# *Zea Mays* Cultivar Behavior as Affected by *Rhizobium radiobacter* Inoculation in Salt-Stressed Environments

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**Abstract:** Response variation for salt tolerance was assessed in two *Zea mays* L. cultivars; Giza 2 (salt tolerant) and Hybrid triple 314 (salt sensitive) amended in presence of *Rhizobium radiobacter* strain (HQ 395610-Egypt) under two N fertilization regimes (75% and 100%). Soil samples tested for enzymatic activities revealed that R. radiobacter inoculation increased nitrogenase and hydrolases activities particularly after 90 and 45 days. The leaf sample analyzed for inorganic osmolytes (potassium and sodium) showed that both cultivars had high K/Na ratio when treated with *R. radiobacter*, while total chlorophyll, soluble sugars and peroxidase activity increased. Proline stress-response was also reduced by *R. radiobacter* inoculation. Substantial variations were observed in the grain quality and yield for both cultivars due to the different treatments. The grain yield increased by 71% in cv. Giza and 48% in cv. Hybrid when *R. radiobacter* treatment was combined with full N fertilization level.

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**Key words:** *Zea mays* L.; *Rhizobium radiobacter*; salinity; N-fertilization; growth parameters.

## 1. Introduction

Corn (*Zea mays* L.) is one of the most important cereal crops cultivated worldwide. It is used as food for human consumption as well as food grain for animals (**Moussa, 2001**). The steady increase in the world population demands an expansion of crop areas to raise food production. In this context, a significant fraction of agricultural crops is cultivated in low quality soils, sometimes affected by salinity (**Allen *et al.*, 1983**). Maize is considered a moderately salt-sensitive plant (**Mass and Hoffman, 1977**).

As salinity inhibition of plant growth is a result of osmotic and ionic effects, different plant species have developed different mechanisms to cope with these effects (**Munns, 2002**). Osmotic adjustment by net solute accumulation to reduce osmotic potential is a mechanism for plant salt tolerance. This includes compatible organic solutes (soluble carbohydrates, proline, etc) accumulations (**Hasegawa *et al.*, 2000**) that contribute to the maintenance of water uptake and cell turgor, allowing physiological processes, such as stomata opening, photosynthesis, and cell expansion (**Serraj and Sinclair, 2002**). They also help in the removal of free radicals, and stabilization of macromolecules and organelles, such as proteins, protein complexes and membranes (**Bray *et al.*, 2000**) and the control of pH in the cytosol and detoxification of excess $NH4^+$ (**Gilbert *et al.*, 1998**).

On the other hand, some plant growth-promoting rhizobacteria (PGPR) may exert a direct stimulation on plant growth and development by providing plants with fixed nitrogen, amino acids, phytohormones, iron that has been sequestered by bacterial siderophores, and soluble phosphate (**Hayat *et al.*, 2010**).

Plants infected with IAA-overproducing PGPR strains showed high antioxidant enzyme activities that contribute to enhance plant protection against salt stress (**Bianco and Defez, 2009**). Degradation of the ethylene precursor 1-Aminocyclopropane-1-carboxylic acid (ACC) exuded from plant roots into 2-oxobutanoate and ammonia by bacterial ACC-deaminase lowers the ethylene concentration in plant roots, relieves the ethylene repression of auxin response factors synthesis, and indirectly increases plant growth. Taken together, the ACC-deaminase function seems to be mutually beneficial between plants and PGPR and as a result, the hydrolyzed ACC products would enhance bacterial growth (**Glick 2005; Glick *et al.*, 2007; Kang *et al.*, 2010**). The PGPR strains can produce exo-polysaccharides to bind cations including sodium (**Geddie and Sutherland,1993**), thus help alleviating salt stress in plants grown under saline environment (**Ashraf *et al.*, 2004**).

According to their residing sites, the PGPR can be divided into two major groups; iPGPR, which live inside plant cells and are localized in specialized structures, the so-called nodules, and ePGPR which live outside plant cells and do not produce organs like nodules, but still prompt plant growth (**Gray and Smith, 2005**). An example of ePGPR is *R. radiobacter* described as non-pathogenic *Agrobacterium* strain, which is capable of $N_2$ fixation and IAA production, used in inoculation of wheat and barley and resulted in crop yield increases (**Bairamov *et al.*, 2001; Zavalin *et al.*, 2001**). *R. radiobacter* also used with Turkish hazelnut cultivars and proved to be the most effective in promoting macro and micro nutrient uptake (**Yasar *et al.*, 2011**). The aim of this study is to evaluate the possible interaction between salinity and *Rhizobium radiobacter* inoculation, ion relations as well as some physiological parameters of maize cvs. Giza2 and Hybrid triple 314 in a field experiment.

## 2. Materials and Methods:

Two *Zea mays* cultivars; salt tolerant Giza 2 (G) and salt sensitive Hybrid triple 314 (H), were provided from Horticulture Research Institute, ARC, Giza, Egypt. Elsarw Agric. Res. Station was the experimental location in two successive summer seasons 2010-2011. The mechanical and chemical profile of the experimental soil is presented in Table (1).

**Table (1): Mechanical and chemical properties in the experimental soil.**

| | | | |
|---|---|---|---|
| Physical properties | Mechanical analysis (%) | Coarse sand | 1.75 |
| | | Fine sand | 9.60 |
| | | Silt | 21.90 |
| | | Clay | 66.70 |
| | Textural class | | Clayey |
| Chemical properties | Organic matter % | | 0.7 |
| | Total nitrogen % | | 0.032 |
| | CaCO3 % | | 2.2 |
| | Available K (ppm) | | 28.7 |
| | pH | | 7.9 |
| | E.C (ds.m-1) | | 5.7 |
| | C.E.C (meq/100 g soil) | | 52 |
| | Cations (meq/100 g soil) | $Ca^{2+}$ | 7.9 |
| | | $Mg^{2+}$ | 9.6 |
| | | $Na^{+}$ | 33.9 |
| | | $K^{+}$ | 0.13 |
| | Anions (meq/100 g soil) | $CO_3^{2-}$ | --- |
| | | $HCO_3^{-}$ | 1.6 |
| | | $Cl^{-}$ | 31 |
| | | $SO_4^{2-}$ | 18.93 |

A *Rhizobium radiobacter* strain isolated from saline soil, obtained from Department of Microbiology/SWERI/ARC, Egypt and having an accession number HQ 395610-Egypt was applied in a field trial as nitrogen fixing and osmoprotectant bacterium. The experiment was carried out in a spilt plot design with four replicates for each treatment. Treatments included two rates of nitrogen fertilizer ($NH_4NO_3$) applied as 100 and 150 kg N.fed$^{-1}$ in combination with or without *R. radiobacter.*

The *R. radiobacter* was used as a seed coat prior to planting then added as soil drench as 10 L.fed$^{-1}$ (ca.10$^{6}$ cfu.ml$^{-1}$) after 15 and 30 days of sowing. Superphosphate ($P_2O_5$) was applied at the rate 200 kg.fed$^{-1}$. Data were recorded for samples taken after 45, 90 and 120 days (harvest) for both seasons.

In the fresh leaf samples; plant chlorophyll (a, b and total) and caroteniods were determined according to **Saric *et al.* (1976)**. Total soluble phenols were determined as recommended by **A.O.A.C. (1980).**

Soluble sugars were estimated using the method described by **Dubois *et al.* (1956)** while antioxidant enzyme activity as peroxidase was measured according to **Maehly and Chance (1955)**.

Determination of free proline in leaf powder was done as mentioned by **Bates *et al.* (1973)**. $K^{+}$, $Na^{+}$ and $Ca^{+2}$ were measured by flamephotometer (**Havre, 1961**), while $Mg^{+2}$ was measured by atomic absorption spectrophotometer (**Anju *et al.*, 2011**).

At harvest, plants were estimated for grain yield, grain quality (weight of 100 grain) and the macronutrients % (N, P, and K). Grain total nitrogen was determined by Kjeldahl digestion method (**Jackson, 1973),** total phosphorus in the acid digest by the method of **Murphy and Riley (1962)**, while total potassium was determined in the same digest by flame photometry according to the method of **Chapman and Pratt (1961)**.

Soil rhizosphere hydrolytic enzymes (lipases, protease, and esterases) expressing *R. radiobacter* activity were collectively assayed by 3,6-diacetylfluorescein (FDA) method as described by **Green *et al.* (2006)**.

The significance of differences between the mean values were statistically evaluated by COSTAT software on the basis of ANOVA statistics for the completely randomized means and calculated through Ducan's Multiple Range test at significance level of 5% for one factor each time (one way analysis) as mentioned by **Metcalfe *et al.* (2002).**

## 3. Results

Based on the mechanical analysis, the soil was classified as clay. Depending on the concentrations of $Na^{+}$, $Ca^{2+}$ and $Mg^{2+}$, the exchangeable sodium percentage (ESP) was 65%. Combining ESP estimate with the EC exceeding 4 and pH lower than 8.5, the soil is saline sodic with normal physical conditions (**Abrol *et al.*, 1988**).

According to leaf analysis of salt-tolerant Giza 2 cultivar (Table 2), the translocated $K^{+}$ and $Na^{+}$ ions significantly reduced with *R. radiobacter* treatment and plant age, regardless of nitrogen fertilization regime. The K/Na ratio reveals the superiority of $Na^{+}$ over $K^{+}$ translocated from roots to leaves after 45 days. With plant age, the decreases in translocated $Na^{+}$ were more obvious than those in translocated K and this significantly raised the ratios of K/Na in most treatments, with control being the highest, while no changes were reported due to *R. radiobacter* treatment and lower N fertilization regimes.

Significant differences in $Mg^{2+}$ and $Ca^{2+}$ levels in leaves were recorded. Both $Mg^{2+}$ and $Ca^{2+}$ ions decreased with plant growth. $Mg^{2+}$ was higher in R. radiobacter treatment with complete N fertilization, while $Ca^{2+}$ was markedly greater in *R. radiobacter* treatment with low N fertilization.

**Table (2): *Zea Mays* cvs. Giza2 and Hybrid triple 314 leaf analysis (ions) as affected by R. radiobacter inoculation and N-fertilization.**

| Treatments | days | K | Na | K/Na | Mg | Ca |
|---|---|---|---|---|---|---|
| cv. Giza2 | | | | | | |
| 150 Kg.fed$^{-1}$ (Full N) | 45 | $1.31^{a}$ | $2.44^{a}$ | $0.54^{d}$ | $1.19^{b}$ | $1.36^{ab}$ |
| | 90 | $0.72^{c}$ | $0.40^{e}$ | $1.80^{a}$ | $0.74^{f}$ | $0.53^{d}$ |
| *R. radiobacter* + 100 Kg.fed$^{-1}$ | 45 | $1.03^{b}$ | $1.45^{c}$ | $0.71^{c}$ | $1.06^{c}$ | $1.70^{a}$ |
| | 90 | $0.62^{c}$ | $0.86^{d}$ | $0.72^{c}$ | $0.95^{d}$ | $0.61^{cd}$ |
| *R. radiobacter* + 150 Kg.fed$^{-1}$ | 45 | $1.10^{b}$ | $1.81^{b}$ | $0.60^{d}$ | $1.27^{a}$ | $1.03^{bc}$ |
| | 90 | $0.68^{c}$ | $0.81^{d}$ | $0.84^{b}$ | $0.84^{e}$ | $0.77^{cd}$ |
| cv. Hybrid Triple 314 | | | | | | |
| 150 Kg.fed$^{-1}$ (Full N) | 45 | $0.89^{b}$ | $1.58^{b}$ | $0.56^{e}$ | $1.05^{ab}$ | $1.28^{a}$ |
| | 90 | $0.62^{c}$ | $0.63^{e}$ | $0.98^{a}$ | $0.96^{bc}$ | $0.96^{b}$ |
| *R. radiobacter* + 100 Kg.fed$^{-1}$ | 45 | $1.22^{a}$ | $2.02^{a}$ | $0.61^{d}$ | $0.95^{bc}$ | $0.70^{c}$ |
| | 90 | $0.69^{c}$ | $1.04^{d}$ | $0.66^{c}$ | $1.14^{a}$ | $0.67^{c}$ |
| *R. radiobacter* + 150 Kg.fed$^{-1}$ | 45 | $0.87^{b}$ | $1.27^{c}$ | $0.69^{b}$ | $0.83^{cd}$ | $0.74^{c}$ |
| | 90 | $0.59^{c}$ | $1.04^{d}$ | $0.57^{e}$ | $0.76^{d}$ | $0.61^{c}$ |

Means in each column denoted by different letter significantly differ at $P < 0.05$ according to Duncan's multiple range tests.

Concerning the salt sensitive Hybrid cultivar, Table (2) shows that the $K^+$ and $Na^+$ contents in leaf were significantly higher in the *R. radiobacter* treatment of low N fertilization level after 45 days. After 90 days, both $K^+$ and $Na^+$ decreased remarkably in all treatments and $Na^+$ was significantly higher in *R. radiobacter* treatments than control. The K/Na ratio revealed the superiority of $Na^+$ over $K^+$ translocation from roots to plant leaves. With plant age, the ratio of K/Na significantly increased in the control treatment but decreased in the *R. radiobacter* treatment with full N fertilization due to the obvious decreases in translocated $Na^+$ in the former treatment than in the latter one.

$Mg^{2+}$ and $Ca^{2+}$ contents were significantly higher in control than *R. radiobacter* treatments after 45 days while their contents varied after 90 days. $Mg^{2+}$ insignificantly decreased after 90 days in most treatments but significantly increased in *R. radiobacter* treatment with lower N fertilization. This treatment recorded the highest $Mg^{2+}$ content than other treatments after 90 days. On the other hand, $Ca^{2+}$ content decreased after 90 days among all treatments and was significantly higher in the control than other treatments after 45 and 90 days.

In salt-tolerant Giza 2 cultivar (Table 3) significant increases in total chlorophyll contents were in the *R. radiobacter* treatments, full N-fertilization and plant age, where *R. radiobacter* treatments with full N-fertilization recorded the highest contents after 90 days due to increases in chlorophyll A than those in B. Consequently, the chlorophyll A/B ratio was mostly higher in *R. radiobacter* treatments and plant age.

**Table (3): *Zea Mays* cvs. Giza2 and Hybrid triple 314 leaf analysis (organic compounds) due to R. radiobacter treatments and N-fertilization.**

| Samples | Days | ChA | ChB | T.Ch | Carot | S.S | Poxase | Phenols | Prolines |
|---|---|---|---|---|---|---|---|---|---|
| cv. Giza2 | | | | | | | | | |
| 150 Kg.fed$^{-1}$ (Full N) | 45 | $1.10^{ab}$ | $4.00^{bc}$ | $5.10^{d}$ | $4.00^{e}$ | $0.035^{b}$ | $8.6^{f}$ | $2303^{a}$ | $1505^{a}$ |
| | 90 | $5.00^{bc}$ | $4.00^{bc}$ | $9.00^{b}$ | $8.00^{d}$ | $0.054^{b}$ | $58.0^{c}$ | $2237^{a}$ | $756^{d}$ |
| *R. radiobacter* + 100 Kg.fed$^{-1}$ | 45 | $3.50^{d}$ | $4.00^{bc}$ | $7.50^{c}$ | $10.00^{bc}$ | $0.058^{b}$ | $22.0^{d}$ | $1712^{d}$ | $1428^{b}$ |
| | 90 | $7.00^{a}$ | $3.00^{c}$ | $10.00^{ab}$ | $9.00^{cd}$ | $0.075^{a}$ | $84.0^{a}$ | $1900^{c}$ | $683^{e}$ |
| *R. radiobacter* + 150 Kg.fed$^{-1}$ | 45 | $4.00^{cd}$ | $6.30^{a}$ | $10.30^{ab}$ | $13.00^{a}$ | $0.071^{a}$ | $20.0^{e}$ | $2103^{b}$ | $1289^{c}$ |
| | 90 | $6.00^{ab}$ | $5.00^{ab}$ | $11.00^{a}$ | $11.00^{b}$ | $0.087^{b}$ | $68.8^{b}$ | $2062^{b}$ | $667^{f}$ |
| cv. Hybrid Triple 314 | | | | | | | | | |
| 150 Kg.fed$^{-1}$ (Full N) | 45 | $1.00^{d}$ | $1.30^{d}$ | $2.30^{e}$ | $2.00^{ab}$ | $0.069^{bc}$ | $2.4^{f}$ | $1353^{f}$ | $1667^{a}$ |
| | 90 | $5.00^{b}$ | $3.00^{c}$ | $8.00^{cd}$ | $2.10^{a}$ | $0.063^{c}$ | $10.4^{e}$ | $1770^{d}$ | $928^{d}$ |
| *R. radiobacter* + 100 Kg.fed$^{-1}$ | 45 | $3.00^{c}$ | $4.90^{b}$ | $7.90^{d}$ | $1.89^{ab}$ | $0.075^{ab}$ | $16.5^{c}$ | $1887^{c}$ | $1556^{b}$ |
| | 90 | $5.00^{b}$ | $4.00^{bc}$ | $9.00^{c}$ | $1.57^{cd}$ | $0.055^{c}$ | $34.0^{b}$ | $1570^{e}$ | $767^{e}$ |
| *R. radiobacter* + 150 Kg.fed$^{-1}$ | 45 | $3.40^{c}$ | $7.00^{a}$ | $10.4^{b}$ | $1.35^{d}$ | $0.093^{a}$ | $12.0^{d}$ | $2003^{b}$ | $1033^{c}$ |
| | 90 | $7.40^{a}$ | $5.20^{b}$ | $12.60^{a}$ | $1.77^{bc}$ | $0.077^{ab}$ | $38.0^{a}$ | $2103^{a}$ | $689^{f}$ |

Means in each column denoted by different letter significantly differ at $P < 0.05$ according to Duncan's multiple range tests.

Ch: chlorophyll, Carot: carotene, S.S: soluble sugars, Poxase: peroxidase

Generally, carotene and soluble sugar concentrations as well as peroxidase activity were significantly higher in *R. radiobacter* treatments after 45 and 90 days of growth, giving highest carotene and soluble sugars contents with full nitrogen fertilization and highest peroxidases with lower nitrogen fertilization level. On the contrary, phenol and proline contents recorded their highest values in the control. Reducing N fertilization decreased phenol contents, while proline content significantly increased due to *R. radiobacter* treatments.

In Hybrid cultivar (Table 3), the total chlorophyll including both chlorophyll A and B contents was significantly higher in *R. radiobacter* treatment with full N fertilization and decreased in presence of low N fertilization level. With plant age, total chlorophyll increased in each treatment individually. It is worthy to notice that the ratio of chlorophyll A/B after 45 days increased remarkably after 90 days and was higher in control and lower in *R. radiobacter* treatments.

Carotene and proline contents were significantly higher in control than in *R. radiobacter* treatment, while soluble sugars, phenols and peroxidases activity were significantly higher in *R. radiobacter* treatment with complete N fertilization than other treatments. Along growth period, the soluble sugar and proline decreased while peroxidase activity increased significantly. With plant age, phenols and carotenes decreased in *R. radiobacter* treatment with lower N fertilization but increased in the other treatments.

Grain yield significantly increased in both cultivars with *R. radiobacter* treatment and full N fertilization than other treatments (Table 4), while grain quality was significantly the highest with cv. Hybrid. It is worthy to notice that N P K levels in grains of both cultivars were on the same trend of grain yield (Table 4).

**Table (4) Zea Mays cvs. Giza2 and Hybrid triple 314 grains quality and yield, due to R. radiobacter treatments and N fertilization after 120 days of growth.**

| Treatments | Grains | | | | |
|---|---|---|---|---|---|
| | N | P | K | Quality (100 grains wt in g) | Yield (ardab/fedan) |
| cv. Giza 2 | | | | | |
| 150 Kg.fed$^{-1}$ (Full N) | $2.4^{b}$ | $0.35^{c}$ | $0.16^{c}$ | $31.80^{b}$ | $13.9^{d}$ |
| *R. radiobacter* + 100 Kg.fed$^{-1}$ | $2.6^{b}$ | $0.41^{b}$ | $0.19^{b}$ | $32.00^{b}$ | $22.45^{b}$ |
| *R. radiobacter* + 150 Kg.fed$^{-1}$ | $3.2^{a}$ | $0.43^{a}$ | $0.21^{a}$ | $33.00^{b}$ | $23.8^{a}$ |
| cv. Hybrid triple 314 | | | | | |
| 150 Kg.fed$^{-1}$ (Full N) | $2.0^{c}$ | $0.35^{c}$ | $0.15^{b}$ | $27.50^{d}$ | $16.5^{c}$ |
| *R. radiobacter* + 100 Kg.fed$^{-1}$ | $2.3^{b}$ | $0.39^{b}$ | $0.15^{b}$ | $29.00^{c}$ | $21.8^{b}$ |
| *R. radiobacter* + 150 Kg.fed$^{-1}$ | $2.9^{a}$ | $0.52^{a}$ | $0.18^{a}$ | $35.00^{a}$ | $24.5^{a}$ |

Means in each column denoted by different letter significantly differ at $P < 0.05$ according to Duncan's multiple range tests.

Soil nitrogenase activity (Nase) for Giza and Hybrid cultivars increased with plant growth up to 90 days then decreased, giving its highest values with *R. radiobacter* treatment and full N fertilization (Table 5).

Hydrolases activity (FDase) for both cultivars decreased with plant growth and was significantly high with *R. radiobacter* treatment in presence of low N fertilization.

**Table (5) Nitrogenase and hydrolase activities of soils cultivated with *Zea Mays* Giza2 and Hybrid triple 314 due to R. radiobacter R. radiobacter treatments and N-fertilization.**

| Treatments | days | soil | | soil | |
|---|---|---|---|---|---|
| | | Nase | FDAase | Nase | FDAase |
| | | cv. Giza 2 | | cv. hybrid triple 314 | |
| 150 Kg.fed-1 (Full N) | 45 | $113^{g}$ | $32.5^{c}$ | $124^{g}$ | $29.8^{b}$ |
| | 90 | $299^{c}$ | $28.6^{e}$ | $520^{c}$ | $27.7^{c}$ |
| | 120 | $79^{h}$ | $26.6^{f}$ | $86^{i}$ | $25.0^{d}$ |
| *R. radiobacter* + 100 Kg.fed-1 | 45 | $157^{e}$ | $36.0^{a}$ | $144^{f}$ | $32.0^{a}$ |
| | 90 | $355^{b}$ | $32.7^{c}$ | $601^{b}$ | $32.0^{a}$ |
| | 120 | $136^{f}$ | $31.5^{d}$ | $102^{h}$ | $26.6^{c}$ |
| *R. radiobacter* + 150 Kg.fed-1 | 45 | $185^{d}$ | $33.7^{b}$ | $231^{d}$ | $32.0^{a}$ |
| | 90 | $395^{a}$ | $31.2^{d}$ | $745^{a}$ | $30.5^{b}$ |
| | 120 | $161^{e}$ | $28.0^{e}$ | $189^{e}$ | $27.0^{c}$ |

Means in each column denoted by different letter significantly differ at $P < 0.05$ according to Duncan's multiple range tests.

The Hybrid triple 314 soil nitrogenase and hydrolase enzyme activities revealed their significantly the highest levels in the *R. radiobacter* treatment than in the control. The increases in nitrogenase activity and decreases in hydrolases activity were parallel to growth propagation.

## 4.Discussion

In both *Zea mays* cultivars; Giza (salt tolerant) and hybrid (salt sensitive), the 100 grain weight and grain N P K contents were significantly the highest in *R. radiobacter* treatment used with full N fertilization. In absence of *R. radiobacter*, the grain NPK contents were the minima due to salinity stress as mentioned by **Azevedo and Tabosa, (2000).**

Actually, *R. radiobacter* supplies the plant with proline that was enough to cause feedback inhibition to plant proline overproduction as a response to salinity stress (**Laszlo and Arnould, 2009**), that obviously predicted from the reduced proline content detected in plant leaves, directing most of the ATP consumed in proline synthesis (41 moles of ATP) to the expense of plant growth (**Munns and Tester, 2008**).

When **Amal *et al.* (2007)** inoculated salt stressed maize with *Azospirillum*, the proline concentration declined significantly in plant leaves. It could be predicted that most of *R. radiobacter* proline produced localizes its effect in root and functions as osmo-regulator. Proline has been proposed to act as a compatible osmolyte, a way to store carbon and nitrogen, may have an antioxidant activity acting as a reactive oxygen species (ROS) scavenger, function as molecular chaperones able to stabilize the structures of proteins and enhance the activity of different enzymes, and its accumulation plays a role in maintenance of cytosolic pH and regulation of intracellular redox potential (**Verbruggen and Herman, 2008**). In plant leaves, proline can protect and stabilize ROS scavenging enzymes and activate alternative detoxification pathways (**Matysik *et al.,* 2002**). Finally, the advantage of produced proline as a stress release factor is reflected on the higher grain quality and yield recorded in both *R. radiobacter* treatments of both cultivars.

Nearly similar to proline levels in the control of both *Zea mays* cultivars, the accumulation of proline was higher in salt sensitive rice cv. Sohag 3, compared to the salt tolerant cv. Giza 168, especially at higher salinity level (**Hamdia and Shaddad, 2010**). On the same trend, soluble sugars recorded highest levels in both cultivars treated with *R. radiobacter*. In this respect, **Murphy *et al.* (2003)** suggested that both proline and soluble carbohydrates act as compatible solutes under high salinity levels. **Kusaka *et al.* (2005)** added that, the observed increase in the osmotic potential might be due to the accumulation of inorganic solutes, several organic components such as sucrose, glucose, quaternary ammonium compounds, and amino acids including proline.

Parallel to stress release was the high level of total chlorophyll contents in both cultivars treated with *R. radiobacter*. The control treatment of tolerant cultivar recorded more total chlorophyll than the salt sensitive one under salt stress. This finding is in agreement with that of **Zhang *et al.* (2012)** during their work on rice genotypes of different salinity tolerance.

The significant correlation between chlorophyll A concentrations and the peroxidase activities in both *Zea mays* cultivars were well explained by **Szekely *et al.* (2008)** who stated that any decrease in antioxidant function lead to hyper-accumulation of $H_2O_2$, enhanced lipid peroxidation and chlorophyll damage.

As stated by **Shucheng (2010)**, $Ca^{2+}$ in maize leaves plays important roles as a universal second messenger in maize leaves via $Ca^{2+}$ target protein calmodulin (CaM) and $Ca^{2+}$ dependent protein kinases (CDPKs) that act between absisic acid (ABA) and $H_2O_2$ as an ROS in ABA-induced antioxidant defense signaling that enhances antioxidant enzymes including peroxidase. This emphasizes the reciprocal correlation between $Ca^{2+}$ content in treatments of both Giza and Hybrid cultivars on one side and the peroxidase activity on the other side. Individually, each treatment increased peroxidases activity and decreased $Ca^{2+}$ content with plant age. In Hybrid cultivar, $Ca^{2+}$ content decreased significantly by *R. radiobacter* treatment while peroxidase activity increased after 90 days. This agrees with the finding of **Shucheng (2010)** that $Ca^{2+}$ calmodulin dependent protein kinase acts both upstream and downstream of $H_2O_2$. On the other hand, $Ca^{2+}$ content in hybrid cultivar was higher in control parallel to the higher content of carotene and chlorophyll A/B ratio that might be explained by the sensitivity of this cultivar to salinity stress that pushed it to elevate carotene as an antioxidant compound (**Scandalios, 1997**) with the need to elevate $Ca^{2+}$ that might acted in controlling stomata closure to reduce transpiration (**Pei *et al.,* 2000**) and shared in ABA-induced antioxidant defense signaling that enhanced antioxidant enzymes (**Shucheng, 2010**).

With full N fertilization treatment, in Hybrid cultivar, it was noticed that the translocated $K^+$ and $Mg^+$ ions determined in leaves were lower than those in the less N fertilization treatment and this might be due to increased salinity stress. This could be explained by the finding of **Ali *et al.* (2011)**, who stated that excessive N application leads to soil salinization and that nitrogen application increased Na to K ratio. That was why **Shenker *et al.* (2003)** studied sweet corn response to combination of nitrogen levels with salinity stresses and stated that at low salinities, the leaf N content, N uptake, and yield increased with increasing N fertilizer up to 75% of N-fertilization recommendations.

As the concentration of salt increased, ammonia accumulation increased in soil and ammonification appeared to be mostly chemical in saline conditions (**El-Shahawy and Mashhady, 1984**). Ammonification seemed to be less sensitive to salts than nitrification process (**Ballmann and Conard, 1998**). On the other hand, nitrification of mineral nitrogen fertilizers was found to decrease with the increase of salinity (**Santoro and Enrich, 2009**). Based on this information, there is a great probability that the $NH_4^+$ ion accumulated in the rhizosphere.

The $NH_4^+$ absorption is faster than $NO_3^-$ due to the more energy required to assimilate $NO_3^-$ compared with $NH_4^+$ (20 ATP vs. 5 ATP), as well as oxygen demand for absorption of $NO_3^-$according to the findings of **Ali *et al.* (2011)**. They stated that corn preferred ammonium uptake under salinity conditions. Based on these findings, it is assumed that most of N fertilizer applied as ammonium nitrate was absorbed by both maize cultivars as ammonium that might increased in soil mostly due to the increase in soil salinity and pH.

Cations such as $K^+$, $Ca^{2+}$ and $Mg^{2+}$ decreased by increasing $NH_4^+$ while $NO_3^-$ has incremental effect on these cations as stated by **Ali *et al.* (2011)**. On the same trend, an apparent decrease happened in the K, Ca and Mg contents in both cultivars in correlation to plant age indicating the possibility of $NH_4^+$ accumulation by time and its negative effect on these cations which was also parallel to N fertilizer concentration.

Also, in saline soil, high pH decreased the availability and absorption of other elements essential for growth. On the other hand, nitrification of mineral nitrogen fertilizers was found to increase with time and to decrease with the increase of salinity (**Santoro and Enrich, 2009**).

The $K^+/ Na^+$ ratio was higher in Giza (salt tolerant) than hybrid (salt sensitive) after 90 days. This was similar to the superiority of salt tolerance of rice cv. Giza 168, compared with the more salt sensitive cv. Sohag 3 (**Hamdia and Shaddad, 2010**).

## Conclusion:

In conclusion, this work strongly reports the necessity of *R. radiobacter* inoculation simultaneously with rational N dressing to secure the proper growth and yield of the salt sensitive Giza cultivar when being cultivated in soils of high salinity levels.

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