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# ORIGINAL ARTICLE

## Responses of Banana Plantlets to Rhizobacteria Inoculation under Salt Stress Condition.

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**ABSTRACT**

The effects of rhizobacteria inoculation in modified MS medium containing sodium chloride (0.2%) on the biochemical components, physiological characteristics and mineral content of the *in vitro* banana plantlets were carried out. The presence of rhizobacteria in the medium supplemented with 0.2% sodium chloride resulted in an improvement in growth and root biomass compared to the control (uninoculated). This rhizobacteria inoculation also produced an increase in protein, nitrate, soluble nitrogen and chlorophyll contents of the plantlets cultured in MS modified medium containing 0.2% sodium chloride. The descending order of effectiveness of the rhizobacteria in medium containing 0.2% sodium chloride was: UPMB11>UPMB10>Sp7. The effectiveness of inoculation is increased when associated with boron, nitrogen or carbon into the medium. Thus these bacterial strains could be used as a bioenhancer for growth of in *vitro* banana plantlets.

***Key words:*** Banana. Rhizobacteria. Salt stress

### Introduction

The ability of plants to absorb macro and micronutrients is often limited by the availability of nutrients at the surface of the roots (Fageria *et al.* 2002). Gupta (1993) showed that shoot growth of plants will be decreased under sodium chloride stress while root growth was not changed. Flower and Yeo (1986) suggested that the effect of sodium chloride on plant growth was a result of osmotic adjustment and ion toxicity. The presence of sodium chloride will cause enzyme activities, nutrient uptake and growth changes in plants. These changes might be related to responses of the tissues or cells to adapt to the new environment (Zahran 1997). The root environment can be modified by the presence of nitrogen fixers. These nitrogen fixers have been thought to colonize the rhizosphere of halophilic and salt-tolerant plants due to the presence of plant exudates, and they may be associated with the roots, or live intracellular in symbiosis with host plants (Zahran 1997). Many PGPR have been isolated and they demonstrate their abilities in enhancing plant growth of the host plants under different growth conditions (Zobel *et al.* 1994). The $N_2$-fixing bacteria have shown promoting plant growth and yield under saline condition and also improve the phosphate nutrition and biomass production

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(Khaled *et al*. 2003). A PGPR may affect plant growth and development by influencing the levels of plant hormones known to enhance the plant growth (Glick 1995).

Therefore, the objectives of this study are to investigate the effects of rhizobacteria inoculation in the presence of sodium chloride on biochemical and physiological changes of banana plantlets cultivars Berangan (AAA) and Novaria (AAA).

## Materials and methods

*Plant Materials:*

Banana plantlets cv. Berangan established in MS solid media as described by Marziah and Roslan (1995) were used. One-month-old plantlets were cultured in 30 mL modified MS liquid medium at pH 5.7 using 100 mL Erlenmeyer flasks. The cultures were incubated on an orbital shaker at 80 rpm and were exposed to continuous fluorescent light at 27 ºC ± 1 ºC for a month. The one-month duration was sufficient for plantlets to absorb all nutrients that were available in the media. The 30 mL MS liquid medium was replenish with fresh medium at two week intervals.

*Bacterial Cultures:*

Three species of rhizobacterial: *Azospirillum brasilense* Sp7, *Bacillus sphaericus* UPMB10 and *Microbacterium oxydens* UPMB11 were used in this experiment. In each treatment inoculated with 1 mL of different species of rhizobacteria into the modified MS liquid media containing NaCl at $1x10^7$-$1x10^8$ cfu/mL ($OD_{600nm}$) concentrations.

*Bacterial Inoculation and Biochemical Analyses:*

Plantlets were cultured in sterile modified liquid MS basal media supplemented with 0.2% of sodium chloride. The medium were inoculated with different species of rhizobacterial: *Azospirillum brasilense* Sp7, *Bacillus sphaericus* UPMB10 and *Microbacterium oxydens* UPMB11. The non-inoculated treatment was used as the control. Based on a preliminary study, the plantlets could grow at the highest NaCl concentration of 0.2%. Most of the plantlets died after one week culture in more than 0.2% NaCl. Physiological parameters recorded include plant growth, number, fresh and dry weight and maximum and total length of roots. Similarly, biochemical changes such as total soluble protein content (Bradford, 1976), soluble nitrogen (Speis 1957), proline (Bates 1973), peroxidase activity (Russell 1990), total soluble phenolic content (Singleton and Rossi 1965), nitrate reductase activity (Andrew *et al.* 1992), nitrate content (Stitt 1999), chlorophyll content (Harbone 1973) and N, P, K, Ca and Mg uptake were assayed after one month of culture.

*Statistical Analysis:*

The experimental were set up with 10 replicates and repeated twice. The result were compared by ANOVA and tested by Duncan's multiple range test to find the differences between treatment means at the 5% (0.05) significant level. Data were analyzed using the general Statistical Analysis system (SAS).

## Results and discussion

*Growth and Biomass:*

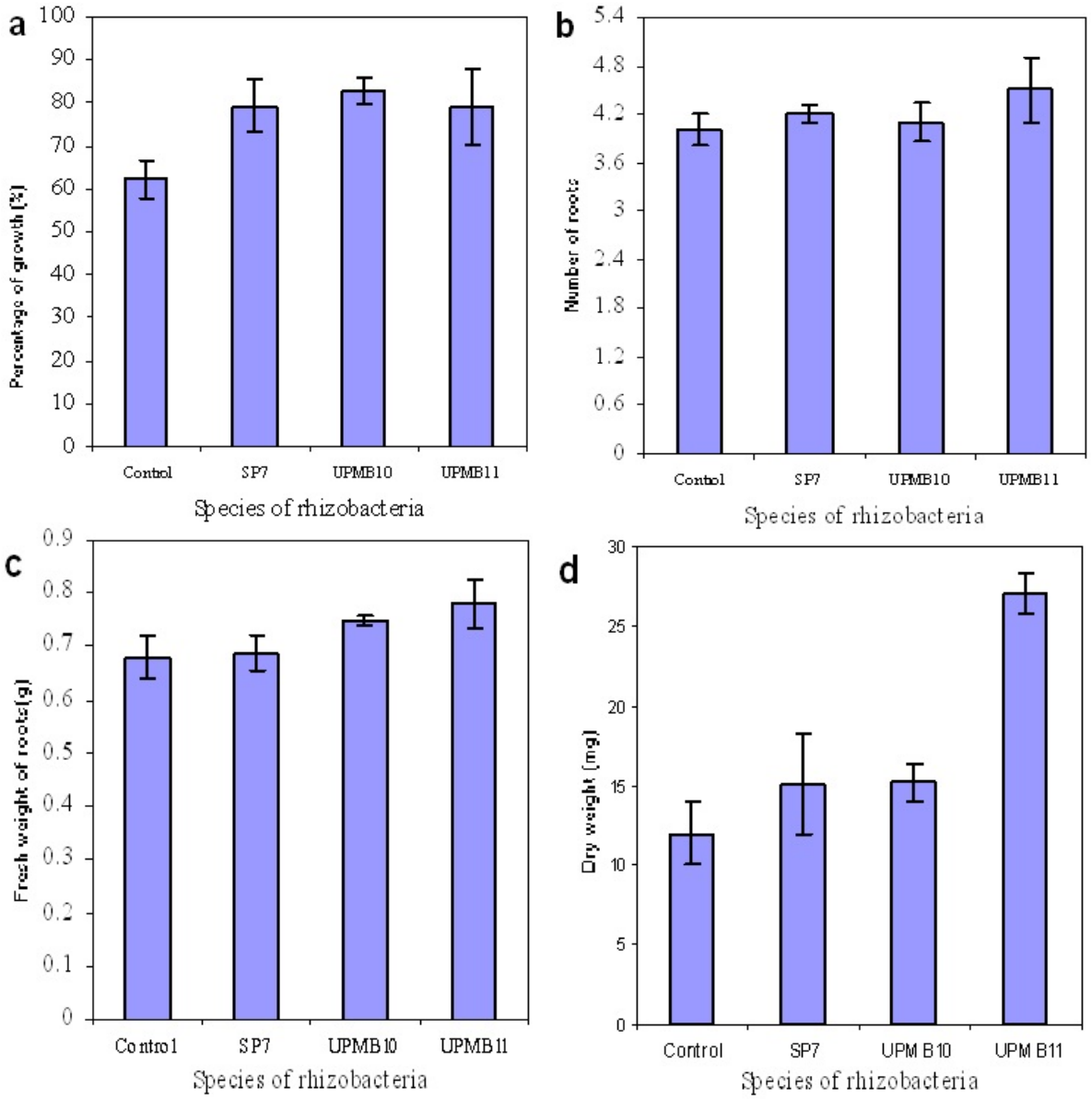
The presence of rhizobacteria in the medium supplemented with 0.2% sodium chloride showed an improvement in plant growth and root biomass of cv. Berangan and cv. Novaria as shown in Fig. 1 (cv. Berangan) and Fig. 2 (cv. Novaria). Results showed that inoculation with rhizobacterial had increase growth of plantlets within a range of 79-83% in Berangan (Fig. 3 [a]) and 87-92% in Novaria (Fig. 3 [b]) when compared to the control only at 62% and 79%, respectively. Inoculation with all three species of rhizobacteria increase in root number of banana plantlets compared to the control within a range of 4.1-4.7 for both cultivars.

The root fresh weight also showed increase when inoculated with rhizobacterial within a range of 0.69-0.78 g in cv. Berangan and 0.51- 0.87g in cv. Novaria, respectively. Inoculation with UPMB11 showed the highest root fresh weight compared to the other treatments. Similar trend was also observed for root dry weight with UPMB11 treatment showed the highest value at 27 mg for cv. Berangan and 31 mg for cv. Novaria. The

maximum root length of cv. Berangan was highest in UPMB11 treatment at 13 cm while cv. Novaria was highest in UPMB10 treatment at 11 cm. Inoculation with UPMB11 showed significant increase in total root length of banana plantlets cv. Berangan at 25 cm but Sp7 and UPMB11 did not produced a significant increase compared to the control. However, when inoculated with all three species of rhizobacteria on cv. Novaria, it showed a significant increase in total root length within a range of 18-19 cm.

*Total Soluble Protein:*

Figure 4 showed the effect of rhizobacteria inoculation in the media containing 0.2% NaCl on plant protein content of banana plantlets cv. Berangan and cv. Novaria. Inoculation with rhizobacteria into media containing NaCl showed a positive response on protein content of the plantlets compared to the control. The average leaf protein content from Sp7, upmb 10 and upmb11 treatments: 11.6 mg/g FW, 22.9 mg/g FW, and 19.7 mg/g FW for cv. Berangan; 13.2 mg/g FW, 12.2 mg/g FW, and 14.23 mg/g FW for cv. Novaria, respectively (Fig. 4). Inoculation with rhizobacteria strains UPMB10 and UPMB11 showed significant increased in leaf protein content cv. Berangan plantlets but Sp7 did not produce a significant increase. Inoculation with UPMB11 had the highest increased in leaf protein content of cv. Novaria plantlets. Inoculation with all three species of rhizobacteria show greater increase in root protein content of banana plantlets by Sp7, upmb 10 and upmb11 treatments at 9.0 mg/g FW, 9.6 mg/g FW and 9.6 mg/g FW for cv. Berangan; 11.0 mg/g FW ,13.6 mg/g FW and 22.3 mg/g FW for cv. Novaria , respectively (Fig. 4).



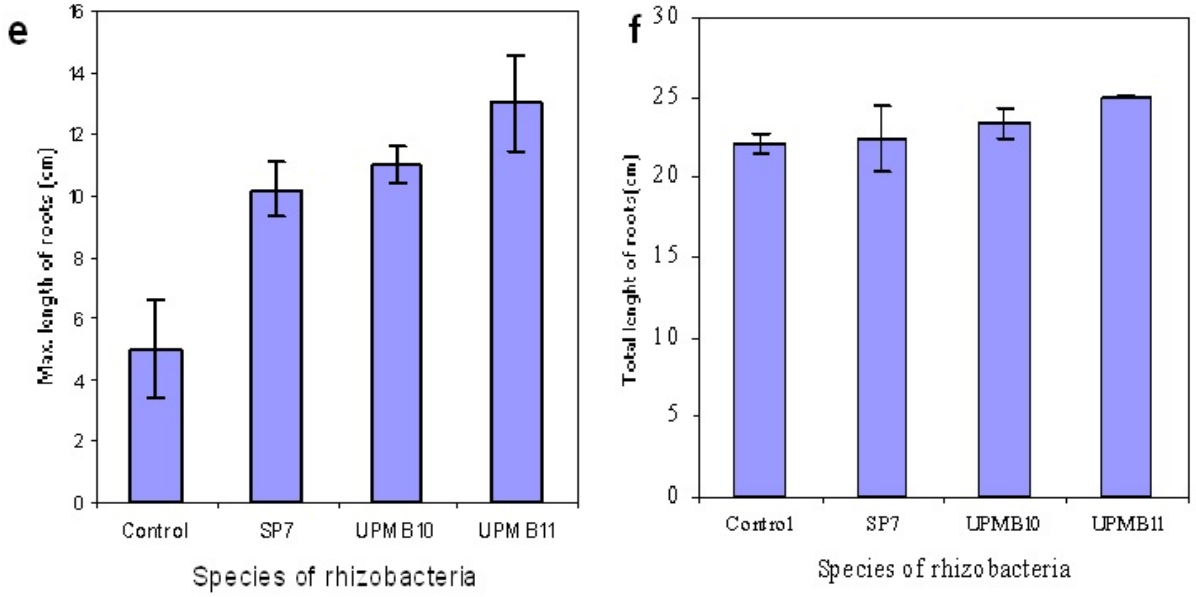
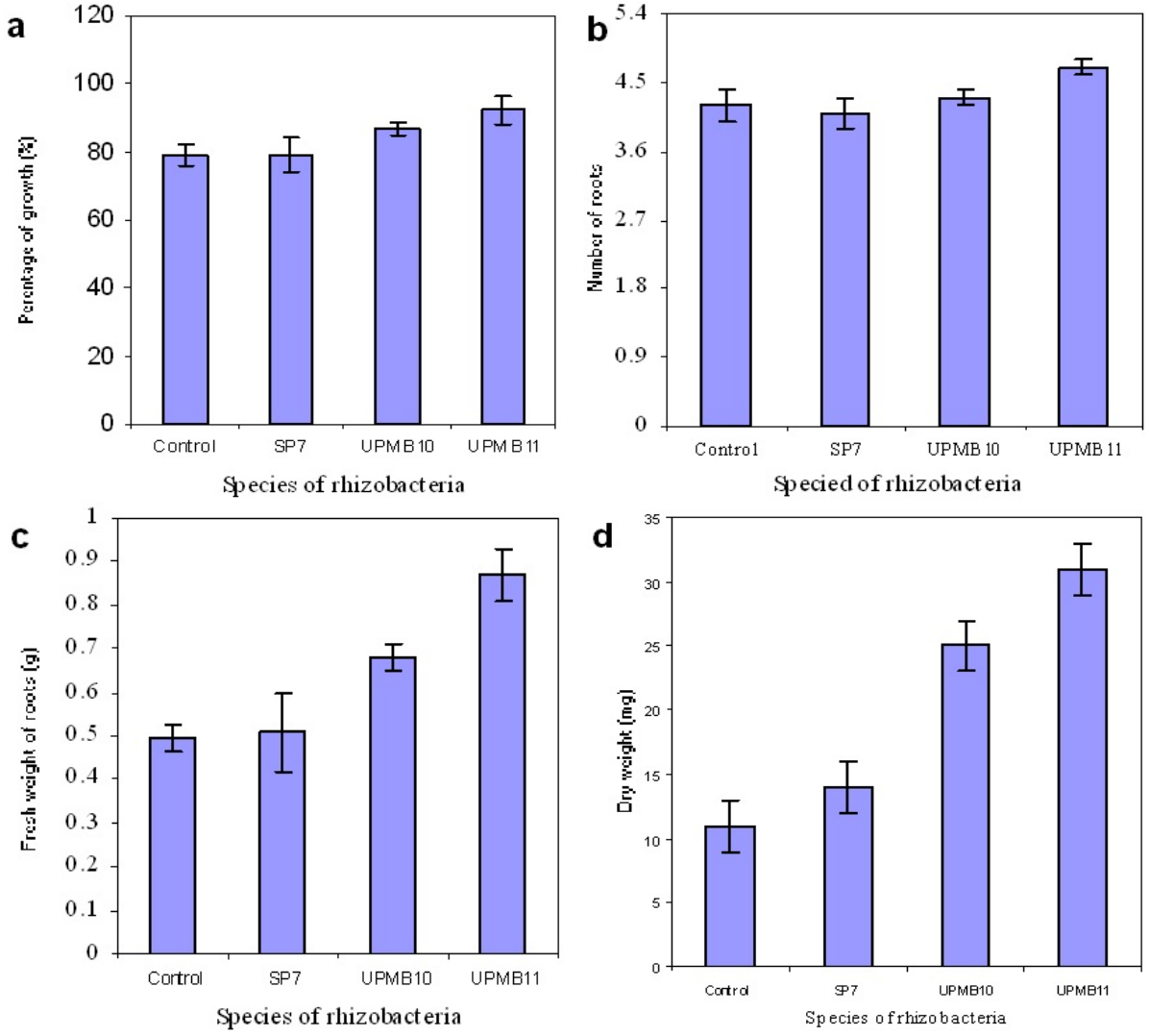



**Fig. 1:** Growth (a) of in vitro banana plantlets and root biomass (b-f) cv. Berangan after one month culture in MS liquid medium containing of NaCl inoculated with rhizobacteria. Bars show mean + SD of ten replicates (P=0.05, n= 10)



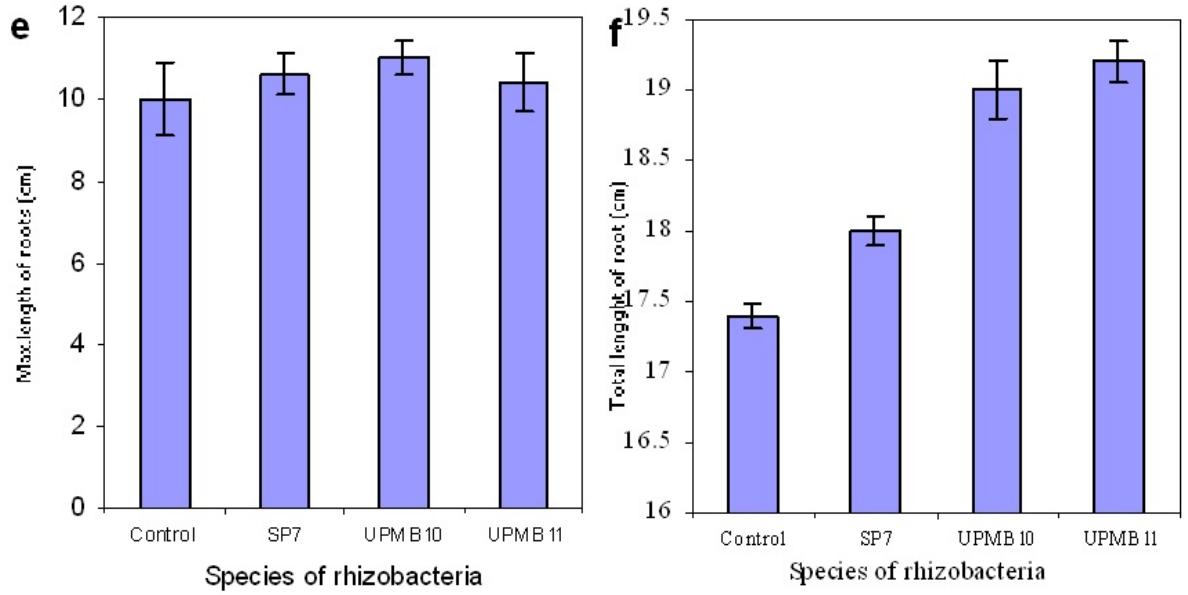



**Fig. 2:** Growth (a) of in vitro banana plantlets and root biomass (b-f) cv. Novaria after one month culture in MS liquid medium containing of NaCl inoculated with rhizobacteria. Bars show mean + SD of ten replicates (P=0.05, n= 10).


**Fig. 3:** (A) Effect of UPMB10 inoculation on growth of in vitro banana plantlets cv. Berangan culture in MS liquid medium containing 0.2% sodium chloride. (B) Effect of rhizobacterial inoculation on growth of in vitro banana plantlets cv. Novaria culture in MS liquid medium containing 0.2% sodium chloride A: Control, B: Sp7, C: UPMB11, D: UPMB10. Bar represents 10mm.

The results showed that the inoculation of rhizobacteria affected the amount of leaf protein content of plantlets, especially when inoculated with UPMB10 and UPMB11 in cv. Berangan and cv. Novaria, respectively, whereby UPMB11 treatment showed the highest root protein content of plantlets in both cultivars used compared to other treatments. The positive effect of rhizobacteria inoculation on plant soluble protein content might be due in part to its lower osmotic pressure and might be correlated by increased growth and rooting system of plantlets.

*Soluble Nitrogen:*

Inoculation with all species of rhizobacteria in medium containing 0.2% NaCl showed there was no significant increase in leaf soluble nitrogen content of plantlets cv. Berangan. Leaves soluble nitrogen content of plantlets cv. Novaria were significantly increased when inoculated with all three species of rhizobacteria within a range 151-164 ug/g FW compared to the control. Root soluble nitrogen content of plantlets cv. Berangan inoculated with all species of rhizobacteria were significantly higher ($P<0.05$) than control. Similar trend was observed on cv. Novaria when inoculated with UPMB10 and UPMB11, but Sp7 did not produce significant increase (Fig. 5).

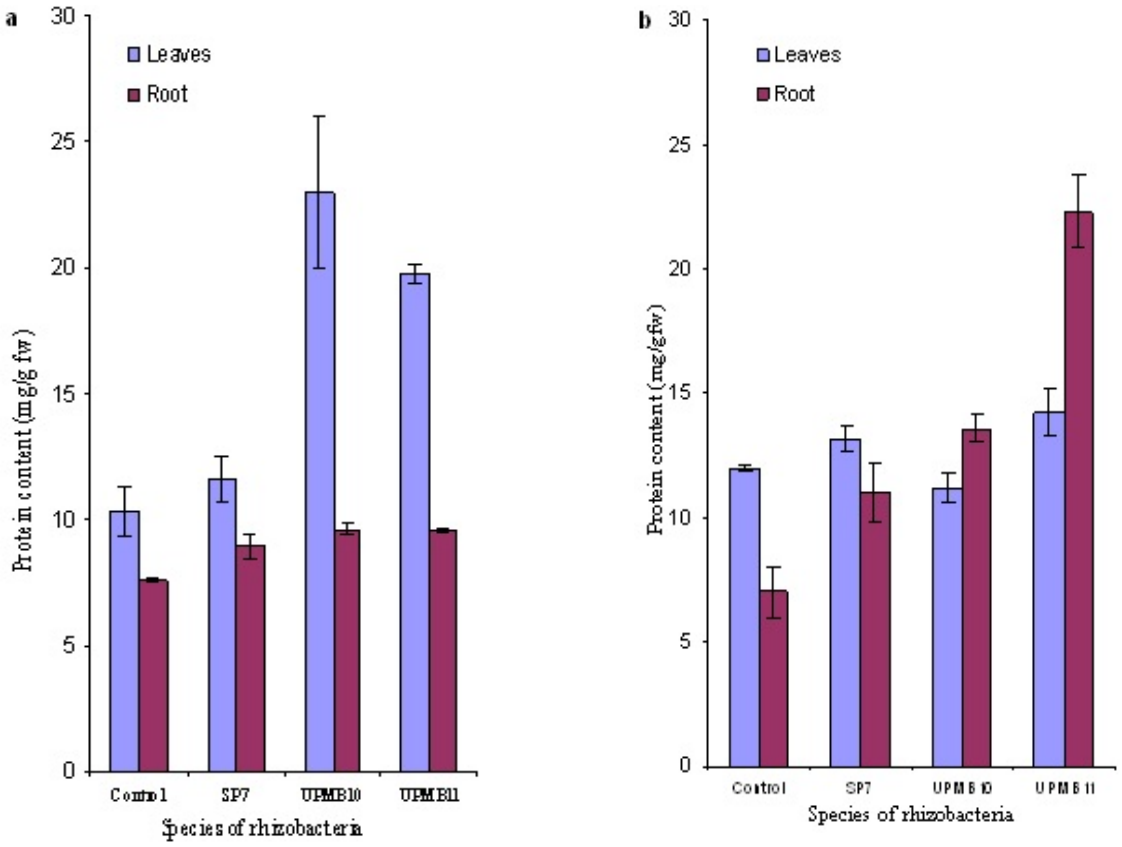



**Fig. 4:** Total soluble protein content of in vitro banana plantlets (a) cv. Berangan and (b) cv. Novaria after one month culture in MS liquid medium containing of NaCl inoculated with rhizobacteria. Bars show mean + SD of ten replicates (P=0.05, n= 10).

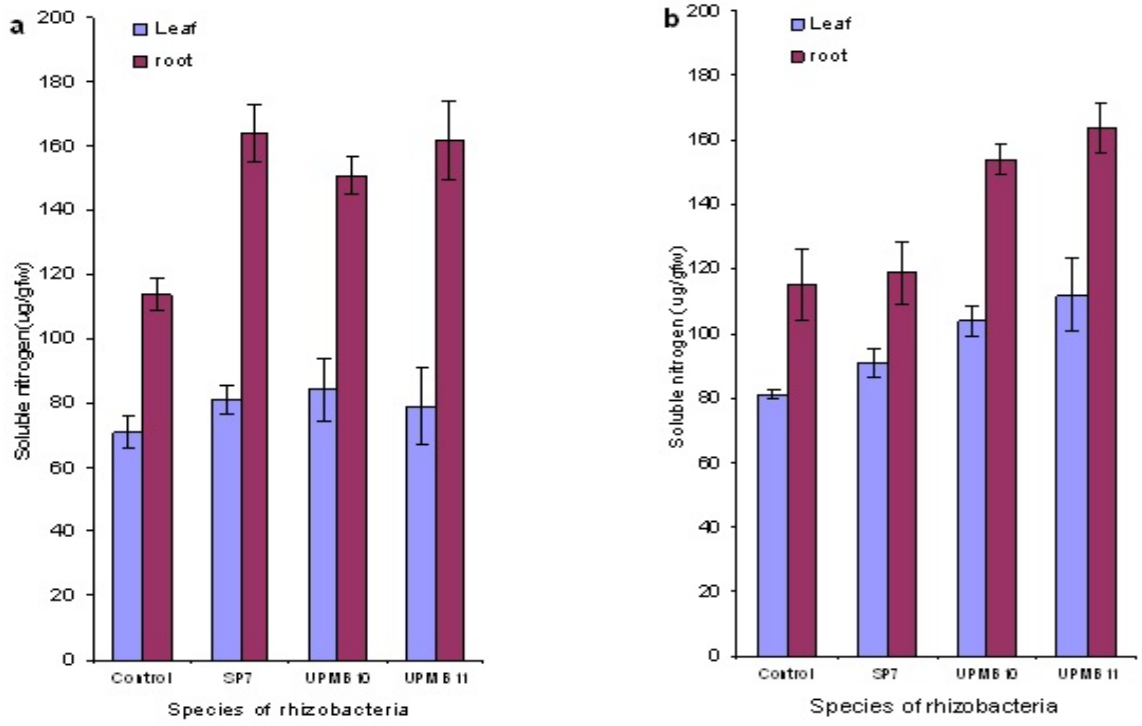



**Fig. 5:** Soluble nitrogen content in part of banana plantlets (a) cv. Berangan and (b) cv. Novaria after one month culture in MS liquid medium containing of NaCl inoculated with rhizobacteria. Bars show mean + SD of ten replicates (P=0.05, n= 10).

The results showed that rhizobacteria species UPMB10 and UPMB11 have the ability to increase soluble nitrogen content of banana plantlets cv. Novaria. All species of rhizobacterial also showed have potential to enhance root soluble nitrogen content cv. Berangan. There was a progressive increment in soluble nitrogen content due to the inoculation treatments in saline media. This is in agreement with earlier finding by Okon (1982) that the root segment from inoculated maize in solution culture exhibited 30-50% greater uptake of nitrate than root segment from uninoculated plants.

*Proline:*

Leaf proline content of plantlets cv.Berangan gave significant decreased in all treatments with rhizobacteria in medium containg 0.2% NaCl. Those inoculated with UPMB10, UPMB11 and Sp7 showed lower leaf proline content than the control at 74 mg/gfw, 69 mg/gfw and 111 mg/gfw, respectively. Similar trend were observed in cv. Novaria which UPMB 11 showed the lowest proline content at 98 mg/gfw compared to other treatments (Fig. 6). Inoculation with all species of rhizobacterial did not show any significant decrease in root proline content of plantlets cv. Berangan. It was observed that there was a significant reduction in root proline content of plantlets cv. Novaria that inoculated with all species of rhizobacterial within a range 141-191 mg/gfw.

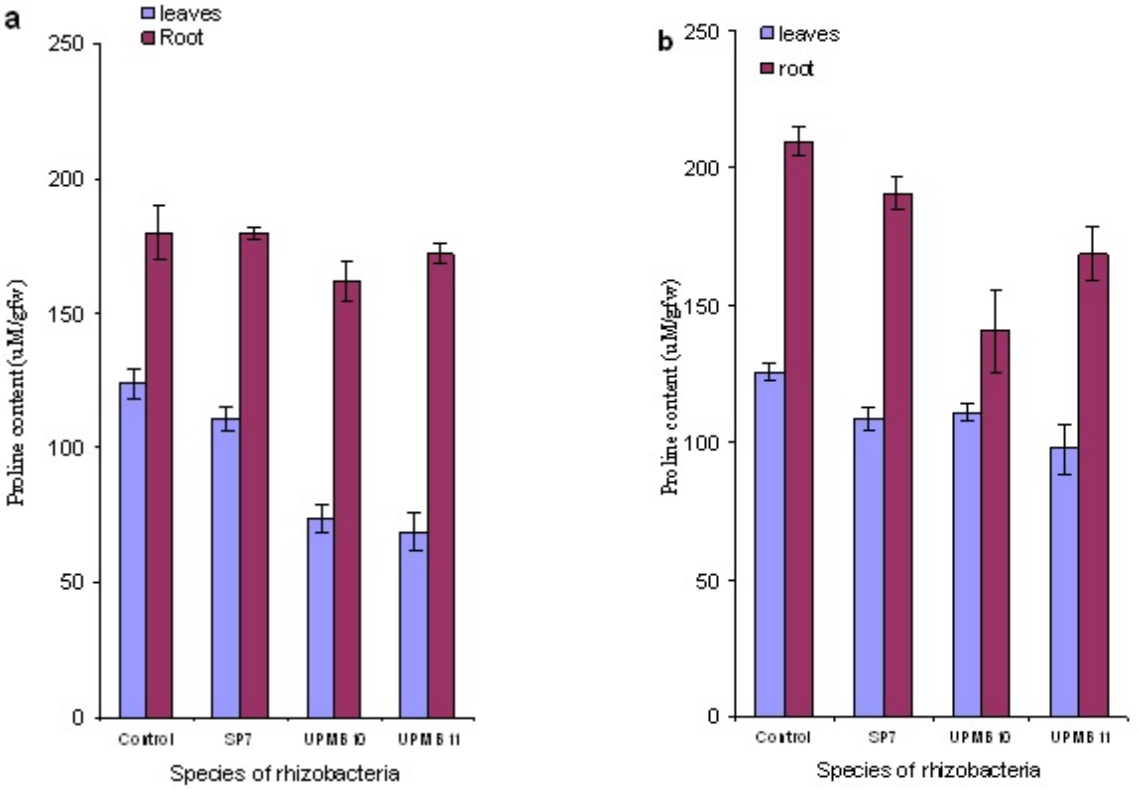



**Fig. 6:** Changes of proline on in vitro banana plantlets (a) cv. Berangan and (b) cv. Novaria after one month culture in MS liquid medium containing of NaCl inoculated with rhizobacteria. Bars show mean + SD of ten replicates (P=0.05, n= 10)

*Peroxidase Activity:*

Total peroxidase activity was assayed on crude extracts of leaves and root of plantlets cv. Novaria and cv. Berangan by using guaiacol as a substrate which reacts with all peroxidases. The results showed that the peroxidase activity in roots is much higher than leaves in both cultivars tested (Fig. 7). Leaf peroxidase activity in media inoculated with rhizobacteria under 0.2% salt treatment were significantly ($P<0.05$) lower than the control in both cultivars used, cv. Berangan and cv. n*ovaria* within a range of 3925 to 7698 U/mg protein and 11192 to 13356 U/mg protein, respectively. Similar trends were observed in roots peroxidase activity of plantlets whereby cv. novaria showed higher activity than the cv. Berangan within a range 66081-91027 U/mg protein and 23697 to 65987 U/mg proteins, respectively.

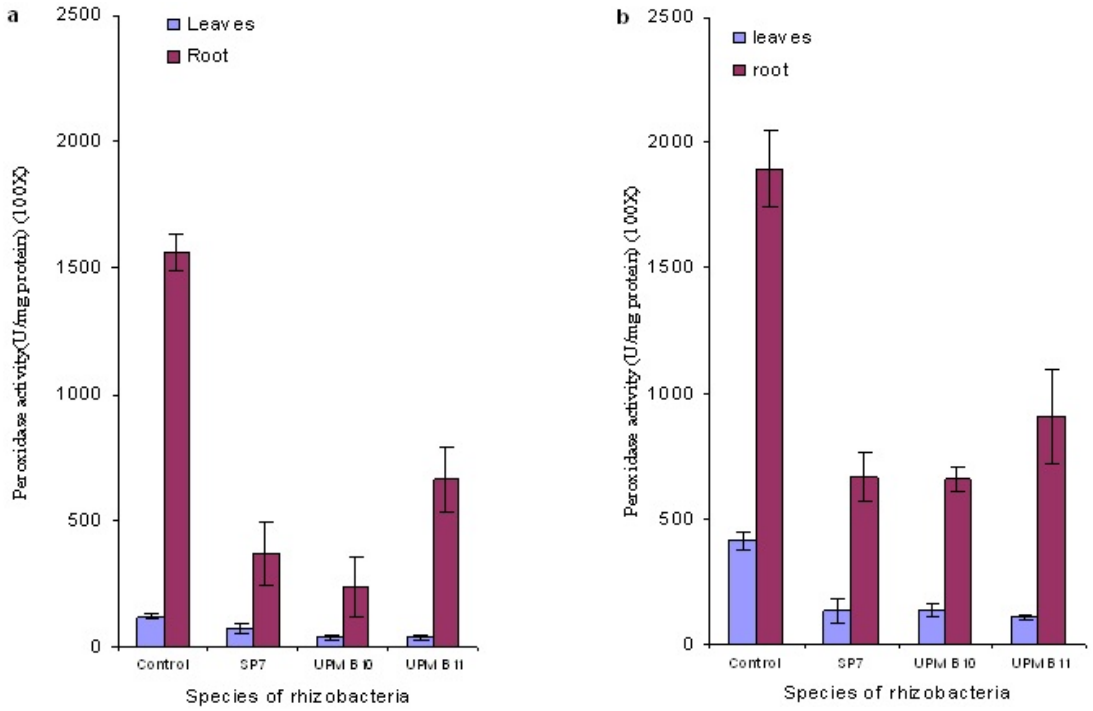



**Fig. 7:** Changes of peroxidase activity on in vitro banana plantlets (a) cv. Berangan and (b) cv. Novaria after one month culture in MS liquid medium containing of NaCl inoculated with rhizobacteria. Bars show mean + SD of ten replicates (P=0.05, n= 10)

The degree of reduction was found to be dependent on the type of rhizobacteria employed and cultivars of the plantlets. upmb11 treatment showed higher root peroxidase activity compared to other treatments. An increase in total peroxidase activity is a common response to various oxidative stress factor (Gaspar 1991). Exposure of cultivars to salinity containing rhizobacteria produced changes in the induction of total peroxidase activity and its quantities vary between cultivar and rhizobacteria that were used.

The results showed that rhizobacteria inoculation caused a reduction in the level of peroxidase activity in both of cultivars used in media containing 0.2% NaCl. However, the degree of accumulation was more in the cv. Novaria than in cv. Berangan indicating this cultivar is more sensitive to salt stress. Therefore, this could be explained the reason for the reduction in stress salinity of plantlets due to the effect of rhizobacterial inoculation. The qualitative and quantitative changes in the activity of several enzymes including peroxidase activity isolated form plant subjected to salinity stress were reported (Bradford 1976; Alberico and Cramer 1993).

*Phenolic Compound:*

Leaf phenolic compound of banana plantlets cv berangan inoculated with rhizobacterial species of UPMB 11 and UPMB10 showed significant increase at 191 mg/gfw and 162 mg/gfw, respectively compared to the control. However, there was no significant change in phenolic compound under the treatment with Sp7. The leaf phenolic compound of banana plantlets cv.novaria showed significant increase only in UPMB11 treatment at 146 mg/gfw. The root phenolic compound of plantlets cv. Berangan inoculated with UPMB11 gave significant increase at 191 mg/gfw while the others showed no significant change. Treatment on cv. novaria showed a less respond on root phenolic content compared to cv. Berangan. Similar trend was showed in UPMB11 treatment where the highest increment in root phenolic content of plantlets cv. Novaria was at 146.2 mg/gfw. Those treatments with UPMB10 and Sp7 gave no significant change, at only 129 mg/gfw and 121 mg/gfw, respectively, compared to the control (Fig. 8).

*Nitrate Reductase Activity (NRA):*

There was not much difference between inoculated treatments and control in leaf NR activity of cv. Berangan in the presence of 0.2% NaCl, although all the values were considerably lower than the control.

Those inoculated with Sp7, upmb10 and upmb11 treatments showed the NRA at 0.09 U/ mg protein, 0.054 U/ mg protein and 0.089 U/ mg protein, respectively and the control at 0.100 U/ mg protein. Leaf NRA activity of plantlets cv. Novaria inoculated with UPMB11 and UPMB10 was significantly lower than the control at 0.45 U/ mg protein and 0.51 U/ mg protein, respectively, while no significant change in SP7 treatment. Root NR activity of plantlets cv. Berangan inoculated with rhizobacteria was significantly lower in treatments UPMB11and UPMB10 at 0.56 U/ mg protein and 0.34 U/ mg protein, respectively, compared to the control, while the Sp7 treatment did not showed any significantly changes. Inoculations with all three species of rhizobacteria were significantly lower than the control within a range of 0.98 and 1.11 U/ mg protein (Fig. 9).

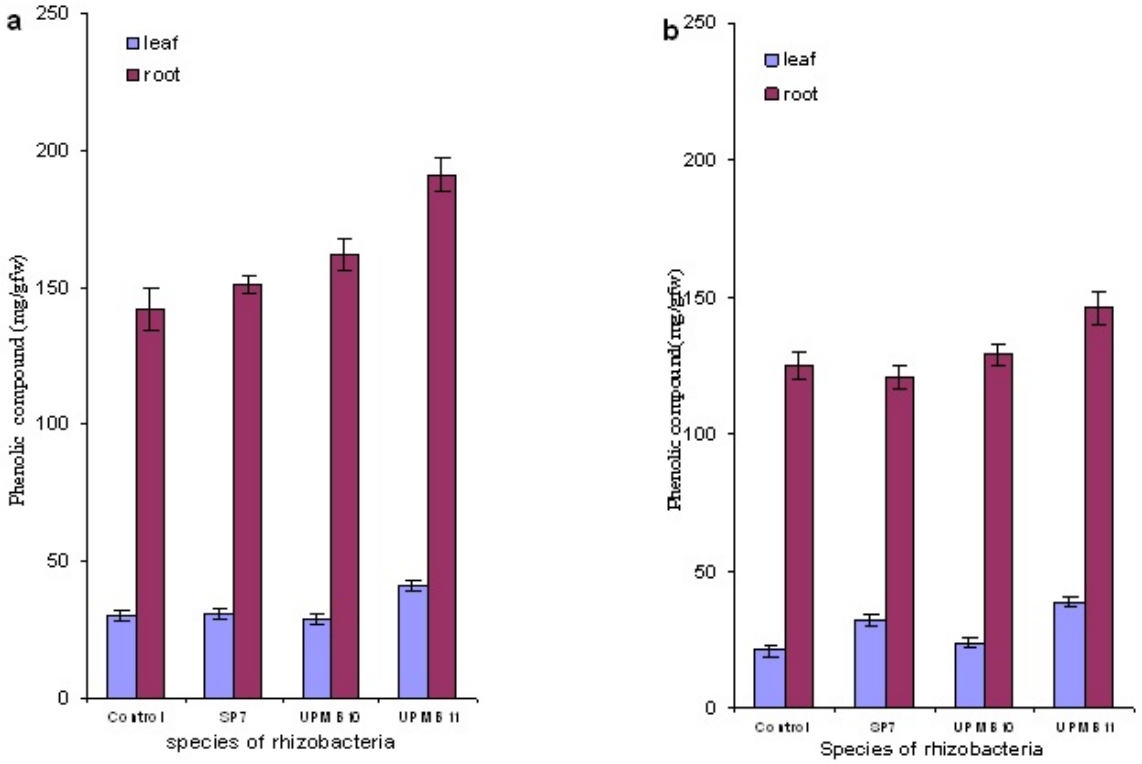



**Fig. 8:** Effect of rhizobacterial inoculation on changes of phenolic content of in vitro banana plantlets (a) cv. Berangan and (b) cv. Novaria after one month culture in MS liquid medium containing of NaCl inoculated with rhizobacteria. Bars show mean + SD of ten replicates (P=0.05, n= 10)

The inoculation process of rhizobacteria in the media containing NaCl showed promising response in NR activity of *in vitro* banana plantlets. Inoculation with UPMB10 and UPMB11 showed lower NR activity of banana plantlets in both cultivars used. NRA activity data in this study indicates that the reduction of NR activity could be due to rhizobacteria inoculation in the media.

*Nitrate:*

Inoculation with rhizobacteria in MS medium containing sodium chloride showed significant increase in nitrate content of plantlets cv. Berangan and cv. Novaria. Nitrate contents of plants were higher in inoculated treatments when compared to the control. Rhizobacteria inoculation affect the leaf nitrate contents of plantlet cv. Berangan, especially in UPMB10 and UPMB11, and both gave similar increment at 21 mg/gfw. Inoculation with Sp7 increased the nitrate content at 15 mg/gfw compared to the control. Similar trend was showed in leaf nitrate contents of plantlet cv. Novaria when inoculated with UPMB11, UPMB10 and Sp7 at 71 mg/gfw, 68 mg/gfw and 49 mg/gfw, respectively. The root nitrate content of plantlets cv. Berangan and cv. Novaria also showed significant increment in all inoculation treatments within a range of 25-34 mg/gfw and 55-91mg/gfw, respectively (Fig. 10). The results showed that inoculation with rhizobacteria in saline conditions is essential to increase nitrate uptake of banana plantlets and for well growth of plants.

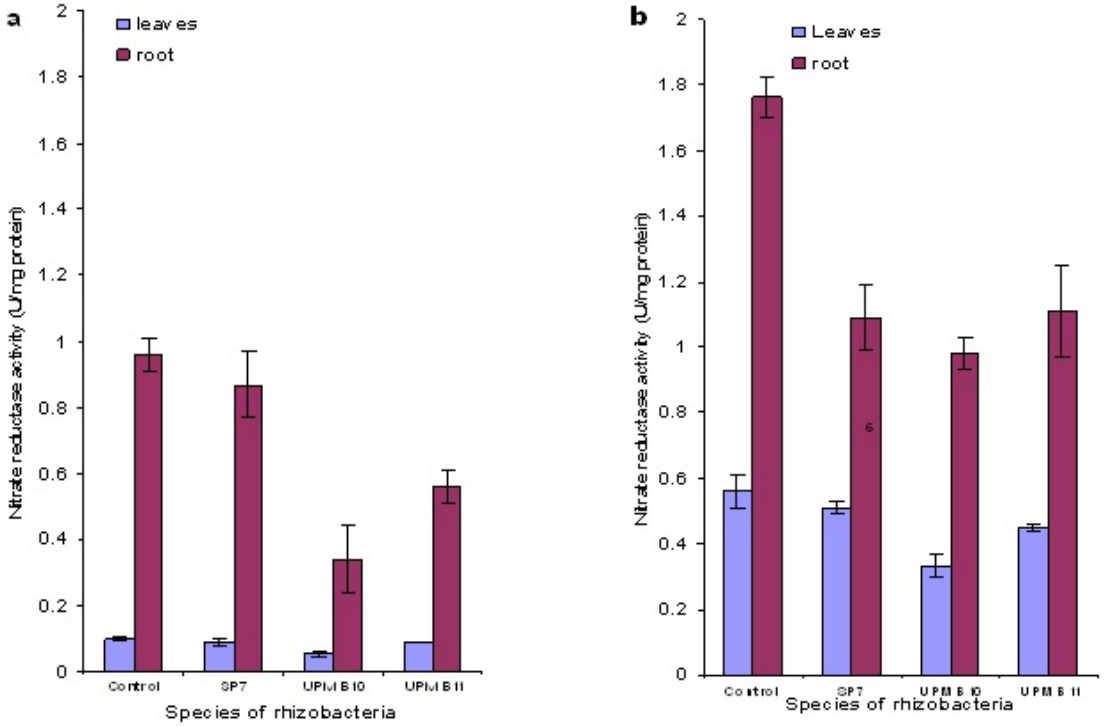



**Fig. 9:** Changes of Nitrate reductase activity on in vitro banana plantlets (a) cv. Berangan and (b) cv. Novaria after one month culture in MS liquid medium containing of NaCl inoculated with rhizobacteria. Bars show mean + SD of ten replicates (P=0.05, n= 10).

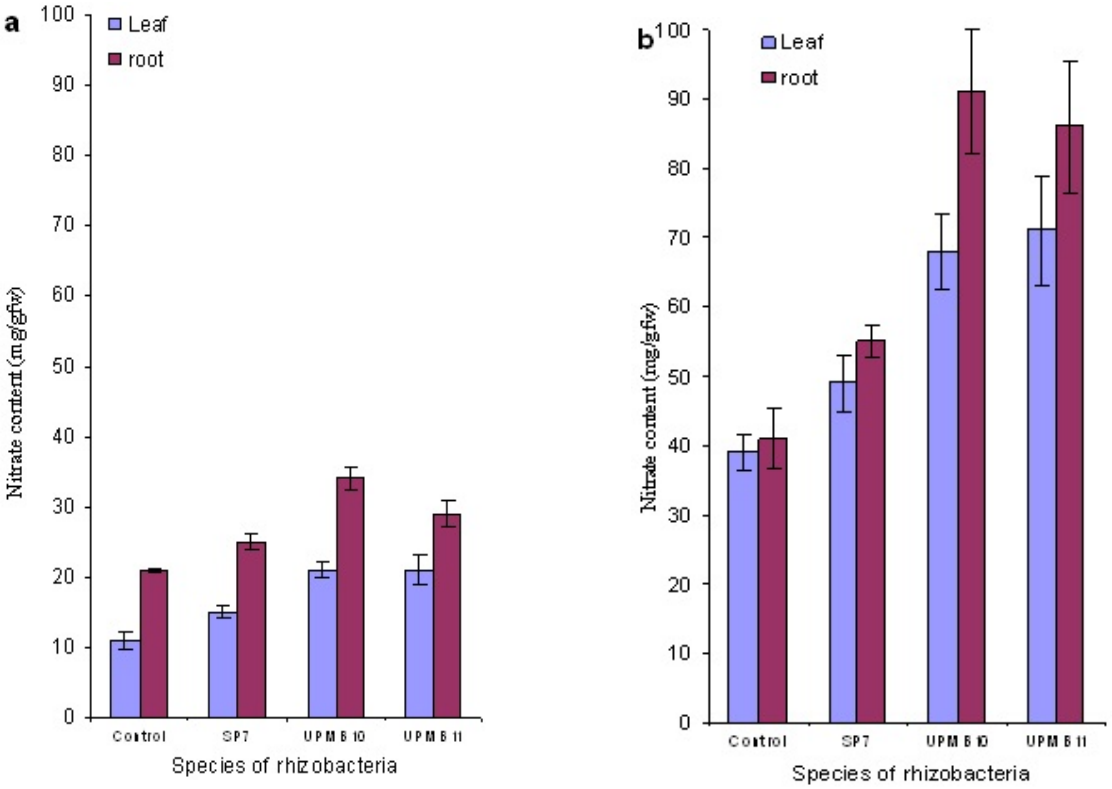



**Fig. 10:** Effect of rhizobacterial inoculation on nitrate content of in vitro banana plantlets (a) cv. Berangan and (b) cv. Novaria after one month culture in MS liquid medium containing of NaCl inoculated with rhizobacteria. Bars show mean + SD of ten replicates (P=0.05, n= 10)

*Total Chlorophyll:*

Leaf chlorophyll content of banana plantlets cv. Berangan and cv. Novaria were higher with inoculation of rhizobacteria compared to the uninoculated in the present of 0.2% NaCl. The highest plants chlorophyll in cv. Berangan was showed in treatment inoculated with UPMB11 followed by UPMB10, SP7 and the control at 2.86 mg/g FW, 2.74 mg/g FW, 2.71 mg/g FW and 2 mg/g FW, respectively. Similar result were observed in cv.Novaria in which upmb11 (3.2 mg/g FW) treatment showed the highest plant chlorophyll content followed by upmb10 (3.1 mg/g FW) and sp7 (2.2 mg/g FW), and the control showed only at 2.1 mg/gfw (Fig. 11).

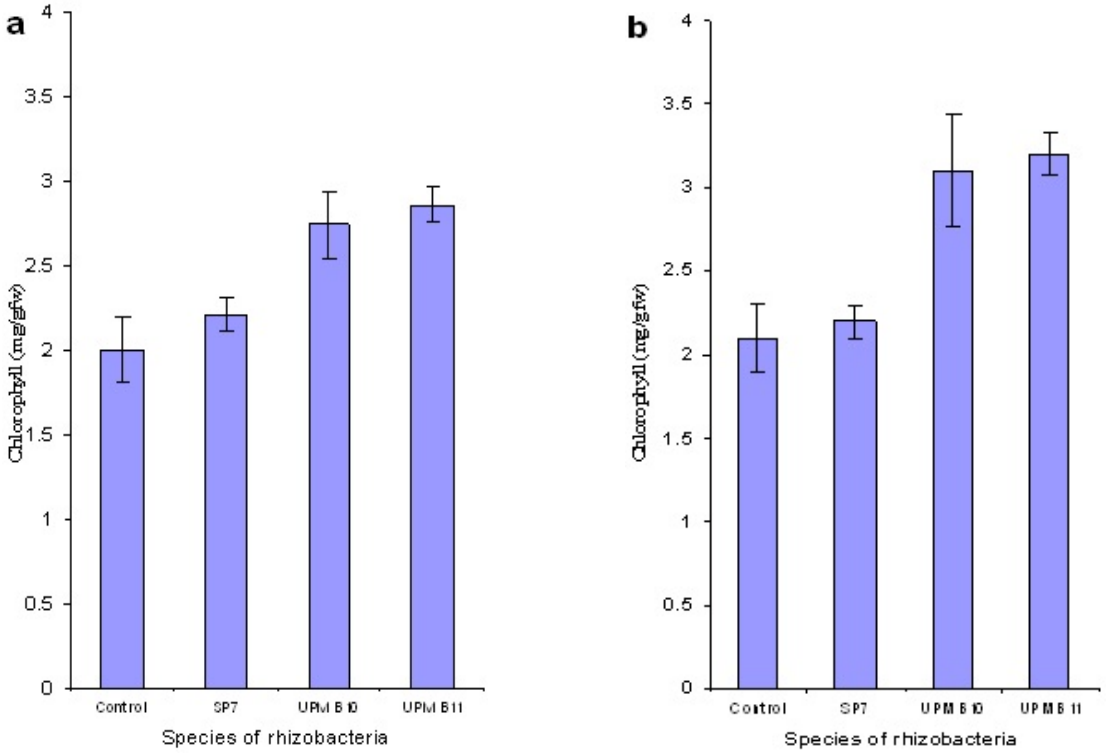



**Fig. 11:** Chlorophyll content of in vitro banana plantlets (a) cv. Berangan and (b) cv. Novaria after one month culture in MS liquid medium containing of NaCl inoculated with rhizobacteria. Bars show mean + SD of ten replicates (P=0.05, n= 10).

*N, P, K, Ca and Mg Content:*

The N, P, K Ca and Mg contents in plantlets inoculated with rhizobacteria in media supplemented with NaCl at 0.2% were presented in Table 1. It has been noticed that inoculation with rhizobacteria showed a positive response on N, P and Ca intake contents of banana plantlets in both cultivars. Inoculated with UPMB10 (2.3 mg/gdw) and UPMB11 (3.4 mg/gdw) showed the highest in N content for cv. Berangan and cv. Novaria, respectively. Positive response of the rhizobacteria inoculation on P content of plantlet was observed for UPMB11 treatment at 0.32 mg/gdw in cv. berangan and 0.34 mg/gdw in cv. novaria. The highest Ca content of the plantlet was recorded in UPMB10 treatment at 0.34 mg/gdw (cv. berangan) and 0.67 mg/gdw (cv. novaria). The inoculation with rhizobacteria in saline media did not produce significant change for K and Mg contents of plantlets when compared to the control.

*Discussion:*

Generally, inoculation with rhizobacteria into the media supplemented with sodium chloride at the concentrations of 0.2% showed an increased in growth and root biomass of the plantlets. Natural saline conditions such as sea and brackish waters have natural bacterial flora, which may play a significant role in the economy of these habitats. This environment may interfere with the uptake of water and may be toxic to a large number of microorganisms (Zahran 1997). Saline environments harbor taxonomically diverse bacterial

groups which exhibit modified physiological and structural characteristics under the prevailing saline condition (Zahran 1997). It is most beneficial to study the effect of salinity to banana explants exposed to PGPR since banana could then be planted in high salinity environments if the experiment proves successful.

**Table 1:** Nutrients content (mg/gdw) of *in vitro* banana plantlets cv. Berangan (a) and cv.Novaria (b) after one month culture in MS liquid medium containing sodium chlorite inoculated with rhizobacteria. Values are means of ten replicates with ± SD (P=0.05, n=10).

| Treatments | N | P | K | Ca | Mg |
|---|---|---|---|---|---|
| (a) cv. Berangan (mg/gdw) | | | | | |
| Control | 1.10±0.20 | 0.04±0.02 | 2.51±0.09 | 0.09±0.01 | 0.11±0.01 |
| SP7 | 2.10±0.30 | 0.10±0.01 | 3.01±0.1 | 0.29±0.05 | 0.12±0.01 |
| UPMB10 | 2.30±0.10 | 0.25±0.01 | 2.14±0.45 | 0.34±0.08 | 0.11±0.02 |
| UPMB11 | 2.10±0.11 | 0.32±0.04 | 2.21±0.23 | 0.25±0.09 | 0.09±0.02 |
| (b) cv. Novaria (mg/gdw) | | | | | |
| Control | 2.40±0.09 | 0.07±0.01 | 2.1±0.08 | 0.13±0.07 | 0.21±0.02 |
| SP7 | 2.60±0.07 | 0.11±0.02 | 2.2±0.06 | 0.59±0.06 | 0.31±0.05 |
| UPMB10 | 2.70±0.10 | 0.21±0.02 | 2.2±0.07 | 0.67±0.10 | 0.19±0.03 |
| UPMB11 | 3.40±0.20 | 0.34±0.08 | 2.5±0.10 | 0.61±0.10 | 0.20±0.01 |

The results indicate that the presence of rhizobacteria in the media containing 0.2% NaCl showed positive response to promote growth and root biomass of *in vitro* banana plantlets in NaCl condition. The data showed that when rhizobacteria inoculated in the media, the growth and root biomass of plantlets increased. Thus, rhizobacteria is beneficial to the plantlet in NaCl-stress conditions through increment of growth and improvement in rooting system. The effectiveness of inoculation to the plantlets in saline condition is probably due to a reduction mechanism in chloride and sodium ions in the root rhizosphere (Romero-Aranda *et al.* 1998). The plant growth is slower in the absence of rhizobacteria under NaCl conditions probably because there is no rhizobacteria to induce growth directly or indirectly (Glick 1995). Indirect promotion of plant growth occurs when a PGPR lessens or prevents the deleterious effect of one or more phytopathogenic organisms. The direct effect on promotion of plant growth usually entails either the provision of a compound that is synthesized by the bacterium or in facilitating the uptake of certain nutrients from the environment. For example, various PGPR strains can fix atmospheric nitrogen, synthesize siderophores that can solubilize iron from the soil and provide it to the plant cells, synthesize phytohormones that can enhance plant growth, solubilize minerals such as phosporus, or synthesize enzymes that can modulate plant growth and development (Glick 1995). The rate of growth is generally linked to the capacity to exclude $Na^+$ ions from the shoot (Gorham e*t al.* 1985; Yeo and Flowers 1986). On the other hand, many authors noticed that a poor correlation exists between shoot Na+ concentrations and inhibition of growth (Alberico and Cramer 1993; Schachtman and Munns 1992). McCoy (1987) reported that sensitivity to NaCl at the cellular level was not related to salinity tolerance of the whole plant. Such a difference in relationship between shoot $Na^+$ concentrations and growth is also observed by Amzallag (1997) and it was reported that shoot turgor is not a limiting factor for growth. A similar conclusion was drawn by Khaled e*t al.* (2003), after observation of growth response of wheat and barley exposed to saline environments. An effect of salt treatments on growth will only slightly affect final phytomass at harvest but NaCl had a significant effect on water content (Zahran 1997). In the present study, there is a decrease in plant growth without inoculation process compared to the treatment inoculated with UPMB10. It may be that plantlet without inoculation is under stress, due to reduced water uptake (McCoy 1987).

The effect of modifications in the nutrient solutions on plant growth adaptation suggests that the root changes the type of response of the plant to salinity, as well as the rate of growth (Amzallag 1997). The control of the plant development by root has been recently proposed: (1) the rate of growth is controlled by the PGRs (Plant Growth Regulator) produced by the root (Peuke *et al.* 1994). (2) the PGR (Plant Growth Regulator) production of the root is influenced by physical and chemical variations of the environment (Wolf *et al.* 1990; Peuke *et al.* 1994). It is possible that rhizobacteria could be applied to saline soils for better growth of plant.

We propose that the principal factor responsible for the well growth in salt condition is the presence of the microorganism. Regarding plant growth and protein content of banana plantlets, it has been recently suggested that salt-induced defoliation may have worse consequences than disturbances of turgor, photosynthesis and enzymatic activity (Munns 1993). Similar results were obtained by Khaled *et al.* (2003), which leaf protein content was improved by rhizobium inoculation. Salt accumulation may be further attributable to reduced growth, as the Na concentrations in an organ or a whole plant depends on the relationship between ion uptake rate and growth rate (Flowers and Yeo 1986). Reduced growth may results in accumulation of ions if ion uptake is reduced less than the growth rate. Accumulation was observed only for the element Na and Cl only (Kahindi *et al.* 1997). The constancy of the concentrations of many essential

elements in plants over a wide range of ion availabilities and growth rate has led to the hypothesis that the rate of ion uptake into the root is regulated by a feedback system so that it matches the growth dependent on demand of roots and shoots (Marschner 1995). Increase or decrease in activities of nitrogen assimilating enzymes of plants under salt stress could be linked to the accumulation of excess $NH_4^+$ and $NO_3^-$ or decrease in total protein (Goyal and Huffaker 1986).

Generally, the present of rhizobacteria in media containing 0.2% NaCl due to reduced in plant proline content within a range 10-80% when compared to the control. The present study showed that there was a positive response of rhizobacteria to reduce stress of host plant due to application of salt condition. According to Pandey and Agarwal (1998), proline accumulation is a common metabolic response of higher plants to water deficits, and salinity stress, and has been the subject of numerous studies over the last 20 years. Therefore, rhizobacteria inoculation in media containing NaCl due to reduced in salinity stress and resulted increase in growth of banana plantlets *in vitro*. In saline conditions, the majority of bacteria can osmoregulate by synthesizing specific compatible organic osmolytes such as glutamine, proline and glycine betaine and a few of them accumulation inorganic solubles such as $Na^+$, $K^+$ ang $Mg^{2+}$ ions (Zahran 1997).

The morphology of the bacteria is usually modified with cells are usually elongated, swollen and showing shrinkage, in addition to changes in the cell and cytoplasmic volume (Zahran 1997). The chemical composition of membranes may also occasionally be modified, and the synthesis pattern of protein, lipids, fatty acids and polysaccharides may change with a moderate increase in salinity (Zahran 1997). Change in proline concentrations have also been found in the evergreen, *Halimione portulacoides*. The changes in proline concentrations are consistent with the rapid metabolism of this compound (Aspinall and Paleg 1981) suggesting that proline might be involve in adjustment of the plant to sudden increases in stress, as in exposure to NaCl. The bacteria which usually grow in non-saline conditions may exhibit a great modification in cell morphology when subject to salt stress. The swelling, elongation and shrinkage (reduction in cell volume) are characteristic features of sensitive bacteria under salt stress (Zahran 1997). However, it has been found (Skjerdal *et al.* 1995) that the cell and the cytoplasmis volume of *Brevibacterium lactofermentum* and *Corynebacterium glutamicum* spontaneously decrease upon hyporosmotic shock.

In *Sorghum bicolor,* the exposure to salinity and addition of cytokinins to the root media during the 3 weeks of NaCl pretreatment prevent the capacity to grow at high salinity (Amzallag *et al.* 1993). Moreover, the absence of DPLs in cytokinin-treated plant indicated that this growth factor is able to prevent the initiation of the adaptation process (Seligmann *et al.* 1993). Similarly, in *Mesembryanthemum crystallium*, the transition from $c_3$ to CAM mode of photosyntheiss following exposure to salinity was prevented by a cytokinin-treatment (Schmitt and Piepenbrock 1992). The cytokinin produced by the root through inoculation process should be the stabilizing factor which prevents the initiation of the adaptation response. The cytokinin production is reduced by salinity and present of the rhizobacteria (Tripathi *et al.* 1998). The interruption in stalinization may prevent the induction of adaptation through an increase in cytokinin production by the root (Amzallag 1997). Net $NO_3$ uptake by root was positively correlated with transpiration rate (Nelson 2001). Similarly, Garabito *et al.* (1998) found that whole plant $NO_3^-$ uptake under saline condition was positively correlated with whole plant transpiration. Further analyses are required in order to confirm this interpretation.

Inoculation with rhizobacteria into media showed an increased in phenolic compound of plantlets compared to the control. Enhancement of phenolic contents of banana plantlets is related to the potential of the inocula to stimulate root hair formation. The phenomena could be related to the potential of the inocula to produce phytohormone even in saline conditions. Similar results were demonstrated by Steenhoudt (2000) with inoculation of *Azospirillum* on plant root. In consequence, it seem that rhizobacteria may induced a factor for non-specific adaptation to stress, or the decrease in production of a root factor required for maintaining the initial mode of development albeit the presence of NaCl in the root environment and this was supported by Amzallag (1997). Salinity is a major environmental stress limiting plant growth and crop productivity. It alters a wide array of metabolic process in growing plants and induced changes in contents and activities of many enzymes (Garabito *et al.* 1998). Genotypes of crop species differing in salt tolerance show different behavior of these enzymes and different levels of metabolites in their tissue when grown under salinity stress. Prolonged water stress which limited photosynthesis led also to loss of SPS activity e.g. in leaves of *Phaseolus vulgaris*, whereas in rapidly stressed spinach leaves a stimulation in SPS activity was observed (Marcelo *et al.* 2000). Metabolism of sugars is adversely affected plants grown under saline conditions. In leaves of glycophytes, content of soluble sugars increase under salinity (Perez-Alfocea *et al.* 1996), water stress and chilling stress (Gomez-cadena *et al.* 1996).

The inhibition of the *in vitro* NRA activity especially in the roots indicated that growth-related indirect effects might be involved as well. NRA activity is considered to be limiting factor for growth, development and protein production in plants (Traore and Maranville 1999). Sturz and Nowak (2000) have proposed that in the association of beneficial associative rhizobacteria, the inoculums could stimulate plant growth, increased disease resistance, improved the plant's ability to withstand environmental stress, or enhanced $N_2$ fixation.

A possible mechanism for enhancement of leaves chlorophyll content through rhizobacteria inoculation in media containing 0.2% NaCl is the beneficial effect of *Azospirillum* in enhancing plant growth and development (Kapulnik, 1991). De Veau *et al.* (1990) have reported that the mean milligram of chlorophyll content per square decimeter of *Bradyrhizobium* inoculated soybean (*Glycine max*) test leaves was about 50% lower than the other group's leaves of control which indicating nitrogen deficiency. The inoculated host plant utilized their chlorophyll more efficiently for photosynthetic $CO_2$ uptake than the control plants. Rhizobacterial inoculation may reduce dehydration and the uptake of $Na^+$ ions in plantlets.

According to Kahindi *et al.* (1997), NaCl stress may result in dehydration and NaCl treatment had a significant effect on water content. The increased dehydration at higher salt treatment might be related to the high foliar Na concentrations. Salt accumulation may be further attributable to reduced chlorophyll and growth, as the Na concentrations in an organ or a whole plant depends on the relationship between ion uptake and growth rate (Flower and Yeo 1986). Reduction in growth may results in accumulation of ions, if ion uptake rate is reduced less than the growth rate.

High accumulation of N, P, Ca were shown in the inoculated plantlets in saline media and could be related indirectly to the inoculation process through enhanced uptake of essential nutrient by stimulation of rooting system, growth and development of the plantlets. According to Lin *et al.* (1983) and Rai and Hunt (1993), *Azospirillum brasilense* inoculation could improve ions uptake of plants and improve the availability and efficiency of use on applied mineral nutrients. The response could be related to production of hormone such as auxin, gibberellin and cytokinin by *Azospirillum* strain, which would stimulate root growth of the host plants (Patriquin *et al.* 1983).

Therefore, the rhizobacteria have the potential to be used to improve growth of *in vitro* banana plantlets cv. Berangan and cv. Novaria under the saline condition (0.2%) through the ability to increase the physiological and biochemical characteristics. Inoculation of rhizobacteria could be beneficial to the plantlets in saline conditions through increment of growth and improvement in rooting system. Results suggested that rhizobacterial plays an important role to enhance plant growth in saline habitats.

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