ABA-overproduction response under salinity
Respuesta a la sobreproducción de ABA en salinidad

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Abstract

With the aim of better understanding the influence of the plan hormone abscisic acid (ABA) in adaptation to saline irrigation, two independent transgenic tomato (*Solanum lycopersicum* L.) lines, sp12 and sp5, overexpressing constitutively *NCED1* (the enzyme that catalyzes a key rate-limiting step in ABA biosynthesis) and the wild type Ailsa Craig, have been studied in experiments either i) as whole plants or ii) as rootstocks under control and salinity conditions. While *NCED* overexpression penalizes growth under control conditions, it minimized the effect of salinity (whole plants) or significantly improved plant growth and yield when used as rootstocks. The analysis of the root xylem sap revealed that the phenotypes resulting under the different conditions were difficult to explain in terms of ABA overproduction. With the aim of explaining these results, the expression of a set of hormone and stress associated genes (analysed by real time PCR) as well as a transcriptomic analysis (by using one-color microarray) were performed in roots. The results suggest that *NCED* overexpression seems to alter several signalling pathways leading to stress adaptive responses that could help to explain the observed phenotypes.

Keywords: phytohormone; transgenic; gene expression; microarray.

Resumen

Con el fin de comprender la influencia de la fitohormona ácido abscísico (ABA) en la adaptación al riego salino, dos líneas transgénicas independientes de tomate (*Solanum lycopersicum* L.), sp12 y sp5, que sobreexpresan constitutivamente el gen *NCED1* (codifica para la enzima que cataliza un paso limitante en la biosíntesis de ABA) y la variedad silvestre Ailsa Craig, se han estudiado en experimentos o bien i) como planta entera o ii) como portainjerto bajo condiciones control y de estrés salino. Aunque la expresión constitutiva de *NCED* disminuye el crecimiento bajo condiciones control, minimiza los efectos producidos por la sal (planta completa) y mejora significativamente el crecimiento cuando se usa como portainjerto. El análisis de la savia xilemática de raíz mostró que los fenotipos resultantes bajo las diferentes condiciones de cultivo eran difíciles de explicar en términos de sobreproducción de ABA. Para intentar explicar estos resultados se llevó a cabo un análisis de expresión de un conjunto de genes relacionados con hormonas y estrés mediante PCR cuantitativa, así como un estudio transcriptómico mediante microarrays en la raíz. Los resultados sugieren que la sobreexpresión de *NCED* parece alterar diversas rutas de señalización, derivando en una respuesta adaptativa al estrés que podría ayudar a explicar los fenotipos observados.

Palabras clave: fitohormona; transgénico; expresión de genes; microarrays.

1. INTRODUCTION

Saline irrigation water can limit plant growth and development of greenhouse tomato crops grown in Mediterranean environments with the plant hormone abscisic acid (ABA) playing a key role in adaptation to abiotic stress. Overexpression of genes that respond to drought or salinity stress is an attractive approach for improving resistance in crops. The recent production of transgenic plants overexpressing ABA biosynthesis genes, specifically *NCED* (*cis*-epoxycarotenoid dioxygenase) provides a new tool to investigate ABA's role in growth regulation, but the responses of these plants to salinity have not been characterized. Understanding the biochemical and physiological responses to salinity provides a framework to identify breeding targets for improving salt tolerance [1].

The aim of this work was to evaluate if the use of ABA-overproducing tomato plants could improve growth when they were used as rootstocks.

2. MATERIALS Y METHODS

The plant material used was the tomato wild-type *Solanum lycopersicum* L. cv Ailsa Craig (AC), and two independent ABA-overproducing transgenic lines, sp12 and sp5, overexpressing the *NCED1* gene expressed constitutively under the control of the Gelvin superpromoter [2]. Seeds of the 3 genotypes were sown in trays filled with a vermiculite moistened regularly with half-strength Hoagland's nutrient solution.

2.1 Short-term hydroponic experiments.

At the stage of 2-3 true leaves seedlings were transferred to a hydroponic culture by using 20 L plastic trays containing half-strength Hoagland nutrient solution. The growth chamber conditions were settle to 16-h day and 8-h night (photon flux density was was 245 μmol m−2 s−1) and 50%-60% relative humidity. After 1 week of acclimatization in control conditions, plants seedlings were exposed to 0 and 100 mM NaCl added to the nutrient solution for 21 days. Shoot fresh weight were determined in 4 replicates for each genotype and salt treatment. Xylem sap was collected using a Scholander-type pressure chamber. Leaves and xylem sap were kept at -80ºC prior to hormonal analyses. Hormone ABA concentrations were analyzed in the xylem sap and in the mature leaf by UHPLC-MS according to Albacete et al. (2008) [3]. Gene expression was analysed according to Ferrández-Ayela et al [4].

2.2 Long-term greenhouse experiments

Seed of AC, sp12 and sp5 were sown and grown as described in above, and used as rootstocks. A commercial cherry tomato variety, Sugar Drop -SD- (Unigenia, Murcia, Spain) was used as a scion. Grafting was performed using the splicing method at the two to three true leaf stages (3–4 weeks after sowing), as is common commercially, and the scion was attached at the first node of the rootstock. When the grafted plants were well established, they were cultivated in a semi-hydroponic system using sand as substrate under commercial greenhouse conditions during autumn-winter in Almería (Spain). Fertilizers and water were supplied by a drip fertirrigation system (as is common in commercial tomato production). From 40 days after transplanting, a low salinity treatment (EC= 3.5 mS cm^{-1}) was applied for a period of 200 days. Shoot fresh weight were determined in 6 replicates for each genotype. Xylem sap was collected using a Scholander-type pressure chamber. Leaves and xylem sap were kept at -80ºC prior to hormonal analyses. Hormone ABA concentrations were analyzed as described in 2.1. Transcriptomic analysis was performed according to Ferrández-Ayela et al. [4].

3. RESULTS AND DISCUSSION

NCED overexpression in the transgenic lines decreased growth under control conditions (Fig. 1A) by 32% and 44% in sp12 and sp5 respectively (compared to WT plants). The salinity treatment applied for 21 days significantly decreased shoot growth in all genotypes (Figure 1A) by 67%, 50% and 53% in WT, sp12 and sp5 respectively. Thus *NCED* overexpression decreased shoot biomass accumulation under control conditions, but maintained it under salt conditions. The decreased in growth was correlated with an increase in ABA according to bibliography [2]. Transgenic lines sp12 and sp5 showed higher ABA concentration, by 1.3 and 1.6-fold of the WT in leaf (Figure 1B), and by 2.2 and 4.3-fold of the WT in root xylem sap (Figure 1C). Salinity increased ABA concentration in all genotypes around 30% as an average (Figures 1B and 1C). The rise in ABA concentration under 100mM NaCl occurs within a day of salinity treatment [1, 3].

When the transgenic lines were used as rootstocks in long-term experiments (Figure 1D), shoot FW was higher by 2.1 and 1.8-fold of the WT for sp12 and sp5 respectively. However this increase in growth was not correlated with a rise in the ABA concentration in the leaf (Figure 1E) or in the leaf xylem sap (Figure 1F), notwithstanding *NCED* was overexpressed (Figure 2). *NCED* overexpression alters the expression of different genes (Table 1) involved in osmotic adjustment and stress protection (upregulation of *TAS14* and *AQP2*), ABA metabolism (downregulation of *ZEP1*, *AOO/FLL* and *CYP707A*) and other hormone associated genes (upregulation of *ACs1a* and *JA2*) which could be involved in the plant response to salinity. Further gene expression analysis in long-term experiments (data not shown) revealed similar results for some genes such as *TAS14* or *JA2*. A comprehensive analysis of physiological and transcriptomic data is currently ongoing to explain the root-ABA signalling mediated phenotypes observed.

4. CONCLUSIONS

The results of this study suggest that *NCED* overexpression and early ABA accumulation in tomato roots seems to alter several plant hormone metabolism and signalling pathways leading to local and systemic stress adaptive responses.

5. ACKNOWLEDGEMENTS

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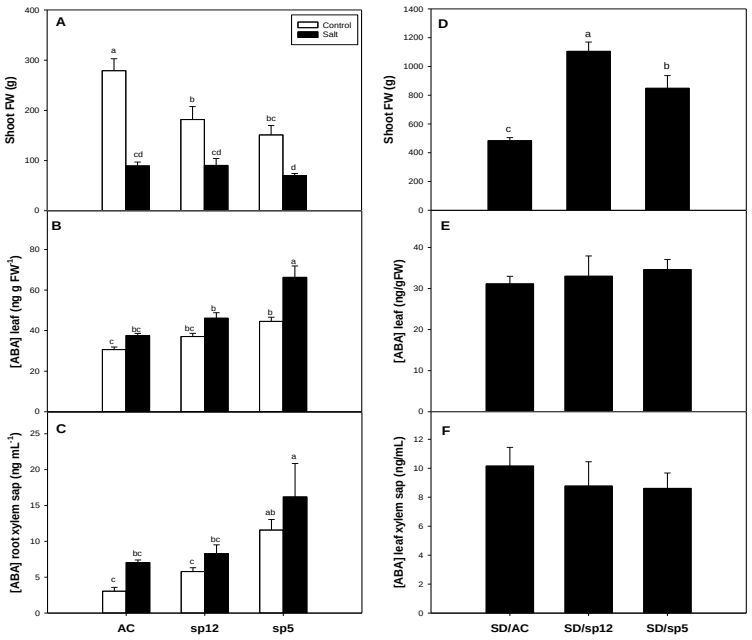

Figure 1. Final shoot FW (A), ABA concentration in the leaf (B) and in the root xylem sap (C) of the WT and the two transgenic lines after 21 days of hydroponic culture under control and salinity (100 mM NaCl) treatment. Final shoot FW (D), ABA concentration in the leaf (E) and in the leaf xylem sap (F) of grafted tomato plants with AC, sp12 and sp5 as rootstocks grown in a commercial greenhouse for 200 days under 3.5dS/m salinity treatment. Data are means ± SE of 4-5 replicates. Letters above bars indicate significant differences as determined by Tukey pair-wise analysis.

Table 1. Gene expression in roots of AC and sp12 tomato plants grown hydroponically under control and salinity (100mM NaCl) during 11 days

Description	Gene annotation	Relative gene expression			
		WT		sp12	
		Control	Salt	Control	Salt
Osmotic adjustment	*TAS14*	1	70	3	94
	AQP2	1	3.2	2.5	3.2
ABA metabolism, homeostasis and signalling	*ZEP1*	1	3	0.8	1.5
	AAO/FLC	1	7.4	3	2
	CYP707A	1	251	1.5	2.5
Ethylene and JA metabolism	*ACs1a*	1	18.4	1	31.8
	JA2	1	285	122	300

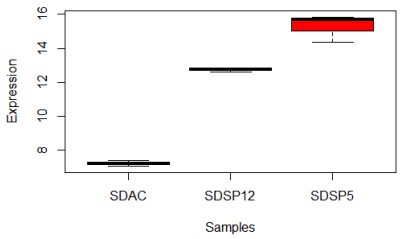

Figure 2. Expression values of Solyc07g056570.1.1, corresponding to *LeNCED1* gene in roots of grafted plants cultivated in commercial greenhouse for 200 days under 3.5 mS/cm EC.