Effect of Jasmonic Acid on Growth and Ion Relations of *Oryza sativa* L. Grown under Salinity Stress

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ABSTRACT

Rice (*Oryza sativa* L.) is considered salt sensitive compared to other cereals. Recent transcriptome studies on salinity stress response in barley revealed indicates that exogenous application of jasmonic acid (JA) can ameliorate growth reductions brought about by salinity stress. It is hypothesized that JA plays a role in adaptation of some plants to salinity stress. In this study, we investigated whether or not application of exogenous JA to salt-sensitive rice (*indica* cultivar IR29) can also ameliorate salinity stress. All three treatment employed, salinity, JA pretreatment, and JA-pretreatment salinity-stress reduced growth as measured by shoot and root dry weight of shoot and root length. JA pretreatment prior to salinity stress did not appear to ameliorate growth reductions due to salinity stress. Gas-exchange responses were also compared after salinity stress, JA pretreatment, and JA pretreatment followed by salinity stress. Consistent with our finding on growth parameter, pretreatment with JA did not alleviated photosynthetic inhibition caused by salinity stress. Also, the JA-pretreated salt stressed plants accumulated dramatically higher levels of Na^+ in the shoot tissue compared to untreated salt-stressed plants. Ions such as Ca^{2+} and Cl^- also increased while other ion such as Mg^{2+} and K^+ decreased. We detected no change in S levels. Analysis of selectivity constants for K:Na, Ca:Na, K:Mg, and Ca:Mg indicated that ion homeostasis was greatly affected by JA pretreatment. We concluded that JA pretreatment in rice does not ameliorate salinity stress, possible due to increased accumulation of toxic ions and changes in ion homeostasis.

INTRODUCTION

High-quality water needed for agriculture is becoming increasingly scarce due to changing environmental standards and rising demands from urban areas. Any future expansion of irrigated agricultural production will need to include the use of waters containing higher levels of soluble salts..

Unfortunately, two of the world major cereals, rice (*Oryza sativa* L.) and corn (*Zea mays* L.) are sensitive to salts in the rootzone. This is somewhat surprising in that most of the major cereals belonging to the grass family show moderate to high salt tolerance. The grass family is very large and contains more physiological, morphological and genetic diversity than just the major cereal crops. Recent investigations focused on the plant genome revealed that there are extensive similarities among the cereals and other grasses such that a gene on the chromosome of one grass species can be anticipated to be present in a predicted location on a specific chromosome of a number of other grass family species. Recognition of the close genetic relationship among the cereals indicates that salt-tolerant cereals may possess valuable genetic information which the plant breeder may exploit in crops such as rice and maize.

Recently, in salt-tolerant barley the role of JA in osmotic/salinity stress treatment has been the focus of several. In fact, a role for JA in improving the adaptive response of plants to salt stress has been proposed. These investigators found that pretreatment of barley plants with JA before salinization ameliorated the inhibitory effects of salinity on both photosynthesis and growth when compared to a salt stress only. The authors suggested that JA pretreatment facilitate adaptation to high salt stress by increasing photosynthetic membrane stability. Salt stress also increased the JA levels in roots of rice plants.

In this study, we focused on the effect of exogenous JA on ion accumulation in a salt-sensitive rice, IR29.

MATERIALS AND METHODS

Plant Material

The experiment was conducted under natural sunlight in a greenhouse located at the U. S. Salinity Laboratory, Riverside, CA (33°, 58', 24" N. latitude; 117°, 19', 12" W. longitude) February and April, 2006. Temperature settings of 35 C day and 25 C night were controlled by evaporative coolers and heaters, while relative humidity in the greenhouse was uncontrolled.

Seven days after initiation of germination, germinated seeds were transferred onto two circular plastic grids with cheese cloth sandwiched between the grids. The grids were placed on plastic tanks (volume 20 liters) containing deionized water. Twelve days later, nutrient solution nutrient solution (Yoshida et al., 1976) was added. The nutrient solution consisted of NH_4NO_3 (1.43 mM), $NaH_2PO_4.2H_2O$ (0.37 mM), K_2SO_4 (0.5 mM), $CaCl_2$ (1.00 mM), and $MgSO_4 7H_2O$ (1.6 mM). Nutrient solution pH was maintained between 5.0 and 6.5 by adding sulfuric acid as needed. Electrical conductivity, pH and solution temperature were monitored daily. Tanks were topped daily with deionized water to replace losses due to evapotranspiration. Reflective insulation material was used to keep the nutrient solution temperature between 21°C and 25 °C.

The jasmonic acid (JA) pretreatment was initiated on day 46 after initiation of germination by transferring the tanks into an adjacent greenhouse maintained at similar conditions and adding jasmonic acid (J2500; Sigma-Aldrich, St. Louis) dissolved in water to a final concentration of 12 µM in the tank. The transfer of the tanks was done to avoid JA-volatiles from affecting the expression of the control and salinity stressed plants. On day 48 after initiation of germination, a salinity stress of ~6.0 dSm^{-1} was imposed by adding enough $CaCl_2$ and NaCl to maintain a molar ratio of Na^+: Ca^{2+} concentration ratio of 5:1. Two days later, $CaCl_2$ and NaCl was again added to achieve a final salinity stress of ~7.5 dSm^{-1} (Na^+:Ca^{2+} concentration ratio of 5:1 on a molar basis). On day 73 after initiation of germination, shoot and root tissue was harvested for fresh and dry weight, and ion analyses. Tissue from 10 plants from each tank constituted a single replicate of a treatment. Two independent biological replicates of the experiment were conducted.

Plant Ion Analysis

The irrigation waters were analyzed weekly using inductively-coupled plasma optical emission spectrometry (ICPOES) to confirm that target ion concentrations were maintained. Plant tissues were washed to remove surface salts, dried at 60 C to a constant weight, and_weighed. Oven-dried shoots were ground and stored in acid-washed vials. Total-S, total-P, Ca^{2+}, Mg^{2+}, Na^+ and K^+ were measured on nitric-perchloric acid digests of shoot tissue by ICPOES. Chloride was determined by coulometric-amperometric titration.

RESULTS

Growth Responses

In our system, IR29 displayed typical salt sensitivity (Table 1). Salinity stressed resulted in a reduction of both shoot and root dry weight.

This reduction was not reversed by pretreatment with JA. In fact, the JA-pretreatment salinity stressed plants had significantly lower shoot dry weights than salinity stress plants. Salinity stress also increased the shoot: root ratio compared with controls. This observation was not reversed by JA pretreatment.

Overall, it appears that all three treatments: salinity stress, JA-pretreatment, and JA-pretreatment salinity-stress reduced growth in IR29.

Gas-exchange Responses

We measured gas-exchange properties 2 days and 12 days after final imposition of salinity stress. Initially, the photosynthetic rate declined in response to all three treatments when compared to control plants (Table 2).
Salt-stressed plants maintained a photosynthetic rate about half that of the controls. We found further decreases in the JA-pretreated (~66% reduction) and JA- pretreated salinity plants (~75% reduction). Salinity stress reduced the stomatal conductance drastically (61%). Jasmonic acid treatment, alone or with salinity, decreased the stomatal conductance of the plants by roughly 73% compared to controls.

The transpiration rate measurements correlated well with the stomatal conductance values for all three treatments and controls. With respect to gas exchange, these data indicate that, initially, JA treatment prior to salinization, with or without subsequent salinization, can have negative effects of salinity stress in rice. However, by day 12 after final salinization, photosynthesis in the JA-pretreated rice recovered to near-control levels with similar increases in stomatal conductance and transpiration (Table 3). However, JA pretreatment did not have an ameliorative effect on gas-exchange properties under salinity stress as all values continued to decline by day 12.

Ion interactions

The shoot Na^+ concentrations were measured 73 days after germination. We found that JA-pretreated salinity stressed plants maintained a higher Na^+ concentration compared with salinity stressed plants.
Also, we found lower K^+ concentrations in the JA-pretreated salinity stressed plants compared with salinity stressed plants.

The K:Na selectivity of the JA-pretreated salinity stressed plants decreased dramatically from 106 in the salinity stressed plants compared to 33 in the JA-pretreated salinity stressed plants.

The changes in ion uptake under salinity after JA pretreatment were not limited to cations. Chloride levels increased by about 50% in JA-pretreated salinity stressed plants compared with salinity stressed plants. JA pretreatment did not increase chloride levels relative to Control plants in the absence of salinity. Apparently, the JA response in rice is not just limited to Na^+ levels, but is the consequence of a more general membrane phenomenon..

As was observed for K-Na selectivity, Ca-Na selectivity of the JA-pretreated salinity-stressed plants also decreased from 1.71 in the salinity-stressed plants compared to 1.27 in the JA-pretreated salinity-stressed plants. In contrast to Ca-Na selectivity, Ca-Mg, selectivity increased from 0.10 in the salinity-stressed plants to 0.25 in the JA-pretreated salinity-stressed plants. This observation appears to be due to an effect of exogenous JA application as Ca-Mg, selectivity increased from 0.47 in Control plants to 0.79 in JA-pretreated rice.

A similar situation appeared to be the case with respect to K:Mg selectivity. Pretreatment with JA increased K:Mg selectivity compared with Control (6.76 vs. 5.75). However, we found no difference in K:Mg selectivity between salinity-stressed plants and JA-pretreated salinity-stressed.

CONCLUSION

The present study investigated the effect of JA pretreatment on the growth of a salt-sensitive cereal, rice (*Oryza sativa* L.). Overall, the effect of JA pretreatment on salt-sensitive rice was different than reported for salt-tolerant barley. Others have reported that JA pretreatment had an ameliorative effect on salinity stress.

Pretreatment of rice with jasmonic acid did not improve the adaptation of rice plants to salinity stress in our experimental set-up. Phenotypic measurements thought to be important in salt tolerance such as photosynthesis, stomatal conductance, were not improved. This may be due, at least in part, to dramatic changes in ion levels, particularly Na+ in JA-pretreated salinity stressed plants. The elucidation of the molecular mechanism(s) involved in the JA-salinity response must await further experimentation.

ACKNOWLEDGEMENTS

The author gratefully thanks Dr. Xuan Liu for excellent analytical services.

Table 1. Growth responses of rice (*O. sativa*), IR29, to salinity stress, JA treatment and JA pre-treatment plus salinity stress.

Treatment	Shoot Dry Weight (g plant^{-1})	Root Dry Weight (g plant^{-1})	Shoot/Root	Shoot Length (cm)	Root length (cm)
Control	0.90	0.37	2.43	34.0	38.4
Salinity	0.45	0.13	3.46	29.4	22.7
JA	0.41	0.21	1.95	24.7	36.4
JA + Salinity	0.32	0.09	3.56	23.5	24.9

Table 2. Phenotypic responses of rice (*O. sativa*), IR29, to salinity stress, JA treatment and JA pre-treatment plus salinity stress. Measurements were taken two days after final imposition of salinity stress.

Treatment	Photosynthesis (µmol m^{-2} s^{-1})	Conductance (mol H_2O m^{-2} s^{-1})	Transpiration (mmol H_2O m^{-2} s^{-1})
Control	26.1	0.47	7.1
Salinity	13.9	0.18	3.3
JA	8.8	0.13	2.2
JA + Salinity	6.6	0.13	2.3

Table 3. Phenotypic responses of rice (*O. sativa*), IR29, to salinity stress, JA treatment and JA pre-treatment plus salinity stress. Measurements were taken twelve days after final imposition of salinity stress.

Treatment	Photosynthesis (µmol m^{-2} s^{-1})	Conductance (mol H_2O m^{-2} s^{-1})	Transpiration (mmol H_2O m^{-2} s^{-1})
Control	20.6	0.58	8.7
Salinity	15.5	0.31	4.9
JA	19.1	0.42	6.8
JA + Salinity	3.1	0.10	1.8

Table 4. Mineral composition in 73-day old whole shoots of rice (*O. sativa*), IR29. Plants were 52-days old at final imposition of salinity.

Treatment	Ca	Mg	Na (mmoles kg^{-1} dry weight)	K	S	Cl
Control	69	234	29	841	157	377
Salinity	121	172	322	699	104	907
JA	90	182	32	767	143	303
JA + Salinity	217	130	936	641	107	1383

Table 5. Effects of salinity stress, JA treatment and JA pre-treatment plus salinity stress on selectivity coefficients calculated according Flowers and Yeo (1988).

Treatment	$S_{K,Na}$	$S_{Ca,Na}$	$S_{K,Mg}$	$S_{Ca,Mg}$
Control	NA[1]	NA	5.7	0.47
Salinity	106.2	1.7	6.5	0.11
JA	NA	NA	6.8	0.79
JA + Salinity	32.9	1.2	6.4	0.25

1 NA, Not Applicable. Sodium was not part of the nutrient solution. Thus, the selectivity constants could not be calculated.