# **UC Davis**

The Proceedings of the International Plant Nutrition Colloquium XVI

### Title

Does jasmonic acid control the maize shoot growth during the first phase of salt stress?

### Permalink

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## **Publication Date**

2009-08-07

Peer reviewed

#### Introduction

Salt stress affects plant growth in two separate phases, osmotic stress and ion toxicity (Munns et al 1993). The reduction in rate of leaf expansion in response to osmotic stress occurs by decreasing the external osmotic potential (low water availability). The leaf expansion depends on maintenance of cell turgor by water uptake and modifications in cell wall components. Regulation of cell turgor and changes in cell wall properties by root-sourced signals (e.g., hormones, pH) in response to salt/drought stress is not completely understood. Jasmonic acid (JA) is involved in plant adaptations to biotic and abiotic stresses and accumulates transiently in response to osmotic/salt stress (Creelman and Mullet 1995, Lehman et al. 1995). We investigated the biosynthesis of JA in response to salt stress and its effect on shoot growth in two maize genotypes with differences in salt-stress resistance.

#### Materials and methods

Two maize genotypes (*Zea mays* L.), the salt-sensitive Across 8023 and resistant hybrid SR 03 (Schubert and Zörb 2005) were cultivated in nutrient solution under controlled conditions for 18 d. The salt-treated plants were gradually adapted to a maximum stress of 100 mM NaCl with 25 mM increments over a period of 48 h. The corresponding control was grown with 1 mM NaCl, and each treatment was run in four replicates. The plants were harvested 84 h after the start of salt treatment for JA analysis.

The analysis of jasmonic acid followed the previously described protocol of Müller et al. (2002). Root (300 mg) and shoot (1 g) tissue samples were extracted with pre-warmed (60 °C) methanol (1 ml each), supplemented with 200 pmol and 50 pmol, respectively, of  $[^{13}C]_2$ -JA (internal standard) and incubated under shaking for 3 min at 60 °C. Incubation was continued for 1 h under shaking at 25 °C. Cell-free supernatants were dried under vacuum for subsequent gas chromatography-tandem mass spectrometry analysis (GC-MS/MS).

To investigate the effect of exogenous JA on shoot growth, 6 d old seedlings were transferred to quarter-strength nutrient solution supplemented with 30  $\mu$ M JA. Extension in shoot growth was measured daily and seedlings were harvested 3 d after the start of treatment.

#### **Results and Discussion**

Salt stress reduced the shoot growth of Across 8023 significantly (by 30 % in comparison to control, Fig. 1). Shoot growth of SR 03 was also reduced (by 17%, Fig. 1), but statistically it was not significant. Root growth of both genotypes was not affected by salt stress. The levels of endogenous jasmonic acid in SR 03 plant tissues (Fig. 2) were not altered by osmotic stress. In Across 8023, shoot JA also did not respond to the stress but an increase of JA by a factor of 2 in roots was observed (Fig. 2). The question arises if differences in JA production in maize genotypes of contrasting salt resistance can contribute to osmotic stress resistance. We speculate that a rise of JA in roots of the salt-sensitive genotype may contribute to salt sensitivity in maize and may control the shoot growth.

Root growth inhibition by exogenous treatment of jasmonic acid is well documented in the literature (Creelman and Mullet 1995), but the effects of exogenous JA-treatment of root medium on shoot growth are not clear. Treatment of 30  $\mu$ M JA in nutrient medium significantly stunted the maize seedling growth (Fig. 3).



Figure 1: Effect of salt stress on (A) shoot fresh weight and (B) root fresh weight of two maize genotypes. Each column is the mean of four replicates. The error bars indicate the 95% CIs for comparison of the two salt treatments within each genotype.



Figure 2: Effect of salt stress on free jasmonic acid concentration of (A) shoot and (B) root of two maize genotypes. Each column is the mean of four replicates. The error bars indicate the 95% CIs for comparison of the two salt treatments within each genotype.

All growth parameters such as shoot extension rate, root length, and shoot and root fresh weights were significantly affected by exogenous JA (Tab. 1). The effects of JA on shoot extension were clearly visible 2 d after treatment (Fig. 3). Results indicate that exogenous JA-treatment of roots inhibits shoot growth, but the role of endogenous JA in the control of shoot growth under osmotic stress is still to be elucidated.

	Shoot extension rate (cm d <sup>-1</sup> )	Root length (cm)	Shoot fresh wt. (g)	Root fresh wt. (g)
Control	4.08 ± 0.21	30.60 ± 0.37	$\begin{array}{c} 1.30 \\ \pm \ 0.05 \end{array}$	$\begin{array}{c} 1.10 \\ \pm \ 0.05 \end{array}$
30 µM JA	$\begin{array}{c} 2.39 \\ \pm \ 0.08 \end{array}$	$\begin{array}{c} 19.36 \\ \pm \ 0.35 \end{array}$	$\begin{array}{c} 0.76 \\ \pm \ 0.02 \end{array}$	$\begin{array}{c} 0.60 \\ \pm \ 0.02 \end{array}$

Table 1: Effect of  $30 \,\mu\text{M}$  JA on maize seedling growth. Data points are means of four replicates of two seedlings each.



Figure 3: Effect of  $30 \mu M$  JA on maize seedling growth (A) and shoot extension (B). Data points are means of four replicates of two seedlings each.

#### Conclusion

The differences in JA biosynthesis in genotypes of contrasting salt resistance indicate that jasmonic acid has a role in plant adaptation to osmotic stress and increased JA in roots of salt-sensitive genotype may be responsible for shoot growth inhibition.

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