Journal of Plant Biology, March 2005, 48(1) : 142-146 **SHORT COMMUNICATION**

Effect of Plant Growth Regulators on Regeneration of Plantlets from Bud Cultures of *Cymbopogon nardus* L. and the Detection of Essential Oils from the *in Vitro* Plantlets

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***Cymbopogon nardus* L. could be propagated via tissue culture using axillary buds as explants. The aseptic bud explants obtained using double sterilization methods produced stunted abnormal multiple shoots when they were cultured on Murashige and Skoog medium (MS) supplemented with 1.0 mg L^{-1} or 2.0 mg L^{-1} benzyladenine (BA). Stunted shoots that cultured on MS + 1.0 mg L^{-1} BA + 1.0 mg L^{-1} N^6-isopentenyl-adenine (2iP) could induce elongation of shoots from about 60% of the stunted shoots. Normal multiple shoots could be induced at the highest (19.7 shoots per bud) from the bud explants within six weeks when cultured on proliferation medium consisted of MS supplemented with 0.3 mg L^{-1} BA and 0.1 mg L^{-1} indole-3-butyric acid (IBA). The separated individual shoot produced roots when transferred to basic MS solid medium. The essential oils that were contained in the mature plants namely citronellal, geraniol and citronellol were also found in the *in vitro C. nardus* plantlets. Citronellal was the main essential oil component in the matured plants while geraniol was the main component in the *in vitro* plantlets.**

Keywords: axillary buds, benzyladenine, *Cymbopogon nardus*, essential oils, indole-3-butyric acid, multiple shoots

Cymbopogan nardus L. is an aromatic grass belonging to *Gramineae* family. It is commonly known as serai wangi in Malaysia and Indonesia. This plant is believed to be originated from Sri Lanka and South India. It has been grown commercially on a large scale in Haiti, Central America, the South Pacific, and tropical Africa. It grows very well in moist alluvium soil and its growth becomes retarded during the dry season (Henderson, 1954). It produces clumped bulbous stems that become leaf blades and branched clusters of stalked flowers when flowering.

C. nardus has long been used in Malaysia as traditional medicine. It contains three main essential oils, citronellal, citronellol and geraniol, which can be used for relieving stomach discomfort, aiding digestion and as antispasmodic agent (Burkill, 1966). The plant was also found to be effective in the treatment of skin aczema, giddiness, and abdominal colic. It has antiseptic properties and also has been used as insect repellant (Ghani et al., 1991). In Europe, the essential oils of the plant have been distilled and used for making perfume, soap, and cosmetics (Ketaran, 1988). It is also found to be effective against *Anopheles stephesi* larvae, a carrier of malaria fever (Kumar and Dutta, 1987).

C. nardus is conventionally propagated by dividing into small clumps and planted in the soil. But this process often results in fungal diseases that can influence the quality of essential oil within the plants (Ketaran, 1988). In view of their medicinal values and the problems of fungal diseases in the field, *in vitro* culture technique can be an attractive alternative for mass production of the plant. Objectives of this study are to establish a tissue culture method for propagating *C. nardus* and to analyze the essential oils in the *in vitro* plantlets.

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MATERIALS AND METHODS

Plant Material

The mature field grown *C. nardus* plants were obtained from Relau Agriculture Station, Penang, Malaysia. Buds at the bulbous stem were cut, washed with detergent, and rinsed with running tap water for 30 min. The explants were then surface-sterilized

with 0.01% mercuric chloride (w/v) for 5 min. After rinsing three times with sterile distilled water, the explants were surface sterilized for 15 min with 15% Clorox® (v/v) with some drops of Tween20. This was followed by second sterilization with 5% Clorox® for 5 min. The explants were then rinsed three times with sterile distilled water. The cut edges of the bud pieces were removed aseptically before inoculating onto basic MS (Murashige and Skoog, 1962) solid medium. After two weeks of culture, the aseptic buds were used as explants for plantlet regeneration.

Induction of Multiple Shoots

Previous study showed that MS medium with BA (0-10 mg L^{-1}) and IBA (2-10 mg L^{-1}) and MS medium with BA (4-10 mg L^{-1}) resulted in all the bud explants necrotic (Chan and Patra, 2000). Thus, in this study, the aseptic buds were cultured only on MS medium supplemented with 1.0 mg L^{-1} BA or 2.0 mg L^{-1} BA for the induction of multiple shoots. Ten explants were used for each culture medium, and the experiment was repeated three times. Results were recorded after four weeks.

Shoot Elongation

Since most of the multiple shoots of *C. nardus* became stunted on MS medium with 1.0 mg L^{-1} BA or 2.0 mg L^{-1} BA, bud explants were cultured on MS medium with BA (1.0-2.0 mg L^{-1}) and N^6-isopentenyl-adenine (2iP) (1.0-2.0 mg/L) for 4 weeks.

Effect of Reduced Growth Regulators on Multiple Shoots Formation

The aseptic bud explants were further cultured on MS medium supplemented with different concentrations of BA (0, 0.1, 0.3, 0.5 mg L^{-1}) and IBA (0, 0.1, 0.3, 0.5 mg L^{-1}) in 4 x 4 factorial block design. The number of shoot formed from each explant was recorded after 6 weeks. The data collected were analyzed by two-way analysis of variance (ANOVA), and comparison of the mean number of shoot produced from each explant was done by LSD test at $p = 0.05$ with SAS program.

Regeneration of *in Vitro* Plantlets

The multiple shoots were then separated as individual shoot and cultured in hormone free MS medium. After four weeks, percentage of the *in vitro* shoots forming roots was noted. The four weeks old *in vitro* plantlets were collected for extraction and detection of essential oils.

Culture Medium and Culture Condition

The basal medium was MS medium salts with 30 g L^{-1} sucrose and 7.5 g L^{-1} agar (chile agar). Culture vessels were 150 ml Erlenmeyer flask or jam jar each containing 30 ml of the medium and test tubes (25 × 150 mm) fitted with plastic caps containing 15 ml of the medium. pH of the media was adjusted to 5.8 and autoclaved in Elite Sterilizer EAC-4000C at 121°C with 1.05 kg cm^{-3} for 13 min. The cultures were placed in a culture room maintained at 25 ± 2°C under continuous light with cool white fluorescent tubes at an intensity of 32.5 µE m^{-2} s^{-1}.

Detection of Essential Oil

The six months old mature field grown mother plants and the four weeks old *in vitro* plantlets were cut into small pieces and crude extracts were obtained via steam distillation with chloroform as separation solvent. Type, relative composition, and amount of essential oils were determined using a gas-liquid chromatography (GLC) (SRI 8610C Alltech Associates, USA) with EC-WAX capillary column of 30 cm in length and 0.32 mm in diameter and flame ionization detector (FID).

RESULTS AND DISCUSSION

Multiple Shoots Formation

From the culture media of MS supplemented with 2.0 mg L^{-1} BA and MS supplemented with 1.0 mg L^{-1} BA, all the multiple shoots formed were stunted and abnormal (Fig. 1A). As reported by Hudson et al. (1997), cytokinin was needed for induction of multiple shoots, but high concentration of cytokinin resulted in abnormality and reduction in height. It also has been reported that the embryo of Douglas pear could form many normal multiple shoots when the embryo tissues were cultured in MS medium supplemented with 0.05 mg L^{-1} BA. However, if the concentration of BA was increased to 0.1 mg L^{-1}, minute stunted shoots were formed around the embryo tissues (Winston and Verhagan, 1977). Our study indicated that addition of BA exceeding 1.0 mg L^{-1} was not suitable for induction of multiple shoots

from *C. nardus* buds.

Shoot Elongation

When the stunted multiple shoots of *C. nardus* were cultured on MS medium supplemented with BA (1.0 or 2.0 mg L^{-1}) and 2iP (1.0 or 2.0 mg L^{-1}), elongation of shoot was induced. About 60% of the stunted shoots that cultured on MS with 1.0 mg L^{-1} BA and 1.0 mg L^{-1} 2iP underwent shoot elongation. Elongation rate of the stunted shoots was only 26.7% at a medium of MS with 1.0 mg L^{-1} BA and 2.0 mg L^{-1} 2iP. On the MS medium with 2.0 mg L^{-1} BA + 1.0 mg L^{-1} 2iP, 33.3% of the stunted shoots elongated, and on the MS medium with 2.0 mg L^{-1}BA and 2.0 mg L^{-1} 2iP, 46.7% of the stunted shoots elongated (Table 1). The elongated shoots were surrounded with the existing stunted shoots (Fig. 1B).

Effect of Reduced Concentration of Growth Regulators on Shoot Proliferation

C. nardus buds were able to produce normal multiple shoots when cultured on MS medium supplemented with 0 - 0.5 mg L^{-1} BA and 0 - 0.5 mg L^{-1} IBA after six weeks of culture. Among the sixteen different culture media of MS supplemented with different combinations of 0 - 0.5 mg L^{-1} BA and 0 - 0.5 mg L^{-1} IBA, MS medium with 0.3 mg L^{-1} BA and 0.1 mg L^{-1} IBA induced the highest number of shoots, i.e. an average of 19.7 shoots, were formed from each bud explant after six weeks of culture (Fig. 2). When the *in vitro* shoots were transferred from the proliferation medium to MS medium free of growth regulators, all the shoots produced roots (Fig. 1C).

Detection of Essential Oils

The essential oils, citronellal, geraniol and citronellol, that were found in the mature plants were also detected in the *in vitro* plantlets. However, the relative composition of the three essential oils was different in the mature plants as compared to the *in vitro* plantlets. Citronellal, geraniol and citronellol were 49%, 30% and 14.2%, respectively, in the mature plants. In the *in vitro* plantlets, the relative composition was 33% citronellal, 47.7% geraniol and 19.1% citronellol. Citronellal was the main essential oil in the mature plants while geraniol was the main compo-

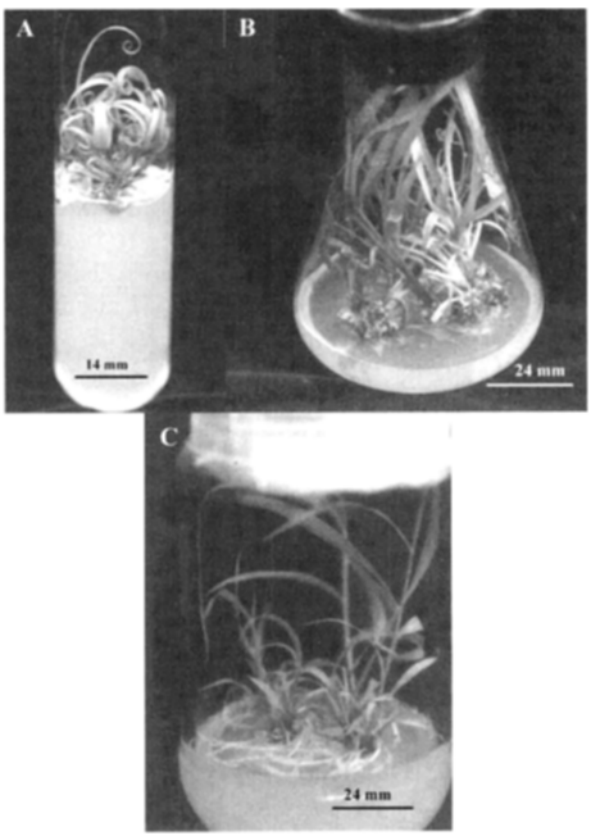

Figure 1. Stunted and abnormal multiple shoots of *C. nardus* formed from bud explants on MS medium supplemented with 1.0 mg L^{-1} BA **(A)**. Shoot elongation of the stunted shoot on MS with 1.0 mg L^{-1} BA and 1.0 mg L^{-1} 2iP. The elongated shoots were surrounded with the stunted shoots **(B)**. Rooting of the *in vitro* shoots on basic MS medium **(C)**.

Table 1. Effect of growth regulators, BA and 2iP, supplemented in MS culture medium on shoot elongation and shoot death of the *in vitro* shoots after 4 weeks of culture.

Culture medium MS + growth regulators (mg L^{-1})		Shoot elongation (%) ± s.d.	Stunted death (%) ± s.d.	Stunted shoots (%) ± s.d.
BA	2ip			
1.0	1.0	60.0 ± 10.0	16.7 ± 7.8	23.3 ± 11.5
1.0	2.0	26.7 ± 5.8	33.3 ± 5.3	40.0 ± 4.0
2.0	1.0	33.3 ± 8.5	30.3 ± 6.0	36.7 ± 5.2
2.0	2.0	46.7 ± 3.8	23.3 ± 7.3	8.8 ± 0.6

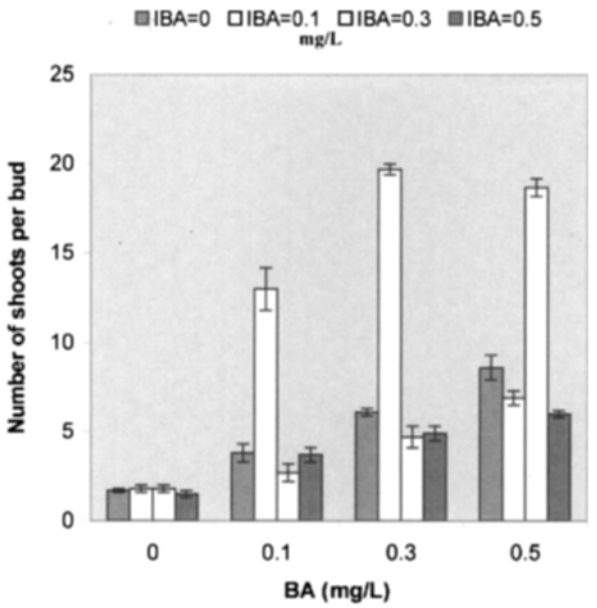

Figure 2. Effect of reduced concentration of BA and IBA in MS medium on *in vitro* shoot proliferation after 6 weeks of culture.

Table 2. Relative composition of citronellal, geraniol and citronellol detected in the 6 months old mature field grown and the 4 weeks old *in vitro* plantlets of *C. nardus*.

Essential oils	Relative composition (%) ± s.d.	
	Mature plants	*In vitro* plantlets
Citronellal	49.0 ± 6.8	33.0 ± 8.5
Geraniol	30.0 ± 4.6	47.7 ± 10.4
Citronellol	14.2 ± 3.5	19.1 ± 6.7

Table 3. Essential oil content in the 6 months old mature field grown and the 4 weeks old *in vitro* plantlets of *C. nardus*.

Type of plant	Essential oil content (mg/100 g ± s.d.)
Mature plants	320 ± 50
In vitro plantlets	90 ± 5

nent of the *in vitro* plantlets (Table 2). Total content of essential oils was higher in the mature plants (320 ± 50 mg per 100 g) compared to the *in vitro* plantlets (90 ± 5 mg per 100 g) (Table 3). Akhila (1986) in his study on biosynthesis of monoterpene from *C. winterianus* showed that geraniol is the precursor for synthesis of citronellal and citronellol with the function of a plant enzyme EK. While citronellol can undergo oxidation to form citronellal in the presence of oxidase EO, the reverse process is not so far detected. Based on these facts, it is tempting to suggest that the mature plants contained higher amount of EK and EO than the *in vitro* plantlets. Hence less geraniol (30%) but more citronellal (49%) were found in the mature plants, and more geraniol (47.7%) was present in the *in vitro* plantlets compared to citronellal (33%) and citronellol (13.1%). Although the *in vitro C. nardus* plantlets contained 3.5 times lower level of essential oils than the mature plants, it took less time to produce the plantlets once the *in vitro* plantlet culture is established. Moreover, the *in vitro* plantlet culture can ensure disease free condition. Hence, *in vitro* mass propagation of *C. nardus* could be an alternative method for the production of quality essential oils.

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LITERATURE CITED

Akhila A (1986) Biosynthesis of monoterpenes in *Cymbopogon winterianus*. Phytochem 25: 421-424

Burkill IH (1966) A Dictionary of the Economic Products of the Malay Peninsula, Vol I. Ministry of Agriculture and Co., Kuala Lumpur, pp 734-738

Chan LK, Patra RD (2000) *In vitro* culture of *Cymbopogon citrstus* and *C. nardus*: problems and prospects, *In* YS Chang, V Subramaniam, AS Zainin, S Khozirah, eds, Medicinal plants: Quality herbal products for healthy living. Proceeding of the conference on forestry and forest products research (CFFPR) series, Kepong, Kuala Lumpur, pp 154-158

Ghani AKA, Rahman AA, Alimon H, Din LB (1991) Kultur tisu dan penghasilan minyak pati daripada *Cymbopogon nardus* Linn. Rendle. *In* KH Neumann, W Barz, E Reinhard, eds, Medicinal products from tropical rain forest. Proceedings of the conference. Kuala Lumpur, pp 69-78

Henderson MR (1954) Malayan Wild Flowers, Monocotyledons. Malayan Nature Society, Kuala Lumpur

Hudson TH, Kester DE, Davies FT, Geneve RL (1997) Plant Propagation: Principles and Practices, Ed 6. Prentice-Hall, New Jersey

Ketaran S (1988) Pengantar Teknologi Minyak Atsiri. Balai Pustaka, Jakarta

Kumar A, Dutta GP (1987) Indigenous plants oils as larvicidal agent against *Anopheles stephensi* mosquitoes. Curr Sci 54: 959-960

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol Plant 15: 473-497

Winton LL, Verhagen SA (1977) Douglas-fir shoots cultures. Can J Bot 55: 1246-1250