การเพาะเลี้ยงเนื้อเยื่อไผ่รวก (*Thyrsostachys siamensis* Gamble) โดยการชักนำให้เกิดยอดทวีคูณ

Tissue culture of *Thyrsostachys siamensis* Gamble by multiple shoot induction

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บทคัดย่อ: การชักนำให้เกิดยอดจากตาข้างของไผ่รวก (*Thyrsostachys siamensis* Gamble) โดยเพาะเลี้ยงข้อของไผ่รวกในอาหารแข็งสูตร Murashige and Skoog (MS) benzyladenine (BA) ที่ระดับ 11.25 ไมโครโมลาร์ ชักนำให้เกิดจำนวนยอดต่อตาข้างอย่างมีนัยสำคัญ 13.3 ยอดต่อตาข้าง สำหรับการเพิ่มขยายจำนวนยอดทวีคูณพบว่า เมื่อนำกลุ่มยอดที่มี 3 ยอดต่อกลุ่มที่ได้จากตาข้าง เลี้ยงในอาหารเหลวสภาพเขย่าสูตร MS ที่ประกอบด้วย BA ความเข้มข้น 44.40 ไมโครโมลาร์ และ kinetin (Kn) ความเข้มข้น 2.32 ไมโครโมลาร์ ให้ผลในการเกิดยอดทวีคูณของไผ่รวกมากที่สุด คือ 36.1 ยอด ในเวลา 8 สัปดาห์ ซึ่งคิดเป็นอัตราการเพิ่มขยาย 12 เท่า การเกิดยอดทวีคูณในอาหารเหลวสภาพนิ่งและสภาพเขย่าให้ผลที่แตกต่างกันอย่างมีนัยสำคัญในช่วงระยะเวลา 8 สัปดาห์ การชักนำให้เกิดรากโดยนำกลุ่มของยอด 3-5 ยอดต่อกลุ่มเลี้ยงในอาหารสูตร MS ที่เติม Naphthaleneacetic acid (NAA) ความเข้มข้น 26.85 ไมโครโมลาร์ เลี้ยงเป็นเวลา 3 สัปดาห์ แล้วย้ายลงในอาหารสูตร MS ที่ไม่เติมสารควบคุมการเจริญเติบโต เพื่อการยืดยาวของราก

Abstract: Nodal explants of *Thyrsostachys siamensis* Gamble were cultured on solid Murashige and Skoog's (MS) medium to induce shoots from axillary buds. Benzyl adenine (BA) at concentration of 11.25 µM significantly induced 13.3 shoots per axillary bud. For multiple shoot induction, a cluster of 3 shoots derived from axillary buds was cultured in agitated liquid MS medium supplemented with BA and kinetin (Kn). The combination of 44.40 µM BA and 2.32 µM Kn provided the best multiple shoots with 36.1 shoots within 8 weeks. The multiplication rate was 12 fold. Numbers of multiple shoots induced from stationary and agitated liquid medium were significantly different within 8 weeks of culturing. Root initiation was induced in a cluster of 3-5 shoots on MS medium added with 26.85 µM of naphthaleneacetic acid (NAA) for 3 weeks, and then transferred to MS medium without plant growth regulator for root elongation.

Introduction: *Thyrsostachys siamensis* Gamble (Pai Ruak) is an important economic bamboo in Thailand, mainly found in Kanchanaburi. It is used for food, constructions, furniture, agricultural implements and raw materials of paper and pulp industry (1). The current level of production is insufficient to satisfy the demand by consumers. Different propagation techniques are available such as seeds, clump division and rhizome cutting. However, these techniques have certain limitations. The development of tissue culture technique will be the best procedure for rapid clonal propagation Therefore, bamboos can be produced year-round with high quality and quantity (2). The techniques have been reported for many species (2, 3, 4, 5, 6). The

present research reports and effective micropropagation system from nodal segments of *T. siamensis*, taken from field-grown plants, through shoot multiplication.

Methodology:

Plant materials Nodes were surface-sterilized in 70% ethanol for 10 min. and 10% (v/v) Hyter with 0.02% (v/v) Tween 20 for 10 min, followed by three rinses in sterile distilled water. Single node about 2.0 cm long, were cultured on MS medium supplemented with 11.25 µM BA to induce shoot clumps from axillary buds. Then, the cluster of 3 shoots was used as explants for multiple shoot induction (Fig. 1a).

Media and culture conditions: The medium used for multiple shoot induction was MS medium and 200 ml/L coconut water supplemented with singly different concentrations of BA and in combinations with 2.32 µM Kn, 30 g/L sucrose and 6.2 g/L agar. All media were adjusted to pH 5.7, prior to adding agar and autoclaving. Explants were cultured for 8 weeks (1b). A Cluster of 3-5 shoots from multiplication medium were separated and cultured on MS rooting medium containing 26.85 µM NAA (3) for 3 weeks and then transferred to MS medium without growth regulator (Fig. 1c). Cultures were incubated at 24 ± 1 ºC with a 16-h photoperiod of 35-40 $\mu mol.m^{-2}.s^{-1}$ provided by cool white fluorescent lights.

Statistical analysis: Number of shoots and number of leaves were recorded. Data were analyzed by using Duncan's New Multiple Range Test (DMRT).

Results, Discussion and Conclusion:

***Effects of BA and Kn on multiple shoot formation*:** Clusters of 3 shoots per cluster, induced from axillary buds, were cultured on MS medium supplemented with 0-44.40 µM BA and 2.32 µM Kn for 8 weeks with subculturing every 2 weeks. Higher concentrations of BA promoted shoot proliferation in liquid medium either in a stationary or agitated condition. Regenerated shoots from solid medium were significantly less than those from liquid medium. The combination of 44.40 µM BA and 2.32 µM Kn provided 5.1, 23.3 and 36.1 shoots per explant from solid (Fig. 2a), stationary (Fig. 2b) and agitated liquid medium (Fig. 2c), respectively, within 8 weeks (Table 1). Between stationary and agitated liquid medium, there was not significant difference in number of shoots obtained from medium supplemented with 0-22.22 µM (MS1-MS5) when cultured for 2, 4 and 6 weeks. Although 44.40 µM BA in stationary liquid medium produced slightly more shoots (7.2 shoots) than that in agitated liquid medium (4.4 shoots) within 2 weeks, significantly higher number of shoots occurred in agitated liquid medium within 8 weeks. 18.0 and 23.3 shoots obtained from 22.22 µM BA (MS 5) and 44.40 µM BA (MS 6) in stationary liquid medium while 25.1 and 36.1 shoots obtained from the same concentrations of BA in agitated liquid medium (Table 1). Similar results were obtained from previous reports for using liquid medium (4, 6) and types of plant growth regulators (2, 3, 4, 5, 6), but different concentrations were used depending on species. The cluster of 3-5 shoots were separated and rooted on MS medium supplemented with 26.85 µM NAA (3) for 3 weeks. Rooted shoots were then transferred to MS medium without plant growth regulator for root elongation to obtain plantlets (Fig. 1c).

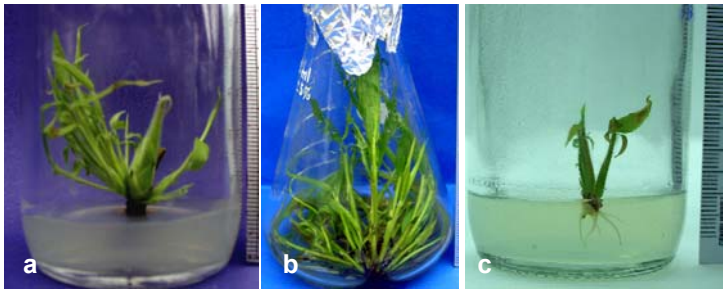

Fig. 1 Process of micropropagation a) shoot induction from axillary bud on MS + 11.25 µM BA; b) shoot multiplication in agitated liquid MS + 44.40 µM BA + 2.32 µM Kn ; c) rooting on MS + 26.85 µM NAA for 3 weeks, then transferred to MS without plant growth regulator

Table 1 Effects of BA in combination with IBA on multiple shoots formation of *T. siamensis* after cultured for 8 weeks

Medium condition	MS medium	Concentration (μM)		multiple shoot formation			
				time (week) [1/]			
		BA	Kn	2	4	6	8
Solid	MS 1	0.0	0.00	0.0 a	0.0 a	0.0 a	0.0 a
	MS 2	0.44	2.32	0.0 a	0.0 a	0.0 a	0.0 a
	MS 3	2.22	2.32	1.0 a	1.4 a	1.9 a	2.2 a
	MS 4	4.44	2.32	1.4 a	2.0 a	2.6 ab	3.3 a
	MS 5	22.22	2.32	1.6 a	2.4 ab	3.3 b	4.3 a
	MS 6	44.40	2.32	1.7 a	2.8 ab	3.7 b	5.1 ab
Stationary Liquid	MS 1	0.0	0.00	0.9 a	1.6 a	2.1 ab	2.5 a
	MS 2	0.44	2.32	1.8 a	2.7 ab	3.5 b	4.5 a
	MS 3	2.22	2.32	3.2 b	5.4 b	7.3 c	9.5 bc
	MS 4	4.44	2.32	2.2 ab	5.6 b	8.7 c	12.2 c
	MS 5	22.22	2.32	4.7 bc	8.7 bc	12.3 cd	18.0 d
	MS 6	44.40	2.32	7.2 d	13.2 d	18.8 d	23.3 e
Agitated Liquid	MS 1	0.0	0.00	0.0 a	0.0 a	0.0 a	0.0 a
	MS 2	0.44	2.32	0.6 a	0.7 a	0.7 a	0.7 a
	MS 3	2.22	2.32	1.5 a	3.3 ab	4.1 b	4.9 ab
	MS 4	4.44	2.32	2.0 a	5.7 b	10.2 c	13.8 cd
	MS 5	22.22	2.32	3.5 b	8.8 bc	15.9 d	25.1 efg
	MS 6	44.40	2.32	4.4 bc	11.3 c	18.1 d	36.1 g

[1/] Values are mean (n = 10). Means followed by the same letters in the same column are not significantly different at $P = 0.05$ by DMRT

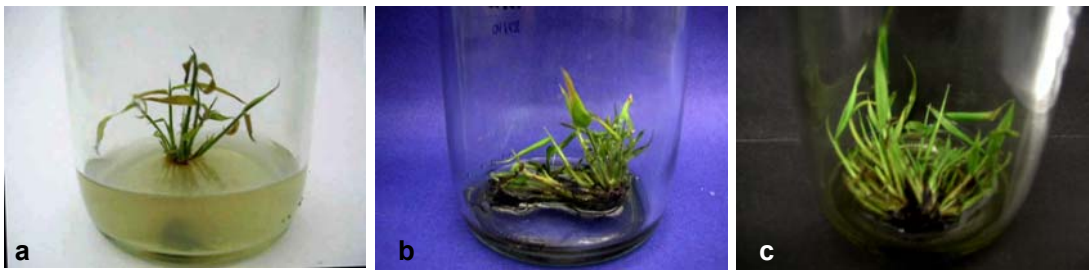

Fig. 2 Multiple shoots regenerated from a cluster of 3 shoots, derived from an axillary bud, cultured on MS + 44.40 μM BA + 2.32 μM Kn for 8 weeks. a) solid medium, b) stationary liquid medium, c) agitated liquid medium

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