Journal of Oil Palm Research Vol. 25 (1) April 2013 p. 100-107

INFLUENCE OF INDOLE-3-ACETIC ACID (IAA) PRODUCED BY DIAZOTROPHIC BACTERIA ON ROOT DEVELOPMENT AND GROWTH OF *in vitro* OIL PALM SHOOTS (*Elaeis guineensis* Jacq.)

NOOR Al'SHAH, O*; THAREK, M*; KEYEO, F*; CHAN LAI KENG*; ZAMZURI, I; AHMAD RAMLI, M Y* and AMIR, H G***

ABSTRACT

The interaction between plant and diazotrophs would influence the synthesis of phytohormone [indole-3-acetic acid (IAA)]. Generally, IAA excreted by the bacteria would increase top and root biomass and lateral root numbers of the host plants. Thus, the objectives of this study were to estimate IAA productivity of free-living diazotrophs and to observe the effects of IAA produced on root development and shoot growth of in vitro *oil palm plantlets. Four diazotrophic bacteria used in the study,* Herbaspirillum seropedicae *(Z78),* Microbacterium *sp. (E7),* Acetobacter *sp. (E9) and* Microbacterium *sp. (E14) successfully produced IAA under free-living conditions. These diazotrophs exhibited optimum productivity of IAA during the log growth phase. Isolates of* Microbacterium *sp. E7 and E14 produced the higher concentration of IAA and also higher overall productivity of IAA compared to the other isolates tested. In order to observe plant growth-promoting effects of the phytohormones produced by the diazotroph, all the tested diazotrophs were inoculated onto the* in vitro *(tissue-cultured) oil palm shoots (*Elaeis guineensis *Jacq.). In associative conditions, inoculation of Z78 showed a significant increment in number of secondary roots, fresh weight, and protein content of oil palm shoots compared to those receiving control treatments. Significant responses of isolates E7 and E14 were also observed in the initiation of secondary roots, protein content and the increment of shoot fresh weight. This study concluded that IAA produced by Z78, E7 and E14 could contribute to enhanced growth and development of* in vitro *oil palm shoots and can be further developed into a potential biofertiliser.*

Keywords: indole-3-acetic acid, plant growth, *in vitro*, tissue-cultured oil palm shoot.

Date received: 9 February 2012; **Sent for revision:** 23 April 2012; **Received in final form:** 15 February 2013; **Accepted:** 18 February 2013.

INTRODUCTION

Diazotrophic plant growth-promoting rhizobacteria (PGPR) have the capabilities to stimulate plant growth by fixation of N_2, solubilisation of minerals and production of phytohormones (Bashan and de Bashan, 2005). The diazotrophs were reported to produce phytohormones such as indole-3-acetic acid (IAA) which is important in promoting plant growth (Arshad and Frenkenberger, 1991; Bashan *et al.,* 2004). IAA is one of the common physiologically active auxins and it is a common product of L-tryptophan metabolism produced by PGPR which may result in pronounced effects on plant growth. Among the PGPR species, *Azospirillum*

* School of Biological Sciences,
Universiti Sains Malaysia, 11800 Minden,
Pulau Pinang, Malaysia.
E-mail: amirhg@usm.my

** Malaysian Palm Oil Board,
P. O. Box 10620,
50720 Kuala Lumpur, Malaysia.

is well-known for its ability to excrete auxins. The phytohormone causes morphological and physiological changes to the inoculated plant roots, which leads to plant-bacteria interaction (Costacurta and Vanderleyden, 1995; Tien *et al*., 1979; Harari *et al.,* 1988; Pedraza, 2008; Malhotra and Srivastava, 2009). The modification, in turn, can affect root growth, leading to better nutrient and water absorption by the plant hosts (Bashan *et al.,* 2008). It may result in bigger and more branched roots, thus providing a larger root surface area by which the host plant can access more nutrients (Vessey, 2003). The diazotrophs offer interesting perspectives for alternative fertilisation approaches which significantly influence plant growth (Baldani *et al*., 1983; Ladha and Reddy, 2000). It has been successfully applied and tested on rice, sugar-cane and oil palm seedling (Elbeltagy *et al.,* 2001; Amir *et al*., 2001; 2003; Muthukumarasamy *et al.,* 2006; Azlin *et al.,* 2007; 2009; Noor Ai'shah *et al.,* 2010; Keyeo *et al.,* 2011). Thus, the objectives of this study were to estimate IAA productivity of free-living diazotrophs and to observe the effects of IAA produced on root development and shoot growth of *in vitro* oil palm plantlets.

MATERIALS AND METHODS

Bacterial Isolates

Three locally-isolated diazotrophic rhizobacteria were tested in this study. These bacteria [*Microbacterium* sp. (E7), *Acetobacter* sp. (E9) and *Microbacterium* sp. (E14)] were isolated from root tissues of oil palm planted under field conditions (Azlin *et al.,* 2005). Identification was done via 16S rDNA sequence analyses. *Herbaspirillum seropedicae* (Z78) (ATCC 35893) was used as a positive control, whereas killed Z78 was the negative control.

The Production of Indole-3-Acetic Acid and Viability of Free-living Diazotrophs

The IAA production by free-living diazotrophs (Z78, E7, E9 and E14) was assayed using high performance liquid chromatography (HPLC) system. The bacteria were cultured in a 250 ml conical flask containing 100 ml N-free liquid medium supplemented with 0.5 g litre^{-1} L-tryptophan as a physiological precursor for biosynthesis of IAA in plants and microbes (Glickmann and Dessaux, 1995). The bacterial cultures were shaken at 160 rpm in room temperature and were harvested at every 12 hr intervals for the production of IAA and the viable cell count (cfu ml^{-1}) until 96 hr of growth. The experiment was laid out in a randomised complete block design (RCBD) with triplicates for each isolate at each harvesting time.

Bacterial cells were separated by filtration through a membrane filter (Whatman ™, 0.20 µm). The filtrate was acidified to pH 2.8 and reconstituted in 1.0 ml acetonitrile (Asghar *et al*., 2002; Patten and Glick, 2002). A total of 20 µl of filtrates were subjected to the HPLC system equipped with UV-VIS variable-wavelength detector (SPD-10 A*VP* Shimadzu) and two Shimadzu LC-10 AT*VP* reciprocating pumps. The analysis was carried out in a low-gradient condition with C-18 reverse phase HPLC column, Supelco (LC 18-DB, 3.3 cm x 4.6 mm ID x 3 µm particle size) at ambient temperature. The analysis was conducted using 1% (v/v) acetic acid adjusted to pH 2.8 as solvent A and 100% acetonitrile as solvent B. The gradient programme for solvent B was adjusted from 13% to 20% and from 20% to 35% within 1.25 min and 5 min, respectively. The run time was 5 min at a flow rate of 1.0 ml min^{-1} with a wavelength of 280 nm. The peak retention time and areas were compared with the IAA standard curve.

The standard curve was prepared from serially diluted 98 µg ml^{-1} (ppm) of 98% purity (Merck) IAA in the range of 0 to 16 µg ml^{-1}. A total of 0.01 g IAA was dissolved in 95% ethanol and the volume was made up to 100 ml with deionised water. The standard solution (20 µl) was injected into the HPLC column. The retention time and peak area for each concentration were recorded (Asghar *et al*., 2002; Patten and Glick, 2002).

Growth of *in vitro* Oil Palm Shoots Inoculated with Diazotrophs

Tissue-cultured oil palm shoots (*Elaeis guineensis* Jacq.) of the clone S69 L436-1/26 T-1/10 were obtained from the Tissue Culture Laboratory of the Malaysian Palm Oil Board (MPOB), Bangi, Selangor. The shoots were individually transferred aseptically into the shoot development medium. Shoots of more than 5 cm in height and at two to three leaf-stages were considered ready for root initiation in the rooting medium supplemented with plant hormone. Selected *in vitro* oil palm shoots with vigorous growth were transferred into N-free MS liquid medium for root induction stage. The inoculation treatments were: 1) + *Herbaspirillum seropedicae* (Z78), 2) + *Microbacterium* sp. (E7), 3) + *Acetobacter* sp. (E9), 4) + *Microbacterium* sp. (E14), 5) + Z78 killed (Z78K) and 6) N-enriched MS medium [+ 1.90 mg litre^{-1} KNO_3, + 1.65 mg litre^{-1} NH_4NO_3, + 16.7 mg litre^{-1} NAA (naphtalene acetic acid)]. The inoculated shoots were monitored for growth and root initiation after 90 days of growth (D_{90}). Shoot height, number of new shoot formation, number of secondary roots initiated, shoot fresh weight and shoot protein content were determined and recorded. The experiment was laid out in a RCBD

with 20 replications for each treatment.

The statistical analysis software (SPSS V.15) was used for data analysis. Analysis of variance (ANOVA) and generalised linear model (GLM) univariate ANOVA were performed to test the significance of differences between means using the least significant difference (LSD) test, $P < 0.05$.

RESULTS AND DISCUSSION

IAA Production and Viability of Free-living Diazotrophs

In this study, the results revealed that all isolates tested were capable of producing IAA (*Figure 1a*). This finding is in line with other studies by Vessey (2003), Bashan *et al*. (2004) and Gravel *et al*. (2007), which reported that diazotrophic bacteria were not only able to fix atmospheric nitrogen but were also capable of producing plant growth regulators such as auxins. IAA is one of the most studied natural auxins, which was reported to be able to stimulate root growth and thus, consequently increases access to more nutrient uptake from the soil.

As shown in *Figure 1a,* isolate Z78, E7, E9 and E14 produced IAA in concentrations ranging from 0.2 to 11.5 µg ml^{-1} within 96 hr of growth incubation. The productivity started to increase rapidly during the log phase growth stages of the isolates tested (*Figures 1a* and *1b*). During this phase, the cells were very well adapted to their environment and were rapidly multiplying. Furthermore, this is a period of balanced bacterial cell growth, in which all components of a cell grow at the same rate (Shuler and Kargi, 2002). This is in agreement with El-Khawas and Adachi (1999), who reported that *Azospirillum brasilense* produced more IAA during the log (exponential) growth stages from 48 hr until 96 hr of incubation. Results have recorded the highest concentration of IAA produced at 11.542 µg ml^{-1} (0.318 µg ml^{-1} hr^{-1} of IAA production rates) after 60 hr of bacterial growth (*Figure 1a, b; Table 1*). In addition, optimal IAA productivity of isolate E7 also propelled the highest maximum overall IAA productivity of 0.185 µg ml^{-1} hr^{-1} (*Table 1*). Similarly, maximum IAA productivity of Z78 was recorded at 0.261 µg ml^{-1} hr^{-1} (10.058 µg ml^{-1} of IAA produced) after 60 hr of incubation (*Table 1*). Subsequently, the IAA was consistantly produced at 72 hr, 84 hr and 96 hr in concentrations ranging from 10.746 to 11.057 µg ml^{-1}. The overall IAA productivity of Z78 was 0.165 µg ml^{-1} hr^{-1}, while the viable cell count of Z78 increased gradually and was stable until the end of the incubation period (96 hr) at 1.05 x 10^{13} cfu ml^{-1} (*Figure 1b*). Whereas, IAA production of other isolates declined, while reflecting the decline in viable cell numbers. The results exhibited that higher viable cell numbers of Z78, E7 and E14 (which was up to 10^{10} cfu ml^{-1}) influenced the production of optimum IAA at 84 hr, 60 hr and 48 hr of incubation, respectively (*Figure 1b*).

Similar studies by Baca and Elmerich (2007) and Bashan *et al*. (2008), found that the amount of IAA obtained varied based on the species and strain as well as on the conditions of their cultivation, including the presence of tryptophan, oxygenation level, pH and growth phase. Similarly, our results also exhibited that the addition of 0.5 g ml^{-1} L-tryptophan into the culture medium stimulated the excretion of the IAA, which implicated the role of exogenous tryptophan as a precursor of the IAA, and supports the existence of a tryptophan dependent route for IAA biosynthesis. Such routes are known to induce multiple enzymatic pathways in both plants and bacteria (Pedraza *et al.*, 2004; Baca and Elmerich, 2007).

Overall, amongst the isolates tested, E14 showed high IAA productivity at 0.432 µg ml^{-1} hr^{-1}, during the mid-exponential growth phase (48-60 h). Concentration of IAA recorded at the maximum productivity rate was 9.066 µg ml^{-1} (*Table 1*), whereas, the overall productivity was 0.180 µg ml^{-1} hr^{-1} which was higher compared to E9 and Z78 (*Table 1*). However, IAA productivity by isolate E9 was much lower even at the maximum cell density (2.55 x 10^{11} cfu ml^{-1}). The IAA productivity for E9 peaked slightly at 36 hr and gradually declined thereafter until 96 hr of incubation, giving a low

TABLE 1. PRODUCTION OF INDOLE-3-ACETIC ACID (IAA) BY FREE-LIVING DIAZOTROPH

Isolate	Maximum productivity (µg ml^{-1} hr^{-1})	Concentration at maximum productivity (µg ml^{-1})	Overall productivity (µg ml^{-1} hr^{-1})
Z78 (*Herbaspirillum seropedicae*)	0.261c	10.058c	0.165c
E7 (*Microbacterium* sp.)	0.318c	11.542cd	0.185c
E9 (*Acetobacter* sp.)	0.117b	2.295b	0.059b
E14 (*Microbacterium* sp.)	0.432c	9.066c	0.180c
Z78K (Control)	0a	0.066a	0a

Note: Means in the same column followed by the same letter do not significantly different at $P < 0.05$.

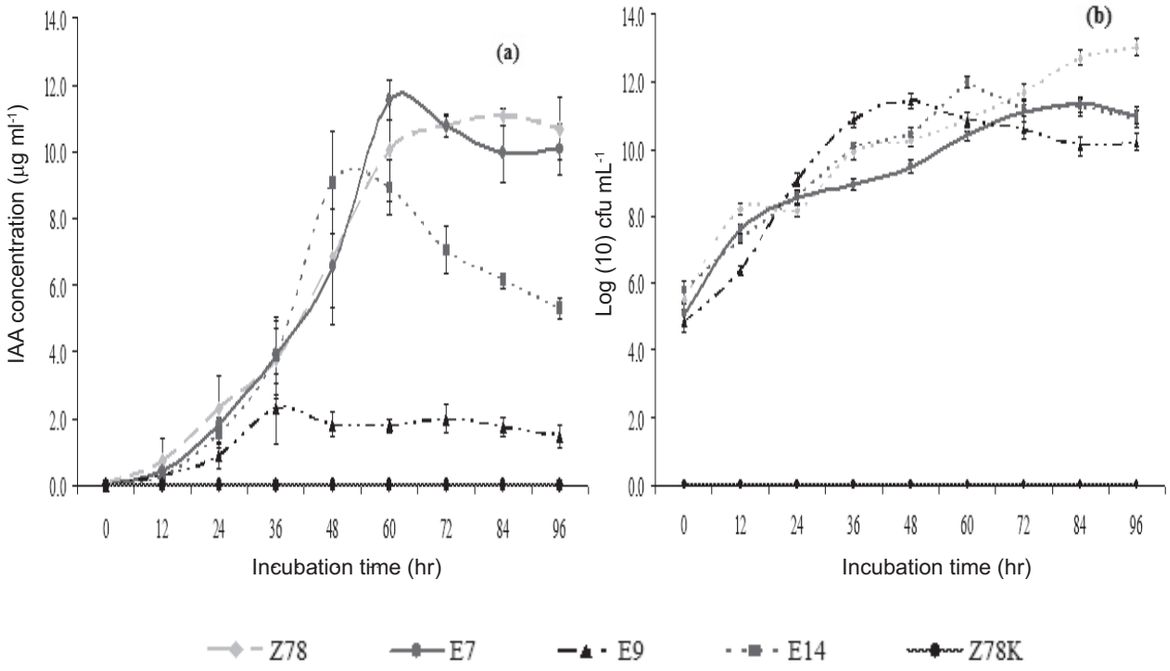

Figure 1. (a) Productivity of indole-3-acetic acid (IAA) by free-living Z78, E7, E9 and E14 and (b) viable cell count of diazotrophs grown in N-free medium supplemented with L-tryptophan during 0 to 96 hr of incubation.

overall productivity of 0.059 µg ml^{-1} hr^{-1} (*Table 1*). In contrast, very low IAA concentration was detected in the bacterial supernatant of control (+Z78K) which ranged from 0.8 x 1 0^{-2} to 6.6 x 10^{-2}.

Effects of Diazotrophs on Growth of *In vitro* Oil Palm Shoots

Plant-bacteria association is mostly found in the rhizosphere, which provides a competitive and *dynamic habitat*. Many factors affect the structure and population of microbial communities on the dynamic environment such as high carbon and nitrogen and less competitors. An association of endophytic diazotrophs with the host plants triggers unique features compared to other nitrogen-fixing associations. In this study, inoculation of Z78, E7 and E14 have shown potential substitution for fertilisers and plant hormones that are essential in promoting plant growth.

In general, the inoculation treatments showed significant increment on percentage in shoot height and fresh weight compared to both control treatments. At D_{90}, the maximum increment percentage in height and fresh weight occurred for oil palm shoots treated with isolate Z78 (52.8% and 36.5% of increment, respectively). This corresponds to 48% and 22% increment in shoot height and fresh weight over Control 1 (+N, +NAA) and Control 2 (+Z78K), respectively. The Z78 also showed the best response on the number of new leaves formation. This was probably because *Herbaspirillum seropedicae* is a facultative endophytic bacterium that is known to promote growth and yield of the host plants due to N_2 fixation and phytohormone production activities. However, no significant differences were recorded between controls (Control 1 and 2) and inoculated treatments (E7, E9 and E14) for two factors observed which was percentage increment of height and also number of new shoot formation (*Table 2*).

The inoculation effect on the development of the root system, such as on root length, and volume, have been frequently observed. The development of root systems may promote water absorption and mineral uptake (Dobereiner *et al*., 1993; Bastian *et al*., 1998). Among the proposed mechanisms for the increase in root development, is the bacterial production of phytohormonal substances such as cytokinins, auxins and gibberellins (GA). A quantitative analysis performed previously by capillary gas chromatography-mass spectrometry also reported that *H. seropedicae* produced 7.0 ng ml^{-1} and 12.5 ng ml^{-1} of IAA and GA_3, respectively (Bastian *et al*., 1998). This shows that both GA_3 and IAA are produced by *H. seropedicae* and further shows the importance of studying phytohormonal production, when inter-relationships between plants and endophytic microorganisms are analysed. These results may also explain, in part, the beneficial effects of endophytic bacteria on the host plant, as demonstrated in this present study.

TABLE 2. GROWTH OBSERVATIONS OF *In vitro* OIL PALM SHOOTS INOCULATED WITH DIAZOTROPH AT D_{90}

Treatment	No. of secondary roots	Shoot protein content	Percent of increment (%)		
			Shoot height	Shoot fresh weight	New shoot formation
Control 1 (+N,+NAA) [(+ 1.90 *mg litre*$^{-1}$ KNO_3, + 1.65 *mg litre*$^{-1}$ NH_4NO_3, + 16.7 *mg litre*$^{-1}$ *NAA* (*Naphtalene acetic acid*)]	2a	17.2c	35.5ab	28.5bc	100a
Z78 (*Herbaspirillum seropedicae*)	4c	23.6de	52.8bcd	36.5d	150b
E7 (*Microbacterium* sp.)	4c	19.6c	51.8bcd	29.0bc	150b
E9 (*Acetobacter* sp.)	2a	13.1b	40.3abc	29.0bc	150b
E14 (*Microbacterium* sp.)	3b	22.8d	46.3bc	30.3c	150ab
Control 2 (+Z78K)	3b	10.3a	34.2ab	23.5a	100ab

Note: Means in the same column followed by the same letter do not significantly different at $P < 0.05$.

The significance of inoculation (+Z78, +E7) response was also observed in the initiation of secondary roots. The results of the treatments (Z78 and E7) were significantly different compared to E9 and E14. More secondary root formation was recorded in the host plants inoculated with Z78 and E7, followed by E14 and Control 2 at D_{90} (*Table 2*). However, lesser number of secondary roots were initiated in the host plants inoculated with E9 and Control 1 at D_{90}. As reported by Tsimilli-Michael *et al.* (2000), the diazotrophs were important for the establishment and growth of the host plants. Inoculation with Z78, E7 and E14 had promoted better growth performance of the host plants at D_{90}. In addition, shoots inoculated with Z78 and E7 showed significant influence on the development of secondary roots compared to E14 and Z78K (control 2) (*Table 2*). This indicated that the endophytic bacteria Z78 (*H. seropedicae*) and E7 (*Microbacterium* sp.), induced better rooting for *in vitro* oil palm shoots. This may be ascertained by the exogenous IAA production by the inocula which altered and improved root growth of host plants (*Tables 1* and *2*). As noted previously, the ability to produce phytohormone as a regulator of growth and development in plants is an important trait of diazotrophic bacteria. This regulatory ability includes modification of root morphology, such as an increase in length and in root branching and density of root hairs and root surface area (Tien *et al.*, 1979; Baca and Elmerich, 2007). This is consistent with the results in the present study where the inoculated roots were bigger in size, while the surface areas were wider than the controls supplied with NH_4NO_3 and KNO_3 as N source and NAA synthetic auxin (Control 1). Consequently, a higher root surface area and a higher number of secondary roots led to enhanced water and mineral uptake efficiency.

Similar beneficial effects were observed in another study on *Herbaspirillum* sp. inoculation on rice and sugar-cane under axenic conditions. These beneficial effects on plants are attributed mainly to an improvement in root development and consequent increase in the rate of water and mineral uptake by roots (Njoloma *et al.*, 2006; Zakria *et al.*, 2007). Better plant growth was observed with increments in fresh weight, height and number of shoot formation of oil palm shoots inoculated with Z78, E7 and E14 at D_{90}. These effects may be attributed to IAA production by the bacteria. These diazotrophs would have affected plant growth through phytohormone synthesis and later through improved nutrient uptake. This result is in agreement with the previous study, which revealed that *in vitro* oil palm plantlets inoculated with *A. brasilense* produces high shoot and root biomass compared to the control treatment supplemented with NAA and inoculated with killed Sp7 (Azlin, 2007). *Azospirillum* sp. is also well-known for its ability to produce plant hormones *in vitro*, mainly IAA and possibly other plant hormones (Azlin *et al.*, 2007).

Similar beneficial effects of PGPR inoculants on *in vitro* plantlets have been noted in potatoes, which have shown significant increases in biomass and the production of more top and root growth than those of non-inoculated plants, owing to sufficient auxin production by the inocula (Bensalim *et al.*, 1998). IAA excretion has been similarly shown to improve plant growth in other micropropagated crops, such as sugar-cane inoculated by *G. diazotrophicus* and *B. vietnamiensis* (Govindarajan *et al.*, 2006). This strong influence of bacterial colonisation on root development via excretion of IAA, gibberellins and other phytohormones, is well documented (Steenhoudt and Vanderleyden, 2000; Martinez-Morales *et al.*, 2003; Mantelin and Tourine, 2004). In addition, better growth of inoculated shoots particularly Z78, E7 and E14 were observed apart from influencing the root development. This might be due to an effective

colonisation of bacterial inocula which had changed root morphology and induced more secondary roots (*Table 2*). Phytohormone production has been cited as the main factor of growth improvement besides N_2-fixing capability of many diazotrophic PGPR strains such as *Azospirillum*, *Herbaspirillum*, *Glucanoacetobacter* and *Burkholderia* (Bashan and Holguin, 1998; Govindarajan *et al.,* 2006; Pedraza, 2008). A positive response of inoculation on the growth of oil palm shoots could also be contributed through the biological nitrogen fixation (BNF) process. The oil palm shoots had increased root initiation (*Table 2*) and fresh weight, when the diazotrophs were used.

From the current study, it was observed that the well developed rooting system of *in vitro* oil palm induced by diazotrophs has influenced the growth of shoots. Stimulation by PGPR has been shown to improve root growth and functions, thus promoting crop yield which leads to an increased uptake of water and minerals (Matiru and Dakora, 2004; Kennedy *et al.,* 2004; Bashan *et al.,* 2008). This evidence can be used to explain the result of total shoot protein content obtained in this experiment *(Table 2).* Higher protein concentration was recorded in plant tissues of oil palm shoots inoculated with Z78 and E14. This may be related to the capacity of phytohormone production by the bacteria which might provoke changes in root morphology after the inoculation. This would later enhance the ability of host plants to absorb more nutrients and subsequently increase the protein content. In other studies, *Azospirillum*-inoculated plants exhibited an enhanced root branching and surface area due to phytohormone production, which in turn, can explain the enhancement of nutrient uptake and water status in both inoculated plants (maize and rice) (Okon and Labendera-Gonzalez, 1994; Tilak *et al.,* 2005; Chi *et al.,* 2005). Shoots inoculated with Z78 showed the highest shoot protein content (23.6 mg BSA ml^{-1} protein) followed by E14, E7 and Control 1 (*Table 2*). In overall, the inoculation treatments, particularly Z78, E7 and E14, successfully improved certain plant growth parameters in this study.

CONCLUSION

The potential of selected diazotrophs (Z78, E7, E9 and E14) in producing phytohormones under free-living conditions was successfully observed. The diazotrophs, Z78 and local isolates E7 and E14 successfully influenced root development of *in vitro* oil palm shoots (clone S69 L436-1/26 T-1/10) at D_{90}. This may be partly due to the excretion of growth hormones by the tested inocula. Inoculation of E7 and Z78 also revealed positive influences on growth of oil palm shoots (height, fresh weight, new shoot formation and total shoot protein content) compared to the controls (+N, +NAA and +Z78K). The study concluded that phytohormone (IAA) produced by *Herbaspirillum* sp. (Z78) and locally-isolated E7 and E14 could in part enhance growth and development of *in vitro* oil palm shoots and may be further developed into a potential biofertiliser.

ACKNOWLEDGEMENT

We thank the School of Biological Sciences, Universiti Sains Malaysia, Pulau Pinang, and the Ministry of Science, Technology and Innovation for the research funding.

REFERENCES

AMIR, H G; SHAMSUDDIN, Z H; HALIMI, M S; RAMLAN, M F and MARZIAH, M (2001). Effects of *Azospirillum* inoculation on N_2 fixation and growth of oil palm plantlets at nursery stage. *J. Palm Oil Res. Vol. 13*: 42-49.

ARSHAD, M and FRENKENBERGER, W T (1991). Microbial production of plant hormones. *Plant and Soil Vol. 133:* 1-8.

ASGHAR, H N; ZAHIR, Z A; ARSHAD, M and KHALIQ, A (2002). Relationship between *in vitro* production of auxins by rhizobacteria and their growth-promoting activities in *Brassica jencea*. *Letter Biology Fertility Soil Vol. 35*: 231-237.

AZLIN, C O; AMIR, H G and CHAN, L K (2005). Isolation and characterisation of diazotrophic rhizobacteria of oil palm roots. *Malaysian Journal of Microbiology Vol. 1:* 31-35.

AZLIN, C O; AMIR, H G; CHAN, L K and ZAMZURI, I (2007). Effect of plant growth promoting rhizobacteria on root formation and growth of tissue cultured oil palm (*Elaeis guineensis* Jacq.). *Biotechnology Vol. 6*: 549-554.

AZLIN, C O; AMIR, H G; CHAN LAI KENG and ZAMZURI, I (2009). Microbial inoculation improves growth of oil palm plants (*Elaeis guineensis* Jacq.). *Tropical Life Sciences Vol. 20*: 71-77.

BACA, B E and ELMERICH, C (2007). Microbial production of plant hormones. *Associative and Endophytic Nitrogen-fixing Bacteria and Cyanobacterial Associations* (Elmerich, C and Newton, W eds.). Springer, Dordrecht, The Netherlands. p. 113-143.

BALDANI, V L D; BALDANI, J I and DOBEREINER, J (1983). Effects of *Azospirillum* inoculation on the root infection and nitrogen incorporation in wheat. *Canadian Journal of Microbiology Vol. 29*: 433-439.

BASHAN, L E; ANTOUN, H and BASHAN, Y (2008). Involvement of indole-3-acetic acid produced by the growth-promoting bacterium *Azospirillum* spp. in promoting growth of *Chlorella vulgaris*. *J. Physiology Vol. 44*: 938-947.

BASHAN, L E; HERNANDEZ, J P; MOREY, T and BASHAN, Y (2004). Microalgae growth-promoting bacteria as 'helpers' for microalgae: a novel approach for removing ammonium and phosphorus from municipal waste water. *Water Research Vol. 38*: 466-74.

BASHAN, Y and DE BASHAN, L E (2005). Bacteria: plant growth-promoting soil. *Encyclopedia of Soils in the Environment* (Hillel, D ed.). Elsevier, Oxford, UK. p. 103-115.

BASHAN, Y and HOLGUIN, G (1998). Proposal for the division of plant growth promoting rhizobacteria into two classifications: biocontrol-PGPB (plant growth promoting bacteria) and PGPB. *Soil Biology Biochemistry Vol. 30*: 1225-1228.

BASTIAN, F; COHEN, A; PICCOLI, P; LUNA, V; BARALDI, R and BOTTINI, R (1998). Production of indole-3-acetic acid and gibberellins A1 and A3 by *Acetobacter diazotrophicus* and *Herbaspirillum seropedicae* in chemically-defined culture media. *Plant Growth Regulation Vol. 24*: 7-11.

BENSALIM, S; NOWAK, J and ASIEDU, S K (1998). Temperature and pseudomonad bacterium effects on *in vitro* and *ex vitro* performance of 18 clones of potato. *Am J Potato Res Vol. 75*: 145-152.

CHI, F; SHEN, S H; CHENG, H P; JING, Y X; YANNI, Y G and DAZZO, B D (2005). Ascending migration of endophytic rhizobia, from roots to leaves, inside rice plants and assessment of benefit to rice growth physiology. *Applied Environmental Microbiology Vol. 71*: 7271-7278.

COSTACURTA, A and VANDERLEYDEN, J (1995). Synthesis of phytohormones by plant associated bacteria. *Critical Review in Microbiology Vol. 21*: 1-18.

DOBEREINER, J; REIS, V M; PAULA, M A and OLIVARES, F (1993). *New Horizons in Nitrogen Fixation.* Kluwer Academic Publishers.

ELBELTAGY, A; NISHIOKA, K; SATO, T; SUZUKI, H; YE, B; HAMADA, T; ISAWA, T; MITSUI, H and MINAMISAWA, K (2001). Endophytic colonisation and *in planta* nitrogen fixation by *Herbaspirillum* sp. isolated from wild rice species. *Applied and Environmental Microbiology Vol. 67*: 5285-5293.

EL-KHAWAS, H and ADACHI, K (1999). Identification and quantification of auxins in culture media of *Azospirillum* and *Klebsiella* and their effect on rice roots. *Biology and Fertility of Soils Vol. 28*: 377-381.

GLICKMANN, E and DESSAUX, Y (1995). A critical examination of the specific of the Salkowski reagent for indolic compounds produced by phytopathogenic bacteria. *Applied and Environmental Microbiology Vol. 61*: 793-796.

GOVINDARAJAN, M; BALANDREAU, J; MUTHUKUMARASAMY, R; REVATHI, G and LAKSHIMINARASIMHAN, C (2006). Improved yield of micropropagated sugarcane following inoculation by endophytic *Burkholderia vietanamiensis*. *Plant and Soil Vol. 280*: 239-252.

GRAVEL, V; ANTOUN, H and TWEDDELL, R J (2007). Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: possible role of indole-3-acetic acid (IAA). *Soil Biology and Biochemistry Vol. 39*: 968-1977.

HARARI, A; KIGEL, J and OKON, Y (1988). Involvement of IAA in the interaction between *Azospirillum brasilense* and *Panicum miliaceum* roots. *Plant and Soil Vol. 110*: 275-282.

KENNEDY, I R; CHOUDHURY, A T M A and KECSKES, M L (2004). Non-symbiotic bacterial diazotrophs in crop-farming systems: can their potential for plant growth promotion be better exploited? *Soil Biology and Biochemistry Vol. 36*: 1229-1244.

KEYEO, F; NOOR AI'SHAH, O and AMIR, H G (2011). The effects of nitrogen fixation activity and phytohormone production of diazotroph in promoting growth of rice seedlings. *Biotechnology Vol. 10*: 267-273.

LADHA, J K and REDDY, P M (2000). *The Quest for Nitrogen Fixation in Rice*. International Rice Research Institute. Makati City, Philippines. p. 1-354.

MALHOTRA, M and SRIVASTAVA, S (2009). Stress-responsive indole-3-acetic acid biosynthesis by *Azospirillum brasilense* SM and its ability to modulate plant growth. *European Journal of Soil Biology Vol. 45*: 73-80.

MANTELIN, S and TOURAINE, B (2004). Plant growth promoting rhizobacteria and nitrate availability: impacts on root development and nitrate uptake. *J. Experimental Botany Vol. 55*: 27-34.

MARTINEZ-MORALES, L J; SOTO URZA, L; BACA, B E and SANCHEZ-AHEDO, J O (2003). Indole-3-butyric acid (IBA) production in culture medium by wild strain *Azospirillum bransilense. FEMS Microbiology Letters Vol. 228*: 167-173.

MATIRU, V N and DAKORA, F D (2004). Potential use of rhizobial bacteria as promoters of plant growth for increased yield in landraces of African cereal crops. *African Journal of Biotechnology Vol. 3*: 1-7.

MUTHUKUMARASAMY, R; GOVINDARAJAN, M; VADIVELU, M and REVATHY, G (2006). N-fertiliser saving by the inoculation of *Gluconacetobacter diazotrophicus* and *Herbaspirillum* sp. in micropropagated sugar-cane plants. *Microbiology Research Vol. 161*: 238-245.

NJOLOMA, J; TANAKA, K; SHIMIZU, T; NISHIGUCHI, T; ZAKRIA, M; AKASHI, R; OOTA, M and AKAO, O (2006). Infection and colonisation of aseptically micropropagated sugar-cane seedlings by nitrogen-fixing endophytic bacterium, *Herbaspirillum* sp. B501*gfp*1. *Biology and Fertility of Soil Vol. 43*: 137-143.

NOOR AI'SHAH, O; AMIR, H G; CHAN LAI KENG and OTHMAN, A R (2010). Influence of various combinations of diazotrophs and chemical N fertiliser on plant growth and N_2 fixation capacity of oil palm seedlings (*Elaeis guineensis* Jacq.). *Thai Journal of Agricultural Science Vol. 42*: 139-149.

OKON, Y and LABENDERA-GONZALEZ, C A (1994). Agronomic applications of *Azospirillum*: an evaluation of 20 years worldwide field inoculation. *Soil Biology and Biochemistry Vol. 26*: 1591-1601.

PATTEN, C L and GLICK, B R (2002). Role of *Pseudomonas putida* indole acetic acid in development of the host plant root systems. *Applied and Environmental Microbiology Vol. 68*: 3795-3801.

PEDRAZA, R O; RAMIREZ, A; XIQUI, M L and BACA, B E (2004). Aromatic amino acid amino transferase activity and indole-3-acetic acid production by associative nitrogen-fixing bacteria. *FEMS Microbiology Letter Vol. 233*: 15-21.

PEDRAZA, R O (2008). Recent advances in nitrogen-fixing acetic acid bacteria. *International Journal of Food Microbiology Vol. 125*: 25-35.

SHULER, M L and KARGI, F (2002). *Bioprocess Engineering: Basic Concepts*. Second edition. Prentice Hall PTR, New Jersey.

STEENHOUDT, O and VANDERLEYDEN, J (2000). *Azospirillum*, a free-living nitrogen-fixing bacterium closely associayed with grasses: genetics, biochemical and ecological aspects. *FEMS Microbiological Reviews Vol. 24*: 487-507.

TSIMILLI-MICHAEL, M; EGGENBERG, P; BORO, B; KOVES-PECHY, K; VOROS, I and STRASSER, R J (2000). Synergystic and antagonistic effects of abuscullar mycorrhizal fungi and *Azospirillum* and *Rhizobium* nitrogen fixer on the photosynthetic activity of alfalfa, probed by polyphasic chrophyll a fluorescene transcients O-J-I-P. *Applied Soil Ecology Vol. 15*: 169-182.

TIEN, T M; GASKINS, M H and HUBBELL, D H (1979). Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.). *Applied Environmental Microbiology Vol. 37*: 1016-1024.

TILAK, K V B R; RAANGANAYAKI, K; PAL, K; DE, R; SAXENA, A K; SHEKAR, N; MITTAL, S; TRIPATHI, A K and JOHRI, B N (2005). Diversity of plant growth and soil health supporting bacteria. *Current Science Vol. 89*: 136-150.

VESSEY, J K (2003). Plant growth promoting rhizobacteria as biofertilisers. *Plant and Soil Vol. 255*: 571-586.

ZAKRIA, M; NJOLOMA, J; SAEKI, Y and AKAO, S (2007). Colonisation and nitrogen-fixing ability of *Herbaspirillum* sp. strain B501 *gfp*1 and assessment of its growth promoting ability in cultivated rice. *Microbes Environments Vol. 3*: 197-206.