

Elevated CO₂ Influences Salt Tolerance of Rice

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1 INTRODUCTION

Due to increases in global population, world agriculture must produce a greater yield per unit land area than ever before. One concern is that nearly one-half of all irrigated land worldwide is seriously affected by salinity or water logging. At present, more agricultural land is not being irrigated due to salinity problems than there is new land coming under irrigation. Irrigated agriculture continues to be of considerable importance in this regard as irrigation minimizes the uncertainties due to weather and enables high yield potential per unit land area. Another concern is that high-quality water needed for irrigated agriculture is becoming increasingly scarce due to changing environmental standards and rising demands from urban areas. Most of the early work on salinity focused on the manifestations of salt-stress. The exact physiological, biochemical, biophysical, and genetic mechanisms remain unresolved.

In an earlier work, Nicolas et al. (1993) used elevated CO₂ levels to alleviate salinity stress by, presumably, increasing carbohydrate supplies. In wheat, elevated CO₂ levels increased dry matter, leaf area, and tillering in salt-stressed plants to a greater extent than in control plants. Recent work on genetic mapping of cereals with common DNA probes indicates a highly conserved gene content and gene order within *Poaceae* (Gale and Devos, 1998). Thus, it would be interesting to determine if rice, a salt-sensitive crop, responds to elevated CO₂ in manner similar to tolerant wheat.

The objectives of this study were: (1) to determine if intermediate levels of elevated CO₂ (about 2X ambient) could alleviate salinity induced growth reductions in rice by measuring changes in biomass (tiller number, leaf area and dry weight, stem and root weight); (2) to determine if there are any changes in leaf gas exchange rates, stomatal conductance, and transpiration; (3) and to identify possible physiological characters which might be affected by elevated CO₂ levels.

2 MATERIALS AND METHODS

Plant growth: Seeds of salt-sensitive rice (*Oryza sativa* L.) cultivar, M202, were sown in buckets with sand medium and supplied with Yoshida nutrient solution. Each bucket served as a replicate and had 12-13 plants. The buckets were placed in two transparent, outdoor SPAR chambers. Three salt treatments used were 0.9 (control), 3.9 and 6.5 dS m⁻¹ and constructed by adding the calculated amount of NaCl and CaCl₂ (5:1 on a molar basis) to the nutrition solution the next day after planting. Elevated CO₂ treatment commenced upon germination and was achieved by releasing pure CO₂ into one of the growth chambers. The elevated CO₂ level was constantly maintained at 800 ppm by the chamber automatic controlling system. The CO₂ concentration in the other chamber was set at 350 ppm and

served as the CO₂ treatment control. Each category of treatment had three replicates. Both growth chambers were illuminated with natural sunlight and had the same temperature and relative humidity controls: 20-23 °C for the night (19:00 to 7:00), 27-33 °C for the day (7:00 to 19:00) and 40-50%, respectively.

Biomass determination: The plants were harvested 61 days after planting, and processed immediately. Biomass dry weights were obtained after the samples were dried in the oven at 55 °C to a constant weight. Leaf area was measured using Li-Cor 3100 Area Meter.

Measurements: Pn, gs, and tr were measured on fully expanded leaves, 52 days after planting using Li-Cor 6400 Photosynthesis System. Freeze-dried and ground tissue (0.05 g) was weighed and its soluble sugars were extracted in 80% (v/v) ethanol in a 80 °C water bath. The ethanol extract was taken to dryness in a concentrator. Dried samples were resuspended in deionized water and filtered and then 10 µL was analyzed by HPLC using a ESA Coulochem II Electrochemical Detector connected to a Dionex CaboPac PA1 column. Starch was assayed as follows. The extracted residues were oven dried at 55 °C and then its starch was gelatinized in 1 mL 2 N in a boiling water for 1 h. Starch was then hydrolyzed to glucose using amyloglucosidase (Sigma). Glucose content was assayed using a Sigma HK20 glucose kit and quantified by comparison to a known glucose standard using Bio-Tek PowerWavex Select Scanning Microplate Spectrophotometer.

Data analysis: Data were analyzed using general linear model (GLM) procedures in SAS statistical package (SAS, 1998). Means were compared using Bonferroni (Dunn) t-tests in the GLM or TTEST.

3 RESULTS AND DISCUSSION

With respect to biomass parameters, salinity significantly ($P \leq 0.05$) reduced tiller number, leaf area, leaf dry weight, stem dry weight, and root dry weight for both 350-ppm and 800-ppm grown plants (Fig. 1 (A)-(E)). The reduction was much less for the 800-ppm grown plants, relative to the 350-ppm grown plants. This is evidenced by the significantly higher tiller number, leaf area and dry weight, stem dry weight, and root dry weight of the 800-ppm grown plants than that of the 350 ppm grown plants under salt stress (Fig. 1(A)-(E)). Interestingly, at harvest (61 days after planting) most of the 350-ppm grown plants had died in the 6.5 dS m⁻¹ treatment. Conversely, at the same salt level, most of the 800-ppm grown plants survived.

Generally, the 800-ppm grown plants had a higher photosynthetic rate compared with the 350-grown plants (Fig. 2 (A)), which may account for the increases in the growth parameters for the 800-ppm grown plants. However, elevated CO₂ significantly ($P \leq 0.05$) reduced both leaf stomatal conductance (gs) and leaf transpiration rate (tr) of the rice grown at 0.9 and 3.9 dS m⁻¹ (Fig. 2 (B); Fig. 2 (C)). Thus, it is possible that elevated CO₂ partially ameliorated salinity stress by reducing the salt load. However, as in wheat, elevated CO₂ had no significant effect on Na⁺ or Cl⁻ levels in leaves. It would appear from these data that some aspects of Na⁺ uptake in rice are similar to wheat. In both species, Na⁺ uptake is largely independent of water uptake since elevated CO₂ reduced transpiration rates but did not change ion accumulation.

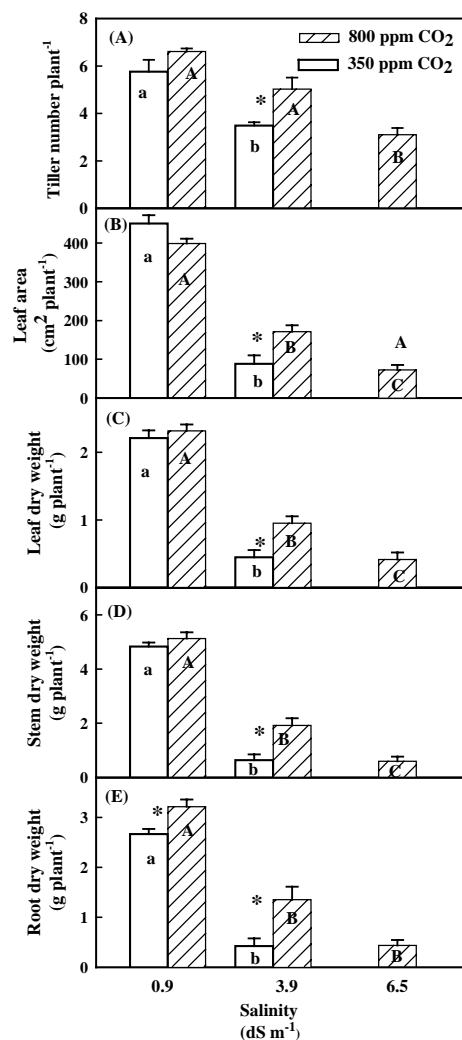


Figure 1. Effects of salinity and ambient CO₂ concentration on (A) tiller number (tiller no.), (B) leaf area, (C) leaf dry weight, (D) stem dry weight, and (E) root dry weight of rice (*Oryza sativa* L.), cultivar ‘M202’, 61 days old. At the highest salinity, most plants grown at 350 ppm CO₂ died and thus no corresponding data were available.

4 CONCLUSIONS

Early work suggested that carbohydrate levels did not limit growth of plants in saline environments. However, further investigations demonstrated elevated CO₂ levels could partially alleviate salt stress. Nicolas et al. (1993) showed that elevated CO₂ levels could enhance growth of salt-stressed wheat, primarily by increasing tillering. Elevated CO₂ did not seem to be acting by reducing transpiration, thereby reducing sodium accumulation. Our work demonstrates that a salt-sensitive grass, rice, responds to elevated CO₂ levels similarly to tolerant wheat. These results are important in light of the recent findings that the gene order is conserved among different grass genomes. Since rice and wheat seem to respond similarly to that elevated CO₂ levels, the use of elevated CO₂ levels may be a useful tool to

identify useful salt-tolerant traits. Once plant breeders map a useful trait in wheat, they could use that information to search for the similar genes in rice.

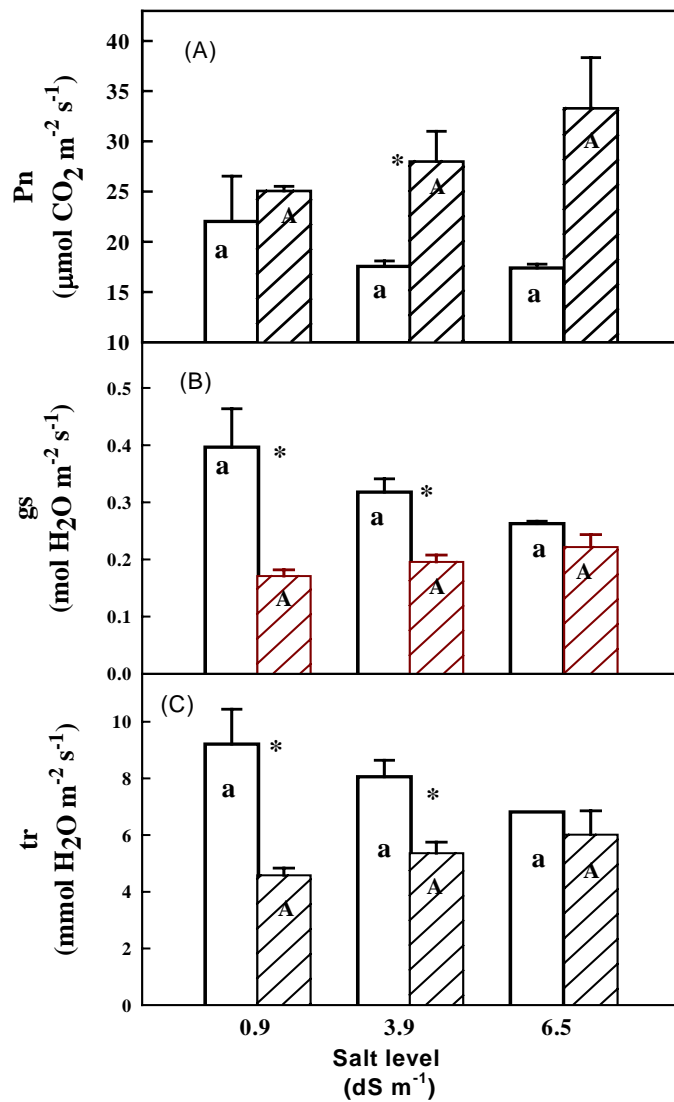


Figure 2. Fully e
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