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Yield and Nutrient Content of Sweet Potato in Response of Plant Growth-Promoting Rhizobacteria (PGPR) Inoculation and N Fertilization

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Abstract

This study was carried out to determine the effects of selected beneficial bacterial isolates with N fertilizer application on yield and nutrient content of sweet potato under field condition. A factorial experiment with two factors (Plant growth-promoting rhizobacteria inoculation and N fertilizer) was positioned with three replications in a Randomized Complete Block Design (RCBD). Three stages of N fertilizer (0, 33, and 100 kg N ha^{-1}) and five strains of bacteria (*Bacillus sphaericus* UPMB10, *Erwinia* sp. UPMSP10, *Klebsiella* sp. UPMSP9, *Azospirillum brasilense* SP7 and Uninoculated control) were used for treatments. Plants were grown on sandy clay soil at the Universiti Putra Malaysia experimental plot. The effect of bacterial population in the soil at different phases of plant growth was significantly stimulated by bacterial inoculation and N fertilization. The soil inoculated with *Klebsiella* sp. applied with 33 kg N ha^{-1} showed highest population of $2.63X10^7$ CFU g (dry wt.)$^{-1}$ soil. However, after the 2nd and 3rd month of inoculation, the number of bacteria in soil dropped. The results of inoculated plants showed significant differences in sweet potato yield, N, P, K, Ca and Mg contents of storage root compared to control. After the field experiment, it was found that plants inoculated with *Klebsiella* sp. applied with 33 kg N ha^{-1} showed the highest storage root yield. Substantial relations among PGPR inoculation and N fertilization was detected on nutrient content of sweet potato storage roots. *Klebsiella* and application of 33kg N ha-^{1} demonstrated the highest N, P, K, Ca, Mg content of storage roots. These results recommended that *Klebsiella* sp. can be used as biofertilizer of sweet potato for decreasing the rates of N fertilizers and giving a stage forward for sustainable agriculture.

Keywords: Sweet potato, Plant growth-promoting rhizobacteria, Nitrogen fertilizer, Yield, Nutrient content

1. Introduction

Sweet potato (*Ipomoea batatas* L.) is one of the major staple foods in several countries including Asia and the Pacific Islands. In Malaysia, sweet potato is the second most important root crop after cassava. It is also a good source of carbohydrate, beta-carotene, thiamine, riboflavin, folic acid, ascorbic acid and minerals. There are many nutritious food items like noodles, bakery products, snacks, breakfast cereals and beverages where the storage root is widely used (O' Sullivan *et al.*, 1997; Zhang *et al*., 2009; Santra Kumawat,2014). The sweet potato production in Malaysia is currently less due to non-availability of land and conversion of agricultural land to industrial uses, labor costs, marketing issues, outbreak of diseases and high input namely fertilizer (Tan *et al*., 2005; Loebenstein, 2009). Excessive nitrogen is not only wasteful but can lead to environmental pollution and increase the cost of crop production. One of the methods to sustain production is through the application of beneficial microorganisms such as plant growth-promoting rhizobacteria (PGPR). These microbes can produce phytohormones. These phyto-hormones improve plant growth by increased uptake of nutrients. Indole-acetic acid (IAA) is regarded as the most important hormone synthesized by PGPR, (Glick., 2012; Umair *et al*.,2018). The IAA produced by bacteria is involved in several types of microorganism-plant interaction. IAA may induce the plant either to grow faster or better due to the stimulation on cell division and differentiation. The excreted IAA can positively influence development of root system (Hagen, 1990; Chaiharn, 2011). The promotion of growth and development of sweet potato by PGPR is probable through secretion of plant growth hormones and increased mineral uptake and mineral accumulation through efficient rooting system (Dawwam *et al.*, 2013). Most soils lack nitrogen, and application of nitrogen fertilizer is essential for good yield. Nitrogen supply plants show elaborate responses at both physiological and morphological dimensions to change their development and improvement. Nitrogen is significant for metabolic activities of bacteria. Presumably, it could positively affect IAA biosynthesis (Frankenberger and Arshad, 1995; Spaepen *et al.*, 2007, Moshira, *et al.*,

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2015). Earlier studies demonstrated that banana and sweet potato inoculated with PGPR and applied 1/3Ni fertilizer produced the highest root yield and shoot growth under field conditions (Radziah and Zulkifli, 2003, Mia *et al.*, 2013). This demonstrates a potential saving on fertilizer cost. Therefore, the following study aimed to assess the beneficial effects of four strains of PGPR and three levels of nitrogen fertilizer on yield and nutrient content of sweet potato under field condition.

2. Materials and Methods

The experiment was conducted at UPM experimental plot (Ladang kongsi). The area was located at $3^0 02'$ N latitude, 101^0 42′ E longitude and about 31 m above sea level. The soil was sandy clay. The land was ploughed with a rotovator, and ridges at 1.0 m apart were then built using a tractor mounted ridge. The size of ridge was 30 cm high and 60 cm wide at the base. Each unit plot measured 3.2 m X 2 m. A factorial experiment with two factors (PGPR inoculation and N fertilizer) was laid out in a Randomized Complete Block Design (RCBD) with three replications. The treatments consisted of five bacterial strains (*Klebsiella* sp. UPMSP9, *Erwinia* sp. UPMSP10, *Azospirillum brasilense* SP7, *Bacillus sphaericus* UPMB10 and uninoculated control) and three levels of N fertilizer (0, 33, and 100 kg N ha^{-1}). Prior to planting, cuttings of sweet potato shoot variety Sepang Oren (30 cm in length with 8 nodes) were soaked in 48 hr old rhizobacterial solution for six hours. Each plant was inoculated with the respective inoculum at planting and one month after planting with 20 mL inoculum per plant (approximately 10^9 CFU mL^{-1}). Control plant received the same volume of sterile media without bacteria.

The field was irrigated regularly by a sprinkler system. Plants were harvested after 3 months of planting. Storage roots were collected for nutrient analysis. The dried storage roots were ground and digested with H_2SO_4 and H_2O_2 using block digestion following the micro-kjeldahl method (Thomas *et al.*, 1967). The clear digested sample was then cooled, diluted to 100ml with distilled water. Approximately 20ml of the sample was kept in test tubes for N, P, K concentration determined by auto-analyzer (Technicon II, Technicon Ltd.). The remaining sample was diluted with Lithium Chloride and analyzed for Ca and Mg using Atomic Absorption Spectrophotometer (Perkin-Elmer, 5100 PC, Perkin Elmer).

The storage root starch content and crude protein were determined according to method of Truong (1992) and Woolfe (1992) respectively. Fresh soil samples from area around plant roots were collected for bacterial counts by using total plate count technique (Parkinson *et al.*, 1971). All data was statistically analyzed by Statistical Analysis System (SAS, version 6.12, 1989). 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.

3. Results

3.1. Sweet potato Yield

There was significant (P<0.05) effect of PGPR inoculation and nitrogen fertilization on the percentage of sweet potato yield and storage root number (Table 1). There was no significant increase in yield at the highest N rate of 100 kg. *Klebsiella* sp. inoculation and N application of 33 showed higher percentage of sweet potato yields compared to the same N rate of the uninoculated treatment.

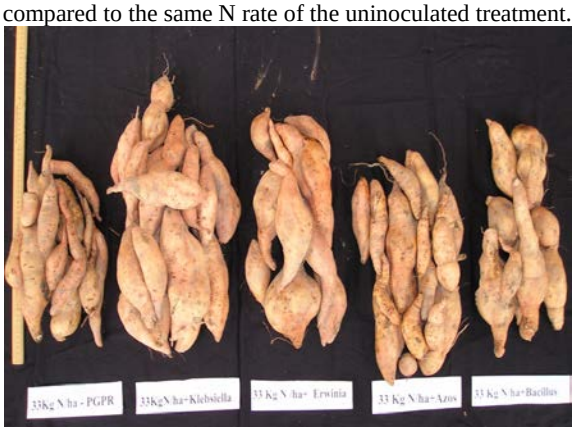

Figure 1. Storage root yield of sweet potato in 33 kg N fertilizer rate with PGPR.

Table 1. Effect of Rhizobacterial Inoculation and N Fertilization on Total Storage Root Number and Yield

Treatments		Storage root yield (%)	Storage root number ($Plot^{-1}$)
Control	0 kg	48.99e	17def
	33 kg	79.93abc	16efg
	100 kg	73.59bcd	16efg
Klebsiella sp.	0 kg	72.53.bcd	14fgh
	33 kg	89.91 a	26b
	100 kg	88.20a	25b
Erwinia sp.	0 kg	71.59bcd	17def
	33 kg	82.63 ab	25b
	100 kg	84.4 8ab	20cd
Azospirillum sp.	0 kg	64.68d	12h
	33 kg	81.47abc	21c
	100 kg	78.21abcd	18cde
Bacillus sp.	0 kg	68.36cd	20cd
	33 kg	81.10abc	32a
	100 kg	74.45bcd	13gh
Significance due to PGPR		* (0.0001)	*(0.0002)
N Fert.		*(0.0001)	*(0.0001)
PGPR * N Fert		*(0.0209)	*(0.0022)

Note: *Significant (P<0.05), Means the values followed with same letter (s) are not significantly different (P>0.05).

3.2. Starch and crude protein concentrations of storage root

PGPR inoculation and nitrogen application significantly (P<0.05) influenced the storage root starch and crude protein contents which increased with the application of 33 kg N ha^{-1} fertilizer (Table 2). There was significant interaction effect of PGPR inoculation and N fertilization on crude protein contents of storage root.

Table 2. Effect of Rhizobacterial Inoculation and N Fertilization on Starch and Crude Protein Content of Storage Root.

Treatments Bacterial Fertilizer	N Isolates (Nha^{-1})	Starch Content (%)	Crude Protein (%)
Control	0 kg	17.09	1.08f
	33 kg	19.88	1.67cde
	100 kg	18.52	1.56de
Klebsiella sp.	0 kg	19.49	1.60cde
	33 kg	22.25	2.23a
	100 kg	21.89	2.13ab
Erwinia sp.	0 kg	20.03	1.51de
	33 kg	20.86	2.22a
	100 kg	20.57	1.68cde
Azospirillum sp.	0 kg	20.09	1.38ef
	33 kg	21.41	2.29a
	100 kg	20.90	1.88bc
Bacillus sp.	0 kg	19.62	1.61cde
	33 kg	21.02	2.13ab
	100 kg	20.06	1.82bcd
Significance due to PGPR		*(0.0001)	*(0.0001)
N Fert.		*(0.0001)	*(0.0001)
PGPR * N Fert		NS (0.2242)	*(0.0027)

Note: *Significant (P<0.05), Means the values followed with same letter (s) are not significantly different (P>0.05).

3.3. Nutrient content of storage root

Nutrient uptake by storage roots were greatly enhanced by PGPR inoculation and N fertilizer. Higher contents of the nutrients were observed in *Klebsiella* inoculated plants. Meanwhile the interaction effect of PGPR inoculation and N fertilization significantly (P<0.05) influenced storage root of N, P, K, Ca and Mg contents. *Klebsiella* and N application of 33 kg N ha^{-1} showed the highest N content of storage root (Table 3).

3.4. Total bacterial population in soil

PGPR inoculation and N fertilization affected the total bacterial population in soil (Figure 2 a, b, c). There was a significant (P≤0.05) interaction effect of PGPR and N fertilization on bacterial population. There were changes in bacterial population during different plant growth stages. In general, the population was high at 30 days after planting and decreased at 60 and 90 days after planting. Inoculated treatments showed higher bacterial population compared to uninoculated control. Highest population (7.42$\log_{10}$ CFU g^{-1} soil) was observed with *Klebsiella sp.* inoculation at 33kg N ha^{-1} fertilization rate.

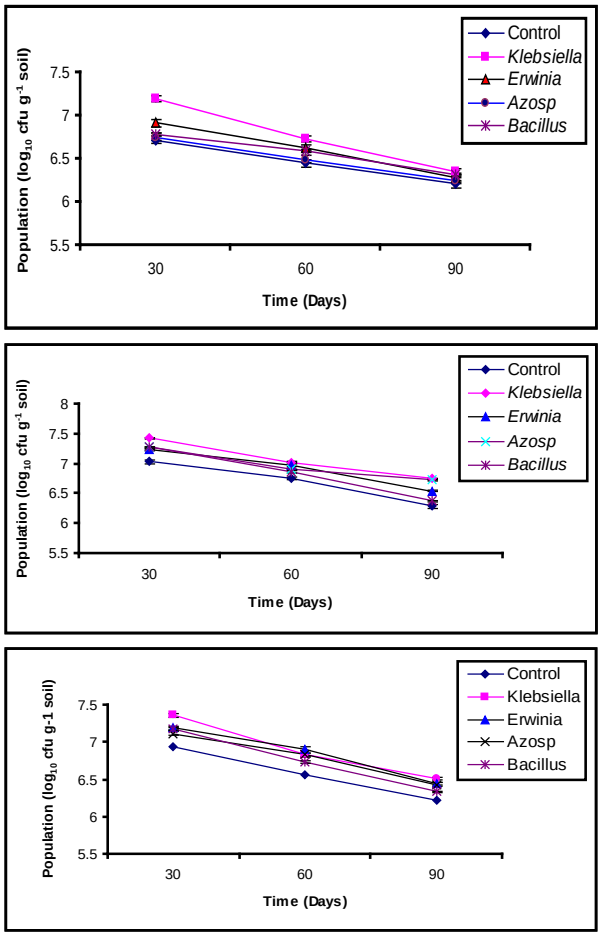

Figure 2. Effect of Rhizobacterial Inoculation and Nitrogen on Soil Bacterial Population at Different Sweet potato Growth Stages; (a) 0 kg Nitrogen, (b) 33 kg Nitrogen and (c) 100 kg Nitrogen.

Table 3. Effect of Rhizobacterial Inoculation and N Fertilization on Nutrient Content of Sweet potato Storage Roots.

Bacterial Treatments	N Isolates	Nutrient Content (mg plant^{-1})				
	Fertilizer (N ha^{-1})	N	P	K	Ca	Mg
Control	0 kg	110.55g	63.87i	623.89i	312.41f	114.91f
	33 kg	304.22c	182.59e	1377.93de	608.67bc	258.52bc
	100 kg	198.67ef	113.76gh	959.60fgh	406.81de	156.07ef
Klebsiella sp.	0 kg	226.74de	132.59fg	1071.41fgh	423.67de	232.74bc
	33 kg	502.89a	310.19a	2329.11a	751.44a	404.11a
	100 kg	447.99b	271.22b	1962.96b	653.72b	394.50a
Erwinia sp.	0 kg	193.01ef	103.50h	1003.56fgh	414.04de	215.02cd
	33 kg	434.60b	215.05cd	1946.49b	639.59bc	403.22a
	100 kg	306.55c	194.44de	1583.35cd	595.70bc	262.37b
Azospirillum sp.	0 kg	152.17fg	104.11h	858.80hi	343.91ef	161.37e
	33 kg	436.28b	196.83de	1815.89bc	602.50bc	265.07b
	100 kg	267.44cd	142.77f	1188.74efg	458.33d	266.93b
Bacillus sp.	0 kg	191.81ef	106.97gh	908.09ghi	380.63def	179.22de
	33 kg	401.08b	226.74c	1889.54b	561.65c	405.06a
	100 kg	257.89cd	143.19f	1234.62ef	451.68d	268.78b
Significance due to PGPR N Fert. PGPR * N Fert		*(0.0001)	*(0.0001)	*(0.0001)	*(0.0001)	*(0.0011)
		*(0.0001)	*(0.0011)	*(0.0021)	*(0.0023)	*(0.0002)
		*(0.0002)	*(0.0021)	*(0.0011)	*(0.0031)	*(0.0022)

Note: NS: non significance, and *: significant difference at (P<0.05). Means the values followed with same letter (s) are not significantly different (P>0.05).

4. Discussion

The PGPR inoculation and N fertilization rates significantly influenced sweet potato storage root yield. Plants inoculated with *Klebsiella sp.* and 33 kg N ha^{-1} fertilizer showed highest yield of sweet potato. The increase could be due to the ability of *Klebsiella* sp. to produce high level of IAA that stimulated plant and root growth. Kloepper *et al*., (1989), Martinez-Viveros *et al*., (2010) and Jordan *et al*., (2013) had recommended that rhizobacteria produced plant growth regulators including IAA that may establish a system for direct plant and root growth promotion by rhizobacterial inocula.

Storage root yield increased with PGPR inoculation compared to the uninoculated control without N fertilizer. This indicated that inoculation and N fertilizer produced a synergistic effect on plant growth and storage root yield. Chela *et al.,* (1993), Saad *et al*., (1999) and Vosawai*et al*., (2015) observed that the use of nitrogen in combinations with PGPR produced significantly higher plant growth and yield than those from fertilization alone under field condition. Also, Helaly *et al*, (2009) found that effect of bio-and mineral fertilizers on yield and tuber quality of potato plants.

PGPR inoculation and N fertilization positively influenced the uptake of nutrients into the storage root. The increase in plant growth could be attributed to the increased uptake of plant nutrients as shown by the higher uptake of N, P, K in storage root of *Klebsiella* inoculated plants. Morphological and physiological changes of plant and enhancement of nutrient content were significantly increased by growth promoting effects of PGPR (Amir *et al.*, 2005; Mia *et al.* 2010; Noor *et al*., 2013). IAA producing PGPR are believed to increase root growth and root length, resulting in greater root surface area which enables the plant to access more nutrients from the soil (Vessey, 2003; Chaiharn, 2011). Nitrogen (N) was considered to be an important factor in determining the production and nutrient composition of root tubers. N may often limit the plant growth and yield among the mineral nutrient elements. For plant development and advancement, N is the most essential mineral nutrient. Therefore, the appropriate management is necessary in an intensive agriculture for plant production and nutrient composition of root tubers (Loebenstein, 2009; Bajshya *et. al*., 2013). The starch and crude protein components of sweet potato appeared to increase by application of Nitrogen. Genotypic and environmental variations are some of the factors that impact the reaction of sweet potato towards Nitrogen fertilizer application (Zhang *et. al*, 2009; Kareem, 2013).

Five sweet potato varieties and four stages of nitrogen fertilizer (N) had been assessed in UPM to examine the yield and nutrient composition of these varieties at estimated optimum N.

Earlier reports have shown that application of nitrogen fertilizer can increase crude protein and starch content in tuber crops. (Ozturk *et al*., 2010; Biruk *et al*., 2014; Vosawai *et al*., 2015).

During plant growth, microbial population density in soil near plant roots was significantly influenced by PGPR inoculation and N fertilizer application. In general, total bacterial population was high at 30 days after planting and reduced with plant age. In the present study, treatment with *Klebsiella* inoculation at 33 kg N ha^{-1} fertilization rate had the highest population of total bacteria in the soil. The inoculated rhizobacteria probably induced the plant growth hormones and other metabolites that encouraged proliferation of other indigenous bacteria. *Klebsiella* inoculation may probably been enhanced root's growth and increased the secretion of root exudates. Kennedy, (1997) and Xing *et al*., (2014) suggested that root exudates contain sugars, amino acids, vitamins, tannins, alkaloids, phosphatides and other unidentified substances. The root exudate sugars give readily available sources of carbon

and energy for the bacterial community in soil. The application of N fertilizer in the soil gives the nitrogen source for bacteria, which improved development.

Liljeroth *et al.,* (1990) and Bashan *et al.,* (2014) observed a significant effect of nitrogen fertilization and PGPR inoculation on microbial populations in rhizosphere soil of barley plants. The decreased population at 60 and 90 days of plant growth could be due to competitions for nutrients and space among the inoculants and other indigenous bacteria in soil. The rhizobacteria probably compete for carbon and energy sources and colonizing space in the rhizosphere.

5. Conclusion

Based on the results obtained in present study, it might be concluded that the application of PGPR and Nitrogen fertilizer improved the yield, microbial population, and also increased the available nutrients (N,P,K) content and quality properties in sweet potato under field condition. Application of 33 kg N ha-1 generally increased yield but at higher application of 100 kg N ha-1 yield was decreased. Field inoculation of *Klebsiella* with 33 kg N ha-1 improved storage root yield which was significantly superior to other treatments, but this treatment reduced the Nitrogen fertilizer rate. Hence, *Klebsiella* has great prospects to be used as biofertilizer for sweet potato production and to sustain soil health under field condition.

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