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Growth and Storage Root Development of Sweetpotato Inoculated with Rhizobacteria Under Glasshouse Conditions

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Abstract: A pot experiment was conducted to determine the effects of rhizobacterial inoculation on growth and storage root development of Sepang Oren sweetpotato. Inoculation positively affected plant growth. The highest growth was observed on plants inoculated with *Klebsiella* sp. which increased shoot dry weight by 23% compared to control. Five of the isolates were able to produce sweetpotato storage roots. *Klebsiella* sp and *Erwinia* sp. produced higher storage root yields of 35.15 and 8.22 g plant^{-1}, respectively, compared to the other treatments. The inoculation significantly increased the uptake of nutrients in plant tissue and the concentrations of nutrients in soil. The results suggest that *Klebsiella* sp and *Erwinia* sp are potentially effective as bioenhancers and biofertilizers for sweetpotato.

Key words: sweetpotato, rhizobacteria, *Klebsiella sp., Erwinia sp*., biofertilizer

INTRODUCTION

Sweetpotato is grown world wide as source of carbohydrates and commercial production requires high input of chemical fertilizers which is costly. Plant growth-promoting rhizobacteria (PGPR) is being used as biofertilizer and bioenhancer for different crop plants as an alternative source of chemical fertilizer which could reduce the input cost on farming. PGPR has been known to improve plant and root growth and plant nutrition (Egmarberdiyeva and Hoflich, 2004). It influences crop growth and development by changing the physiological status and morphological characteristics of inoculated roots which favour improved nutrient uptake. Inoculation of PGPR increases the growth of plants by making nutrients more available through mechanisms such as N_2 fixation process and solubilization of phosphates, or increasing plant access to nutrients by increasing root surface area. Locally isolated PGPR strains were observed to enhance the uptake of nutrient and growth in Gendut sweetpotato (Lusi *et al.,* 1999). Single or dual inoculation of sweetpotato with different PGPR species in sterilized soil significantly stimulated plant growth and increased the concentrations of nutrient in sweetpotato shoot (Radziah and Tan, 1999). Earlier studies by Lusi (2000) observed an increased uptake of nutrient and plant growth in Gendut sweetpotato plants inoculated with PGPR. Associative diazotrophic rhizobacteria e.g. *Azospirillum brasilense* SP7 and *Bacillus sphaericus* UPMB10 have been reported to fix N_2 and enhance growth with several non-leguminous crops such as oil palm seedling and banana production (Amir *et al*., 1999, 2002; Mia *et al*., 1998).

Although the beneficial traits of the PGPR have been shown in vitro (Farzana *et al*., 2007), the effect of the bacteria on plants grown in soil need to be evaluated. Selection of bacteria that has the potential to improve growth of sweetpotato is important for successful commercial application. The present study was conducted to determine the effect of selected rhizobacterial isolates on growth and storage root formation of sweetpotato under glasshouse condition.

MATERIALS AND METHODS

Preparation of Soil and Planting Materials:

A pot experiment was conducted in the glasshouse of Universiti Putra Malaysia, Serdang Selangor. Seven kg of sterilized mixed soils (sand and mineral soil,1:3) were filled in ceramic pots of 21 cm height and 25.4 cm inner diameter. The soil contained 65.8 % sand, 5.8% silt and 28.4% clay, 0.17% total N, 2.03 mg kg^{-1} extractable P, 0.46 cmol (+) kg^{-1} K, 1.64 cmol (+) kg^{-1} Ca, 0.45 cmol (+) kg^{-1}Mg and pH 4.84. The surface of soil in each pot was covered with black polythene bag to prevent contamination of the soil. The experiment

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was carried out in a Completely Randomized Design (CRD) with three replications per treatment. Healthy apical cuttings of Sepang Oren sweetpotato cultivar with a length of 30cm and 8 nodes were used.

Preparation of Inoculum and Planting:

The study consisted of nine bacterial isolates from sweetpotato rhizosphere and two known bacteria *(Azospirillum brasilense* SP7 and *Bacillus sphaericus* UPMB10) for comparison. The twelve treatments were 1) *Pseudomonas* sp. UPMSP2, 2) *Serratia* sp. UPM SP3, 3) *Klebsiella* sp. UPMSP9, 4) *Erwinia* sp. UPMSP10, 5) UPMSP11, 6) *Acinetobacter* sp. UPMSP12, 7) *Pseudomonas* sp. UPMSP13, 8) *Paenibacillus* sp. UPMSP18, 9) *Pseudomonas* sp. UPMSP20, 10) *A. brasilense* SP7, 11) *B. sphaericus* UPMB10 and 12) Control (uninoculated).

All bacterial isolates used were local except *A. brasilense* SP7 which was originally obtained from Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA), Brazil. *A. brasilense* SP7 and *B. sphaericus* UPMB10 (Amir *et al.,* 2002) were sub-cultured in 100 mL Okon medium (Okon *et al.,* 1977) and the others in King's B (King *et al.*, 1954) medium in 250 ml Erlenmeyer flasks, and were shaken continuously (150 rpm) for 48 hours at 28 ± 2^0C. Sweetpotato Sepang Oren cuttings were cleaned using sterilized water and were then dipped into the respective rhizobacterial cultures for six hours. The inoculated plant cuttings were then planted into the respective pots containing sterile soil. Each pot was inoculated every two weeks with 20 mL (approximately 10^9 CFU mL^{-1}) of the respective bacterial isolates. The control pot received the same volume of sterile media without bacteria. Hoaglands solution (Hoagland, 1950) was added into each pot every two days to supply plants with the essential plant nutrients. All plants were watered daily with distilled water and grown for 110 days.

Plant Tissue Analysis:

Sweetpotato shoots, fibrous roots and storage roots fresh and dry weights were recorded and the root volume was determined by using the water displacement method (Gliessman, 1999). Dried shoots were ground using electric grinder and passed through 0.5 mm sieve. Shoot samples were digested with concentrated sulphuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) following the micro-kjeldahl method (Thomas *et al.,* 1967). N, P, K concentrations were determined using autoanalyser and Ca, Mg by using Atomic Absorption Spectrophotometer.

Soil Analysis:

Fresh soil samples from area around plant roots collected for nutrient concentrations. A modified method of Sarwar *et al*., (1992) was used to determine the concentrations of indole acetic acid (IAA) in soil. The total nitrogen was determined following the micro-Kjeldahl method (Bremner, 1965). The available phosphorus measured by Bray-2 method (Bray and Kurtz, 1945). Concentrations of exchangeable K, Ca and Mg were determined using the shaking method (Schollenberger and Simon, 1945)

Statistical Analysis:

The data were analyzed by Statistical Analysis System (SAS, version 8.2, 2006). Following the analysis of variance procedure (ANOVA), differences among treatment means were determined using Tukey's Studentized Range test (HSD) comparison method at p=0.05. Correlation analysis was performed for the estimation of relationships between different traits such as sweetpotato yield, soil IAA, and nutrient uptake of sweetpotato plants .

RESULTS AND DISCUSSION

Plant Growth and Storage Root Formation:

There was significant effect of bacterial inoculation on growth of Sepang Oren Sweetpotato. In general, most of the inoculated plants showed positive plant growth (Table 1). Plants treated with *Klebsiella* sp. UPMSP9 and *Erwinia* sp. UPMSP10 showed significantly high shoot and root dry weights and root volume compared to the uninoculated control.

Although good top biomass was observed in most plants, there was no significant influence of bacterial inoculation on the production of the storage roots. Only five of the treated plants produced storage roots while the other treatments only produced fibrous roots. Plants inoculated with *Klebsiella* sp. UPMSP9 and *Erwinia* sp. UPMSP10 showed higher storage root yield compare to other treatments. Results indicated that plant growth promotion by the isolates was probably due to the ability of the isolates to produce IAA.

Table 1: Effect of Rhizobacterial Inoculation on Growth and Storage Root Formation of Sweetpotato

Bacterial Treatments	Shoot Dry Weight (g Plant^{-1})	Fibrous Root Dry Weight (g Plant^{-1})	Fibrous Root Volume(cm^3 Plant^{-1})	Shoot to Root Ratio(S/R)	Storage Root Fresh Weight (g plant^{-1})
Pseudomonas UPMSP2	37.12^{abc}	3.85^{abc}	31.83^{c}	9.79^{c}	1.30^{bc}
Serratia UPMSP3	33.88^{bc}	2.88^{de}	20.88^{ef}	11.81^{bc}	0^{c}
Klebsiella UPMSP9	39.54^{a}	4.41^{a}	36.28^{ab}	9.07^{c}	35.15^{a}
Erwinia UPMSP10	38.81^{ab}	4.44^{a}	38.29^{a}	8.86^{c}	8.22^{b}
UPMSP11	33.51^{c}	2.96^{de}	21.91^{def}	11.38^{bc}	0^{c}
Acinetobacter UPMSP12	38.66^{ab}	4.24^{ab}	31.11^{c}	9.26^{c}	1.34^{bc}
Pseudomonas UPMSP13	35.45^{abc}	3.68^{bc}	24.22^{de}	9.65^{c}	0^{c}
Paenibacillus UPMSP18	33.96^{bc}	3.47^{cd}	24.36^{de}	9.84^{c}	0^{c}
Pseudomonas UPMSP20	38.71^{ab}	3.86^{abc}	32.66^{bc}	10.05^{bc}	1.48^{bc}
Azospirillum SP7	36.55^{abc}	3.70^{bc}	25.87^{d}	9.92^{c}	0^{c}
Bacillus UPMB10	32.43^{c}	2.52^{ef}	18.63^{fg}	12.94^{b}	0^{c}
Control	32.15^{c}	1.83^{f}	14.78^{g}	17.74^{a}	0^{c}

Note: Means in column followed with same letter (s) are not significantly different (P>0.05)

Nutrient Uptake in Shoots:

The accumulation of N, P, K, Ca, and Mg in plant shoots were significantly (P≤0.05) influenced by bacterial inoculation (Table 2). Plant inoculated with *Klebsiella* sp. UPMSP9 and *Erwinia* sp. UPMSP10 showed significantly higher N, P, K, Ca, and Mg uptake compared to the uninoculated plants. Inoculation with *Klebsiella* sp. UPMSP9 and *Erwinia* sp. UPMSP10 increased nitrogen uptake compared to the control by 170% and 134% respectively.

Table 2: Effect of Rhizobacterial Inoculation on Uptake of N, P, K, Ca, and Mg in Sweetpotato Shoots

Bacteria Treatments	Nutrients Uptake (mg plant^{-1})				
	N	P	K	Ca	Mg
Pseudomonas UPMSP2	818.03^{cd*}	100.92^{d}	916.49^{de}	283.36^{cd}	114.19^{de}
Serratia UPMSP3	559.33^{fg}	108.73^{cd}	537.65^{gh}	264.04^{de}	73.21^{gh}
Klebsiella UPMSP9	1154.47^{a}	162.99^{a}	1860.12^{a}	481.31^{a}	154.32^{ab}
Erwinia UPMSP10	998.93^{b}	131.37^{b}	1608.39^{b}	367.67^{b}	158.06^{a}
UPMSP11	517.50^{fg}	100.47^{d}	553.52^{fgh}	218.38^{e}	96.43^{ef}
Acinetobacter UPMSP12	909.14^{bc}	124.83^{bc}	977.95^{d}	340.37^{bc}	122.45^{cd}
Pseudomonas UPMSP13	730.23^{de}	100.11^{d}	759.65^{ef}	289.61^{cd}	106.30^{def}
Paenibacillus UPMSP18	638.44^{ef}	91.31^{d}	717.61^{efg}	282.45^{cd}	91.34^{fg}
Pseudomonas UPMSP20	944.54^{bc}	136.64^{b}	1323.77^{c}	366.46^{b}	137.98^{bc}
Azospirillum SP7	794.67^{cd}	109.91^{cd}	815.57^{de}	288.69^{cd}	104.77^{def}
Bacillus UPMB10	440.40^{g}	91.41^{d}	502.18^{h}	207.10^{ef}	100.39^{ef}
Control	427.22^{g}	64.94^{e}	405.90^{h}	154.98^{f}	70.00^{h}

Note: Means in column followed with same letter (s) are not significantly different (P>0.05)

Nutrient Concentration in Soil:

There were significant (P≤0.05) differences among N, P, K, Ca, and Mg concentrations in soil with different inoculation treatments (Table 3). Soil inoculated with *Klebsiella* sp. UPMSP9 and *Erwinia* sp. UPMSP10 significantly increased N, K Ca and Mg concentrations. The concentration of IAA in soil was also found to be significantly (P≤0.05) influenced by the rhizobacterial isolates (Table 3). Concentration of IAA in soil inoculated with *Klebsiella* sp. UPMSP9 was significantly higher than the uninoculated control soil.

Table 3: Effect of Rhizobacterial Inoculation on Soil chemical properties

Bacterial Treatments	Nutrients Concentration					
	N (%)	P mg kg^{-1}	K cmol (+) kg^{-1}	Ca cmol (+) kg^{-1}	Mg cmol (+) kg^{-1}	IAA in Soil (mg kg^{-1})
Pseudomonas UPMSP2	0.14^{abcd}	4.94^{cd}	0.60^{a}	2.15^{bc}	0.36^{bcd}	0.70^{e}
Serratia UPMSP3	0.11^{de}	4.14^{de}	0.56^{ab}	1.75^{d}	0.30^{cd}	1.14^{c}
Klebsiella UPMSP9	0.17^{ab}	11.86^{a}	0.63^{a}	2.55^{a}	0.51^{a}	1.34^{a}
Erwinia UPMSP10	0.18^{a}	6.86^{b}	0.65^{a}	2.41^{ab}	0.50^{a}	1.24^{b}
UPMSP11	0.11^{de}	4.17^{de}	0.54^{ab}	1.31^{e}	0.27^{cd}	0.29^{h}
Acinetobacter UPMSP12	0.15^{abc}	5.31^{c}	0.62^{a}	2.06^{cd}	0.40^{abc}	0.77^{de}
Pseudomonas UPMSP13	0.13^{bcde}	4.35^{de}	0.62^{a}	1.85^{cd}	0.30^{cd}	0.61^{f}
Paenibacillus UPMSP18	0.14^{abcd}	4.33^{de}	0.55^{ab}	1.79^{d}	0.35^{bcd}	0.39^{g}
Pseudomonas UPMSP20	0.16^{ab}	6.30^{b}	0.63^{a}	2.45^{ab}	0.44^{ab}	0.81^{d}
Azospirillum SP7	0.13^{bcde}	4.51^{cde}	0.60^{a}	2.06^{cd}	0.33^{bcd}	0.61^{f}
Bacillus UPMB 10	0.11^{cde}	3.76^{ef}	0.53^{ab}	1.40^{e}	0.26^{d}	0.14^{i}
Control	0.09^{e}	3.19^{f}	0.45^{b}	1.24^{e}	0.23^{d}	0.10^{i}

Note: Means in column followed with same letter (s) are not significantly different (P>0.05).

Correlation Analysis:

Storage root yield correlated positively with soil IAA and nutrient uptake in plant tissue (Table 4). The results showed significant positive correlation between storage root yield and the soil IAA, N, P, K, Ca and Mg uptake in plant tissue. All parameters tested correlated with each other. This indicates that inoculation with rhizobacteria to plant could probably increase IAA production, which may have stimulated root growth and subsequently increased uptake of nutrients for increased yield.

Table 4: Correlation between Sweetpotato yield, soil microbial population soil IAA, and nutrient uptake of Sweetpotato Plants

	IAA	SN	SP	SK	SCa	SMg
Yield	0.60 ***	0.62 ***	0.69 ***	0.73 ***	0.71 ***	0.59 **
IAA		0.78 ***	0.82 ***	0.78 ***	0.82 ***	0.64 ***
SN			0.88 ***	0.93 ***	0.94 ***	0.87 ***
SP				0.88 ***	0.92 ***	0.82 ***
SK					0.92 ***	0.92 ***
SCa						0.84 ***

Note: *: significant (p<0.05), **: significant at (p<0.01) and ***: significant at (p<0.001) SN: Shoot N, SP: Shoot P, SK: Shoot K, SCa: Shoot Ca, SMg: Shoot Mg, IAA: Concentration IAA, Yield: Storage root yield.

Discussion:

Sweetpotato growth was affected by PGPR inoculation. Although inoculated plant showed increased in top and root growth, only five of the bacteria were able to produce storage roots. *Klebsiella* sp. UPMSP9 and *Erwinia* sp. UPMSP10 inoculation resulted in highest plant growth and storage root development. Plant top growth increased by 23% and 21% compared to uninoculated control. The ability of bacteria to produce IAA could be one of the possible mechanisms involved in promoting the growth of sweetpotato. Rhizobacteria has been known to stimulate plant growth through mechanisms such as increase nutrient uptake, solubilization of phosphates, and production of plant growth promoting substances (Glick *et al.,* 1999). Inoculation of plants with PGPR results in a significant change in various plant growth parameters which may affect crop yield. The response to PGPR inoculation of sweetpotato showed significant positive effect on the plant growth and yield compared with uninoculated plants (Saad and Norhayati, 1995) and act as a bioenhancer through the production of phytohormone, N_2 fixing process and other beneficial mechanisms.

The study showed inhibitory effects of rhizobacterial treatments on storage root development under glasshouse condition. It could be due to suppression of hormone that regulates translocation of photosynthtate from the leaves to tuber formation. It was not clear of the reasons for such observation. It seemed that the bacterial isolates were able to stimulate only the fibrous roots for increased shoot growth.

Significant effects of growth and yield of plants were obtained from an interaction between plant genotype and bacterial strain (Garcia de Salomone and Dobereiner, 1996). The ability of the bacterial isolates to produce IAA could be the mechanism for stimulating plant growth. IAA is known to be involved in cell division, cell enlargement, stem growth, root initiation on stem cuttings and the development of branch roots (Salisbury, 1994). Previous studies have concluded that the positive growth response of inoculated cereals and vegetables was due to production of plant growth regulators (Beyeler *et al*., 1997; Salamone *et al*., 1997). Rhizobacterial inoculation in the present study significantly affected nutrient contents and uptake by plants. Plants inoculated with *Klebsiella* sp. UPMSP9 showed significantly higher uptake of N, P and K compared to the uninoculated control plants.

The microorganisms are very important in the biochemical reactions of nutrients in the soil and maintenance of soil health. According to Paula *et al.,* (1992) the magnitude of the plant response to any microbial inoculation can be greatly affected by the nutrients content of soil. Inoculation of rhizobacteria significantly increased the IAA concentration in soil compared to those without inoculant. Treatment with *Klebsiella* produced the highest IAA like compounds in soil. The bacteria probably synthesized IAA through TRP pathways (Frankenberger and Arshad, 1995) by utilizing L-TRP excreted from the roots. Other studies have shown that single inoculation of sweetpotato with PGPR in sterilized soil resulted in significantly increased concentrations of IAA and nutrient contents in soil (Lusi, 2000).

Conclusion:

PGPR inoculation significantly increased the growth of the sweetpotato plants under glasshouse condition. The inoculation had stimulated root growth and enhance the uptake of N, P and K. Two of the isolates, *Klebsiella* sp. UPMSP9 and *Erwinia* sp. UPMSP10 significantly improved growth and storage root of development Sepang Oren sweetpotato. Improvement in growth was associated with the production of high IAA and other beneficial traits.

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