

# Interactive Effect of *Rhizobium* Inoculation, Sodium Benzoate and Salinity on Performance and Oxidative Stress in Two Fababean Varieties

M.S. GABALLAH<sup>1</sup> AND A.M. GOMAA<sup>†</sup>

Water Relations and Field Irrigation Department and <sup>†</sup>Agricultural Microbiology Department, National Research Centre, Cairo, Egypt.

<sup>1</sup>Corresponding authors email address: msgaballa54@yahoo.com

## ABSTRACT

A pot trial was designed to investigate the impact of *Rhizobium* inoculation and/or sodium benzoate application on performance of two contrasting fababean varieties i.e., Giza Blanka (salt tolerant) and Giza 634 (salt sensitive) grown in sandy soil under two levels of salinity (3000 and 6000 ppm). High salinity (6000 ppm) greatly reduced nodules formation in both varieties. *Rhizobium* inoculation reduced the inhibitory effect of salinity and plants were able to survive better. Giza Blanka, surpassed the other variety in all characteristics. Leaf area,  $Ka^+ : Na^+$  ratio, protein content, malondialdehyde and superoxide dismutase activity were greatly affected by increasing salinity in both varieties. Hydroxyl scavenger, (sodium benzoate) positively improved leaf area reduced transpiration rate, malondialdehyde content (MDA) in plant leaves and showed a slight increase in the activity of enzyme superoxide dismutase (SOD). *Rhizobium* inoculation increased plant leaf area, reduced MDA content in plant leaves and did not show a significant effect on SOD activity in plant roots, but the interaction between *Rhizobium* inoculation and sodium benzoate resulted in a significant increase in SOD enzyme activity in plant roots.

**Key Words:** Salinity; Fababean; *Rhizobium* inoculation; Chemical constituents; Oxidative stress.

## INTRODUCTION

Earth is a salty planet, with most of its water containing about 30 g of sodium chloride  $L^{-1}$ . This is continually affecting the land on which crops are or will be grown. Although the salt affected land (about  $900 \times 10^6$  ha) is imprecisely known, its extent is sufficient to pose a threat to agriculture (Flowers & Yeo, 1995; Munns, 2002), since most plants and certainly most crop plants will not grow under high concentrations of salts (Flowers, 2004). Consequently, salinity is a threat to food supply and we need to increase the food production. Therefore, it seems imperative to determine the salt tolerance potential of crops.

Work on salinity in relation to legume-*Rhizobium* symbiosis using small seeded legumes has shown that *Rhizobium* is more tolerant of salts than the host. Plasma membrane injury induced by salt stress is related to an increased production of highly toxic oxygen free radicals (Liu *et al.*, 1987; Hernandez *et al.*, 1993; Singha & Choudhuri, 1990). Under salt stress, both superoxide dismutase (SOD) and catalase (CAT) activities decline in plants (Hernandez *et al.*, 1993; Singha & Choudhuri, 1990) and malondialdehyde (MDA) accumulates rapidly (Hernandez *et al.*, 1993; Lutts *et al.*, 1996), which results in an increase in permeability of plasma membranes. Therefore, sodium benzoate is an efficient hydroxyl radical scavenger in plants (Minotti & Aust, 1987). Legumes are important constituents of vegetarian diets; especially

fababean has lipid reducing effects and may also be a good source of antioxidants and chemopreventive (Madar & Stark, 2003). Fababean (*Vicia faba* L.) is a crop under recent consideration in saline water strategies because it is a major protein-rich food for both human and animals in many parts of the world where poor quality irrigation waters are used (Bond *et al.*, 1994). Growers cultivate fababean as a cover crop for water strategies because of high boron tolerance and ability to fix atmospheric nitrogen (Maas, 1997). Some legumes are reported to be resistant to salinity (Singh *et al.*, 1997) and show ion-exclusion (Lauchli, 1984). Thus the aim of the present work was to evaluate the effect of *Rhizobium* inoculation and/or sodium benzoate application on fababean plant growth and oxidative stress under saline conditions.

## MATERIALS AND METHODS

Two contrasting fababean (*Vicia faba* L.) cultivars Giza 634 (salt sensitive) and Giza Blanka (salt tolerant), were grown at the Greenhouse of the National Research Centre, Dokki, Cairo, Egypt during two successive seasons; 2002 and 2003. Fababean seeds were sown in plastic pots of 30cm diameter. Each pot was filled with 10 kg uncultivated low fertile sandy soil with the following characteristics: clay 8.19%; silt 7.04 %; sand 84.58%, organic matter 0.09%; total nitrogen 0.015%; total phosphorus 0.038%; pH 7.41; EC  $3.7 dS m^{-1}$  and total microbial count  $9.6 \times 10^2$  CFU/gm

dry soil. Five fababean seeds were sown in each pot. *Rhizobium* treated pots were inoculated with 10 ml liquid culture of *Rhizobium sp.* containing  $6.4 \times 10^7$  cell mL<sup>-1</sup> were added directly on the seeds and then covered with thin layer of the soil (Gomaa, 1989). Seeds were soaked either in 1mM sodium benzoate or tap water for 48 h before cultivation. Irrigation was made according to plant need either with tap or salinized water with a mixture of NaCl and CaCl<sub>2</sub> (1:1 w/w) to give the concentration of 3000 & 6000 ppm. Detail of experimental treatments is as follows:

- control S<sub>1</sub> (tap water)
- salinity S<sub>2</sub> (3000 ppm)
- salinity S<sub>3</sub> (6000 ppm)
- S<sub>1</sub> + sodium benzoate
- S<sub>2</sub> + sodium benzoate
- S<sub>3</sub> + sodium benzoate
- S<sub>1</sub> + *Rhizobium*
- S<sub>2</sub> + *Rhizobium*
- S<sub>3</sub> + *Rhizobium*
- S<sub>1</sub> + *Rhizobium* + sodium benzoate
- S<sub>2</sub> + *Rhizobium* + sodium benzoate
- S<sub>3</sub> + *Rhizobium* + sodium benzoate

These treatments were applied to both varieties and each treatment was replicated five times in a randomized complete block design. Boosted doses (half the recommended ones per fed.) of mineral fertilizers i.e. N, P and K were applied. Seedlings were thinned to three per pot after two weeks of cultivation. Plant samples were taken at different harvests and data were recorded for different parameters. Leaf area plant<sup>-1</sup> was measured using the disk method (Watson & Watson, 1953). Rate of transpiration of plant (30 days after germination) was estimated as described by Bozcuk (1975). The ion concentrations of Na<sup>+</sup> and K<sup>+</sup> were determined at flame photometer in the plant samples dried at 70°C for 48 h using nitric-perchloric acid digestion.

Soluble protein was measured by the method of Bradford (1976). Superoxide dismutase (SOD) enzyme activity was detected by the photochemical method described by Giannopolitis and Ries (1977). TBA (thiobarbitric acid) test was adopted to determine malondialdehyde (MDA) following the method of Heath and Packer (1968). Pooled data of the two growing seasons were statistically analyzed (Snedecor & Cochran, 1982), where the means of studied treatments were compared using L.S.D. test at 5% significance level.

## RESULTS AND DISCUSSION

Increasing salinity levels, severely decreased number of nodules per plant and nodules dry weight (Table I). This effect was significantly limited by the application of sodium benzoate and *Rhizobium* inoculation in Giza Blanka, but insignificantly in Giza 634. Abdel Ghaffar (1987) found that water stress caused a marked reduction in nodulation. Salinity badly affected growth and yield but nodulation and N-fixation were less affected. Also, these data get support

from previous data (Abd-Alla, 1992), showing that under salinity *Rhizobium* formed an effective symbiosis with *V. faba* and a decrease in N and nitrogenase activity was due to nodule senescence. Al-Mutawa (2003) suggested that poor symbiotic performance was not due to inhibition of the growth of *Rhizobium* by salinity, rather plant growth was moderately inhibited by 300mM NaCl. Slatery *et al.* (2001) proved that salinity reduced populations of *Rhizobium* in the soil and that high number of naturally occurring *Rhizobium* populations were poor in their ability to fix nitrogen and can compete strongly with introduced *Rhizobium* inoculum.

The leaf area was generally reduced by increased salinity (Table I) and Giza 634 was more affected. Sodium benzoate application was more effective than *Rhizobium* inoculation for increasing leaf area at high salinity level particularly for the sensitive variety (Giza 634). Munns (1993) and Tammam (2003) proposed that the death of old leaves due to ion-toxicity might prevent the supply of nutrients and hormones to young leaves, reducing leaf area.

The transpiration rate was severely affected by salinity (Table I). *Rhizobium* inoculation showed insignificant effect on plant transpiration rate of both varieties. Hydroxyl scavenger (sodium benzoate) application resulted in a significant reduction in the transpiration rate compared to the control, proving that the sodium benzoate application stimulated water conservation via stomatal movements and/or decreasing water loss. As noted in this study, Katerji *et al.* (2002) showed that fababean had a clear inability to adjust osmotically. And as a result, the effect of osmotic potential on stomatal opening was negligible, although, the plants maintained high stomatal conductance.

The K<sup>+</sup>:Na<sup>+</sup> ratio in fababean leaves decreased with increasing salinity and *Rhizobium* inoculation had no significant effect on this ratio for both the varieties. Sodium benzoate application showed a slight increase in this ratio for both the varieties (Table I). Shukry & El-Bassiouny (2002) found that plants under saline conditions, showed a decrease in K<sup>+</sup>:Na<sup>+</sup> ratio. Also Baalbaki *et al.* (2000) found that in salt tolerant plants the K<sup>+</sup> contents of nodulating plants were lower than those of non-nodulating ones at low salinities. There may be different implications for a relation between K<sup>+</sup> and Na<sup>+</sup> in plant parts. Demidchik *et al.* (2002) opined that the mechanism by which sodium enters plants is still poorly understood. At low external concentrations, K<sup>+</sup> may enter roots through its carriers, while at higher concentrations, non-selective cation channels may be the means of transport. However, Dvorak *et al.* (1994) stated that the K<sup>+</sup>:Na<sup>+</sup> ratio in plant is determined at a single locus with a given number of proteins that might contribute to Na<sup>+</sup> and K<sup>+</sup> transport from root to leaves.

High salinity levels severely reduced protein content of fababean seeds (Table I) where this reduction was noted up to 50.6% in Giza Blanka at 6000 ppm while plants of Giza 634 failed to survive. Although application of sodium benzoate curtailed the reduction in protein content by 11.8% at 6000 ppm and due to *Rhizobium* inoculation by 16.4% in

**Table I. Effect of sodium benzoate and *Rhizobium* inoculation on leaf area, leaf transpiration rate, and nodulation, K<sup>+</sup>:Na<sup>+</sup> ratio, total proteins, MDA concentration and SOD activity of fababean grown under various levels of salinity**

| Varieties          | Treatments                      | Leaf area (cm <sup>2</sup> plant <sup>-1</sup> ) | Transpiration rate (mg cm <sup>-2</sup> d <sup>-1</sup> ) | Number of nodules per plant | of Nodules per weight (g plant <sup>-1</sup> ) | dry K <sup>+</sup> :Na <sup>+</sup> ratio | Protein (%) | MDA conc. (mMmg <sup>-1</sup> protein) | SOD activity (U.mg <sup>-1</sup> protein) |      |
|--------------------|---------------------------------|--|---|-----------------------------|--|---|-------------|--|---|------|
| Giza               | S <sub>1</sub> (tap water)      | 239.20   | 91.07   | 20.3                        | 0.42   | 0.84                                      | 22.55       | 14.6                                   | 110.51                                    |      |
| Blanka             | S <sub>2</sub> (3000 ppm)       | 177.44   | 69.20   | 18.8                        | 0.35   | 0.77                                      | 14.63       | 18.2                                   | 98.11                                     |      |
|                    | S <sub>3</sub> (6000 ppm)       | 73.49  | 36.15   | 4.5                         | 0.08   | 0.66                                      | 11.16       | 21.4                                   | 71.40                                     |      |
|                    | S <sub>1</sub> + sod. Benz      | 210.34   | 79.25   | 22.7                        | 0.41   | 0.91                                      | 23.30       | 12.4                                   | 114.59                                    |      |
|                    | S <sub>2</sub> + sod. benz      | 193.76   | 55.20   | 19.2                        | 0.36   | 0.89                                      | 19.71       | 20.7                                   | 121.23                                    |      |
|                    | S <sub>3</sub> + Sod. benz      | 91.53  | 36.12   | 5.4                         | 0.09   | 0.75                                      | 15.42       | 33.5                                   | 134.33                                    |      |
|                    | S <sub>1</sub> + Rh             | 222.14   | 88.13   | 95.0                        | 1.59   | 0.81                                      | 19.56       | 10.5                                   | 110.32                                    |      |
|                    | S <sub>2</sub> + Rh             | 109.88   | 62.87   | 85.7                        | 1.33   | 0.63                                      | 14.92       | 14.7                                   | 97.12                                     |      |
|                    | S <sub>3</sub> + Rh             | 69.52  | 38.20   | 53.0                        | 1.02   | 0.41                                      | 11.56       | 18.6                                   | 61.94                                     |      |
|                    | S <sub>1</sub> + Rh + sod. benz | 199.71   | 87.94   | 98.5                        | 1.72   | 0.88                                      | 22.91       | 11.4                                   | 123.71                                    |      |
|                    | S <sub>2</sub> + Rh + sod. benz | 121.34   | 54.41   | 79.7                        | 1.33   | 0.81                                      | 19.63       | 13.8                                   | 130.11                                    |      |
|                    | S <sub>3</sub> + Rh + sod. benz | 88.11  | 23.65   | 46.3                        | 0.62   | 0.67                                      | 14.11       | 20.7                                   | 101.34                                    |      |
|                    | L.S.D (p<0.05)                  | S  | 12.70   | 7.20                        | 3.11   | 0.91                                      | 0.12        | 0.9                                    | 2.1                                       | 9.80 |
|                    |                                 | S x Rh   | NS  | NS                          | 4.8  | 1.12                                      | NS          | 0.88                                   | NS  | NS   |
| S x sod. benz      |                                 | 10.61  | 6.69  | 1.12                        | 0.86   | 0.18                                      | NS          | NS                                     | 7.20                                      |      |
| S x Rh x sod. benz |                                 | 9.71   | 6.31  | 3.15                        | 0.71   | 0.18                                      | 0.71        | 0.18                                   | 4.46                                      |      |
| Giza 634           | S <sub>1</sub> (tap water)      | 188.22   | 52.15   | 19.5                        | 0.40   | 0.78                                      | 18.32       | 30.70                                  | 105.61                                    |      |
|                    | S <sub>2</sub> (3000 ppm)       | 96.19  | 43.90   | 15.0                        | 0.21   | 0.54                                      | 9.60        | 45.8                                   | 67.11                                     |      |
|                    | S <sub>3</sub> (6000 ppm)       | FS   | FS  | FS                          | FS   | FS  | FS          | FS                                     | FS  |      |
|                    | S <sub>1</sub> + sod. benz      | 201.73   | 63.11   | 20.0                        | 0.37   | 0.83                                      | 21.15       | 31.5                                   | 106                                       |      |
|                    | S <sub>2</sub> + sod. benz      | 100.67   | 48.17   | 18.0                        | 0.25   | 0.61                                      | 17.12       | 44.9                                   | 81.17                                     |      |
|                    | S <sub>3</sub> + Sod. Benz      | FS   | FS  | FS                          | FS   | FS  | FS          | FS                                     | FS  |      |
|                    | S <sub>1</sub> + Rh             | 192.12   | 55.46   | 60.0                        | 0.76   | 0.66                                      | 18.11       | 28.6                                   | 97.11                                     |      |
|                    | S <sub>2</sub> + Rh             | 166.32   | 41.76   | 47.0                        | 0.85   | 0.58                                      | 14.43       | 45.2                                   | 101.30                                    |      |
|                    | S <sub>3</sub> + Rh             | 71.09  | 20.95   | 34.5                        | 0.34   | 0.51                                      | 10.11       | 55.0                                   | 55.89                                     |      |
|                    | S <sub>1</sub> + Rh + sod. benz | 179.81   | 69.30   | 51.5                        | 0.87   | 0.74                                      | 20.30       | 32.5                                   | 108.42                                    |      |
|                    | S <sub>2</sub> + Rh + sod. benz | 100.55   | 58.13   | 27.0                        | 0.51   | 0.69                                      | 15.35       | 36.9                                   | 71.56                                     |      |
|                    | S <sub>3</sub> + Rh + sod. benz | FS   | FS  | FS                          | FS   | FS  | FS          | FS                                     | FS  |      |
|                    | L.S.D (p<0.05)                  | S  | 10.15   | 7.20                        | 2.71   | 0.56                                      | 0.12        | 0.9                                    | 2.1                                       | 9.80 |
| S x Rh             |                                 | NS   | NS  | 1.81                        | 0.61   | NS  | 0.88        | NS                                     | NS  |      |
| S x sod. benz      |                                 | 9.11   | 6.69  | NS                          | NS   | 0.18                                      | NS          | NS                                     | 7.20                                      |      |
| S x Rh x sod. benz |                                 | 9.81   | 6.31  | NS                          | NS   | 0.18                                      | 0.71        | 0.18                                   | 4.46                                      |      |

S: salinity; Rh: *Rhizobium* inoculation; sod. benz: sodium benzoate; FS: plants failed to survive

Giza Blanka and by 6.5% in Giza 634, the interactive effect of sodium benzoate and *Rhizobium* inoculation was significant in maintaining protein content. Protein was reduced by 12.2% in Giza Blanka at 6000 ppm and 25.9% in Giza 634 at 3000 ppm. Similar results were reported by Gaballah & Gomaa (2000).

The data for malondialdehyde (MDA) concentration (Table I) showed that for both fababean varieties, the MDA levels of salt stressed plants were significantly higher compared to the control. The extent of membrane damage was monitored by measuring MDA concentration which increased at moderate salinity by 24.6% in Giza Blanka, 49.1% in Giza 634 and by 46.4% in Giza Blanka at high salinity compared to control. However, inclusion of sodium benzoate as a hydroxyl scavenger reduced MDA concentration of salt stressed plants in comparison with those subjected to salinity stress only, although sensitive fababean variety (Giza, 634) induced high lipid peroxidation (inferred by MDA concentration) than the tolerant variety (Giza Blanka). Conversely, *Rhizobium* inoculation did not show a significant change in MDA concentration for both

chosen varieties under different salinity levels. The results of this study are in line with previous findings concluding that salt stress creates oxidative stress through the formation of reactive oxygen species (ROS) which attack lipids, proteins and nucleic acids causing lipid peroxidation and protein denaturation. Also, legume root nodules have a high potential for producing ROS because they contain abundant oxygen-labile proteins. Liang *et al.* (2003) stated that the induction of lipid oxidative damage was closely related to the decline and non-enzymatic antioxidants in plants roots. A clear decline in SOD activity in fababean roots was observed with increasing salinity stress (Table I). The SOD was reduced by 35.5% at 6000 ppm in Giza Blanka and by 36.4% at 3000 ppm in Giza 634 compared to the control. However, the inclusion of sodium benzoate as a hydroxyl scavenger significantly improved the SOD content in roots of both the cultivars. Contrarily, the *Rhizobium* inoculation under saline condition showed an insignificant effect on the SOD activity, although the interactive effect of salinity, *Rhizobium* and sodium benzoate on SOD level was significant particularly in Giza 634. Salt tolerant plants had

higher SOD level than sensitive plants. These data is supported by the previous findings showing that the cooperation between hydroxyl scavenging enzymes or non-enzymes and SOD plays an important role in the resistance of plants to different environmental stresses (Gaballah & Gomaa, 2000; Liang *et al.*, 2003).

## CONCLUSION

*Rhizobium* inoculation, was beneficial in increasing productivity, but it did not show protection against oxidative damage caused by salinity may be due to earlier oxidative damage which might occur in the period over which the symbiosis become established. The application of sodium benzoate under saline condition is recommended as a good hydroxyl scavenger which increased the SOD activity thus protecting the plant against oxidative damage.

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(Received 22 January 2005; Accepted 26 March 2005)