

Interaction between ABA and cytokinins in salinized tomato plants

A. Martínez-Pérez¹, G. Bahar Oztekin², A. Albacete¹, A.J. Thompson³,
M.P. Sánchez-Iglesias¹, F. Pérez-Alfocea¹ C. Martínez-Andújar¹

¹ Consejo Superior de Investigaciones Científicas, CEBAS-CSIC, Murcia, Spain.

e-mail: amperez@cebas.csic.es

²Ege University. Faculty of Agriculture. Department of Horticulture. 35100. Bornova-Izmir. Turkey

³Cranfield Soil and Agri-Food Institute, Cranfield University, United Kingdom.

Abstract

Abscisic acid (ABA) and cytokinins (CKs) are phytohormones that mediate plant responses to abiotic stress and are mainly considered to be produced in roots. These two plant hormones have long been considered antagonists, but the role of this antagonism in mediating abiotic stress resistance remains unknown. In this study we have used reciprocal grafting of transgenic lines with altered hormone synthesis (ABA or CK synthesis) with the aim of assessing the importance of ABA and CK interaction in mediating plant salinity responses in tomato. Preliminary results have shown a negative effect in ABA and CK on tomato plant biomass under salinity. Results have also indicated that jasmonic acid (JA) might be involved in the negative impact on tomato growth caused by ABA and CK interaction under salt conditions.

Keywords: Transgenics; phytohormones; grafting; hydroponic.

1. Introduction

Salinity is a major factor limiting plant growth and crop productivity. Major effects of salt stress on plants include partial stomatal closure, nutrient imbalances, altered metabolism to divert assimilates to the roots, growth inhibition, leaf senescence and ultimately abscission. As sessile organisms, plants must sense their environment and generate systemic signals (by the tissues exposed to stress) to coordinate plant metabolic and developmental adjustments. Salinity does not only change root hormone concentration [1], but it also alters root to shoot hormone signaling [2] which affects shoot physiology. This group of hormones includes abscisic acid (ABA) and cytokinins (CKs), which are mainly considered to be produced in roots. ABA plays a role to control leaf stomatal conductance, plant growth and adaptation to water deficit and salinity, while CKs play an active role in the control of leaf growth, leaf senescence, shoot and root balance and nutritional signaling. Most studies about the role of plant hormones in stress physiology have focused on the "stress hormones" ABA and ethylene and have ignored other possible interactions. ABA and CKs have long been considered antagonists. CKs increase stomatal aperture and/or delay ABA-induced stomatal closure [3]. ABA-mediated stomatal closure decrease cytokinin transport to the shoot under salinity [4]. ABA can enhance CKs catabolism by increasing cytokinin oxidase (CKX) activity [5] [6].

Transgenics overexpressing *IPT* decreased or had no effect [7] on ABA concentrations, while the CKs status of transgenic overexpressing *NCED* have not been measured. In this work, we have used reciprocal grafting between transgenic tomato plants overproducing either ABA or CK, with the aim of determining whether ABA-CK interaction is important in mediating plant biomass under salinity.

2. Materials & Methods

Two independent sets of tomato seeds (*Solanum lycopersicum* L.) either overexpressing genes encoding enzymes involved in ABA biosynthesis (*NCED*, line termed *sp12* [8]) or cytokinin biosynthesis (*IPT*, line termed *ipt*) and the respective WT (cv Ailsa Craig (AC) for *NCED* and cv UC82B (UC82) for *IPT*) were sown. Between 3 and 4 weeks after sowing (depending of germination performance of each genotype), reciprocal grafting between different transformant was performed resulting in the following 8 scion-rootstock combinations: AC/UC82, AC/*ipt*, *sp12*/*ipt*, *sp12*/*sp12*, UC82/AC, UC82/*sp12*, *ipt*/AC and *ipt*/*sp12*. One month later, when grafted plants were well established, they were transferred to a hydroponic culture by using 20 L plastic trays containing half-strength Hoagland nutrient solution. After 1 week of acclimatization in control conditions, plants seedlings were exposed to 100 mM NaCl added to the nutrient solution for 21 days. Root and

shoot fresh weights were determined in 4 replicates for each graft combination. Total leaf area was analyzed and gas exchange parameters (transpiration rate, stomatal conductance and photosynthesis) were monitored. Xylem sap was collected by applying a pneumatic pressure slightly greater (0.2 MPa) than leaf water potential using a Scholander-type pressure chamber. Leaves and xylem sap were kept at -80°C for ionic and hormonal analyses. Ionic quantification (K^+ and Na^+) in xylem sap was performed in convenient dilutions using an atomic absorption spectrophotometer and hormone concentrations (abscisic acid (ABA), jasmonic acid (JA), the cytokinins zeatin (Z), zeatin-riboside (ZR), and isopentenyl adenine (iP)) were analyzed in the xylem sap and in the mature leaf by UHPLC-MS.

3. Results and Discussion

Results have shown that regardless the transgenic line or WT used as scion; the leaf area and total fresh weight of grafted plants with background UC82B as rootstocks were significantly higher than those with background AC. The use of transgenic, either as a rootstock or as a scion alone, did not cause any significant effect of growth. The reciprocal grafting between transgenic plants (*sp12* and *ipt*) resulted in a significant growth reduction (Figure 1).

Sp12/ipt and *ipt/sp12* graft combinations exhibited the highest ABA concentrations in leaf and xylem sap, respectively. Leaf and xylem CK concentrations were increased when AC was used as rootstock onto UC82 and when *sp12* was grafted onto UC82, in comparison with the remaining graft combinations. Interaction between *sp12* and *ipt* was not found on both leaf and xylem CK concentrations. The highest JA concentrations in xylem sap were registered in reciprocal grafting between *sp12* and *ipt* (Table 1). Furthermore, a negative correlation between xylem JA concentrations and total fresh weight ($r=-0.63$, $p<0.01$, $n=28$), and a positive between leaf JA and total fresh weight were found ($r=0.37$, $p<0.1$, $n=28$).

PCA was performed in order to gain insights about the contribution of the physiological parameters to the plant biomass-related attributes. The principal components accounted for 43.9% of the total variance. Fresh weight parameters (TFW, RFW and SFW) were explained by the main principal component (PC1), in which leaf area (LA) and JA concentrations in leaf were associated. The PCA also showed that gas

exchange parameters (transpiration rate and stomatal conductance) covaried with ABA and JA concentration in xylem sap (PC2) which accounted for 17.90 % of the total variance (Figure 2).

Previous works have indicated that selective *IPT* overexpression in roots improved the growth of tomato plants under salinity conditions [4]. Moreover, in a separate experiment, we have found a growth improvement under salinity when *sp12* is used as rootstock (data not shown). However, in this study, the use of transgenic, either as a rootstock or as a scion alone, did not cause any significant effect of growth, this may be due to the fact that different WT genetic backgrounds were used in this experiment. What remains clear in our experimental conditions is that a negative interaction between ABA and CKs seems to occur. The positive correlation found between JA concentration in leaf and SFW points out the JA involvement in the plant biomass reduction found in reciprocal *sp12* and *ipt* grafting. In line with these results, LA, TFW, SFW and RFW were associated to JA concentration in mature tomato leaf as regards the PCA. It has been described that salt tolerant tomato plants increased endogenous level of JA [9]. Moreover, Zhao et al. [10] reported that *AOC* constitutive expression, an enzyme involved in JA synthesis, induced JA contents and improved salinity tolerance in both wheat and *Arabidopsis*. These authors also provided evidence for the existence of a JA-related pathway responsible for salinity tolerance. Our study has also shown that ABA content in xylem sap was correlated with transpiration and stomatal conductance, in contrast to what was expected. Interestingly, reciprocal grafting between transgenic *sp12* and *ipt* plants registered the highest JA concentration in xylem sap, which was correlated with xylem ABA content and gas exchange parameters. In addition to the ABA-CK interaction, a cross talk between ABA and JA pathway signaling may occur in reciprocal grafting between *sp12* and *ipt* plants. Indeed, it has been suggested that there is a link between ABA and JA-mediated salinity response pathways [11].

4. Conclusions

The effects of the NCED and IPT overexpression separately reported in previous works have shown positive effects, but maybe the fact of using different genetic backgrounds in the reciprocal grafting can make these results not completely conclusive. Further research using

similar genetic backgrounds is still being necessary in order to understand the mechanisms underlying ABA and CK interaction and the possible JA involvement in this process under salinity conditions.

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Tables and Figures

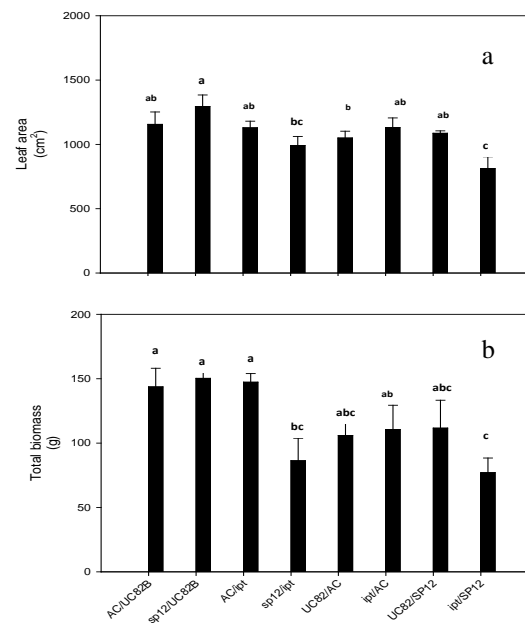
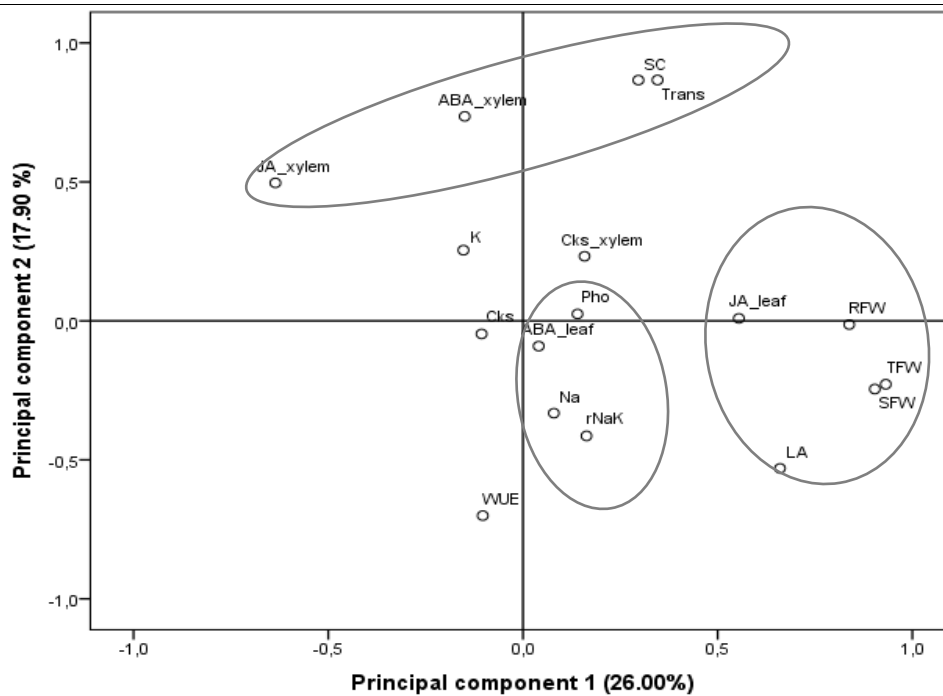


Fig. 1. Leaf area (a) and total fresh weight (b) in reciprocal grafting between UC82, AC, ipt and sp12 plants after 21 d under salinity (100 mM NaCl). Data are means of four plants. Different letters indicate that differences between grafting combinations are significant according to Tukey's test at $P < 0.05$.

Table 1. Concentrations of abscisic acid (ABA), total cytokinin (CKs; cytokinins zeatin (Z) + zeatin-riboside (ZR) + isopentenyl adenine (iP)) and jasmonic acid (JA) in the xylem sap and mature leaf of the reciprocal grafting between UC82, AC, ipt and sp12 plants after 21 d under salinity (100 mM NaCl). Data are means of four plants. Different letters indicate that differences between graft combinations are significant according to Tukey's test at P<0.05.

	ABA		CKs		JA	
	Xylem	Leaf	Xylem	Leaf	Xylem	leaf
AC/UC82	11.22 ab	50.49 ab	97.11 a	54.14 ab	110.50 bc	46.80 ab
Sp12/UC82	13.00 ab	45.05 abc	63.13 abc	46.67 bc	43.52 b	28.96 ab
AC/ipt	10.10 b	46.33 abc	69.20 abc	40.88 bc	33.57 b	72.52 a
Sp12/ipt	17.10 ab	57.43 a	53.45 bc	47.39 bc	225.21 ab	32.58 ab
UC82/AC	18.08 ab	46.68 abc	60.85 abc	31.19 c	212.63 ab	38.99 ab
ipt/AC	11.17 ab	35.34 bc	45.95 c	50.44 ab	100.39 bc	30.35 ab
UC82/sp12	21.27 ab	34.25 c	91.85 ab	66.90 a	90.56 bc	49.06 ab
ipt/sp12	23.77 a	40.75 bc	86.58 ab	45.33 bc	317.83 a	26.66 b

Fig. 2. Two principal components analysis (PCA) plot showing the relationship between physiological variables. The x-axis represents Principal component 1 (26.00%) and the y-axis represents Principal component 2 (17.90%).



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