

# The Effects of Plant Growth Promoting Rhizobacteria on Healthy Plant Growth of Tomato Affected by Soil Sickness.

Yusran<sup>A</sup>, Markus Weinmann<sup>B</sup>, Volker Roemheld<sup>B</sup>, Torsten Mueller<sup>B</sup>

<sup>A</sup>Forestry Faculty, Tadulako University, Palu, Sulawesi Tengah, Indonesia, Email yusran\_ysrn@yahoo.ca

<sup>B</sup>Institute for Plant Nutrition, University of Hohenheim, Stuttgart, Germany, Email weinmark@uni-hohenheim.de

<sup>B</sup>Institute for Plant Nutrition, University of Hohenheim, Stuttgart, Germany, Email roemheld@uni-hohenheim.de

<sup>B</sup>Institute for Plant Nutrition, University of Hohenheim, Stuttgart, Germany, Email tmuller@uni-hohenheim.de

## Abstract

The use of antagonistic microorganism for the biological control of root disease is becoming an important alternative or supplement to chemical pesticides. In the present study, we investigated the potential of beneficial rhizobacteria to interact synergistically with indigenous arbuscular mycorrhiza fungi (AMF), furthermore for improvement plant growth of two tomato varieties using replant disease. Soil inoculation with *Pseudomonas* sp. "Proradix<sup>®</sup>" and *Bacillus amyloliquefaciens* FZB42 significantly improved the root and shoot biomass production of the two tomato varieties growing on pathogen-infected soil. Roots of both tomato varieties were not only healthier but also showed a significantly higher colonization by AMF. Root lesions was significantly lower in the rhizosphere soils of both tomato varieties when inoculated with *P. sp.* "Proradix<sup>®</sup>" or *B. amyloliquefaciens* FZB42 compared to the untreated control. The concentration of macro and micronutrients in tomato shoots was higher in the *P. sp.* "Proradix<sup>®</sup>" and *B. amyloliquefaciens* FZB42 treated plants when compared to the untreated control. The result obtained suggest an important role of rhizosphere interactions for the expression of bio-control mechanisms by inoculation with effective *Pseudomonas* and *Bacillus* strains independent of simple antagonistic effects.

## Key Words

Plant Growth-Promoting Rhizobacteria (PGPR), arbuscular mycorrhiza fungi (AMF), soil sickness, Tomato.

## Introduction

Establishing of the same crops in long term at the same site can cause a problem known as replant disease (Utkhede 2006). Mono-cropping systems lead to decreases in the abundance of beneficial microorganisms such as *Pseudomonas* and to increases in the population of soilborne pathogens in the soil (Joshua-Otieno and Jingguan 2006). Soilborne plant pathogen control by fumigation, chemical pesticide and soil solarization is intensively investigated. However, research is needed to develop cultural and biological control methods to induce resistance of host plant. The use of plant growth promoting rhizobacteria (PGPR) to protect crops from soilborne disease eventually inducing healthy growth of plants with high yields at lower cost and minimum risk to humans and environment is a promising strategy. In the present study, we conducted a pot experiment to investigate the potential of beneficial rhizobacteria (*Pseudomonas* sp. "Proradix<sup>®</sup>" (DSMZ 13134) (Proradix<sup>®</sup>, Sourcon Padena, Tübingen-Germany) and *Bacillus amyloliquefaciens* FZB42 (RhizoVital<sup>®</sup>, ABiTEP, Berlin, Germany) to interact synergistically with indigenous, site specific and adapted AMF. It was hypothesized that these rhizobacteria improve indigenous AMF infection, nutrient acquisition and growth of two tomato varieties, and suppression of soilborne pathogens under high pathogen pressure induced by replant disease soil from the rooting zone of a tomato plantation with known replant disease problems.

## Methods

### *Plant and microbial inoculum's preparation*

Tomato seeds (*Lycopersicon esculentum* Mill., varieties Money Maker and Hellfrucht Hillmar) were surface sterilized by first shaking them in a 75% Ethanol solution for 1 min and then in a 1.5% Sodium hypochloride (NaOCl) solution for 3 min. First, the tomato seeds were cultivated in pots containing 50 g substrate (Einheitserde Type P, Einheitserde und Humuswerke Gebr. Patzer, Sinntal-Jossa, Germany). After two weeks, the seedlings were transplanted to pots containing 1 kg replant disease soils/sand mixture (3:1). Before, the plants were treated by dipping of the roots into preparations of *Pseudomonas* sp. "Proradix<sup>®</sup>" (DSMZ 13134) (Proradix<sup>®</sup>, Sourcon Padena, Tübingen-Germany) ( $1.5 \times 10^{10}$  cfu/l sterile distilled water) and *Bacillus amyloliquefaciens* FZB42 (RhizoVital<sup>®</sup>, ABiTEP, Berlin, Germany) (100 g/l sterile distilled water). The non-treated control plants were dipped into pure sterilized distilled water. Before planting the soil was fertilized with 100 mg N, 50 mg P, 150 mg K, 50 mg Mg, 0.06 mg Fe per kg replant disease soils. The

replant disease soil was collected from the root zone of tomato field at the horticultural experimental station of the Universität Hohenheim (Stuttgart-Germany) with known replant disease problems. Before use, the soil was passed through a 2 mm sieve, moistened to about field capacity and stored in a closed plastic box for about one week. The pots were arranged in a completely randomized design pattern in the greenhouse. A heating-cooling system adjusted the soil temperature conditions to a day/night cycle: 14 h at 25°C/10 h at 19°C. The relative humidity in the greenhouse was about 75%. The plants were irrigated when required and harvested four weeks after transplanting.

*Plant harvest, nutrient concentration analysis, mycorrhizal root colonization and root lesions*

At harvest, roots were thoroughly washed and blotted. A subsample was taken for the assessment of mycorrhiza formation. Shoots and roots were dried 72 hours at 65°C for dry weights were determined. Mineral elements were determined by atomic absorption spectrophotometry (Mn, Zn and Cu), flame-photometry (Mg and K) and photo-spectrophotometry (P) after wet digestion. Assessment of mycorrhizal root colonization was based on Koske and Gemma (1989) and Kormanik and McGraw (1984) and root lesion was based on Tennant (1975).

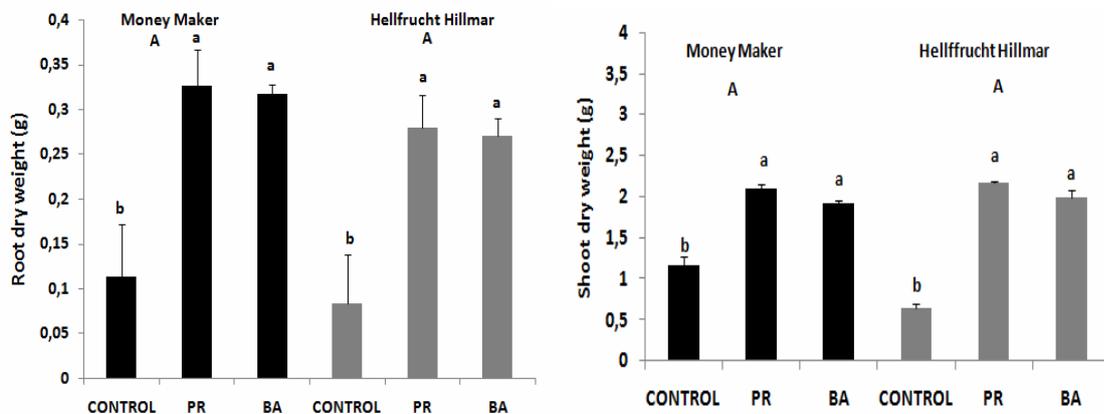
*Statistical analysis*

The experimental design was a completely randomized 2 (tomato varieties) x 3 (PGPR) factorial design with 4 replicates. Data of relative percentage of mycorrhiza infection and root lesions were normalized by arcsin√% transformation before being subjected to a two way analysis of variance (ANOVA) (Gomez and Gomez, 1984). A Tukey test at a significance level of P<0,05 was conducted on the transformed data after the ANOVA to distinguish between differences among the treatments. The results in tables, text and figures are given as means. All statistical analyses were performed using Sigma Stat version 2.03 statistical software (SPSS Inc. Chicago. IL. USA).

**Results and Discussion**

In general, Soil inoculation with *Pseudomonas* sp. “Proradix®” (DSMZ 13134) (Proradix®, Sourcon Padena, Tübingen-Germany) and *Bacillus amyloliquefaciens* FZB42 (RhizoVital®, ABiTEP, Berlin, Germany) significantly increased the root and shoot biomass production of the two tomato varieties growing on pathogen-infected soil (Figure 1). Roots of both tomato varieties were not only healthier but also showed a significantly higher colonization by AMF and lower root lesions caused by soilborne pathogens (Figure 2). The percentage of AMF infected roots was significantly higher in the tomato variety Hellfrucht Hillmar than in the variety Money Maker.

The concentration of macro and micronutrients in tomato shoots was higher in the *P. sp.* ”Proradix®” and *B. amyloliquefaciens* FZB42 treated plants when compared to the untreated control (Table 1). This is in accordance with authors (Siddique *et al.* 2001; Barea *et al.* 2002; Akkopru and Demir 2005; Yusran *et al.* 2008) who reported that AMF colonization in the roots of many crops greatly enhanced the uptake of phosphorus and micro nutrients and reduce severity of plant diseases. This indicates that the AMF infection potential in the soils was not generally low but rather suppressed directly by pathogens or indirectly as consequence of poor root development.



**Figure 1. Root dry weight (left) and shoot dry weight (right) of two tomato varieties 6 weeks after planting with *Pseudomonas* sp. “Proradix®” (PR), *Bacillus amyloliquefaciens* FZB42 (BA) on replant disease soil. Vertical bars indicate standard errors of the mean (n=4). Different small letter above the bars indicate significant differences between the treatments within one tomato variety and capital letter indicate significant differences between the two tomato varieties (Tukey test, p<0,05).**

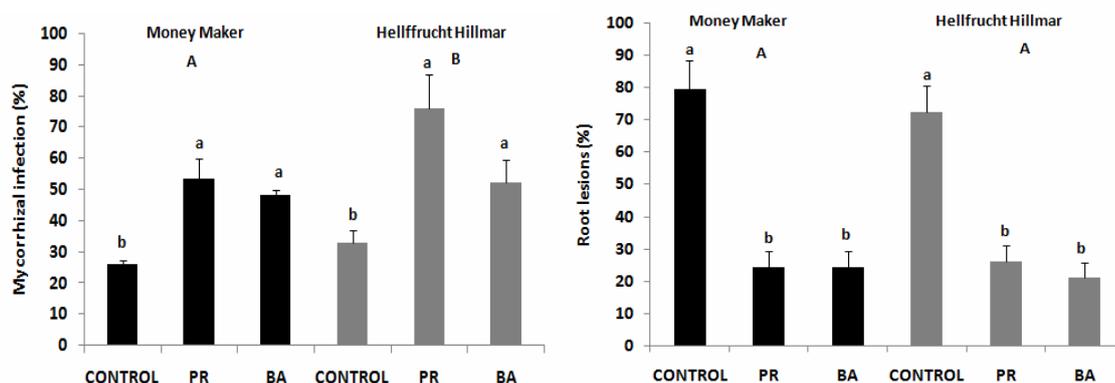


Figure 2. Percentage of roots infected by indigenous AMF (left) and root lesions (right) 6 weeks after planting with *Pseudomonas* sp. “Proradix<sup>®</sup>” (PR) or *Bacillus amyloliquefaciens* FZB42 (BA) on replant disease soil. Vertical bars indicate standard errors of the mean (n=4). Different small letters above the bars indicate significant differences between the treatments within one tomato variety and different capital letters indicate significant differences between the two tomato varieties (Tukey test, p<0,05).

Table 1. Macro- and micronutrient concentration in the shoot dry matter of two tomato varieties six weeks after planting with *Pseudomonas* sp. “Proradix<sup>®</sup>” (PR) or *Bacillus amyloliquefaciens* FZB42 (BA) in replant disease soil. Different letters in the same row indicate significant differences between all treatments within each variety and asterisk following nutrient symbol indicate significant differences between two variety (Tukey test, p<0,05). NS = No significant difference.

Nutrient concentration in the shoots	Tomato variety					
	Money Maker			Hellfrucht Hillmar		
	CONTROL	PR	BA	CONTROL	PR	BA
P (mg/g)*	2,4 b	3,5 a	3,2 a	1,6 c	3,7 a	2,9 b
K (mg/g)*	21,4 b	33,6 a	29,7 a	18,9 b	24,8 a	24,4 a
Mg (mg/g) ns	2,8 b	4,0 a	3,8 a	3,0 b	3,7 a	3,5 a
Mn(mg/kg)*	7,8 c	27,5 a	14,9 b	5,3 c	20,8 a	18,9 b
Zn (mg/kg)*	7,3 c	32,5 a	16,8 b	4,5 c	11,9 a	9,8 b
Cu(mg/kg)*	3,7 c	5,6 a	5,1 b	4,5 c	9,5 a	7,7 b

## Conclusion

In conclusion, the result obtained suggest an important role of rhizosphere interactions for the expression of bio-control mechanisms by inoculation with effective *Pseudomonas* and *Bacillus* strains in addition to simple antagonistic effects. The use of PGPR may provide immediate benefits as improving mycorrhization, supporting healthy growth of plants and suppressing soilborne pathogens. However, further field based research is necessary to ensure a high efficiency and reliability of those products under field conditions.

## Acknowledgements

Yusran thanks the Asia link – European Commission project ASIA LINK/008/110-005 for a postgraduate scholarship. We also express our thanks to Sourcon Padena GmbH and Co. KG. Tübingen (Germany) for providing *Pseudomonas* sp. “Proradix<sup>®</sup>” inoculums (Proradix<sup>®</sup>) and ABiTEP, Berlin (Germany) for providing *Bacillus amyloliquefaciens* FZB42 inoculums (RhizoVital<sup>®</sup>).

## References

- Akkopru A, Demir S (2005) Biological control of Fusarium wilt in tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* by AMF *Glomus intraradices* and some rhizobacteria. *Journal of Phytopathology* **153**, 544–550.
- Barea JM, Azcón R, Azcón-Aguilar C (2002) Mycorrhizosphere interactions to improve plant fitness and soil quality. *Antonie van Leeuwenhoek* **81**, 343-351.
- Gomez KA, AA Gomez (1984) Statistical procedures for agricultural research (2<sup>th</sup> edn.) John Willey and Sons, New York, pp. 680.
- Joshua-Otieno O, Yu J (2006) Autotoxic potential in soil sickness: A re-examination. *Allelopathy Journal* **18**, 93-102.
- Kormanik PP, McGraw AC (1984) Quantification of Vesicular-arbuscular mycorrhizae in plant roots. In ‘Methods and Principles of Mycorrhizal Research’. (Ed NC Schenck) pp. 37-45. (The American Phytopathological Society. St. Paul Minn).

- Koske RE, Gemma JN (1989) A modified procedure for staining roots to detect VA mycorrhizas. *Mycological Research* **92**, 486–505.
- Siddique ZA, Iqbal A, Mahmood I (2001) Effects of *Pseudomonas fluorescens* and fertilizers on the reproduction of *Meloidogyne incognita* and growth of tomato. *Applied Soil Ecology* **16**, 179–185.
- Tennant D (1975) A test of modified line-intersect method for estimating root length. *Journal of Ecology* **63**, 995-1001.
- Utkhede RS (2006) Soil sickness, replant problem or replant disease and its integrated control. *Allelopathy Journal* **18**, 23–28.
- Yusran, Weinmann M, Roemheld V, Mueller T (2008) Suppression of soilborne pathogens in onion (*Allium ascalonicum* L.) by rhizobacteria; related mechanism. In 'International Symposium Proceedings, Land use after the tsunami, supporting education, research and development in the Aceh region' (Eds H Perner, A George, Syahabuddin Zaitun) pp. 122-130. (Banda Aceh, Indonesia).