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Microbial Inoculation Improves Growth of Oil Palm Plants (*Elaeis guineensis* Jacq.)

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Abstrak: Pengenalan rhizobakteria diazotrofik kepada tisu kelapa sawit ketika proses mikropropagasi secara *in vitro* membolehkan wujud interaksi asosiatif awal di antara sel tumbuhan dan bakteria. Di dalam hubungan asosiatif ini, diazotrof membekalkan tumbuhan perumah dengan fitohormon dan nitrogen terikat. Kajian ini dijalankan untuk menilai pertumbuhan tisu kultur kelapa sawit yang sedia dikolonisasi oleh bakteria (*bacterised*) dalam keadaan *ex vitro* selepas 280 hari pertumbuhan. Berat kering akar, berat kering pucuk, isipadu akar, kolonisasi bakteria dan kandungan protein serta klorofil daun tumbuhan perumah telah diperhatikan. Keputusan menunjukkan bahawa inokula telah berjaya mengkolonisasi akar tumbuhan perumah tersebut. Tumbuhan yang telah diinokulasi dengan *Acetobacter diazotrophicus* (R12) mempunyai lebih berat kering akar dan isipadu berbanding tumbuhan diinokulasi oleh *Azospirillum brasilense* (Sp7). Kandungan protein dan klorofil daun adalah lebih tinggi bagi tumbuhan yang telah diinokulasi (*bacterised*) berbanding tumbuhan Kawalan 2 (diinokulasi dengan Sp7 mati). Keputusan ini mencadangkan bahawa diazotrof tersebut berjaya meningkatkan pertumbuhan perumah (kelapa sawit) dan mengurangkan jumlah baja N yang diperlukan untuk pertumbuhan.

Kata kunci: Tumbuhan Sedia Dikolonisasi oleh Bakteria, Pengikatan Nitrogen, Fitohormon, Penggalak Pertumbuhan Pokok, Kolonisasi Akar, Tisu Kultur Kelapa Sawit

Abstract: Introduction of diazotrophic rhizobacteria to oil palm tissues during the *in vitro* micropropagation process establishes an early associative interaction between the plant cells and bacteria. In the association, the diazotrophs provide the host plants with phytohormones and fixed nitrogen. This study was conducted to observe growth of bacterised tissue cultured oil palm plants under *ex vitro* conditions after 280 days of growth. Root dry weight, shoot dry weight, root volume, bacterial colonisation, leaf protein and chlorophyll content of the host plants were observed. The results revealed that the inocula successfully colonised roots of the host plants. Plants inoculated with *Acetobacter diazotrophicus* (R12) had more root dry weight and volume than plants inoculated with *Azospirillum brasilense* (Sp7). Leaf protein and chlorophyll content were higher in the bacterised plants compared to Control 2 plants (inoculated with killed Sp7). These results suggest that the diazotrophs successfully improved the growth of the host plant (oil palm) and minimised the amount of N fertiliser necessary for growth.

Keywords: Bacterised Plants, Nitrogen Fixation, Phytohormones, Plant Growth Enhancer, Root Colonisation, Tissue Cultured Oil Palm

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INTRODUCTION

The high nutrient demands and high cost of chemical fertilisers (e.g., urea) for oil palm plants have encouraged growers to find less expensive alternatives that facilitate greater nutrient use efficiency. As oil palm plants require large quantities of fertiliser, greater profit could be gained through extensive biofertiliser applications. To meet the nutrient demands of oil palm, the recommended annual application of N fertiliser is 4.2 kg nitrogen (ammonium sulphate) palm^{-1} year^{-1} (Tarmizi & Mohd Tayed 2006). However, use of high levels of chemical fertilisers and plant growth hormones has several drawbacks. High inorganic nitrogen fertiliser usage can contribute to health hazards and environmental pollution (Stoltzfus *et al*. 1997). In addition, high concentrations of IAA input can inhibit the hypersensitive response and may suppress expression of plant defence genes. This leads to problems such as epinasty, tumour formation and plant organ deformation (Maor *et al*. 2004). As many agricultural chemicals are implicated in human toxicity and negative environmental impacts, the use of biofertiliser is becoming increasingly important and appealing to industry.

Biofertiliser use in oil palm could be performed by applying diazotrophic rhizobacteria (biofertiliser) through tissue culture techniques at any stage in oil palm production. Sturz and Nowak (2000) reported that the beneficial diazotrophic rhizobacteria is maintained within the tissues of the host plants after introduction during *in vitro* propagation. The rhizobacteria could be introduced as early as the embryoid and shoot production stages of the plant. Several studies have been performed in which the bacteria were established within tissues of the host plants (Preininger *et al*. 1997; de Mayolo *et al*. 2003; Kumria *et al*. 2001). Early attachment of microbes within the root cells can help achieve full establishment of diazotrophs within the host plants, thus offering protection. Pandey *et al*. (2000) tested for hardening of tissue culture in tea plants and found that inoculants of *Bacillus subtilis*, *Bacillus* sp. and *Pseudomonas corrugata* enhanced survival of the host plants up to 100%, 96% and 88%, respectively, compared to the control plants.

Inoculation of selected diazotrophic rhizobacteria to oil palm tissues during *in vitro* micropropagation would enable early associative interactions between the bacteria and the host plants. These associations would enable better adaptation of the host plants to environmental conditions and a higher survival rate for the host plants (Sturz & Nowak 2000; Vestberg *et al*. 2004; Azlin *et al*. 2007). In addition, the bacterised plants require minimal nitrogen fertiliser, as the diazotroph can fix nitrogen *in situ* (Mantelin & Touraine 2004; Azlin *et al*. 2007). Finally, the diazotrophs produce plant growth hormones that can enhance plant growth and root development (Kefalogianni & Anggelis 2002).

Application of *A. brasilense* Sp7 and locally isolated *A. diazotrophicus* R12 may induce benefits in oil palm development. Thus, the objective of this study was to evaluate growth performance of bacterised tissue culture of oil palm inoculated with *A. brasilense* (Sp7) and locally isolated *A. diazotrophicus* (R12) under glasshouse conditions.

MATERIALS AND METHODS

The experiment was a randomised design with five replicates of bacterised oil palm plants as previously described (Azlin *et al*. 2007). The plant treatments were: i) + nitrogen fertiliser (urea) (Control 1), ii) + *A. brasilense* (Sp7) (20 ml/plant), iii) + locally isolated *A. diazotrophicus* (R12) (20 ml/plant) and iv) + killed *A. brasilense* (Sp7k) (Control 2) (20 ml/plant). Bacterised plants were grown in polybags (10 x 15 cm) containing 30 g of planting media (soil:sand:coconut husk in a ratio of 1:1:1) that were placed in a glasshouse and watered twice daily. After 30 days of growth, the bacterised plants were transferred to larger polybags (15 x 23 cm) containing 1 kg of soil mixture. The bacterised plants were reinoculated at monthly intervals for 280 days (d_{280}). Each plantlet was supplied with phosphorus and potassium in the form of Triple Superphosphate (TSP) and Muriate of Potash (MOP), respectively (Table 1).

Table 1: Basal fertiliser rates based on recommended fertilisers for oil palm seedlings.

Days	Reformulated fertiliser rate (mg/plant)		
	**$(NH_4)_2SO_4$	KH_2PO_4	
64–94	49.5	60.9	
	**Urea	TSP	MOP
125	23.3	21.9	7.0
156–280	47.0	44.0	14.0

Notes: **Supplied only to Control 1 treatment. [TSP (Triple Superphosphate) and MOP (Muriate of Potash)]. The amount of urea, TSP and MOP supplied were equivalent to 7 g/100 palm of NPK yellow 15:15:6:4 for d_{64} – d_{94}, 7 g/palm of NPK yellow 15:15:6:4 for d_{125} and 14 g/palm of NPK yellow 15:15:6:4 for d_{156} – d_{280}.

Bacterised plants were evaluated at d_{280} for shoot dry weight, root dry weight, root volume, leaf protein (Lowry *et al*. 1951) and chlorophyll content (Minolta™ SPAD meter). For bacterial colonisation experiments, the primary and lateral root samples were fixed using McDowell fixatives containing 0.1 M phosphate buffer at pH 7.2 at 4°C (McDowell & Trump 1976). The samples were washed in the same buffer (3 x 10 min) followed by postfix in 1% osmium tetroxide (prepared in 0.1 M phosphate buffer; pH 7.2) for 2 hours, and then washed and dipped in distilled water twice. The tissues then underwent a series of dehydration steps: 50% ethanol for 15 minutes, 75% ethanol for 15 minutes, 95% ethanol for 15 minutes (twice) and 100% ethanol for 20 minutes (twice) (Glauert 1980). The dehydrated samples were then immersed in 1–2 ml of hexamethyldisilazane (HMDS) for 10 minutes (Nation 1983). The HMDS solution was decanted and the samples were air dried in the desiccator. Then, the specimens were mounted on SEM specimen stubs and covered with aurum (20 nm) by Sputter Coater (Polaron SC515). Finally, the samples were ready for viewing under a Field Emission Scanning Electron Microscope (Leo Supra 50 VP).

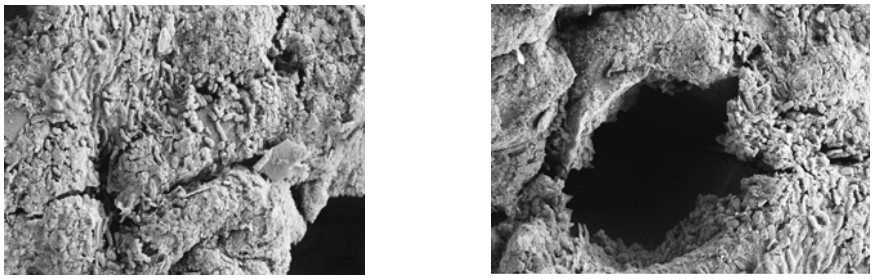
RESULTS AND DISCUSSION

Under nitrogen elimination conditions, the bacterised plants inoculated with R12 had greater root dry weight and volume than those inoculated with Sp7 or the control plants (Table 2). The plants inoculated with Sp7 and R12 both had higher leaf protein and chlorophyll content than Control 2 plants (+Sp7k) (Table 2). Better plant development occurred in host plants inoculated with R12, which was isolated originally from oil palm roots (Azlin *et al*. 2005). The positive influence of R12 on plant growth was due to the compatibility of the inocula tested with the host plants. In addition, the bacteria attached and formed aggregates on primary and lateral roots of the host plants (Fig. 1).

Table 2: Effects of inoculation of *A. brasilense* (Sp7) and locally isolated *A. diazotrophicus* (R12) on root and shoot dry weight, root volume, leaf protein and chlorophyll content of bacterised oil palm plants at d_{280}.

Treatments	Dry weight (g)		Root volume (cm^3)	Leaf protein content (mg BSA/protein)	Leaf chlorophyll content (mg chlorophyll mg^{-1} leaf fresh weight)
	Root	Shoot			
+N (Control 1)	10.1 a (0.911)	16.9 a (0.613)	30.0 ab (3.000)	9.61 a (1.426)	0.25 a (0.003)
+Sp7 (*A. brasilense*)	4.5 b (0.646)	7.0 c (0.841)	27.0 ac (3.000)	8.53 a (0.685)	0.24 b (0.005)
+R12 (*A. diazotrophicus*)	10.5 a (1.963)	10.0 b (0.305)	43.0 a (5.831)	8.44 a (0.827)	0.23 b (0.004)
+Sp7k (Control 2)	4.5 b (0.595)	7.6 c (0.493)	26.0 c (2.449)	5.27 b (0.573)	0.20 c (0.101)

Standard errors are given in parentheses. For each variable, means with a common letter are not significantly different (Least Significant Different Test at $P<0.05$).



(a) (b)

Figure 1: Scanning electron micrograph showing colonisation of *A. brasilense* (Sp7) on primary and lateral roots of oil palm plants at d_{280} (a and b).

Bacterised oil palm plants inoculated with R12 survived the acclimatisation process at a higher rate (100% survival) than plants inoculated with Sp7 (86% survival) (Azlin *et al*. 2007). Higher survival rates and better growth performance of the inoculated plants may have resulted from the elimination of transplant shock, minimised environmental stress or protection against disease (Compant *et al*. 2005). Vestberg *et al*. (2004) proposed that microbial inoculants may be used to protect plants against environmental stress and to improve crop health, yield and quality. The direct influence of bacterial inoculation on root development and plant growth is likely triggered by phytohormone production (Steenhoudt & Vanderleyden 2000; Mantelin & Touraine 2004) and nitrogen fixation (Torres-Rubio *et al*. 2000; Amir *et al*. 2003). Successful colonisation of the roots by the inocula influenced growth and development of oil palm plants. Previous studies by Bashan and Holguin (1997) found that secure attachment of inocula to the root surface is essential for long-term association of rhizobacteria with the host plant. In addition, secure attachment of the rhizobacteria ensures that the substances excreted by the bacteria reach the plant instead of diffusing into the rhizosphere, where other microorganisms may consume them. Our study shows that the locally isolated *A. diazotrophicus* (R12) is a better growth promoter and is more compatible with the host plants compared to *A. brasilense* (Sp7). These results are consistent with those of Rengel (2002), who reported that the proper selection criteria for inoculant should include competitiveness against native rhizobacteria. Thus, selection for inocula for enhancement of plant growth should be conducted under natural conditions in competition with indigenous rhizobacteria.

In conclusion, our results suggest that the diazotrophs tested improve growth of bacterised oil palm plants. The plants inoculated with R12 (*A. diazotrophicus*) and Sp7 (*A. brasilense*) grew better than the control plants. In addition, our experiment suggests that R12 is a better plant growth enhancer than Sp7 for bacterised oil palm plants. Inoculating oil palm plants with diazotrophs may reduce nitrogen fertiliser costs, thus making the oil palm industry more profitable.

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