J Plant Growth Regul (2010) 29:63–72
DOI 10.1007/s00344-009-9114-7

Contrasting Effects of GA_3 Treatments on Tomato Plants Exposed to Increasing Salinity

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Received: 31 March 2009 / Accepted: 9 June 2009 / Published online: 6 August 2009

Abstract The role of plant hormones under saline stress is critical in modulating physiological responses that will eventually lead to adaptation to an unfavorable environment. Nevertheless, the functional level of plant hormones, and their relative tissue concentration, may have a different impact on plant growth and stress tolerance at increasing salinity of the root environment. Vigorous plant growth may counteract the negative effects of salinization. In contrast, low gibberellin (GA) levels have been associated with reduced growth in response to salinity. Based on these facts and considering that the physiological basis of the cause-effect relationship between functional growth control and stress adaptation/survival is still a matter of debate, we hypothesized that exogenous applications of the plant hormone GA_3 may compensate for the salt-induced growth deficiency and consequently facilitate tomato plant adaptation to a saline environment. GA_3 application (0 or 100 mg GA_3 l^{-1}) was compared under four salinity levels, obtained by adding equal increments of NaCl:$CaCl_2$ (2:1 molar basis) (EC = 2.5, 6.8, 11.7, 16.7 dS m^{-1}) to the nutrient solution. GA_3 treatment reduced stomatal resistance and enhanced plant water use at low salinity. These responses were associated with an increased number of fruit per plant at harvest. However, moderate and high salinity nullified these differences. The fruit carotenoid level was generally lower in GA_3-treated plants, indicating either an inhibitory effect of GA_3 treatment on carotenoid biosynthesis or a reduced perception of the stress environment by GA_3-treated tomato plants.

Keywords Abscisic acid · Carotenoids content · Cl^- and Na^+ accumulation · Leaf water potentials · Stomatal regulation

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Introduction

Root zone salinization, a common phenomenon in irrigated agriculture, may expose crop plants to ionic/osmotic stress and ultimately affect both final yield and quality (Flowers 1999). The process of plant adaptation to salinity is mostly under hormonal control and involves the activation of stress response mechanisms, which mediate ionic/hydraulic re-equilibrium, reactive oxygen species (ROS) detoxification, and modulation of cell growth/division (Hasegawa and others 2000; Zhu 2001; Ruggiero and others 2004; Achard and others 2006). Although plant hormones have been proven to directly or indirectly control key aspects of plant growth and adaptation to adverse environments (Zhu 2002), the main features underlying the complex interactions between these metabolites are mostly unknown and only recently many important physiological cross-talks have begun to be unravelled (Nemhauser and others 2006).

The most characterized stress hormone is abscisic acid (ABA), which is involved in pivotal physiological responses required for salt stress adaptation, including ion and water homeostasis. ABA may directly control cell enlargement and division (Ruggiero and others 2004), or indirectly modulate plant growth by increasing stomatal resistance,

which restricts both water loss and CO_2 uptake. These events are essential in overcoming temporary or long-term physiological perturbations and they contribute to both plant adaptation and survival. In response to hyperosmotic stress, other hormones that primarily affect cell enlargement and growth, such as gibberellins (GA), must also coordinately interact with ABA and possibly other stress metabolites, including antioxidants and ROS scavengers (Achard and others 2006). It has been documented that ABA and GA play antagonistic roles in controlling many developmental processes, including germination, growth, and flowering (Razem and others 2006). On a biochemical basis, upstream regulation of the biosynthesis and balance between these two hormones may reside in the common precursor geranylgeranyl diphosphate (Hedden and Proebsting 1999; Ren and others 2007). Downstream checkpoints of their mode of action may involve the activation or inhibition of hydrolytic enzymes that have been proven to be critical for embryo development (Rogers and Rogers 1992; Gubler and others 1995). Transcriptional regulation of ABA-mediated suppression of GA responses has also been described (Xie and others 2006; Weiss and Ori 2007). Less clear are the antagonistic functions of GA and ABA in terms of stomatal regulation and their functional role in response to a hyperosmotic environment.

On a whole-plant basis and under field conditions, compelling evidence indicates that vigorous plants may better cope with salinity (Munns and others 2006), possibly by delaying the onset of the salinity tolerance threshold (Dalton and others 2000). In contrast, both ABA- and GA-dependent growth reductions have been reported to be critical in stress adaptation and/or survival (Ruggiero and others 2004; Achard and others 2006; Magome and others 2008). Although the independent roles of ABA and GA have been well documented (Zeevart and Creelman 1988; Olszewski and others 2002), it remains uncertain how these two hormones coordinately regulate plant growth and stress adaptation (Ross and O'Neill 2001). In this respect, there is a clear need to unravel the physiological bases and genetic determinants that control plant adaptation versus survival to link functional tolerance traits to specific agricultural contexts (Maggio and others 2002). In a previous experiment we demonstrated that tomato plants respond to increasing salinity by activating metabolic/morphological adaptation mechanisms in a quite specific functional sequence, which involves the control of plant growth and transition from vegetative to reproductive stages (Maggio and others 2007). In the present study we further analyzed the functional role of GA and ABA in stress adaptation. Here we demonstrate that exogenous GA applications may benefit plant growth and yield at low to moderate salinity, whereas it may enhance stress sensitivity at moderate- to high-salinity levels.

Materials and Methods

Growth Conditions

The experiment was carried out at the Department of Agricultural Engineering and Agronomy of the University of Naples Federico II experimental greenhouse, Portici (Naples), Italy (40°49′ N, 14°20′ E). Seeds of cherry tomato (Diamante F1—ESASEM 99-125) were germinated in styrofoam flats containing a mixture of sand and peat moss (1:1) and subsequently transferred, at the stage of two fully expanded leaves (September 10), to 15-l buckets filled with perlite (Agrilit 3 Ø 2–5 mm) with one plant per bucket at the crop density of 3.5 plants m^{-2}. The buckets were covered to avoid evaporative loss and equipped with two drippers with a nominal discharge of 2 l h^{-1}. Plants were fertilized with nutrient solution [electrical conductivity at 25°C (EC) = 2.5 dS m^{-1}; pH = 6.0] containing (in mmol l^{-1}): 13.5 NO_3^-, 1.5 NH_4^+, 1.25 PO_4^{3-}, 8.75 K^+, 4.25 Ca^{2+}, 2.0 Mg^{2+}, 3.75 SO_4^{2-}, 3.0 Na^+, and 4.0 Cl^-, plus micronutrients (B, 0.03; Mn, 0.01; Fe, 0.015; Zn, 0.005; Cu, 0.00075; Mo, 0.0005). The nutrient solutions were pumped from reservoir tanks (one 200-L tank per 15 plants) into the buckets. The surplus drained solution was then sent back to the tanks based on a recirculating system. The number of pulses ranged from 3 to 6 per day (3-5 min/pulse). The reservoir tanks were refilled with new nutrient solution every week.

Salt Stress Treatments

Two weeks after transplanting, the plants were divided into two groups of 180 single-plant buckets. One group was irrigated with plain nutrient solution ($-GA_3$), whereas the second group of plants ($+GA_3$) was irrigated with nutrient solution containing gibberellic acid (Gibrelex, 100 mg GA_3 l^{-1}) for 1 week (Levent Tuna and others 2008). Three weeks after transplanting, four salinity treatments were imposed on both groups ($+/-GA_3$). To avoid NaCl-induced calcium deficiencies, equal increments of NaCl:$CaCl_2$ (2:1 molar basis) were added to reach four different EC levels (Maggio and others 2007): 2.5 (nonsalinized control = S0), 6.8 (S1), 11.7 (S2), 16.7 (S3) dS m^{-1}, corresponding to 28 (S1), 55 (S2), 88 (S3) mM Na and 55 (S1), 111 (S2), 177 (S3) mM Cl. The experimental design was a split-plot with three replications. The GA_3 treatments were assigned to the main plots and different salinity treatments were assigned to the subplots, randomized within the main plots. Each salinity treatment consisted of 45 buckets (15 buckets per replication). Photosynthetic photon flux density (PPFD), relative humidity (RH), and air temperature (T) were continuously monitored during the experiment. EC and pH and the amount of the nutrient solution collected weekly from each bucket also were measured and recorded. Plant water

use was calculated by measuring the difference between the nutrient solution applied and the corresponding volume of percolate collected for each bucket. Water use was expressed as cumulative water consumption per plant at 141 days after transplanting (DAT), whereas water use efficiency (WUE) was estimated as the ratio of the leaf area per plant and their relative cumulative water consumption at 50, 92, and 141 DAT.

Plant Water Relations

Stomatal resistance and leaf water potentials were measured on the youngest, fully expanded leaf of 9 plants per treatment (3 per each replication). Plants were sampled three times during the crop cycle: 50, 92 (at harvest of the II truss), and 141 DAT (at harvest of the V truss).

Measurements were performed at 3-h intervals from 09:00 a.m. to 03:00 p.m. Stomatal resistance was measured on the abaxial surface of the youngest, fully expanded leaves with a diffusion porometer (AP-4, Delta-T Devices, Cambridge, UK). Water potentials were measured on tissue discs punched from the first, uppermost, fully expanded, healthy and sun-exposed leaf (Slavik 1974). Leaf total water potentials (Ψ_{tot}) were measured using a dew-point psychrometer (WP4, Decagon Devices, Pullman, WA). The osmotic potential (Ψ_{π}) was measured after freezing and thawing leaf samples and the pressure potential (Ψ_{p}) was estimated as the difference between Ψ_{tot} and Ψ_{π}, assuming a matric potential equal to zero (Hsiao 1973). At the end of each measurement day, plant samples were collected for measuring leaf area and dry mass yield. Leaf area was measured on green leaves using a Li-Cor 3000 area meter (Li-Cor, Lincoln, NE). Fresh weight and dry weight were measured separately on leaves, stems, fruits, and roots after drying them at 60°C.

Ion Accumulation and Carotenoid Content

Sodium and chloride concentrations were measured on dried and ground tissue subsamples from young, fully expanded leaves by atomic absorption spectrophotometry (Walinga and others 1995). Fruits were collected at full ripeness in five harvests from December 12 (93 DAT) to January 31 (143 DAT). On January 7 (119 DAT) and January 31 (143 DAT), samples of red fruits were collected in each plot from the II truss and from the V truss, respectively. Total soluble solids (TSS) and carotenoid contents were measured. TSS were measured on tomato juice samples with a refractometer and expressed as °Brix. Carotenoid contents were determined according to the method of Leonardi and others (2000). High-performance liquid chromatography (HPLC) separation was carried out at a flow rate of 0.8 ml min^{-1} using a Shimadzu HPLC with diode array detection and a Supelcosil LC_{18} (250 × 4.6 mm i.d.). Carotenoid elution was achieved using the following linear gradient: starting condition, 82% A and 18% B, 20 min; 76% A and 24% B, 30 min; 58% A and 42% B, 40 min; 39% A and 61% B, where A was CH_3CN and B was methanol-exane-CH_2Cl_2 1:1:1 v/v. Carotenoid quantification was done using a standard curve

Table 1 Leaf area, leaf dry matter (DM) percentage, total aboveground dry mass, and water use in response to GA_3, and salinity treatments at 141 DAT

Treatment	Leaf area (dm^2 $plant^{-1}$)	Leaf DM (%)	Total DM (g $plant^{-1}$)	Water use (L $plant^{-1}$)	WUE[a] (cm^2 cm^{-3})
GA_3					
$-GA_3$	30.9	11.1	101.1	47.5	0.118
$+GA_3$	33.7	10.6	117.5	53.4	0.109
	ns	ns	ns	ns	ns
Salinity					
S0	45.6	10.0	150.7	62.0	0.128
S1	34.0	10.5	113.1	50.6	0.116
S2	31.2	10.9	97.3	47.1	0.117
S3	18.4	12.1	76.1	42.3	0.091
	**	**	**	**	*
LSD	10.7	0.9	27.9	6.4	0.024
Interaction GA_3 × salinity	ns	ns	ns	*	*

EC of the nutrient solution = 2.5 (S0), 6.8 (S1), 11.7 (S2), and 16.7 (S3) dS m^{-1} at 25°C. Significant interactions between GA_3 and salinity are displayed in Fig. 1a, b. Mean values from the salinity treatments include non-GA_3-treated and GA_3-treated plants

$-GA_3$ non-GA_3-treated plants, *$+GA_3$* plants treated with 100 mg GA_3 l^{-1}, *LSD* least significant difference at $P \leq 0.05$, *ns* not significant

*, ** Significant at $P \leq 0.05$ and $P \leq 0.01$, respectively

[a] Water use efficiency (WUE) calculated as the average of three sampling days (50, 92, and 141 DAT)

based on commercial β-carotene purchased from Fluka or on HPLC-purified lycopene. The concentrations of the standards were calculated using the extinction coefficient and corrected for the estimated recovery. At 143 DAT, ABA measurements were performed on dehydrated samples of the youngest, fully expanded leaves using HPLC according to the method of Kelen and others (2004).

Data were analyzed by the analysis of variance method (two-way ANOVA) and means were compared by the least significant difference (LSD) test.

Results

Plant Growth and Water Relations

General plant development was not significantly affected by GA_3 treatment, which in some respects only moderately increased plant vigor. In contrast, increasing salinization (EC) of the nutrient solution caused a reduction of leaf area, leaf dry matter percentage, and total dry matter. Plant water use and water use efficiency both mirrored this response, with approximately 30% reduction from S0 to S3 plants (Table 1). For these parameters significant interactions between salinity (EC) and GA_3 treatments were observed (Fig. 1). At low salinity, GA_3-treated plants used 30% more water compared with non-GA_3-treated plants, whereas higher salinization nullified these differences (Fig. 1a). Similarly, salinization gradually reduced both total (-1.65 vs. -2.14 MPa at S0 and S3, respectively) and osmotic (-2.13 vs. -2.87 MPa at S0 and S3, respectively) water potentials, whereas it moderately increased the pressure potential. There was no significant GA_3 effect on these parameters (Table 2). Therefore, the overall plant water status was relatively similar in GA_3-treated and non-GA_3-treated plants and was not affected by the reduced stomatal resistance of the former, which was always lower than non-GA_3-treated plants ($-GA_3$) at any tested salt concentration (Table 3). Comparative analysis of plant water use (Table 1) and stomatal resistance (Table 3) indicated that the observed differences in terms of water consumption in response to salinization could be explained only partially by stomatal resistance because this was consistently higher in plants not exposed to GA_3 treatment, even when no differences in terms of plant water use were observed. The combined effect of GA_3 on both stomatal resistance and leaf area development was revealed in terms of water use efficiency (Fig. 1b). Consistent with most published literature, the leaf area was reduced by salt stress relatively more than transpiration in control plants ($-GA_3$). In contrast, the GA_3 contribution to cell enlargement and leaf expansion partially compensated for the salinity-induced growth reduction. As a consequence, upon

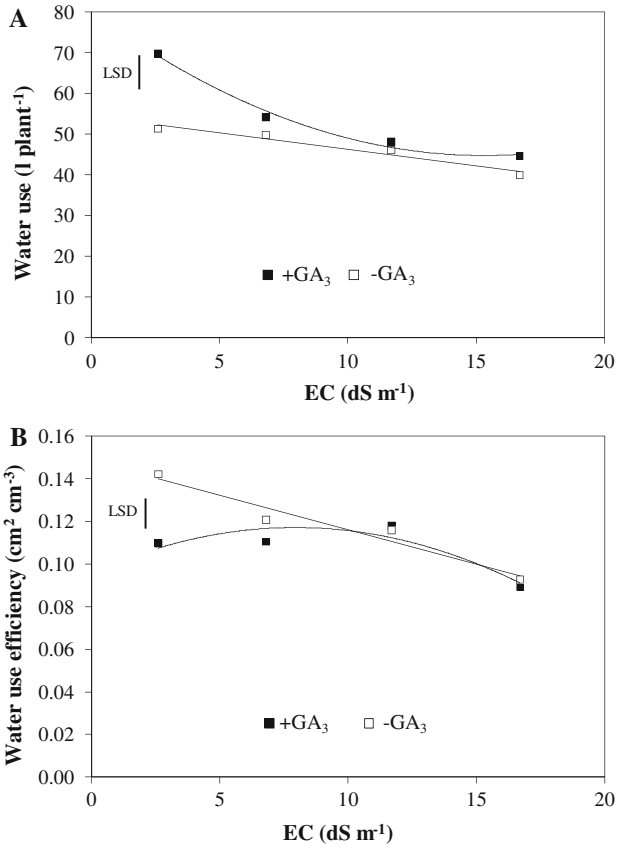

Fig. 1 Interaction GA_3 ($-GA_3$ non-GA_3-treated plants, $+GA_3$ plants treated with 100 mg GA_3 l^{-1}) × salinity treatments [as electrical conductivity (EC) of the nutrient solution (dS m^{-1} at 25°C)] on plant water use (**a**) and on water use efficiency (**b**). *LSD* least significant difference at $P \leq 0.05$. **a** $-GA_3$: $y = 0.246x + 6.358$ ($R^2 = 0.949$); $+GA_3$: $y = 0.1514x^2 - 4.6385x + 80.242$ ($R^2 = 0.987$). **b** $-GA_3$: $y = -0.0032x + 0.1484$ ($R^2 = 0.946$); $+GA_3$: $y = -0.0003x^2 + 0.0042x + 0.1033$ ($R^2 = 0.821$)

salinization, WUE decreased in non-GA_3-treated plants whereas it was rather stable in GA_3-treated plants.

Na^+ and Cl^- Contents

The leaf Cl^- and Na^+ accumulation in response to salinity was remarkably higher in GA_3-treated plants (Fig. 2), especially for Cl^-, whose concentration was doubled relative to the non-GA_3-treated plants at the highest salinity (EC = 16.7 dS m^{-1}). This was most likely associated with a reduced stomatal resistance to the transpirational water flux of GA_3-treated plants and, consequently, to a faster accumulation of Cl^- ions, which typically follow the transpirational stream (Hasegawa and others 2000). The relatively higher accumulation of Cl^- in $+GA_3$ with respect to $-GA_3$ leaves (Fig. 2) may have reduced transpiration on a whole-plant basis by anticipating leaf senescence and/or counteracting the positive effect of GA_3 on plant growth. This would explain the similar leaf area

Table 2 Leaf total (Ψ_{tot}), osmotic (Ψ_{π}), and pressure (Ψ_p) potentials in response to GA_3 and salinity treatments

Treatment	Ψ_{tot} (MPa)	Ψ_{π} (MPa)	Ψ_p (MPa)
GA_3			
$-GA_3$	−1.93	−2.51	0.58
$+GA_3$	−1.87	−2.37	0.50
	ns	ns	ns
Salinity			
S0	−1.65	−2.13	0.49
S1	−1.87	−2.30	0.44
S2	−1.96	−2.47	0.52
S3	−2.14	−2.87	0.73
	**	**	*
LSD	0.20	0.31	0.15
Interaction GA_3 × salinity	ns	ns	ns

EC of the nutrient solution = 2.5 (S0), 6.8 (S1), 11.7 (S2), and 16.7 (S3) dS m^{-1} at 25°C. Mean values of three sampling days (50, 92, and 141 DAT)

$-GA_3$ non-GA_3-treated plants, *$+GA_3$* = plants treated with 100 mg GA_3 l^{-1}, *LSD* least significant difference at $P \leq 0.05$, *ns* not significant

*, ** Significant at $P \leq 0.05$ and $P \leq 0.01$, respectively

Table 3 Leaf stomatal resistance (R_s) during the day in response to GA_3 and salinity treatments

Treatment	R_s (s cm^{-1})		
	Morning	Midday	Afternoon
GA_3			
$-GA_3$	1.88	1.88	2.29
$+GA_3$	1.55	1.62	1.93
	**	**	*
Salinity			
S0	1.26	1.32	1.77
S1	1.60	1.71	2.23
S2	1.86	1.90	2.08
S3	2.15	2.08	2.38
	**	**	*
LSD	0.30	0.28	0.24
Interaction GA_3 × salinity	ns	ns	ns

EC of the nutrient solution = 2.5 (S0), 6.8 (S1), 11.7 (S2), and 16.7 (S3) dS m^{-1} at 25°C. Mean values of three sampling days (50, 92, and 141 DAT)

Morning = 09:00 a.m., midday = 12:00 a.m., afternoon = 03:00 p.m

$-GA_3$ non-GA_3-treated plants, *$+GA_3$* plants treated with 100 mg GA_3 l^{-1}, *LSD* least significant difference at $P \leq 0.05$, *ns* not significant

*, ** Significant at $P \leq 0.05$ and $P \leq 0.01$, respectively

and shoot biomass of non-GA_3-treated versus GA_3-treated plants (Table 1), which we found despite their relatively lower stomatal resistance (Table 3).

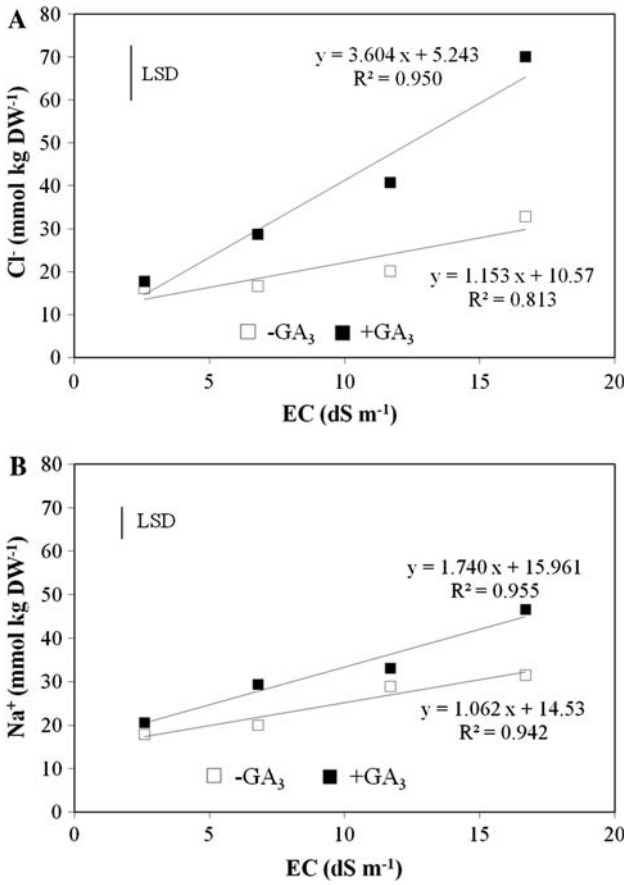

Fig. 2 Relationship between leaf Cl^- concentration (**a**) or leaf Na^+ concentration (**b**) and electrical conductivity of the nutrient solution [EC (dS m^{-1} at 25°C)] as affected by GA_3 treatment (*$-GA_3$* non-GA_3-treated plants, *$+GA_3$* plants treated with 100 mg GA_3 l^{-1}). *LSD* least significant difference at $P \leq 0.05$. **a** $-GA_3$: $y = 1.153x + 10.57$ ($R^2 = 0.813$); $+GA_3$: $y = 3.604x + 5.243$ ($R^2 = 0.950$). **b** $-GA_3$: $y = 1.062x + 14.53$ ($R^2 = 0.942$); $+GA_3$: $y = 1.740x + 15.961$ ($R^2 = 0.955$)

Tomato Yield

Salinity and GA_3 treatments significantly affected both the final tomato yield and its components (Table 4). The number of fruits and the total weight of tomatoes per plant were reduced at increasing EC of the nutrient solution, whereas these parameters were not affected by the GA_3 treatment itself. The significant interaction between GA_3 and salinity treatments revealed a positive effect of GA_3 on the number of fruits per plant (+14) but only at very low EC (Fig. 3). In contrast, salinity and GA_3 both reduced the fruit mean weight. GA_3 did not affect fruit quality parameters such as total soluble solids and dry matter percent, which instead were both increased at increasing salinity (Table 4).

Carotenoid and ABA Accumulation

The carotenoid content of tomato fruits was approximately 20% lower in GA_3-treated plants (Table 5). However, the

Table 4 Tomato yield, fruit weight, total soluble solids (TSS), and fruit dry matter percentage (DM) in response to GA_3 and salinity treatments

Treatment	Fruit yield		Fruit weight (g)	TSS[a] (°Brix)	DM[a] (g/100 g)
	(g plant^{-1})	(n. plant^{-1})			
GA_3					
$-GA_3$	825.9	68.7	12.1	9.7	12.2
$+GA_3$	767.6	72.1	10.5	9.7	10.9
	ns	ns	**	ns	ns
Salinity					
S0	989.4	76.7	13.2	8.8	10.9
S1	870.5	74.3	11.8	9.4	11.2
S2	747.0	65.8	11.2	10.1	11.8
S3	580.1	64.9	9.0	10.6	12.2
	**	**	**	**	**
LSD	102.2	6.0	1.2	0.4	0.5
Interaction GA_3 × salinity	ns	**	ns	ns	ns

EC of the nutrient solution = 2.5 (S0), 6.8 (S1), 11.7 (S2), and 16.7 (S3) dS m^{-1} at 25°C. The significant interaction between GA_3 and salinity is displayed in Fig. 3

$-GA_3$ non-GA_3-treated plants, *$+GA_3$* plants treated with 100 mg GA_3 l^{-1}, *LSD* least significant difference at $P \leq 0.05$, *ns* not significant

*, ** Significant at $P \leq 0.05$ and $P \leq 0.01$, respectively

[a] TSS and dry matter (DM) percentage are mean values of two harvests: January 7 (119 DAT) and January 31 (143 DAT)

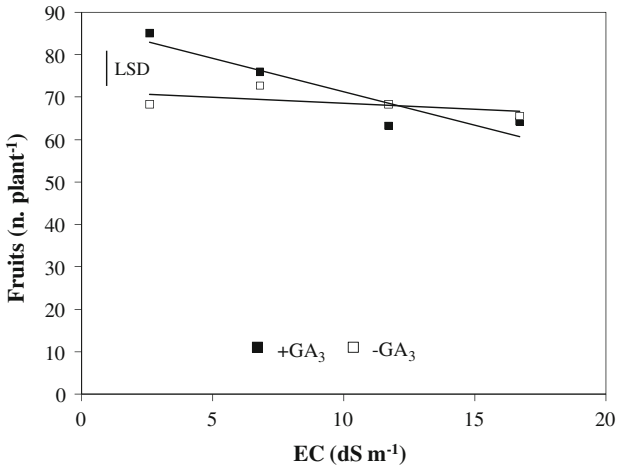

Fig. 3 Interaction of GA_3 (*$-GA_3$* non-GA_3-treated plants, *$+GA_3$* plants treated with 100 mg GA_3 l^{-1}) × salinity [as electrical conductivity of the nutrient solution (EC dS m^{-1} at 25°C)] on the number of fruits per plant. *LSD* least significant difference at $P \leq 0.05$). $-GA_3$: $y = -0.283x + 71.40$ ($R^2 = 0.346$); $+GA_3$: $y = -1.573x + 86.95$ ($R^2 = 0.858$)

patterns of carotenoid accumulation in response to salinity were quite different between the two sets of plants (Fig. 4). In non-GA_3-treated plants, the carotenoid concentration was relatively stable in response to salinity, with the exception of a slight reduction at the highest EC level. Conversely, the amount of carotenoids in GA_3-treated

Table 5 Carotenoid content of tomato fruits in response to harvest, GA_3, and salinity treatments

Treatment	Total carotenoids (mg 100 g^{-1} FW)
Truss[a]	
II	13.45
V	9.87
	**
GA_3	
$-GA_3$	12.90
$+GA_3$	10.43
	**
Salinity	
S0	11.64
S1	11.65
S2	11.90
S3	11.47
	ns
Interaction GA_3 × salinity	*

EC of the nutrient solution = 2.5 (S0), 6.8 (S1), 11.7 (S2), and 16.7 (S3) dS m^{-1} at 25°C. The significant interaction between GA_3 and salinity is displayed in Fig. 4

$-GA_3$ non-GA_3-treated plants, *$+GA_3$* plants treated with 100 mg GA_3 l^{-1}, *LSD* least significant difference at $P \leq 0.05$, *ns* not significant

[a] II truss harvested at 119 DAT; V truss harvested at 143 DAT

*, ** Significant at $P \leq 0.05$ and $P \leq 0.01$, respectively

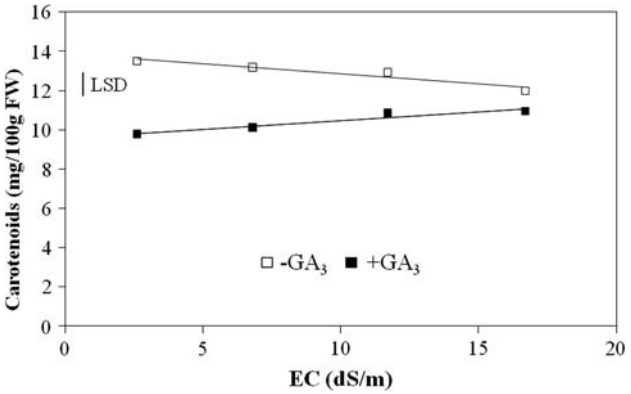

Fig. 4 Interaction of GA_3 ($-GA_3$ non-GA_3-treated plants, $+GA_3$ plants treated with 100 mg GA_3 l^{-1}) × salinity [as electrical conductivity of the nutrient solution (EC dS m^{-1} at 25°C)] on fruit carotenoid content. Mean values of two harvests: 119 and 143 DAT. *LSD* least significant difference at $P \leq 0.05$. $-GA_3$: $y = -0.102x + 13.86$ ($R^2 = 0.912$); $+GA_3$: $y = 0.0895x + 9.58$ ($R^2 = 0.920$)

plants, which was generally lower compared to non-GA_3-treated plants, gradually increased with increasing salinity. At 16.7 dS m^{-1} the carotenoid concentrations of $+GA_3$ and $-GA_3$ plants were similar.

The leaf ABA concentration increased with increasing salinity. However, the mean ABA concentration was generally lower in GA_3-treated plants, with significant differences at 16.7 dS m^{-1} (Fig. 5).

Yield Response to Salinity

Yield response to salinity was described according to the Maas and Hoffman linear model (Maas and Hoffman 1977): $Y_r = 100 - S(EC - T)$, where Y_r is the relative yield expressed as the percentage of the yield obtained in the nonsalinized control (S0; EC = 2.5 dS m^{-1} at 25°C); EC is the electrical conductivity of the nutrient solution; T is the EC threshold expressed in dS m^{-1} at 25°C, corresponding to the maximum value of EC that does not reduce yield; S is the slope expressed in % per dS m^{-1} that indicates the yield reduction percentage per unit increase in EC above the threshold.

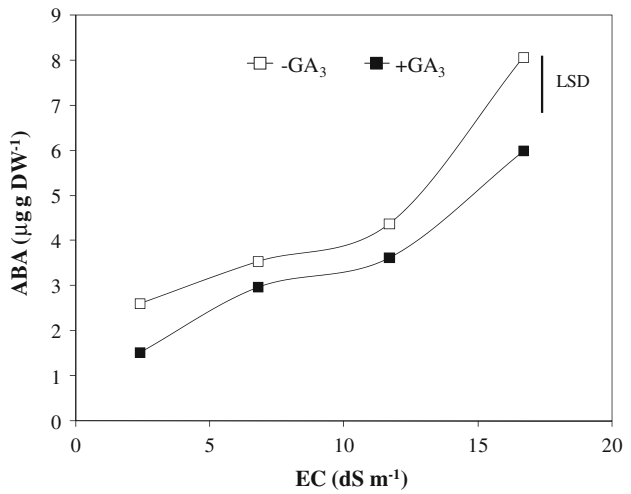

Fig. 5 Relationship between leaf abscisic acid (ABA) content on dry weight basis (DW) and electrical conductivity of the nutrient solution (EC dS m^{-1} at 25°C) as affected by GA_3 ($-GA_3$ non-GA_3-treated plants, $+GA_3$ = plants treated with 100 mg GA_3 l^{-1}). LSD = least significant difference at $P \leq 0.05$; ** = significant at $P \leq 0.01$ (the main effect of GA_3 treatment was significant at $P \leq 0.01$; mean values: $-GA_3$ = 4.65 mg g DW^{-1}; $+GA_3$ = 3.53 mg g DW^{-1})

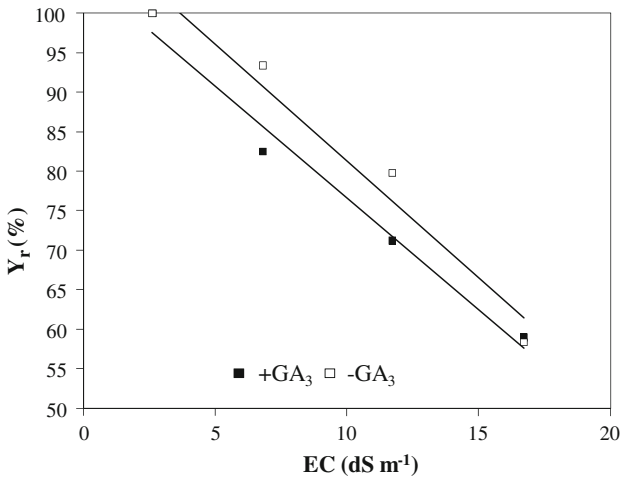

Fig. 6 Relative yield [Y_r (%)] response at increasing electrical conductivity (EC dS m^{-1} at 25°C) of the nutrient solution [$Y_r = 100 - S(EC - T)$; where Y_r is the relative yield expressed as percentage of the yield obtained in the nonsalinized control (S0; EC = 2.5 dS m^{-1} at 25°C); EC is the electrical conductivity of the nutrient solution; T is the EC threshold expressed in dS m^{-1} at 25°C, corresponding to the maximum value of EC that does not reduce yield; S is the slope expressed in % per dS m^{-1} at 25°C, that indicates the yield reduction percentage per unit increase in EC above the threshold] as affected by GA_3 treatment ($-GA_3$ non-GA_3-treated plants, $+GA_3$ plants treated with 100 mg GA_3 l^{-1}). $-GA_3$: $Y_r = 100 - 2.956(EC - 3.66)$; $R^2 = 0.962$; $+GA_3$: $Y_r = 100 - 2.833(EC - 1.74)$; $R^2 = 0.98$

GA_3 feeding through the irrigation water decreased the salinity tolerance threshold by approximately 50% (3.66 vs. 1.74 dS m^{-1}), but it did not significantly alter the yield reduction percentage per unit increase in EC above the threshold (Fig. 6).

Discussion

GA_3 Treatment Does not Mitigate Salinity-Induced Growth Reduction

Abiotic stresses such as drought and salinity are recurrent causes of reduced crop yield and quality in arid and semi-arid regions. Physiological responses to abiotic stresses are mediated by ABA, which accumulates and/or mobilizes to different tissues and organs to activate functional metabolic

components essential for plant adaptation to different developmental stages, including germination and vegetative growth (Zeevaart and Creelman 1988; Verslues and Zhu 2005). Although it has been demonstrated that cross-talk between ABA and other plant hormones mediates plant responses to different environmental conditions (Nemhauser and others 2006), control of their relative balance may have greater physiological significance compared to their absolute values (Ross and O'Neill 2001). We hypothesized that a controlled increase of the endogenous GA levels in tomato plants, obtained by feeding GA_3 to the plants through nutrient solution, could alleviate the growth reduction associated with both a stress-induced increase of ABA (Ruggiero and others 2004) and a decrease of GA levels (Magome and others 2008) without excessively impairing the adaptation process (Achard and others 2006).

Salinization of the root environment reduced plant growth and, consequently, plant water use. Despite a slight increase in total dry matter, the GA_3 treatment did not mitigate the salinity effects for the analyzed growth parameters (Table 1). These results partially disagree with those of Kaya and others (2006), who reported significant GA_3 protection of the shoot of drought-stressed maize plants. Because we observed a GA_3-dependent decrease in stomatal resistance, it is conceivable that salt-stressed plants may have been affected by a toxic ion accumulation in the shoots, which may not have occurred upon drought exposure (Kaya and others 2006). This possibility was confirmed both directly, by the interaction between GA_3 and salinity in terms of water use (Fig. 1), and indirectly, by the pattern of ion accumulation (Fig. 2). We found greater water use in GA_3-treated plants at low EC values, which is consistent with decreased stomatal resistance of these plants (Table 3). It would have been interesting to compare our data with those of Kaya and others (2006) for maize. Unfortunately, this was not possible because a GA_3 nonstressed control was not provided in their experiment. The constant difference in terms of stomatal resistance that we measured was apparently in contrast with the water use results that were relatively similar in GA_3-treated and non-GA_3-treated plants at high salinity (Fig. 1). This could be explained by considering that water use measurements on a whole-plant basis do not allow differentiation between fully versus partially functional or nonfunctional leaves, whereas stomatal resistance measurements are performed on the youngest, fully expanded leaves. The lower stomatal resistance of GA_3-treated plants also enhanced the leaf accumulation of Na^+ and Cl^- (Fig. 2). The accumulation of these ions in GA_3-treated plants reasonably explains the reduced plant water use observed at high salinity levels. Leaves of comparable age may have accumulated more ions in GA_3-treated plants compared with non-GA_3-treated plants due to their higher transpirational rates. This in turn may have anticipated the leaf senescence and/or loss of functionality (increased stomatal resistance) of $+GA_3$ plants compared with $-GA_3$ plants (Tables 1 and 3). The different stomatal resistances and transpirational water fluxes, however, did not alter the water potentials of young, fully expanded leaves (Table 2). This indicates that the control of stomatal closure is not the only mechanism for maintaining high leaf water potentials in hyperosmotic environments. Additional components, including the effect on cell enlargement and plant growth, may have contributed to the preservation of tissue hydration in GA_3-treated plants (Zhu 2001), as is also demonstrated by the relatively more stable WUE of these plants compared with non-GA_3-treated control plants (Fig. 1B).

Different results have been recently obtained by Levent Tuna and others (2008), who found that foliar applications of GA_3 in maize partially reversed the effects of salt stress. It must be considered that foliar spraying of GA_3 may cause partial and/or temporary stomatal closure that would delay the upload of toxic ions to the shoots and, in turn, the appearance of toxicity symptoms. In this case, the "induced" adaptation mechanism may be beneficial in early vegetative stages, yet it will likely have a negative impact on the final yield due to subsequent reduced photosynthetic activity.

Establishing a cause-effect relationship between growth reduction and plant stress adaptation is a longstanding challenge. It has been demonstrated recently that *Arabidopsis* plants actively reduce endogenous GA levels to repress growth during stress adaptation (Magome and others 2004; Achard and others 2006; Magome and others 2008). By using transgenic *Arabidopsis* plants, it has also been shown that a constitutive increase of tissue GA_3 may enhance growth under high salinity compared to wild-type plants. Although the GA feeding approach that we used functionally mimics the constitutive overproduction obtained via transgene technology, we did not observe a beneficial effect under saline stress. Our results may appear to contrast with the findings of Magome and co-workers, but they are actually in line with them, and, moreover, they provide a physiological basis for a coordinated role of GA and ABA in this process. In our experimental conditions, plant growth was assessed on actively transpiring plants and not under water vapor-saturated air as occurs in Petri plates or tissue culture boxes (Achard and others 2006; Magome and others 2008). In such microenvironments, the ABA-mediated stomatal contribution to plant growth and/or adaptation to hyperosmotic stress is marginal if not irrelevant (Ruggiero and others 2004). Consistent with our results, the improved growth of GA-overproducing transgenic plants (Magome and others 2008), earlier observed in *Arabidopsis* mutants with inactive downstream GA-regulated growth repressors (Achard and others 2006), may not

have been observed in soil experiments and/or in actively transpiring plants. This is expected if we consider that the phenotype of the quadruple DELLA mutant, described by Achard and others (2006), was similar to the ABA-insensitive *abi1-1* with respect to relative root growth. We have demonstrated previously that the ABA-deficient mutant *sto1/nced3* was salt tolerant under a saturated atmosphere (Petri plates), whereas it was salt sensitive in soil experiments conducted in a growth chamber (Ruggiero and others 2004).

An Altered Hormonal Balance Affects Fruit Quality and Stress Adaptation in Tomato Plants

Carotenoid concentration was higher in fruits harvested from the II truss compared with that from the V truss (Table 5). This was possibly because of both decreasing temperature and decreasing light intensity during the growing season (Dumas and others 2003). In terms of fruit quality, GA_3 affected the nutritional value of tomato fruits by reducing the carotenoid concentration (Table 5, Fig. 4). Carotenoid accumulation in response to salinity has been found to increase (De Pascale and others 2001), to remain rather stable, or to decrease (De Pascale and others 2007) depending on the environmental and experimental conditions and the level of stress imposed. The reduced carotenoid content of $+GA_3$ plants may be attributed to metabolic competition for common intermediates of carotenoid and GA biosyntheses (Hedden and Proebsting 1999; Olszewski and others 2002; Kopsell and Kopsell 2006), both stemming from the precursor geranylgeranyl diphosphate. At advanced salinization, the recruitment of part of the carotenoid pool for the biosynthesis of ABA (Botella-Pavia and others 2004) (Fig. 5) may have stimulated the biosynthesis of carotenoids that in GA_3-treated plants responded positively to salinization (Fig. 4).

The observed pattern of ABA accumulation in response to salinity (Fig. 5) was consistent with the earlier results of Wilkinson and Davies (2002). The sharp increase of ABA accumulation rates at EC greater than 10 dS m^{-1} mirrored that previously reported by Maggio and others (2007). This confirmed that an enhanced biosynthesis of ABA and/or an increased translocation from the roots to the shoots (Zeevaart and Creelman 1988) may occur after a specific stress threshold (Maggio and others 2007). The pattern of salt stress-induced ABA accumulation did not change in response to GA_3 treatment, although the latter caused a slight yet consistent reduction in leaf ABA concentration (Fig. 5). The generally lower ABA level of GA_3-treated plants delayed most ABA-mediated stress responses, including the activation of stomatal closure (Table 3). This event turned out to be beneficial in the absence of salinity stress (Fig. 3), yet it was detrimental at increasing salinity because it favored a faster transpiration-mediated accumulation of toxic ions to the shoot (Fig. 2). In previous experiments with salinized tomato plants, we showed that an increased leaf Cl^- concentration is associated mainly with a larger root biomass and a reduced leaf area rather than an increased transpiration flux because the latter generally decreases upon salinization (Maggio and others 2007). Although we did not provide a detailed analysis of root-to-shoot biomass distribution of GA_3-treated plants, we demonstrated that hormonal control of water fluxes may anticipate ($+GA_3 - ABA$) or delay ($-GA_3 + ABA$) the onset of the salt tolerance threshold (Fig. 6). For practical purposes, manipulation of the hormonal balance to improve plant salt stress tolerance should refer to the actual stress levels that the crop may realistically experience because an increased tissue GA level may have positive effects at low and moderate salinity, whereas it may have undesirable effects at moderate to high salinity.

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