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Full Length Research Paper

Prolific plant regeneration through organogenesis from scalps of *Musa* sp cv. Tanduk

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A prolific plant regeneration system using scalps derived from shoot tips of *Musa* spp. cv. Tanduk was developed. Highly proliferating scalps, produced after four monthly subcultures of shoot tip explant on Murashige and Skoog (MS) medium supplemented with 100 µM BAP and 1.0 µM IAA, were placed on MS basal medium supplemented with 1.0, 2.5 and 5.0 µM BAP. Rooting of shoots was assessed on hormone-free half strength and full strength MS media and on MS medium supplemented with 1.0, 5.0 and 10 µM IBA. Four types of potting media comprising of sand, peat, sand + top soil + goat dung (3:2:1 v/v) and top soil + sand (1:1 v/v) were evaluated during acclimatization of the plantlets. Prolific shoot regeneration from scalps was obtained on MS medium containing 2.5 µM BAP, at 9.61 and 40.6 shoots per explant after 4 and 8 weeks of culture, respectively. Meanwhile, the highest mean shoot height of 2.19 cm was attained on MS medium with 1.0 µM BAP after 8 weeks of culture. Full-strength MS medium supplemented with 5.0 µM IBA produced the highest mean number of roots per explant at 15.08, while the highest mean root length of 11.07 cm was obtained on hormone-free half strength MS medium at week 4 of culture. The highest plant survivability of 77.5% was achieved in potting medium consisting of top soil + sand + goat dung after 6 weeks of acclimatization. The plants were morphologically normal with vigorous stems and broad green leaves.

Key words: *In vitro*, shoot tip, scalps, regeneration.

INTRODUCTION

Bananas and plantains (*Musa* sp.) are perennial monocotyledonous giant herbaceous plants grown in many tropical areas. The fruits are used both as staple food (cooking banana) and dietary supplement (dessert banana) (Assani et al., 2001). The total world production of banana reached 70.8 million metric tonnes in the year 2006 (FAO, 2007).

Musa sp cv. Tanduk is a cooking banana, having the largest fruit among the banana cultivars, with a light creamy orange pulp, fine in texture but firm. The fruit has excellent keeping quality, remains starchy when fully ripe and requires cooking to be palatable (Valmayor et al., 1990). In Malaysia, banana chips are among the favourites of the locals and it is an up coming cottage industry. Chips made from banana cv. Tanduk is very tasty and in high demand. However, there is shortage of fruit supply from the cultivar for processing into chips due to limited areas under cultivation.

Large scale cultivation requires mass supply of planting materials and this could be achieved through *in vitro* propagation. The current practice of *in vitro* propagation of banana planting materials using shoot tip culture is still insufficient to cater for large scale cultivation. Alternative pathways that can be exploited for *in vitro* mass production of banana plants include the use of male inflorescences and scalps as the starting materials. *Musa* sp. cv. Tanduk does not produce male inflorescences, there-

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Abbreviations: BAP, benzyl amino purine; **IAA,** indole-3-acetic acid; **IBA,** indole-3-butyric acid; **MS,** Murashige and Skoog (1962) medium.

fore, for this cultivar scalps are the appropriate alternative starting materials for propagation. Scalps are cauliflower-like highly proliferating meristems derived from shoot tip culture, first established by Dhed'a et al. (1992). Scalps are genetically stable; therefore they are also suitable materials for cryopreservation which is certainly beneficial for germplasm conservation of banana (Panis et al., 2004). Thus, an efficient regeneration system is necessary for the recovery of plants after cryopreservation.

The banana cultivars in Malaysia including cv. Tanduk are under serious threat from several diseases which include the panama disease or Fusarium wilt (*Fusarium oxysporum f.* sp. *Cubense* and the black sigatoka (*Mycosphaerella fijiensis* Morlet). The diseases cause a devastating effect on banana production, resulting in substantial loss in yield and cultivated areas. The development of disease resistant varieties is urgently needed. However, the sterility and polyploidy of the edible bananas and plantains, constitute an important barrier for conventional breeding programmes (Gomez et al., 2000; Grapin et al., 2000). Genetic engineering methods are the alternatives in overcoming the barrier and for such methods to be successful an efficient plant regeneration protocol is a requirement. This paper describes prolific shoot regeneration from scalps of *Musa* sp. cv. Tanduk through organogenesis, followed by rooting and acclimatisation of plantlets. The regeneraration protocol could be applied for mass propagation of planting materials for commercial cultivation, for recovery of regenerants after cryopreservation and as a tool in genetic improvement programmes of the cultivar.

MATERIALS AND METHODS

Shoot tips of *Musa* sp. cv. Tanduk were placed on MS (Murashige and Skoog, 1962) medium containing 100 µM BAP, 1.0 µM IAA, 2.0 mgl^{-1} glycine, 0.4 mgl^{-1} thiamine HCl, 0.5 mgl^{-1} nicotinic acid, 0.5 mgl^{-1} pyridoxine, 10 mgl^{-1} ascorbic acid, 30 gl^{-1} (w/v) sucrose and 2.0 gl^{-1} gelrite for scalp formation. After four monthly subcultures on fresh medium of the same constituents, highly proliferating scalps consisting of numerous white fleshy bulbous structures bearing tiny meristems were obtained. The scalps were excised along the break lines in-between clumps and transferred to MS medium supplemented with 1.0, 2.5 and 5.0 µM BAP for shoot regeneration. Subculture was performed at 4 weeks interval. The number of shoots produced per explant and the shoot height attained were determined for two consecutive subcultures.

Rooting of shoots was assessed on semi-solid hormone-free MS medium at full and half strengths and on full strength MS medium supplemented with 1.0, 5.0 and 10.0 µM IBA. The number of roots produced per explant and the root length attained were measured after 4 weeks of culture. In both shoot regeneration and rooting experiments, each treatment was replicated three times and each replication per treatment contained 20 explants. All cultures for scalps induction, shoot regeneration and rooting were incubated at 27 ± 2°C under a 16/8 h (light/dark) photoperiod supplied by cool-white fluorescent lamps and a relative humidity of 70%.

Plantlets produced were cleaned, dipped in 2% Benlate solution for 1 min and then transferred to four types of potting media, which were sand, peat, sand + top soil + goat dung (3:2:1 v/v) and top soil + sand (1:1, v/v). Plantlets were acclimatised in the greenhouse for 6 weeks under 50% shade and mist spray irrigation. Each treatment was replicated four times and each replication per treatment contained 10 explants. The percentage of plant survival, plant height (cm) attained and numbers of leaves per plant were recorded after 6 weeks of culture.

All experiments were arranged in a Completely Randomized Design and data were analyzed using the Statistical Analysis System (SAS) version 8.2. Copyright 1999-2001. Differences between treatment means were analysed based on Tukey's Studentized Range (HSD) test.

RESULTS

Through selection of the right meristematic clumps, along with monthly subculture, good quality scalps (Figure 1A) were obtained on MS medium supplemented with 100 µM BAP and 1.0 µM IAA after 4 months. A week after the scalps were transferred to regeneration media containing 1.0, 2.5 and 5.0 µM BAP, they increased in size and their colour changed from whitish to green. By the second week, whitish shoot-buds emerged from the scalps. By the fourth week, spiral greenish shoots but still protected by leaf sheaths were clearly observed elongating from the explant and to enhance the regeneration capacity the scalps were subcultured onto fresh medium of the same composition. By the eight week more shoots emerged (Figure 1B).

The results in Table 1 show highly significant difference ($p < 0.01$) in shoot formation between the BAP treatments in two subsequent subcultures. BAP at 2.5 µM produced the highest mean number of shoots per explant for the two subcultures, at 9.61 and 40.36 respectively. There was no significant difference in shoot production between 1.0 and 5.0 µM BAP in the first subculture, each producing 5.72 and 7.44 shoots per explant, respectively. Similarly in the second subculture the mean number of shoots produced per explant were 18.93 and 29.08 at 1.0 and 5.0 µM BAP, respectively, which did not differ significantly. In terms of shoot height, there was no significant difference observed among the BAP treatments in the first subculture. Nevertheless, in the second subculture the mean shoot height showed highly significant difference ($p < 0.01$) as indicated in Table 2. The highest mean height of 2.19 cm was obtained on medium with 1.0 µM BAP, followed by 1.52 and 1.01 cm on medium with 2.5 and 5.0 µM BAP, respectively.

Rooting occurred in MS medium with or without the addition of hormone. However the response was obviously different in the mean number of roots induced and their lengths (Table 3). Overall, the three IBA concentrations promoted abundant root formation (Figure 1C) while half and full strength hormone-free MS media showed lower capacity of root production. By the fourth week, the highest number of roots per shoot (32.73) was produced in MS medium with 5.0 µM IBA, which differed significantly ($p \leq 0.01$) from all other treatments except with MS medium containing 10 µM IBA (Table 3). The rooting treatments caused highly significant difference ($p \leq 0.01$)

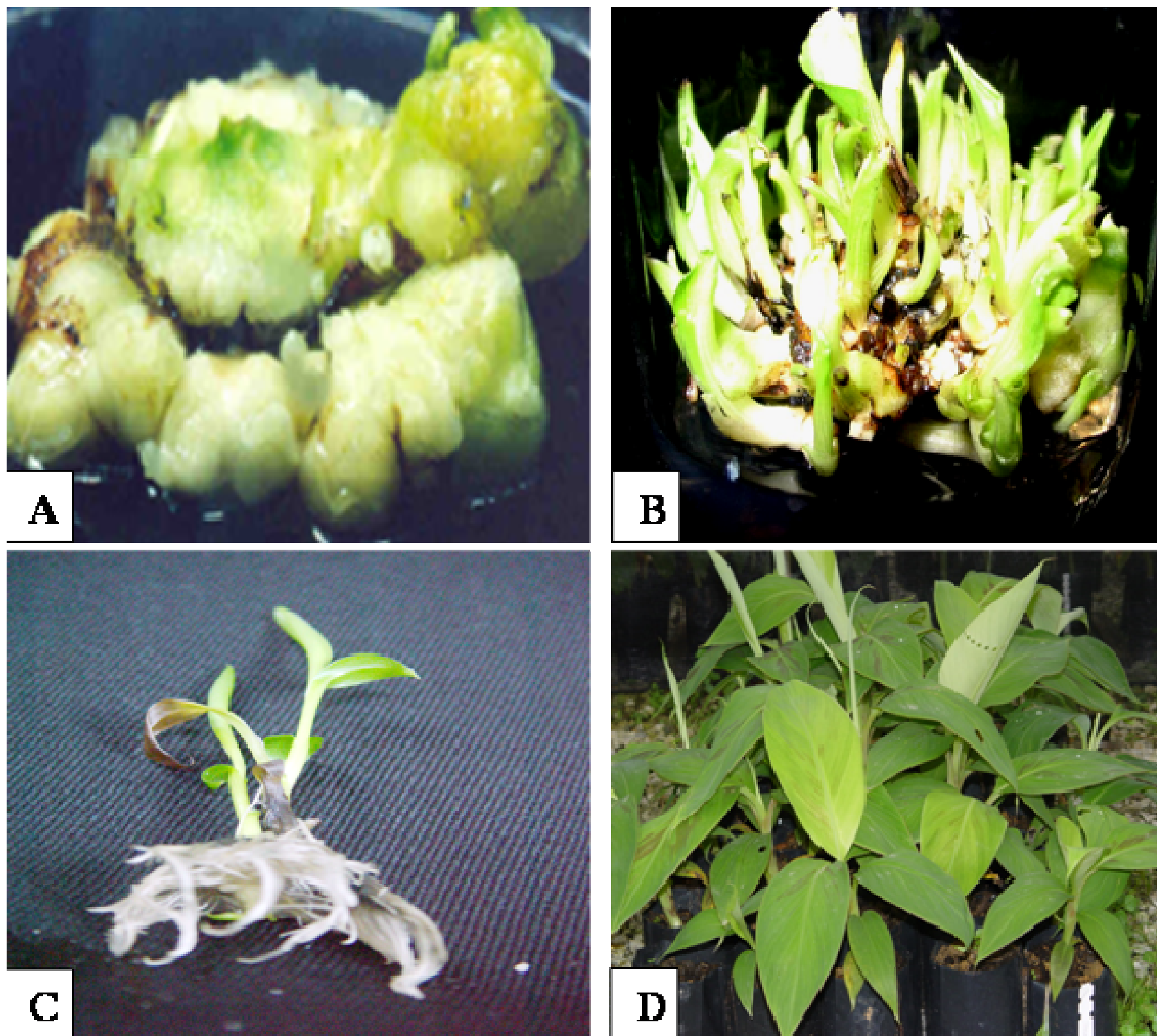

Figure 1. Plant regeneration from scalps of *Musa* spp. cv. Tanduk. (A) Scalps produced after four subcultures on MS medium supplemented with 100 µM BAP and 1.0 µM IAA. Bar = 6.7 mm. (B) Regeneration from scalps on MS medium with BAP. Bar = 9 mm. (C) Well rooted shoots obtained after 4 weeks of culture on full strength MS medium with 5.0 µM IBA. Bar = 10 mm. (D) Plants growing under 50% shade in medium consisting of top soil + sand + goat dung. Bar = 40 mm.

in the root length attained. The highest root length (11.07 cm) was produced in half strength hormone-free MS medium while the lowest root length (2.89 cm) was obtained in MS medium with 10 µM IBA The root lengths were affected by IBA concentration in the medium. As the IBA concentration increased the root length decreased.

Plantlets of *Musa* sp. cv. Tanduk were transferred directly from the *in vitro* state to 50% shade house for acclimatization. By the third day of acclimatization, the plantlets showed wilting symptoms, with yellowing, shrivelling and shedding of older leaves. After two weeks the plantlets thrived and by week 3 of acclimatization the plantlets were fully recovered and exhibited normal growth with newly formed leaves. The highest survive-ability of 77.5% was in potting medium consisting of top soil, sand and goat dung followed by 67.5% survivability in both sand and peat media by week 6 of acclimatization (Table 4). The treatments showed highly significant difference ($p \leq 0.01$) in number of leaves produced and plant height attained. Medium containing top soil, sand and goat dung also produced the highest number of leaves per plant at 7.17 and a mean plant height of 11.41 cm. There was no significant difference in the number of leaves produced per plant as well as in the plant height attained between sand, peat and medium comprising of top soil + sand. Plants grown in medium containing top soil, sand and goat dung were morphologically normal with vigorous stems and broad green leaves (Figure 1D).

Table 1. Number of shoots obtained from scalps of *Musa* sp. cv. Tanduk on MS medium supplemented with 1.0, 2.5 and 5 µM BAP.

Treatment	Number of shoots per explant	
	Subculture 1	Subculture 2
1.0 µM BAP	5.72 b	18.93 b
2.5 µM BAP	9.61 a	40.63 a
5.0 µM BAP	7.44 b	29.08 ab

Means followed by the same letter in the same column are not significantly different based on Tukey's Studentized Range (HSD) test ($p \leq 0.05$).

Table 2. Height of shoot (cm) obtained from scalps of *Musa* sp. cv. Tanduk on MS medium supplemented with 1.0, 2.5 and 5 µM BAP.

Treatment	Shoot height (cm)/explants	
	Subculture 1	Subculture 2
1.0 µM BAP	0.87 a	2.19 a
2.5 µM BAP	0.77 a	1.52 ab
5.0 µM BAP	0.64 a	1.01 b

Means followed by the same letter in the same column are not significantly different based on Tukey's Studentized Range (HSD) test ($p \leq 0.05$).

Table 3. Effect of half strength and full strength MSO and MS medium supplemented with 1.0, 5.0 and 10 µM IBA on number of roots produced per explant and root length (cm) attained at week 4 of culture.

Treatment	Number of roots per explant	Root length (cm)
Half strength MSO	8.27 b	11.07 a
Full strength MSO	8.73 b	10.44 a
MS+1.0 µM IBA	15.17 b	10.33 a
MS+5.0 µM IBA	32.73 a	7.07 b
MS+10.0 µM IBA	24.27 a	2.89 c

Means followed by the same letter in the same column are not significantly different based on Tukey's Studentized Range (HSD) test ($p \leq 0.05$).

At this stage the plants were successfully transplanted to the field.

DISCUSSION

The scalps of cv. Tanduk, induced on medium of high concentration of 100 µM BAP, resulted in high multiple shoot production upon transfer to a medium of low BAP concentration of 2.5 µM. From one shoot tip explant, an average of 3-5 scalps clumps were obtained after four subcultures (data not shown) and from each clump cultured on medium with 2.5 µM BAP 40.63 shoots were produced after two subcultures (Table 1). This means that about 122-203 shoots could be produced from one shoot tip of banana cv. Tanduk after six months, using the scalp method. Whereas, only an average of 43 shoots could be produced via direct regeneration from one shoot tip of the same cultivar after five months (data not shown). Reduction of BAP to a lower concentration had triggered prolific shoot regeneration from scalps of cv. Tanduk. Schoofs et al. (1997) also observed that when BAP concentration was reduced from 100 µM to 10 µM in three cultivars of banana, multiple shoots were induced from the scalps.

The formation of roots in *Musa* sp. cv Tanduk could be obtained as early as one week in culture but to have a strong root system the shoots were maintained for two or more weeks on the rooting media. Cronauer and Krikorian (1984) managed to get shoots of banana to root in as less as four days. Kanchanapoom (2000) stated that auxin is not necessary for *in vitro* root formation in

Table 4. Effect of potting media on percentage of survivability, number of leaves produced per plant and plant height (cm) by week 6 of acclimatization in the shade house.

Treatment	Survivability (%)	No. of leaves produced	Height (cm) attained
Sand	67.5 b	5.58 b	4.46 b
Peat	67.5 b	5.85 ab	7.18 b
Top soil + Sand + Goat dung	77.5 a	7.17 a	11.41 a
Top soil + Sand	65.0 b	4.68 b	6.08 b

Means followed by the same letter in the same column are not significantly different based on Tukey's Studentized Range (HSD) test ($p \leq 0.05$).

banana, but the root system produced was poor to sustain the plantlets in the outside environment. Nevertheless, Azad and Amin (2001) rooted their banana shoots on half strength MS medium fortified with 0.1 - 1.0 mg/L NAA, IBA or IAA. In this study, the addition of IBA produced plantlets with vigorous root system that could ensure high survival rate during acclimatization, even though both root length and shoot height were slightly affected. It was also observed that at low IBA concentrations of 1 and 5 µM, there was a tendency to produce 1-2 additional adventitious shoot-buds by the third week. Cronauer and Krikorian (1984) reported that earlier root induction lessen the additional shoot production in their banana cultures.

Acclimatization of micropropagated plantlets is a crucial step in tissue culture systems. Consequently, the transplantation stage continues to be a major bottleneck in the micropropagation of many plants (Hazarika, 2003). Plantlets or shoots that have grown *in vitro* have been continuously exposed to a unique microenvironment that has been selected to provide minimal stress and optimum conditions for plant multiplication. The physiological and anatomical characteristics of micropropagated plantlets necessitate that they should be gradually acclimatized to the environment of the greenhouse or field. In this study, all plantlets on the acclimatization media showed slight wilting in the beginning in response to a decrease in the air humidity and an increase in photon flux density outside which directly influenced the physiological activity of the plantlets. The medium combination of top soil, sand and goat dung was found to be the best for acclimatization of *Musa* sp. cv. Tanduk in the shade house. The superiority of this medium combination over the other types of potting media could be due to its improved physical and chemical properties as a result of a mixture of three different components.

The highest survivability rate of 77.5% obtained in this study was less than 81.71% obtained in banana cultivar Kluai Bep reported by Chinsuk and Silayoi (2001) and 95% reported in *Musa* sp. by Azad and Amin (2001). Although there was no pest and disease infection observed in the shade house during acclimatization the loss of plantlets could be attributed to environmental stress and therefore a good microenvironment should be provided before transferring the plantlets from an *in vitro* condition to the shade house.

Conclusion

Regeneration of plantlets from scalps of *Musa* sp. cv. Tanduk proved to be very efficient and cost effective. Using a low concentration of 2.5 µm BAP, shoots could be produced in high numbers from the scalps, rooted, acclimatized and plants are ready for field planting within 4 - 5 months.

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