

Characterization of Rice (*Oryza sativa* L.) Physiological Responses to α -Tocopherol, Glycine Betaine or Salicylic Acid Application

Abdul Razack Mohammed (Corresponding author)

Texas AgriLife Research and Extension Center

1509 Aggie Drive, Beaumont, Texas 77713, USA

Tel: 1-409-752-2741 ext 2319 E-mail: abdulrazack@neo.tamu.edu

Lee Tarpley

Texas AgriLife Research and Extension Center

1509 Aggie Drive, Beaumont, Texas 77713, USA

Tel: 1-409-752-2741 ext 2235 E-mail: ltarpley@tamu.edu

Abstract

The impacts of α -tocopherol, glycine betaine (GB) and salicylic acid (SA) applications on higher plants have been the subject of many studies, with special emphasis on oxidative stress tolerance under adverse conditions. However, little work has been carried out on rice responses to α -tocopherol, GB or SA under non-stress conditions, in which yield could potentially increased. This study determined the effects of α -tocopherol (2.3 kg ha⁻¹), GB (2.0 kg ha⁻¹) or SA (12.9 g ha⁻¹) application on rice morphology, phenology and physiology under non-stress conditions. The applications did not affect production of tillers, biomass, phenology, or pollen germination; however, plant height, leaf characteristics and physiology, spikelet fertility (SF), panicle and grain characteristics and yield were affected. Plants treated with α -tocopherol, GB or SA showed 6%, 13% and 13.5% increases in grain yield as a result of decreased respiration and increased membrane integrity and SF.

Keywords: α -Tocopherol, Glycine betaine, Grain characteristics, Rice, Rice physiology, Salicylic acid

1. Introduction

Plants exposed to environmental stresses, such as heat, cold, drought and salinity, produce ROS, which damage macromolecules and cell membranes (Zhang & Kirkham, 1996). Angiosperms possess several enzymatic and non-enzymatic scavenging systems to minimize deleterious effects of ROS. These include lipid-soluble antioxidants (e.g. α -tocopherol and β -carotene), water-soluble reactants (e.g. ascorbic acid and glutathione), and enzymatic antioxidants (e.g. superoxide dismutase, catalase and enzymes of the ascorbate and glutathione cycles) (Zhang & Kirkham, 1996). Salicylic acid (SA) and glycinebetaine (GB) are synthesized in plants and provide important but different, roles in preventing oxidative damage to the membranes (Bowler *et al.*, 1992; Bohnert & Jensen, 1996; Demiral & Turkman, 2004; Larkindale & Huang, 2004).

Alpha-tocopherol is a small molecule that is synthesized in the plants, mainly concentrated in plastids, and is one of the most effective single-oxygen quenchers (Fryer, 1992). Tolerance to salt stress, chilling stress, UV-B stress and pollutant stress is partly correlated with tocopherol content (Munné-Bosch & Alegre, 2002). Alpha-tocopherol is a strong antioxidant that assists in maintaining membrane stability (Munné-Bosch & Falk, 2004), intracellular signaling, and transport of electrons in the photosystem-II system (Munné-Bosch & Alegre, 2002) and is photoprotective in nature.

Glycine betaine is an important osmoprotectant and an amino-acid derivative that is synthesized in many plant families including Chenopodiaceae, Amaranthaceae, Gramineae, Compositae and Malvaceae (Rhodes & Hanson, 1993; Demiral & Turkman, 2004). It enhances tolerance to high temperatures (Paleg *et al.*, 1981), low temperatures (Farooq *et al.*, 2008a), freezing temperatures (WeiBing and Rajashekar, 2001), drought stress (Måkelä *et al.*, 2000) and salinity (Harinasut *et al.*, 1996; Rahman *et al.*, 2002). Glycine betaine induces tolerance by protecting the Rubisco enzyme (Paleg *et al.*, 1981) and photosystem II (Allakhverdiev *et al.*, 1996), maintaining turgor pressure (Wyn Jones, 1984) and ion homeostasis, and through alteration of parts of signal transduction (Ashraf & Foolad, 2007). Plants under various stress conditions showed improvement in growth,

survival, leaf water potential, ion uptake and stress tolerance due to exogenous application of GB (Diaz-Zorita *et al.*, 2001).

Salicylic acid, a phenolic compound, is associated with stress tolerance in plants. Previous studies report that SA can induce tolerance against high and low temperatures, drought, salinity, ultraviolet light, heavy metal toxicity, diseases and pathogens (Raskin, 1992; Yalpani *et al.*, 1994; Dat *et al.*, 1998; Metwally *et al.*, 2003; Sakhabutdinova *et al.*, 2003; Hayat & Ahmad, 2007; Horváth *et al.*, 2007; Farooq *et al.*, 2008b; Hussain *et al.*, 2008). In addition, SA plays an essential role in preventing oxidative damage in plants by detoxifying superoxide radicals (Bowler *et al.*, 1992) and is also involved in calcium signaling (Kawano *et al.*, 1998). Plants treated with SA showed increased vigor of early seedling growth (Farooq *et al.*, 2008b), increased photosynthetic rates and water use efficiency, and decreased stomatal conductance and transpiration rate (Khan *et al.*, 2010). Moreover, there is evidence that exogenous application of SA can alter antioxidant capacity in plants (Rao *et al.*, 1997), thereby providing protection against oxidative damage (Larkindale & Huang 2004), thereby inducing stress tolerance.

Alpha-tocopherol, GB and SA – the chemicals used in this study – are all important in plant growth and development and are also associated with stress tolerance in plants. Plants pre-treated with α -tocopherol (Fryer, 1992), GB (Diaz-Zorita *et al.*, 2001), or SA (Larkindale & Knight, 2002) showed induced stress tolerance and protection against oxidative damage due to various stresses. Despite the importance of these chemicals in stress tolerance, little is known about their effects on rice morphology, phenology and physiology. The research presented herein addresses the effects of α -tocopherol, GB or SA on rice morphology, phenology and physiology, under non-stress conditions.

2. Materials and Methods

2.1 Plant culture

Two experiments were conducted in the greenhouse at the Texas AgriLife Research and Extension Center at Beaumont, Texas, USA. ‘Cocodrie’, a common tropical japonica-type U.S. rice cultivar, was used in both the experiments. Plants were grown in 3L pots that were placed in a square box (0.84 m²), 10 pots per box. The boxes were lined with black plastic (thickness = 6.0 mm; FILM-GARD, Minneapolis, Minnesota, USA) that served as a water reservoir. Pots were filled with a clay-rich soil that is common to rice farms in the area. At 20 days after emergence (DAE), the boxes were filled with water to approximately 3 cm above the top of the soil in each pot. A three-way split application of nitrogen was performed as described by Mohammed *et al.*, (2007) in both the experiments. Nitrogen was applied in the form of urea and ammonium sulfate, and phosphorus in the form of P₂O₅. Nitrogen was applied at the rate of 113.5 kg ha⁻¹ along with 45.4 kg ha⁻¹ of phosphorus (P₂O₅) at planting. The remaining nitrogen fertilizations (both 79.5 kg ha⁻¹ of nitrogen in the form of ammonium sulfate) were applied 20 DAE and at the panicle-differentiation stage. Mean day temperature and humidity were monitored independently of the controlled experimental temperatures using standalone sensor/loggers (HOBOS, Onset Computer Corporation, Bourne, Massachusetts, USA). The mean day temperatures and absolute humidity in the greenhouse ranged between 27-35 °C and 13-14 g/m³, respectively. The light intensity and CO₂ in the greenhouse during the day were measured using a light quantum meter (Quantum Meter, Apogee Instruments, Logan, Utah, USA) and LI-6400 (LI-6400, LI-COR Inc., Lincoln, Nebraska, USA), respectively. The light intensity and CO₂ concentration in the greenhouse ranged between 600-800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 360-380 ppm, respectively, depending upon the time of the day.

2.2 Chemical treatments

The α -tocopherol was applied at the rate of 2.3 kg a.i. ha⁻¹ (2.5% v/v); GB was applied at the rate of 2.0 kg ha⁻¹ (580 mM), and SA at the rate of 12.9 g ha⁻¹ (1 mM). The chemicals were applied at the rate of 100 μL per application to the leaves using a pre-calibrated perfume-bottle sprayer. Each plant was treated three times (on a single day; 20 DAE) to enable thorough coverage prior to imposing heat stress, for a total 300 L per treatment. All the chemicals were dissolved in de-ionized water with 0.5% (v/v) surfactant (Latron AG-98 spreader activator, Rohm and Haas Company, Philadelphia, Pennsylvania USA). The α -tocopherol and SA were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). The GB was supplied by Capstone Food Ingredients (Marion, Massachusetts, USA).

2.3 Growth and development

Plant height (from base of the stem to tip of the uppermost-reaching leaf) and the numbers of productive tillers were recorded at harvest. Daily observations were made for the appearance of panicles. The number of leaves was counted at the early grain-fill stage (EGF), the individual penultimate leaf length (from the point of contact of the leaf blade and the stem to the tip of the leaf) and leaf breadth (widest part of the leaf) was also measured at EGF

stage. The final harvest was carried out at 100 DAE. Destructive sampling procedures were adopted: plants were dissected, productive tillers were counted, plant height and leaf areas were measured, and dry weights were determined. Leaf area was measured using a CI-251 area meter (CID Inc., Camas, Washington, USA). In this study, biomass per plant was calculated by summing up the values of stem dry weight, leaf dry weight and root dry weight of the individual plant.

2.4 Pollen germination

The percent pollen germination (PPG) was determined through in vitro germination on a culture medium. The culture medium was prepared in accordance with the media of Song *et al.*, (2001), with minor modifications. The components of the culture medium in the present study were 15 g sucrose, 0.03 g calcium nitrate, and 0.01 g boric acid dissolved in 100 ml deionised water. Agar, 0.06 g, was added to this solution, which was heated on a hot plate to dissolve the agar. When the agar was dissolved in the solution, 10 ml medium was poured per Petri dish (diameter 8.5 cm) and allowed to cool for 15 minutes. The pollen from different treatments was dusted on this medium as soon as the floret opened in the morning. Pollen was dusted on Petri dishes and incubated at 27 °C for 24 hours in the dark. Pollen was considered as germinated when the pollen tube length was at least equal to the grain diameter as determined using a microscope. The total numbers of pollen grains, germinated and non-germinated pollen in each Petri dish were counted, and the PPG was recorded.

2.5 Spikelet fertility (SF), panicle and grain characteristics

The main-stem panicles were tagged at panicle emergence and harvested at physiological maturity separately from the tiller panicles. The main-stem panicle length, number of primary branches, total grain number, filled grain number and unfilled grain number per panicle were determined at harvest. Spikelet fertility was defined as the ratio of filled grains to total number of grains in the panicle. Each floret was pressed between the thumb and forefinger to determine if the grain was filled or not. Spikelet fertility was expressed as percentage.

Main-stem panicle length and number of primary branches on the panicle were recorded. In both experiments, grain length and width of brown (dehulled) rice were determined using a Winseedle (Regent Instruments, Inc. Quebec, Canada), which uses image analysis of scanned color images of the grain to calculate these parameters. Ten grains per replication were used to determine grain length, width and chalkiness. The weight of 100 filled-grains (dehulled grain) was also determined.

2.6 Leaf photosynthesis

Leaf net photosynthetic rates (P_n) were measured at the early grain-fill (EGF) stage. The P_n was measured on the penultimate leaves using a LI-6400 portable photosynthesis system (LI-COR Inc., Lincoln, Nebraska, USA) between 1000 h and 1200 h. Three leaves per replication were used to measure P_n . When measuring P_n , the photosynthetic photon flux density (PPFD), provided by a 6400-02 LED light source, was set to 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The temperature and CO_2 concentration in the leaf cuvette were set to 25 °C and 360 ppm (ambient CO_2 concentration in the greenhouse), respectively.

2.7 Leaf total chlorophyll content

Chlorophyll was determined on the penultimate leaves at the EGF stage. Three leaf discs (0.196 cm^2 each) were obtained from mid-blade while avoiding the mid-vein and placed in a vial (2.5-ml) with 1-ml dimethyl sulphoxide (DMSO) and incubated at room temperature for 24 h in darkness to allow for complete extraction of chlorophyll into the solution. The absorbance of the extract was measured in microtiter plates of polypropylene material using a PowerWave microplate spectrophotometer (Bio-Tek Instruments, Inc., Winooski, Vermont, USA) at 648 and 664 nm to calculate the chlorophyll a and chlorophyll b concentrations (Chappelle *et al.*, 1992). Using the method of Chappelle *et al.*, (1992), which utilizes a DMSO extraction, the concentrations of the pigments were calculated from the absorbance values at 648 and 664 nm using equations described by Lichtenthaler (1987). The pigment concentrations were expressed on a leaf area basis, $\mu\text{g cm}^{-2}$. Total chlorophyll was calculated by summing up the values of chlorophyll a and chlorophyll b.

2.8 Leaf nitrogen content (LNC) and grain nitrogen content (GNC)

For both LNC and GNC, N was measured using an FP-528 Nitrogen/Protein analyzer (LECO Corporation, St. Joseph, Michigan, USA). For LNC analysis, the penultimate leaf was harvested at EGF stage. Leaf nitrogen content was expressed as percentage (%; w/w) and GNC was expressed as mg g^{-1} .

2.9 Leaf respiration rates

Leaf-level respiration was measured using an LI-6400 portable photosynthesis system (LI-COR Inc., Lincoln, Nebraska, USA). Respiration was measured on the penultimate leaf during the EGF stage. Three leaves per

replication were used to measure leaf respiration rates. The area of the LI-6400 chamber was set to 6 cm² and the middle portion of the leaf was preferred for use while measuring respiration rates. While measuring respiration rates, the photosynthetic photon flux density (PPFD) was set at 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (dark environment). The temperature and CO₂ concentration in the leaf cuvette were set at 25°C and 360 μmol , respectively. Respiration was measured during the nighttime between 2300h and 0002h.

2.10 Leaf membrane thermal stability

Leaves were harvested at EGF stage for the leaf membrane thermal stability assay. The leaf membrane thermal stability of leaves was measured using the procedure described by Martineau *et al.*, (1979). Each sample assay consisted of two sets of five leaf discs cut with a 1-cm diameter punch from the penultimate leaves. The sets were placed into two separate test tubes with 10-mL de-ionized water, after rinsing them three or four times with de-ionized water. One set of test tubes was submerged in a water bath at 55 °C for 20 minutes to a depth equal to the height of water in the tubes. The other set of test tubes was held at room temperature (25 °C). After that, both sets of test tubes were incubated at 10 °C for 12 h. Conductance was measured using a conductivity meter (SensION5 Conductivity Meter, Hach Company, Loveland, Colorado, USA) after standardizing with standard KCl solutions. Test tubes were then autoclaved for 20 min at 120 °C at 0.15 Mpa and conductance was measured again as an indication of maximum potential leakage from a given sample (Ibrahim & Quick, 2001). The relative injury (RI) was calculated using the equation $RI = (1 - [1 - (C_{55,i}/C_{55,f})] / [1 - (C_{25,i}/C_{25,f})]) * 100$ where C_{55} and C_{25} refer to the conductance at 55 °C and 25 °C, and the subscript 'i' and 'f' refers to the initial and final conductance.

2.11 Total antioxidant capacity of leaf

Total antioxidant capacity of the rice leaf was measured using the procedures (DPPH [2,2-diphenyl-1-picrylhydrazyl] assay) from Goffman & Bergman (2004) with modifications. For the DPPH assay, five leaf discs (0.0785 cm² each) were obtained from mid-blade while avoiding the mid-vein, and their weights recorded. Leaf discs were placed in a test tube (4-ml) with 1.5-ml methyl alcohol (MeOH) then sealed. The test tubes were incubated at room temperature for 24 h in darkness to allow for complete extraction of antioxidants into the solution. Rice leaf extract of 40 μL was added to 960 μL DPPH (80 ppm) solution and the optical density determined at 515 nm after 4 h. The antiradical efficiency of the rice leaf methanolic extracts was determined after 4 h by monitoring the reduction in the absorbance (515 nm) of the methanolic solution of DPPH with the extract. The values of DPPH after adding the extract was compared with those obtained from a blank solution of DPPH (zero antiradical activity). Trolox (6-Hydroxyl-2,5,7,8-tetramethylchroman-2-carboxylic acid) and DPPH standard curves were developed and the values were expressed in μmol trolox equivalents (TE) g⁻¹ leaf (fresh weight basis) using these standard curves. The DPPH and trolox were purchased from Sigma-Aldrich (St. Louis, MO). Total antioxidant capacity was measured at mid-dough stage of the rice plant.

2.12 Experimental design and data analysis

The experiments were laid out in a complete randomized design, and were repeated twice. In each experiment, there were 20 plants, five plants per chemical treatment. To test the significance of experiments, the chemical treatments, and their interactive effects on growth, development and physiology parameters, the data was statistically analyzed using analysis of variance (ANOVA; SAS statistical analysis package version 9.2, SAS Institute, Cary, North Carolina, USA). The means were separated using Tukey's Least Significant Difference (LSD) at an alpha level of 0.05. If there was no significant difference between the experiments for a parameter, then the values from both the experiments for that parameter (e.g., n=10) were used to obtain the mean and error. The standard errors of the mean were also calculated and presented in the graphs as error bars.

3. Results

3.1 Effects of α -tocopherol, GB or SA on rice morphology, phenology and physiology

There were no differences among the experiments or for any interaction among the experiments and chemical treatments for the studied variables (Table 1). In addition, application of α -tocopherol, GB or SA application did not affect the number of productive tillers per plant, days for 1st panicle emergence, pollen germination or biomass per plant (Table 1). However, plant height, leaf characteristics, leaf physiology, spikelet fertility, grain characteristics and grain yield of rice plants were affected (Table 1).

3.2 Effects of α -tocopherol, GB or SA on plant height, spikelet fertility and grain yield

There were no significant differences among untreated plants, α -tocopherol-treated plants and SA-treated plants with respect to plant height. However, GB-treated plants were 5% shorter than the untreated plants (Table 2). There was no significant difference between untreated plants and α -tocopherol-treated plants with respect to SF. However, GB-treated plants and SA-treated plants showed 14% and 18% increase in SF, respectively, compared

to untreated plants (Table 2). There was no significant difference between untreated plants and α -tocopherol-treated plants with respect to grain yield. However, GB-treated plants and SA-treated plants showed 13% and 13.5% increase in grain yield, respectively, compared to untreated plants (Table 2).

3.3 Effects of α -tocopherol, GB or SA on leaf characteristics

Application of α -tocopherol, GB or SA affected the number of viable leaves per plant at EGF stage, viable leaf area per plant at harvest, penultimate leaf total chlorophyll content and penultimate leaf nitrogen content measured at EGF stage. However, penultimate leaf length and leaf width measured at EGF stage, were not affected as a result of α -tocopherol, GB or SA application. There was no significant difference among untreated plants, GB-treated plants and SA-treated plants with respect to leaf number per plant. However, α -tocopherol-treated plants showed 11% increase in number of leaves per plant compared to untreated plants (Fig. 1). Plants treated with α -tocopherol showed 10% decrease and SA-treated plants showed 11% increase in penultimate leaf total chlorophyll content measured at EGF stage, compared to untreated plants (Fig. 1). Plants treated with α -tocopherol, GB and SA showed 22%, 21% and 28% increase in penultimate leaf nitrogen content measured at EGF stage, compared to untreated plants (Fig. 1).

3.4 Effects of α -tocopherol, GB or SA on leaf physiology

There were no significant differences among untreated plants, α -tocopherol-, GB- and SA-treated plants with respect to leaf photosynthetic rates, stomatal conductance and leaf total antioxidant capacity (Fig. 2). Plants treated with α -tocopherol, GB and SA showed 34%, 43% and 62%, 19%, 18% and 31% and 45%, 55% and 48% decrease in loss of carbon, leaf transpiration rates and leaf membrane injury measured at EGF stage, respectively, compared to untreated plants (Fig. 2). Plants treated with α -tocopherol and SA showed 18% and 30% decrease in internal CO_2 concentration measured at EGF, compared to untreated plants (Fig. 2). In addition, plants treated with α -tocopherol, GB and SA showed 38%, 50% and 56% increase in water use efficiency, compared to untreated plants (Fig. 2).

3.5 Effects of α -tocopherol, GB or SA on main stem panicle and grain characteristics

There were no significant differences among untreated plants, α -tocopherol-, GB- and SA-treated plants with respect to panicle length, number of primary branches per panicle and total grains per panicle (Table 3). However, plants treated with α -tocopherol, GB and SA showed 16%, 22% and 32% increase in the number of filled grains per panicle and GB-treated plants and SA-treated plants showed 29% and 44% decrease in the number of unfilled grains per panicle (Table 3). Plants treated with α -tocopherol, GB and SA showed 2%, 3% and 2% decrease in grain length, and 1%, 1% and 1% increase in grain width, compared to untreated plants (Table 3). There were no significant differences among untreated plants, GB-treated and SA-treated plants with respect to grain chalkiness and grain nitrogen content. However, α -tocopherol-treated plants showed 12% increase in grain chalkiness and 5% decrease in grain nitrogen content, compared to untreated plants (Table 3).

4. Discussion

The present study was conducted to improve our understanding of rice responses to α -tocopherol, GB or SA application. Our results indicated beneficial effects of GB (2.0 kg a.i. ha^{-1}) and SA (12.9 g a.i. ha^{-1}) application. However, in the present study, application of α -tocopherol (2.3 kg a.i. ha^{-1}) at 20 DAE was not beneficial for rice production. The α -tocopherol, GB or SA application did not affect production of productive tillers, rice phenology, pollen germination or biomass production. In addition, application of GB decreased plant height. This differs from the findings of Farooq *et al.*, (2008a, b), which state that seed priming of maize (*Zea mays* L.) with GB or SA increased shoot length, root length and biomass under optimal conditions. Application of α -tocopherol, GB or SA did not alter rice phenology. This differs from findings of Oota (1975), and Datta & Nanda (1985), which state that SA application induces early flowering in duckweeds (*Lemna gibba* L.) and chenna millet (*Panicum miliaceum* L.). Salicylic acid induces flowering in plants by acting as a chelating agent (Oota, 1972).

In the present study, GB or SA application did not affect leaf photosynthetic rates or stomatal conductance. In previous studies, plants treated with GB under optimal conditions showed similar results with respect to rice leaf photosynthetic rates and stomatal conductance (Farooq *et al.*, 2008a). Application of SA did not alter leaf photosynthetic rates in mungbean (*Vigna radiate* L.) plants with respect to leaf photosynthetic rates. However, stomatal conductance decreased with SA application in mungbean (Khan *et al.*, 2010) and corn (*Zea mays* L.) (Zhou *et al.*, 1999). In the present study, the leaf respiration rate, transpiration rate and relative injury to the leaf membrane decreased with application of α -tocopherol, GB or SA. In previous studies, exogenous application of SA had an antitranspiration effect on the leaves of bean (*Phaseolus vulgaris* L.) and mungbean

(Larque-Saavedra, 1978; Khan *et al.*, 2010). Moreover, previous studies also suggest the possible roles of α -tocopherol, GB and SA in maintaining membrane integrity (Fryer, 1992; Bohnert & Jensen, 1996; Farooq *et al.*, 2008a, b). In the present study, application of α -tocopherol, GB or SA increased membrane stability, which might be due to decreased generation of ROS. The ROS reacts with the proteins, lipids and DNA causing oxidative damage and impairing normal function of the cells (Foyer & Fletcher, 2001). Increased membrane stability as a result of α -tocopherol, GB or SA application decreased respiration rates. Amthor & McCree (1990) reported that plants increase their maintenance respiration to support repair mechanisms of their damaged membranes as a result of oxidative stress. In the present study, increased membrane integrity was not associated with total antioxidant capacity.

Application of GB or SA did not affect the total number of grains per panicle however did have a profound effect on number of filled grains. Application of SA increases mobilization of reserve food materials to the grain during the grain filling process by increasing the activities of hydrolyzing and oxidative enzymes (Sawhney *et al.*, 1979), hence increasing the number of filled grains. Application of GB increases the synthesis of compatible solutes (Farooq *et al.*, 2008a), whereas application of SA detoxifies superoxide radicals (Bowler *et al.*, 1992), which in turn decreases levels of ROS, thus increasing the integrity of cellular membranes. In addition, GB protects transcriptional and translational machinery, acting as a molecular chaperone in the refolding of enzymes (Rhodes & Hanson, 1993), whereas SA stabilizes trimers of heat shock transcription factors (Larkindale & Knight, 2002), thus rendering protection to membranes and enzymes. Decreased respiration rates and increased membrane integrity as a result of GB and SA application might have increased the amount of photosynthates transported to the grains, thereby increasing the number of filled grains per panicle, hence increased spikelet fertility.

In conclusion, yield, the final manifestation of all the physiological processes, increased as a result of GB or SA application under non-stress conditions. In the present study, application of α -tocopherol, GB or SA did not affect production of photosynthates, however they decreased consumption of photosynthates and increased membrane stability which might have aided in transport of photosynthates, thereby increasing yield. Decreased membrane stability can disrupt water, ion, and organic-solute movement across plant membranes, thus affecting production, consumption, transport and accumulation of photosynthates (Christiansen, 1978). Moreover, previous studies have also shown a negative association between respiration rates and yield (Lambers, 1985) and a positive association between membrane stability and yield (Ismail & Hall, 1999). Further field research is required to justify the benefits of these chemical applications for rice farming.

Acknowledgements

Partial support for this study was generously provided by the Texas Rice Research Foundation and Texas Rice Belt Warehouse. We would also like to thank Dr. Ming-Hsuan Chen, Janis T. Delgado, James Medley and Huma F. Mohammed for their help with various aspects of the grain analyses.

References

- Allakhverdiev, S.I., Feyziev, Y.M., Ahmed, A., Hayashi, H., Aliev, J.A., Kimlov, V.V., Murata, N., & Carpentier, R. (1996). Stabilization of oxygen evolution and primary electron transport reactions in photosystem II against heat stress with glycinebetaine and sucrose. *Journal of Photochemistry and Photobiology*, 34, 149-157.
- Amthor, J.S., & McCree, K.J. (1990). Carbon balance of stressed plants: a conceptual model for integrating research results. In: R.G. Alscher & J.R. Cummings (Eds), *Stress responses in plants: adaptation and acclimation mechanisms* (pp.1-15). New York: Wiley-Liss.
- Ashraf, M., & Foolad, M.R. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany*, 59, 206-216.
- Bohnert, H.J., & Jensen, R.G. (1996). Strategies for engineering water-stress tolerance in plants. *Trends in Biotechnology*, 14, 89-97.
- Bowler, C., Montagu, M.V., & Inze, D. (1992). Superoxide dismutase and stress tolerance. *Annual Review of Plant Physiology and Plant Molecular Biology*, 43, 83-116.
- Chappelle, E.W., Kim, M.S., & McMurtrey III, J.E. (1992). Ratio analysis of reflectance spectra (RARS): An algorithm for the remote estimation of the concentration of chlorophyll a, chlorophyll b, and carotenoids in soybean leaves. *Remote Sensing of Environment*, 39, 239-247.
- Christiansen, M.N. (1978). The physiology of plant tolerance to temperature extremes. In: G.A. Jung, (Ed.), *Crop tolerance to suboptimal land conditions* (pp. 173-191). Madison: ASA.

- Dat, J.F., Lopez-Delgado, H., Foyer, C.H., & Scott, I.M. (1998). Parallel changes in H_2O_2 and catalase during thermotolerance induced by salicylic acid or heat acclimation in mustard seedlings. *Plant Physiology*, 116, 1351-1357.
- Datta, K.S., & Nanda, K.K. (1985). Effects of phenolic compounds and gibberellic acid on growth and development of cheena millet (*Panicum milliaceum* L.). *Indian Journal of Plant Physiology*, 3, 298-302.
- Demiral, T., & Turkman, I. (2004). Does exogenous glycinebetaine affect antioxidative system of rice seedlings under NaCl treatment? *Journal of Plant Physiology*, 161, 1089-1100.
- Diaz-Zorita, M., Fernandez-Canigia, M.V., & Grosso, G.A. (2001). Application of foliar fertilizers containing glycinebetaine improved wheat yields. *Journal of Agronomy and Crop Science*, 186, 209-215.
- Farooq, M., Aziz, T., Hussain, M., Rehman, H., Jabran, K., & Khan, M.B. (2008a). Glycinebetaine improves chilling tolerance in hybrid maize. *Journal of Agronomy and Crop Science*, 194, 152-160.
- Farooq, M., Aziz, T., Basra, S.M.A., Cheema, M.A., & Rehman, H. (2008b). Chilling tolerance in hybrid maize induced by seed priming with salicylic acid. *Journal of Agronomy and Crop Science*, 194, 161-168.
- Foyer, C.H & Fletcher, J.M. (2001). Plant antioxidants: colour me healthy. *Biologist*, 48, 115-120.
- Fryer, M.J. (1992). The antioxidant effects of thylakoid vitamin E (α -tocopherol). *Plant Cell Environment*, 15, 381-392.
- Goffman, F.D., & Bergman, C.J. (2004). Rice kernel phenolic content and its relationship with antiradical efficiency. *Journal of the Science of Food and Agriculture*, 84, 1235-1240.
- Harinasut, P., Tsutsui, K., Takabe, T., Nomura, M., Takabe, T., & Kishitani, S. (1996). Exogenous glycinebetaine accumulation and increased salt-tolerance in rice seedlings. *Bioscience, Biotechnology and Biochemistry*, 60, 366-368.
- Hayat, S., & Ahmad, A. (2007). *Salicylic Acid – A Plant Hormone*. Dordrecht: Springer.
- Horváth, E., Pal, M., Szalai, G., Paldi, E., & Janda, T. (2007). Exogenous 4-hydroxybenzoic acid and salicylic acid modulate the effect of short-term drought and freezing stress on wheat plants. *Biologia Plantarum*, 51, 480-487.
- Hussain, M., Malik, M.A., Farooq, M. Ashraf, M.Y., & Cheema, M.A. (2008). Improving drought tolerance by exogenous application of glycinebetaine and salicylic acid in sunflower. *Journal of Agronomy and Crop Science*, 194, 193-199.
- Ibrahim, A.M.H., & Quick, J.S. (2001). Heritability of heat tolerance in winter and spring wheat. *Crop Science*, 41, 1401-1405.
- Ismail, A.M., & Hall, A.E. (1999). Reproductive-stage heat tolerance, leaf membrane thermostability and plant morphology in cowpea. *Crop Science*, 39, 1762-1768.
- Kawano, T., Sahashi, N., Takahashi, K., Uozumi, N., & Muto, S. (1998). Salicylic acid induces extracellular superoxide generation followed by an increase in cytosolic calcium ion in tobacco suspension culture: the earliest events in salicylic acid signal transduction. *Plant Cell Physiology*, 39, 721-730.
- Khan, N.A., Syeed, S., Masood, A., Nazar, R., & Iqbal, N. (2010). Application of salicylic acid increases contents of nutrients and antioxidative metabolism in mungbean and alleviates adverse effects of salinity stress. *International Journal of Plant Biology*, 1, e1.
- Lambers, H. (1985). Respiration in tack plants and tissues: its regulation and dependence on environmental factors, metabolism and invaded organism. In: R. Douce & D.A. Day (Eds.), *Higher plant cell respiration. Encyclopedia of plant physiology* (Volume 18). Berlin: Springer-Verlag.
- Larkindale, J., & Huang, B. (2004). Thermotolerance and antioxidant systems in *Agrostis stolonifera*: Involvement of salicylic acid, abscisic acid, calcium, hydrogen peroxide and ethylene. *Journal of Plant Physiology*, 161, 405-413.
- Larkindale, J., & Knight, M.R. (2002). Protection against heat stress-induced oxidative damage in Arabidopsis involves calcium, abscisic acid, ethylene, and salicylic acid. *Plant Physiology*, 128, 682-695.
- Larque-Saavedra, A. (1978). The Antitranspirant Effect of Acetylsalicylic Acid on *Phaseolus vulgaris*. *Plant Physiology*, 43, 126-128.

- Lichtenthaler, H.K. (1987). Chlorophylls and carotenoids: pigments of photosynthesis, in *Methods in Enzymology*, 148, 350-352.
- Mäkelä, P., Karkkainen, J., & Somersalo, S. (2000). Effect of glycine betaine on chloroplast ultrastructure, chlorophyll and protein content, and RUBPCO activities in tomato grown under drought or salinity. *Biologia Plantarum*, 3, 471-475.
- Martineau J.R., Specht, J.E., Williams, J.H., & Sullivan, C.Y. (1979). Temperature tolerance in soybeans. I. Evaluation of a technique for assessing cellular membrane thermostability. *Crop Science*, 19, 75-78.
- Metwally, A., Finkemeier, I., Georgi, M., & Dietz, K.J. (2003). Salicylic acid alleviates the cadmium toxicity in barley seedlings. *Plant Physiology*, 132, 272-281.
- Mohammed, A.R., Rounds, E.W., & Tarpley, L. (2007). Response of rice (*Oryza sativa* L.) tillering to sub-ambient levels of ultraviolet-B radiation. *Journal of Agronomy and Crop Science*, 193, 324-335.
- Munné-Bosch, S., & Alegre, L. (2002). The function of tocopherols and tocotrienols in plants. *Critical Reviews in Plant Sciences*, 21, 31-57.
- Munné-Bosch, S., & Falk, J. (2004). New insights into the function of tocopherols in plants. *Planta*, 218, 323-326.
- Oota, Y. (1975). Short-day flowering of *Lemna gibba* G3 induced by salicylic acid. *Plant Cell Physiology*, 16, 1131-1135.
- Paleg, L.G., Douglas, T.J., van Daal, A., & Keech, D.B. (1981). Proline, betaine and other organic solutes protect enzymes against heat inactivation. *Australian Journal of Plant Physiology*, 8, 107-114.
- Rahman, M.S., Miyake, H., & Takeoka, Y. (2002). Effects of exogenous glycine betaine on growth and ultrastructure of salt-stressed rice seedlings (*Oryza sativa* L.). *Plant Prod. Science*, 5, 33-44.
- Rajasekaran, L.R., Kriedemann, P.E., Aspinall, D., & Paleg, L.G. (1997). Physiological significance of proline and glycinebetaine: maintaining photosynthesis during NaCl stress in wheat. *Photosynthetica*, 34, 357-366.
- Rao, M.V., Paliyath, G., Ormrod, P., Murr, D.P., & Watkins, C.B. (1997). Influence of salicylic acid on H₂O₂ production, oxidative stress, and H₂O₂-metabolizing enzymes. *Plant Physiology*, 115, 137-149.
- Raskin, I. (1992). Role of salicylic acid in plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, 43, 439-463.
- Rhodes, D., & Hanson, A.D. (1993). Quaternary ammonium and tertiary sulfonium compounds in higher-plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, 44, 357-384.
- Sakhubudinova, A.R., Fatkhutdinova, D.R., Bezrukova, M.V., & Shakirova, F.M. (2003). Salicylic acid prevents the damaging action of stress factors on wheat plants. *Bulgarian Journal of Plant Physiology*, 21, 314-319.
- Sawhney, S., Sawhney, N., Kumar, S., & Nanda, K.K. (1979). Enzyme and electrophoretic pattern of isoenzymes of amylase, catalase, and peroxidase in photo- and gibberellin induced plants of *Impatiens balsamina* L. *New Phytologist*, 82, 41-47.
- Song, Z.P., Lu, B.R., & Chen, J.K. (2001). A study of pollen viability and longevity in *Oryza rufipogon*, *O. sativa*, and their hybrids. *International Rice Research Notes*, 26, 31-32.
- WeiBing, X., & Rajashekar, C.B. (2001). Glycine betaine involvement in freezing tolerance and water stress in *Arabidopsis thaliana*. *Environmental and Experimental Botany*, 46, 21-28.
- Wyn Jones, R.G. (1984). An assessment of quaternary ammonium and related compounds as osmotic effectors in crop plants (photochemical aspects of osmotic adaptation). *Recent Advances in Phytochemistry*, 18, 55-58.
- Yalpani, N., Enyedi, A.J., Leon, J., & Raskin, I. (1994). Ultraviolet light and ozone stimulate accumulation of salicylic acid, pathogen-related proteins and virus resistance in tobacco. *Planta*, 193, 372-376.
- Zhang, J., & Kirkham, M.B. (1996). Antioxidant responses to drought in sunflower and sorghum seedlings. *New Phytologist*, 132, 361-373.
- Zhou, X.M., Mackeuzie, A.F., Madramootoo, C.A., & Smith, D.L.J. (1999). Effects of some injected plant growth regulators, with or without sucrose, on grain production, biomass and photosynthetic activity of field-grown corn plants. *Journal of Agronomy and Crop Science*, 183, 103-110.

Table 1. Effects of experiment and chemicals (control, α -tocopherol, GB or SA) on rice morphology, phenology and physiology

Parameters	Experiments	Chemicals	Experiments $\times$ Chemicals
Plant height	NS	*	NS
Productive tillers	NS	NS	NS
Days for 1 st panicle emergence	NS	NS	NS
Spikelet fertility	NS	**	NS
Pollen germination	NS	NS	NS
Biomass	NS	NS	NS
Yield	NS	*	NS
Leaf number per plant	NS	*	NS
Leaf length	NS	NS	NS
Leaf width	NS	NS	NS
Leaf area	NS	*	NS
Leaf total chlorophyll content	NS	*	NS
Leaf nitrogen content	NS	*	NS
Panicle length	NS	NS	NS
Number of 1 ^o branches	NS	NS	NS
Total grains per panicle	NS	NS	NS
Filled grains per panicle	NS	*	NS
Unfilled grains per panicle	NS	**	NS
Grain length	NS	*	NS
Grain width	NS	*	NS
Grain nitrogen content	NS	*	NS
Grain chalkiness	NS	*	NS
100-seed weight	NS	NS	NS
Leaf photosynthetic rate	NS	NS	NS
Leaf respiration rate	NS	***	NS
Leaf conductance	NS	NS	NS
Leaf internal CO ₂	NS	*	NS
Leaf transpiration rate	NS	**	NS
Leaf membrane stability	NS	*	NS
Leaf total antioxidant capacity	NS	NS	NS
Water use efficiency	NS	*	NS

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels respectively.

NS, not significant

Table 2. Effects of α -tocopherol, glycine betaine or salicylic acid on morphology, phenology and yield of rice plants

Parameters	Untreated	α -tocopherol	Glycine betaine	Salicylic acid
Plant height (cm) (n = 10)	94.50 $\pm$ 1.27 a ¹	95.10 $\pm$ 1.80 a	89.40 $\pm$ 2.71 b	91.10 $\pm$ 2.20 a
Productive tillers per plant (n = 10)	8.80 $\pm$ 0.56 a	8.60 $\pm$ 0.64 a	9.60 $\pm$ 0.36 a	9.50 $\pm$ 0.45 a
Days for 1 st panicle emergence (n=10)	73.00 $\pm$ 0.54 a	73.00 $\pm$ 0.45 a	72.70 $\pm$ 0.76 a	72.70 $\pm$ 0.70 a
Spikelet fertility (%) (n = 10)	75.53 $\pm$ 4.20 c	80.15 $\pm$ 2.48 c	86.02 $\pm$ 1.00 b	89.28 $\pm$ 1.03 a
Pollen germination (%) (n = 10)	30.05 $\pm$ 3.80 a	26.29 $\pm$ 3.19 a	30.50 $\pm$ 2.54 a	26.67 $\pm$ 3.84 a
Biomass per plant (g) (n = 10)	30.12 $\pm$ 1.11 a	32.00 $\pm$ 1.64 a	30.78 $\pm$ 2.02 a	29.90 $\pm$ 1.07 a
Grain yield per plant (g) (n = 10)	22.25 $\pm$ 0.86 b	23.56 $\pm$ 1.24 ab	25.14 $\pm$ 1.29 a	25.26 $\pm$ 0.53 a

Each value is the mean with standard error ($\pm$ S.E.). ¹Mean values within a row followed by a different letter differed significantly (P < 0.05).

Table 3. Effects of α -tocopherol, glycine betaine or salicylic acid on main-stem panicle and grain (dehulled) characteristics of rice plants

Parameters	Untreated	α -tocopherol	Glycine betaine	Salicylic acid
Panicle length (cm) (n = 10)	22.66 $\pm$ 0.57 a ¹	22.33 $\pm$ 0.50 a	21.52 $\pm$ 0.41 a	22.07 $\pm$ 0.52 a
Number of 1 ^o branches per panicle (n = 10)	14.20 $\pm$ 0.99 a	14.80 $\pm$ 0.63 a	13.30 $\pm$ 1.73 a	15.40 $\pm$ 0.64 a
Total grains per panicle (n=10)	154.20 $\pm$ 17.92 a	170.80 $\pm$ 19.91 a	170.70 $\pm$ 6.92 a	177.90 $\pm$ 8.66 a
Filled grains per panicle (n = 10)	120.20 $\pm$ 16.72 b	139.90 $\pm$ 18.21 ab	146.50 $\pm$ 5.30 a	159.00 $\pm$ 8.32 a
Unfilled grains per panicle (n=10)	34.00 $\pm$ 6.14 a	30.90 $\pm$ 3.39 a	24.20 $\pm$ 2.31 b	18.90 $\pm$ 1.87 c
Grain length (mm) (n = 100)	7.51 $\pm$ 0.04 a	7.36 $\pm$ 0.03 b	7.29 $\pm$ 0.07 b	7.34 $\pm$ 0.03 b
Grain width (mm) (n = 100)	2.17 $\pm$ 0.01 b	2.20 $\pm$ 0.01 a	2.20 $\pm$ 0.01 a	2.21 $\pm$ 0.01 a
Grain nitrogen content (mg g ⁻¹) (n = 10)	11.06 $\pm$ 0.17 a	10.54 $\pm$ 0.24 b	10.85 $\pm$ 0.16 ab	11.00 $\pm$ 0.16 a
Grain chalkiness (%) (n = 100)	25.64 $\pm$ 0.96 b	28.77 $\pm$ 0.87 a	26.03 $\pm$ 0.88 b	27.15 $\pm$ 0.83 ab
100-seed weight (g) (n = 10)	2.7 $\pm$ 0.4 a	2.3 $\pm$ 0.1 a	2.2 $\pm$ 0.2 a	2.3 $\pm$ 0.1 a

Each value is the mean with standard error ($\pm$ S.E.). ¹Mean values within a row followed by a different letter differed significantly ($P < 0.05$).

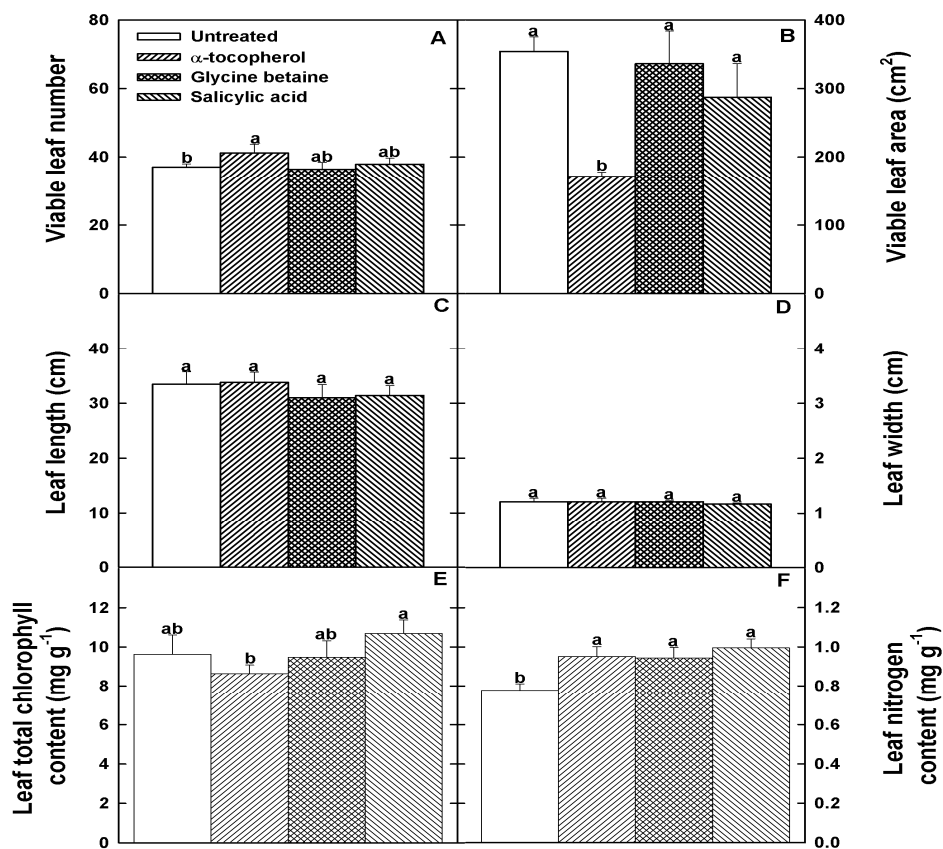


Figure 1. Effects of α -tocopherol, glycine betaine or salicylic acid on rice leaf characteristics. Bars with different letters for a particular parameter differed significantly at $P \leq 0.05$

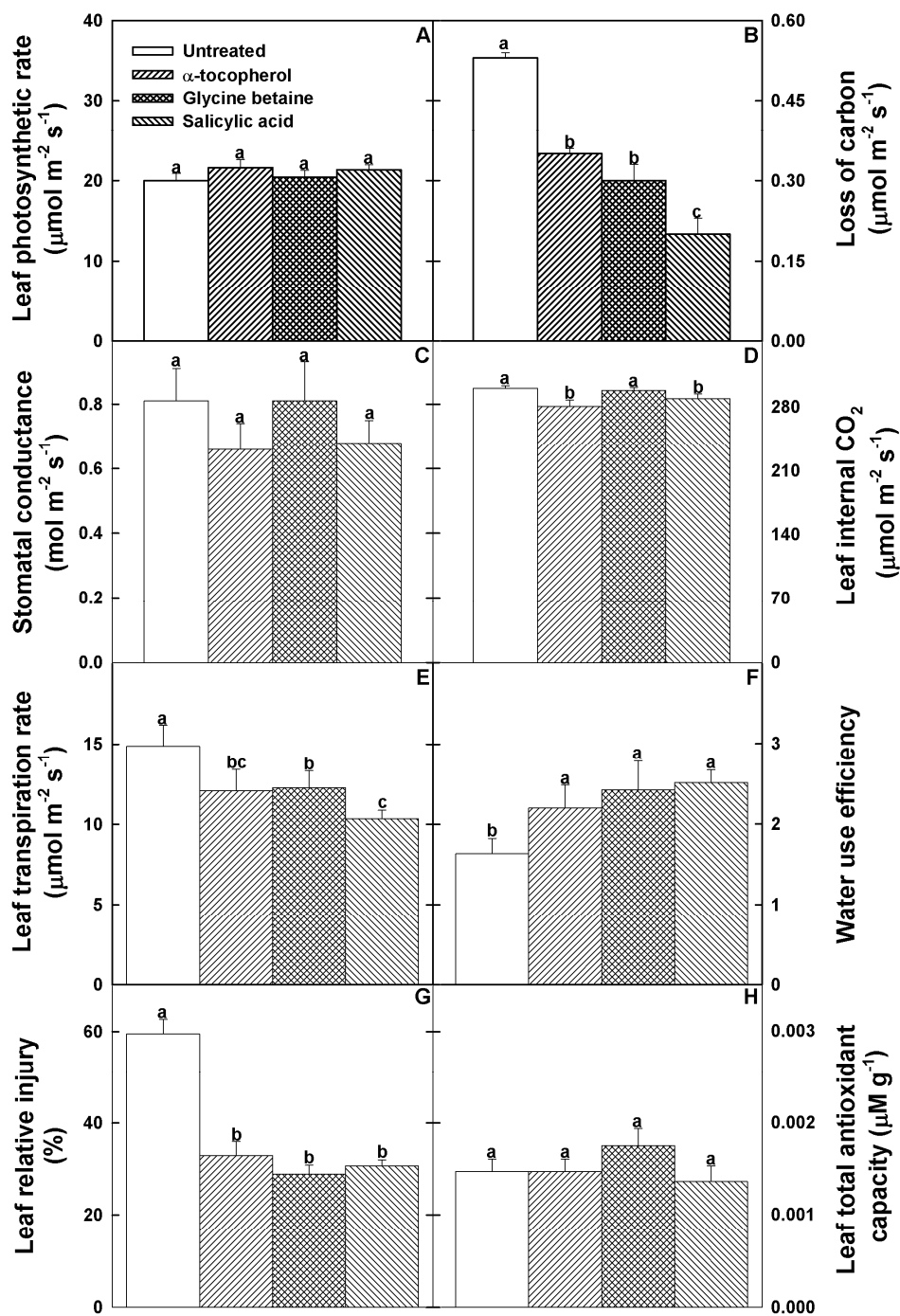


Figure 2. Effects of α -tocopherol, glycine betaine or salicylic acid on rice leaf physiology. Bars with different letters for a particular parameter differed significantly at $P \leq 0.05$