2, Fig. 2a and b). Interestingly, the common factor to these treatments was an osmotic potential of −0.75 MPa generated by PEG in the medium, but while lowered leaf Na^+ and Cl^- contents were registered in the plants incubated with the pure osmotic component (C3, C5, C8), an increase in these toxic ions was found in the plants incubated in the presence of 10 mM NaCl in the medium (D5), suggesting an interaction of both osmotic and ionic components on ion homeostasis (exclusion or inclusion in vacuole) and adaptation to salinity (Matsumoto et al., 2002). Hence, Na^+ and Cl^- contents were not clearly related to fruit yield (Fig. 2a), such as it occurred with the relative growth rate in the short-term experiment (unpublished results), although the behaviour of the worst treatment (B5, −1 MPa + 10 mM NaCl × 5 days) could be explained by the specific toxicity of saline ions (Tables 1 and 2). The high variability induced by some treatments in shoot Na^+ concentration and the low variability for K^+ (Fig. 2a and c) have also been observed in rice and *Shorghum bicolor* following exposition to sublethal levels of NaCl (Flowers and Yeo, 1981; Amzallag, 1999). In the latter species, the variability regarding different physiological responses in the mode of ionic regulation (Na^+-excluder and -includer) differentiated during the induction of salt adaptation was linked to the self-organised process of individuation (Amzallag, 1999). It seems that the growth response under salinity is both a function of the mode of response and of the Na^+ ions accumulated in the shoot (Amzallag, 2002). This differentiation could be related to the diffuse line dividing the regulatory mechanisms for ion homeostasis in glycophytes and halophytes (Rus et al., 2001a; Zhu, 2001). Although this variability could help to explain the different behaviour among treatments and the fact that the responses observed are highly variable among independent experiments (Seligmann, 1998; Amzallag, 1999), as stated by these authors, a general positive trend exists in the improvement of salt tolerance and related physiological responses in the different experiments and different adaptive treatments. As a consequence, it seems to exist a positive correlation between the frequency of specific reactions and the tolerance level of the adapted plants (Seligmann, 1998) and, therefore, the crop salt tolerance. However, since none of the measured physiological parameters are able to explain the different agronomical behaviour, especially in the most productive plants, it is possible that the improvement in plant vigour under the suboptimal conditions itself would be enough to explain the induced tolerance, with independence of other induced mechanisms.

In short-term experiments, the best results regarding globally growth-related parameters, root/shoot ratio and K^+/Na^+ regulation have also been obtained with an osmotic potential of −0.75 MPa applied between 12 h and 8 days (Balibrea et al., 1999; Parra, 2002). In this experiment, the treatments of −1 and −0.75 MPa applied for 3 days were the most effective in the exclusion of Na^+ and Cl^- from the young leaves and in the K^+ uptake and, thereafter, in the K^+/Na^+ selectivity (Table 2, Fig. 2). However, considering the seedling viability after the application of the treatments, the shoot biomass and fruit yield and the results obtained in short-term experiments, the osmotic potential of −0.75 MPa seems to be the most adequate. The addition of 10 mM NaCl to the incubation medium strongly reduced seedling viability over 1 day of application and only a small positive effect was observed on fruit yield and Na^+ and K^+ contents

respect to the pure osmotic treatments applied for 1 day (A1, C1 and E1). Its application at lower concentrations or incubation time should be tested in order to minimise the antagonism between the positive and negative effects observed.

4.2. Horizontal transmission of the induced tolerance

According to these and previous results (Balibrea et al., 1999; Pérez-Alfocea et al., 2002; Parra, 2002), the method of haloconditioning seems to increase salt tolerance in tomato throughout the induction of the adaptive component. Although the method is easy to apply homogeneously to a high number of plants, some of the treatments provoked low seedling viability and the possibility exists that the most tolerant individuals are being selected, which should be rare since the original population was a commercial F1 hybrid. The study of the horizontal transmission of the observed salt tolerance could help to explain the genetic or epigenetic basis of the presumably induced adaptation. The H_0-plants evaluated under greenhouse conditions showed an improved salt tolerance with respect to the SC_0 plants in terms of fruit yield, shoot biomass, leaf water content, regulation of the toxic ions (Na^+, Cl^-) and K^+ concentrations. These H_0-plants were selected for their vigorous aspect and were more productive than the mean of their respective populations, in general. As a consequence, it was expected that the H_1 plants obtained by vegetative propagation would show a higher performance than the SC_1 population in the following cases: if the induced adaptation has (i) a genetic basis or (ii) epigenetic basis manifested in the shoot tissues; and (iii) if the haloconditioning treatments do not induce any adaptation but select the genetically more tolerant individuals of the population. The results have shown that the H_1 plants did not manifest the plant vigour observed in the mother plants. No significant differences were found for any of the parameters tested in all the plants evaluated under salinity, not even in those that were more representative in other experiments, such as the root/shoot ratio, the relative growth rate and the leaf water content (Balibrea et al., 1999; Parra, 2002). The differences found in plant biomass before the imposition of salinity were maintained during the evaluation period, without changes in the relative growth rate or the root/shoot ratio. The only parameter that showed a similar tendency to the mother plants and to other haloconditioned plants was the leaf K^+ concentration, with 5–21% of increases respect to the SC_1 plants. In this case, it could be due to the lower vegetative growth registered in the H_1 plants, while in the greenhouse experiment the leaf K^+ content was linked to a higher fruit yield and shoot biomass under salinity, such as occurred with the relative growth rate in short-term experiments (unpublished results). Although the clones were only evaluated in a short-term experiment, the data do not point to the possibility of an enhanced salt tolerance of these plants in long-term experiments and, in principle, the selection of the genetically most tolerant individuals of the population by high selection pressure during haloconditioning should be discarded (according to their condition of F1 hybrids), but it is possible that these individuals were more receptive in undergoing the epigenetic modifications required for increasing salt tolerance. This statement connects with the third source of variability responsible for the process of individuation involved in the physiological adaptation to salinity described in *S. bicolor* (Amzallag, 1999). Bourgeais et al. (1990) reported that the character inducible of the adaptation by progressive salinization in vitroplants from *L. esculentum* was mainly related to the K^+/Na^+ selectivity, such as occurred when the most tolerant individuals were selected by applying a strong selection pressure. In both cases the salt tolerance was horizontally transmitted during six cycles of shoot apex culture in a saline medium. In our experiment the plants were propagated in a non-saline medium and it is likely that epigenetic changes happening in root and shoot tissues during haloconditioning were responsible for the higher salt tolerance observed. However, these were mostly lost or diluted during the rooting process of the adventitious shoots without salt, in a similar way that described in plants regenerated from salt-adapted cell lines (Binzel and Reuveni, 1994). However, even if epigenetic modifications are the responsible for the induced tolerance, the main advantage of this method of seedling haloconditioning is that agronomic salt tolerance can be directly improved by inducing adaptation in whole plants and large populations.

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