MICROPROPAGATION OF SANG NUAN (*Dendrocalamus membranaceus*), SANG MON (*D. sericeus*) AND KAB DANG (*Cephalostachyum pergracile*)

JANYAPACH RATANAKOM

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การเพาะเลี้ยงเนื้อเยื่อไผ่ซางนวล (*Dendrocalamus membranaceus*) ไผ่ซางหม่น (*D. sericeus*) และไผ่กาบแดง (*Cephalostachyum pergracile*) เพื่อการขยายพันธุ์

นางสาวจรรยพัชร รัตนคม

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for a Master's Degree.

Thesis Examining Committee

(Asst. Prof. Dr. Thitiporn Machikowa)
Chairperson

(Asst. Prof. Dr. Arak Tira-umphon)
Member (Thesis Advisor)

(Dr. Sukanya Aiamlaor)
Member

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Member

(Assoc. Prof. Dr. Chatchai Jothityangkoon)
Vice Rector for Academic Affaire and Quality Assurance

(Prof. Dr. Neung Teaumroong)
Dean of Institute of Agricultural Technology

จรรยพัชร รัตนคม : การเพาะเลี้ยงเนื้อเยื่อไผ่ซางนวล (*Dendrocalamus membranaceus*) ไผ่ซางหม่น (*D. sericeus*) และไผ่กาบแดง (*Cephalostachyum pergracile*) เพื่อการขยายพันธุ์ (MICROPROPAGATION OF SANG NUAN (*Dendrocalamus membranaceus*), SANG MON (*D. sericeus*) AND KAB DANG (*Cephalostachyum pergracile*)) อาจารย์ที่ปรึกษา : ผู้ช่วยศาสตราจารย์ ดร.อารักษ์ ธีรอำพน, 100 หน้า.

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ไผ่ เป็นพืชที่มีความต้องการใช้มากในอุตสาหกรรมเฟอร์นิเจอร์ อย่างไรก็ตามการขยายพันธุ์ไผ่มีข้อจำกัดจากสภาพแวดล้อม ไฟป่า และแมลง การขยายพันธุ์ทั่วไปได้ต้นจำนวนน้อย และใช้ระยะเวลานาน การวิจัยในครั้งนี้มีวัตถุประสงค์เพื่อพัฒนาเทคนิคการเพาะเลี้ยงเนื้อเยื่อที่เหมาะสมสำหรับไผ่ซางนวล และไผ่ซางหม่น โดยใช้ส่วนข้อแขนง และไผ่กาบแดงโดยใช้เมล็ด โดยศึกษาวิธีการฟอกฆ่าเชื้อ สูตรอาหารในการชักนำยอด ทวีคูณยอด และการชักนำรากตลอดจนพัฒนาไปเป็นต้นอ่อนที่สมบูรณ์ ผลการศึกษาพบว่าไผ่ซางนวล ให้ผลการปนเปื้อนเชื้อน้อยที่สุด 18.01% โดยผ่านการฟอกฆ่าเชื้อด้วยแอลกอฮอล์ 70% นาน 1 นาที แล้วแช่ในไฮโดรเจนเปอร์ออกไซด์ 30% นาน 15 นาที และแช่ในโซเดียมไฮโปคลอไรท์ 10 และ 5% นาน 10 และ 5 นาที ตามลำดับ และล้างด้วยน้ำกลั่นที่นึ่งฆ่าเชื้อ หลังจากชักนำยอดนาน 2 สัปดาห์ ใน MS ที่เติม BA ความเข้มข้น 6 มก./ล. มีจำนวนยอดที่พัฒนาสูงสุด เท่ากับ 6.73 ยอด และมีความยาวยอดเท่ากับ 4.20 ซม.จากนั้นทำการทวีคูณยอดในอาหารเหลวสูตร MS ที่เติม BA ความเข้มข้น 3 มก./ล. ร่วมกับ NAA ความเข้มข้น 0.5 มก./ล. สามารถชักนำยอดได้มากที่สุด 8.80 ยอด นำเลี้ยงกลุ่มยอดมาเพาะเลี้ยงบนอาหารกึ่งแข็งสูตร MS ที่เติม NAA ความเข้มข้น 4 และ 5 มก./ล. สามารถเกิดตุ่มราก และรากฝอยมีความยาวประมาณ 1-2 ซม. ลักษณะรากอ้วน สั้น มีสีขาว หลังเพาะเลี้ยงเป็นเวลา 12 วัน ไผ่มีอาการต้นเหลืองปนน้ำตาลและตายในที่สุด สำหรับไผ่พันธุ์ซางหม่น พบการปนเปื้อนเชื้อน้อยที่สุด 25.89% จากการฟอกฆ่าเชื้อด้วยสบู่และล้างน้ำให้สะอาด จากนั้นแช่ในแอลกอฮอล์ 70% นาน 2 นาที แช่ในสารโซเดียมไฮโปคลอไรท์ 10 และ 5 % นาน 15 และ 10 นาที ตามลำดับ และแช่ในสารเคมีป้องกันเชื้อราความเข้มข้น 1.5 กรัมต่อลิตร นาน 1 ชั่วโมง และมาล้างด้วยน้ำกลั่นที่นึ่งฆ่าเชื้อ ข้อแขนงไผ่ซางหม่นที่ปลูกนอกโรงเรือนเมื่อนำมาชักนำยอดบนอาหารสูตร MS ที่เติม BA ความเข้มข้น 4 มก./ล. มีจำนวนยอดที่พัฒนาสูงสุด เท่ากับ 4.03 ยอด และมีความยาวยอดเท่ากับ 2.37 ซม. ในการทวีคูณยอดพบว่าการใช้อาหารเหลวหรืออาหารแข็ง ที่เติม BA ความเข้มข้น 2 และ 4 มก./ล., BA ความเข้มข้น 3 มก./ล. ร่วมกับ NAA 0.5 มก./ล. และ MS ไม่เติมสารควบคุมการเจริญเติบโต สามารถชักนำยอดได้ไม่

แตกต่างกัน โดยมีจำนวนยอดเฉลี่ย 7.30 ยอด และพบการเกิดรากจากการเพาะเลี้ยงบนอาหารกึ่งแข็งสูตร MS ที่เติม NAA ความเข้มข้น 4 และ 5 มก./ล. แต่ต้นไม่สมบูรณ์เช่นเดียวกับไผ่ซางนวล และการเพาะเมล็ดไผ่พันธุ์กาบแดง โดยการแกะเปลือกหุ้มเมล็ดออกและห่อด้วยผ้าขาวบางที่ผ่านการฆ่าเชื้อ จากนั้นแช่ในแอลกอฮอล์ 70% นาน 1 นาที โซเดียมไฮโปคลอไรท์ 10 และ 5% นาน 30 และ 20 นาที ตามลำดับ จากนั้นล้างด้วยน้ำกลั่นนึ่งฆ่าเชื้อ พบว่าให้ผลการปลอดเชื้อมากที่สุด (54.03%) และการเพาะเลี้ยงเมล็ดบนอาหารกึ่งแข็งสูตร MS ที่ไม่เติมสารควบคุมการเจริญเติบโต เป็นเวลา 3 สัปดาห์ สามารถชักนำให้เกิดยอดจำนวน 2.60 ยอด มีจำนวนราก 4.00 ราก ความยาวราก 5.71 ซม. จำนวนใบ 3.60 ใบ และความสูงต้น 7.50 ซม. จากนั้นกล้าไผ่พันธุ์กาบแดงที่มีรากสมบูรณ์ย้ายออกจากห้องปฏิบัติการสู่เรือนเพาะชำในวัสดุปลูกแบบต่างๆ พบการแตกหน่อมากที่สุดใน SUT planting soil เท่ากับ 3.00 หน่อ มีอัตรารอดชีวิต 87.03% และมีความสูงต้น 14.60 ซม. อย่างไรก็ตาม การเพาะเลี้ยงส่วนข้อแขนงของไผ่ การชักนำให้เกิดราก และการเกิดต้นใหม่ยังไม่สมบูรณ์ จึงควรศึกษาเพิ่มเติมโดยการหาสูตรอาหารที่เหมาะสมต่อการพัฒนาไปเป็นราก ต้นอ่อน และปัจจัยอื่น ๆ ที่เกี่ยวข้องในการเพาะเลี้ยงต่อไป

สาขาวิชาเทคโนโลยีการผลิตพืช ลายมือชื่อนักศึกษา จารุพัชร โตนกลาง

ปีการศึกษา 2564 ลายมือชื่ออาจารย์ที่ปรึกษา

JANYAPACH RATANAKOM : MICROPROPAGATION OF SANG NUAN (*Dendrocalamus membranaceus*), SANG MON (*D. sericeus*) AND KAB DANG (*Cephalostachyum pergracile*) ADVISOR : ASST. PROF. ARAK TIRA-AUMPHON, Ph.D., 100 PP.

Keyword: *Dendrocalamus membranaceus*, *D. sericeus and Cephalostachyum pergracile* /Plant hormones/Shoot induction/Shoot multiplication/Root induction/Transplanting

Bamboo is a plant that is in great demand in the furniture industry. However, bamboo propagation is limited by environmental conditions, forest fires, and insects. General propagation produces a small number of plants and takes a long time. This research aims to develop suitable tissue culture techniques for Sang nuan (*D. membranaceus*) and Sang mon (*D. sericeus*) using the branch nodes and Kab dang (*C. pergracile*) using seeds by studying methods of bleaching and sterilization, medium formulas for shoot induction, shoot multiplication, and root induction developing into matured seedlings. The study results found that Sang nuan showed the least contamination, at 18.01%, by being sterilized with 70% alcohol for 1 minute, then soaked in 30% hydrogen peroxide for 15 minutes, and soaked in sodium hypochlorite for 10% and 5% for 10 minutes and 5 minutes, respectively, and subsequently rinsed with sterile distilled water. After 2 weeks of shoot induction, in MS medium supplemented with BA at a 6 mg/L concentration, the maximum developed number of shoots were 6.73 with a shoot length of 4.20 cm. After that, the shoots were multiplied in liquid medium formula MS supplemented with BA at a concentration of 3 mg/L and were able to induce the greatest number of shoots were 8.80 shoots. The shoots cultured on MS semi-solid medium formula with the addition concentration of 4 and 5 mg/L of NAA, potentially formed blisters and fibrous roots with a shoot length of 1-2 centimeters. The roots were succulent, short, and white. After 12 days of cultivation, the bamboo showed signs of yellowing brown and eventually died. For the Sang mon variety, the infection rate was found to be the least contaminated at 25.89%, resulting from sterilizing with soap and rinsing thoroughly before being immersed in 70% alcohol for 2 minutes and in 10 and 5% sodium hypochlorite for 15 and 10

minutes, respectively. After that, they were soaked in anti-fungal chemicals at a concentration of 1.5 grams per liter for 1 hour and washed with sterile distilled water. For branch nodes planted outside the greenhouse, when the shoots were induced on MS medium with a BA concentration of 4 mg/L, they had the highest number of shoots developed at 4.03 shoots per node, with a shoot length of 2.37 cm. In shoot multiplication, the use of liquid medium or solid medium with the addition of BA concentrations of 2 and 4 mg/L, supplemented with NAA 0.5 mg/L and non growth regulator-added MS, potentially induced shoots with no difference. The plants had an average number of 6.90 shoots and showed root formation when cultured on MS semi-solid medium with the addition of 4 and 5 mg/L of NAA, but the plants were not as mature as Sang nuan. The seeding cultivation of Kab dang was conducted by removing the seed coats and wrapping the seeds in a cloth filter. They were immersed in 70% alcohol for 1 minute, sodium hypochlorite 10% and 5% for 30 and 20 minutes, respectively, and washed with distilled water for sterilization, providing the most sterile results (54.03%). The seeds cultured on MS semi-solid medium without the addition of growth regulators for 3 weeks were able to induce 3.35 shoots with 4.00 roots, 5.71 cm. in length, and a number of leaves of 3.60, and a plant height of 7.50 cm. The seedlings with intact roots were then transferred from the laboratory to the greenhouse in different planting materials. The highest germination of 2.60 shoots was found in SUT planting soil, with a survival rate of 87.03% and a plant height of 14.60 cm. However, root induction, and the new seedlings were not yet completed. Therefore, further studies should be undertaken by finding suitable formulas for shoot induction and seedlings as well as several other factors in branch node tissue culture.

School of Crop Production Technology Student's Signature Janyapch Kotamkam

Academic Year 2021 Advisor's Signature Arak Tisa-umphon

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Janyapach Ratanakom

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LIST OF ABBREVIATIONS

BA	=	6-benzylaminopurine
H_2O_2	=	Hydrogen peroxide
$HgCl_2$	=	Mercuric chloride
IAA	=	Indoel-3-acetic acid
KIN	=	kinetin (6-furfurylaminopurine)
NAA	=	1-naphthaleneacetic acid
NaOCl	=	Sodium hypochlorite
TDZ	=	Thidiazuron (N-phenyl-N-(1,2,3-thidiazol-5-yl) urea)
2,4-D	=	2,4-Dichlorophenoxyacetic acids

CHAPTER I

INTRODUCTION

1.1 The background and importance of bamboo

The bamboo is a monocot plant that evolved from a grass family plant in the family *Poaceae* (from *Gramineae*), subfamily *Bambusoideae*, and can thrive in a variety of environments. As a result, it is naturally found in almost every part of the world, including the cold climate zone, temperate climate zone, and tropical climate zone. There are tremendous bamboo species, which are held as the original of the bamboo in the south and southeast, in the areas where there are compact breeds distributions (Pattanavibool, 2006). There are currently 47 bamboo families with 1,250 species. For Thailand, which is in tropical zones where bamboos grow up very well. According to the evidence gathered, there are 13 families of bamboos, with approximately 60-90 species. Furthermore, the distribution of bamboo species breeds varies. Some varieties develop in dense forests. Some of them grow up in tropical or mixed deciduous forests. Despite being of the same species, the size of bamboos varies depending on their origin. Bamboos differ depending on where they are fertilized (Henpithaksa *et al.,* 2007). Bamboos are important to humans because they can completely meet four basic human needs. They have benefited both human and animal food, handicrafts, the construction industry, the paper industry, the furniture industry, the charcoal industry, and the charcoal carbon. The culm of Bamboo can be converted into high-quality charcoal with higher energy content than conventional charcoal. Bamboo charcoals and charcoal carbon can be used to purify the air and filter water because of their smooth but porous skin, which has the properties of good absorption and poisonousness reduction (Saranrom, 2006). As a result, the quantity of bamboo culms has steadily increased. Bamboo is a plant that effectively addresses global warming because it grows quickly and can be planted in all regions of the country. Bamboo forests absorb carbon dioxide, produce oxygen, and store carbon in the form of wood

at a rate that is 33, 35, and 70% higher than natural forests, respectively (Phungchik et al., 2013). Furthermore, they absorb twice as much water and humidity as natural forests. Bamboos they absorb twice as much water and humidity as natural forests. Bamboos grown along riverbanks can help to protect against soil erosion along. The riverbank bamboo forest areas can retain moisture and potentially form forested watersheds because bamboo roots can absorb and retain water up to three times their weight. This provides year-round water flows, such as the upstream bamboo forests at Sai Yok and Erawan waterfalls in Kanchanaburi province (Aksornniam, 2011). Bamboo is another plant that can be grown in an empty space. Because bamboo is a plant that nourishes soil fertility by dropping leaves on the ground as fertilizer in dry weather, it can help improve ecological conditions in degraded forest areas. When bamboo roots, which have a fibrous root system that spreads across the soil surface, die, they become fertilizer (Soithongkham, 2004). According to the exportation data of bamboo and rattan products in the world market in 2012, China was ranked the first exporter with a value of up to 1,238 million US dollars, while Thailand was ranked 8th with a value of 18 million US dollars, Regarding the importation data, EU imported them as the first place with a value of 607 million US dollars, followed by the United States with 288.6 million US dollars during the past 6 years (2012-2017). In the past, Thailand has exported bamboo processed products, approximately 3,939 million baht, which has a tendency of declination, while the importation of bamboo products value was about 4,671 million baht, with an increasing tendency. The information from the public hearing A Draft National Bamboo and Rattan Resource Management Master Plan on February 16, 2018, by the Thailand Environment Institute. The potential of bamboo was analyzed as detailed below.

Strengths

- The diversity of bamboo species in the natural forest
- The suitability of weather and geography
- The availability of widespread uses of bamboo
- The domestic bamboo species can be used in diverse ways
- There are several case studies of bamboo utilization and sources of

the bamboo species collection

- Bamboo can be planted together with other economic crops of Thailand

Weaknesses

- Attitudes, negative beliefs towards bamboo, and low perceptions of the uses and values of bamboo
- Lack of good management of using bamboo from natural forests
- The insufficient bamboo resources for serving its utilization economically
- Farmers' lack knowledge of bamboo care and management in Plantations
- Lack of good data management and very limited research for business extension
- The unclear investments supporting the bamboo business and industry development

Opportunity

- The continuously growing demand for bamboo products in the global
- The advanced production technology providing a wide range of bamboo uses
- Using bamboo as a natural material in ecosystems protection and preservation
- International goals and national policies toward sustainable development
- Environmental conservation trends of the world society
- Being a member of the International Network for Bamboo and Rattan (INBAR) of Thailand

Threat

- Reliance on innovation/production technology from abroad
- Unclear and unsystematic policies driven by the country's executives
- Without direct agency responsible for and lack of mechanisms for integrating cooperation between agencies

- Legal restrictions, and regulations which obstruct commercial use

In addition to deforestation in Thailand, which is an important problem rapidly causing the decrease in various bamboo species, the problem of bamboo' s flowering, known as "monocarp" also reduces their quantities. Monocarp means the flowering of bamboo that leaves the entire clump of bamboo dead. However, the age of each bamboo species flowering is still questionable for further study. In general, bamboo has a relatively long-life cycle of 30-100 years depending on each species (Austin *et al*., 1983; Banik 1980). The flowering of bamboo, which is a feature of the bamboo life cycle, is more interesting than the flowering of other plants as it blooms once and then dies without a second chance. Although the flowering period of each bamboo species is not the same, it does not exceed 3 years before it subsequently dies. Despite remaining rhizomes that will continue to grow, they will develop into inflorescences. Even though the bamboo has new shoots, they are of deteriorating quality and do not grow as usual such as thinner wood and eventually develop into inflorescences. Bamboo is believed to have recorded physiological age through its stem. When planting bamboo from rhizomes or cuttings or cuttings or branching, etc., those cultivars will bloom at the same time as the mother plant. Later, the stem will turn yellow and die at the same time as the mother plant (Phutthaphong, 1995). Thailand had a problem with monocarps and catastrophically died off in 1994-1995 due to a large number of Phai tong (*Dendrocalamus asper*)' s monocarps covering 250,000 rai from a total bamboo plantation nationwide of 340,000 rai (Cusack, 2000). In addition, bamboo propagation is limited due to the environment, humans, forest fires, and insects as well as animals eating seeds or bamboo shoots.

For propagation, it can be done by seeding, cutting, or grafting but there are often limitations on the quality of buds or intact branches as well as pathogens entering the plant while propagating. Due to taking time for plant growth, a large number of plants cannot be proliferated; therefore, plant tissue culture techniques were applied to reduce the propagation period for plant tissue culture. Besides, it is one of the ways to produce plants in large quantities, which are germ-free with consistent plant size (Kaveeta, 1997). According to a report (Songsoem, 2018), Sang mon was able to induce

shoots on MS medium supplemented with 2 mg/L BA, while Phai hok, Phai ruak, and Phai tong were able to proliferate well on MS medium supplemented with 2.5 mg/L BA (Godbole *et al.*, 2002), (Dongmanee, 2006) and 5 mg/L (Prutpongse and Gavinlertvatana, 1992) respectively.

1.2 Research objectives

1.2.1 The purpose of this study was to micropropagation of *Dendrocalamus membranaceus* and *D. serviceus* using bamboo nodes explant.

1. 2. 2 The purpose of this study was to micropropagation of *Cephalostachyum pergracile* using bamboo seeds explant.

CHAPTER II

LITERATURE REVIEWS

2.1 Bamboo's general traits and botanical characteristics

Bamboo is native to Southeast Asia. It has a branch of a large-sized and straight stem. The bamboo that has the originality in the temperate zone is a branch off small-sized and has a large stem (Banjomyut, 2000).

Bamboo has an underground trunk called "Rhizome" while the base of the culm above the soil is large and gradually tapering towards the tip of the culm. New shoots will grow out of the bud or bud of rhizome in the underground. Each bamboo culm is composed of part of the segment. The culm is a hollow tube, and the node has a characteristic of a solid flat sheet. The diameter of the culm depends on the species of bamboo which has a diameter from 0.5-20 cm. It was also found that the diameter of the culm depends on the size of the young shoots that grow from rhizomes underground, too. The segment in the middle of the culm is often longer than that located at the base of the culm or the terminal of the culm and has scars of the leaf sheath falling out of the vicinity node of the culm as well. The node of the bamboo culm of some species may distend and may find special roots that grow out of the node near the base of the culm (Banjomyut, 2000).

The bamboo leaf consists of blade, leaf sheath, ligule, and auricles which are in different sizes and shapes according to the species of bamboo as well as the color of the leaf sheath covering the young shoot, having thorns wool or the luster of the leaf sheath which also depend on bamboo species.

The branching of bamboo is found from the base of the culm until the culm end in some bamboo species, but some types of bamboo have branching, only at the culm end (Banjomyut, 2000).

The bamboo flowers are like a bouquet but have different characteristics based on their species. This can be used for classifying bamboo species. In general, a spike

has many groups of spikelets. the flowering of bamboo can be divided into two major categories (Anantachote, 1990) as follows;

- Gregarious flowering: This type of bamboo will be simultaneously flowering such as Pai Ruak (*Thyrsostachys siamensis*), Pai Sang (*D. strictus)*, Sang nuan (*D. membranaceus*) etc.
- Sporadic flowering: This type of bamboo will be sparsely flowering in the area. It may be a clump flowering, or a small group and the flowering often occurs in different times such as Pai Hok (*Dendrocalamus hamiltonii*) (Pattanavibool *et al.,* 2011).

Fruit of bamboo is caryopsis like other plants which are in the grass family. The wall connected to the part of the seed coat. Seeds contain embryo, endosperm, and one cotyledon called "Scutellum". When the seed germinates to be a seedling it will have primary roots developed from the radicles of embryos. For plumule, it will grow up to be a trunk emerging above the ground with tissue wrapping coleoptile at the top of the seedlings (Ban Chom Yut, 2000).

Dendrocalamus bamboo is a medium-sized to large-sized bamboo without thorns. The culm is standing upright with the large-sized culm sheath often falling out rapidly. The sheath blade lis a triangular shape with swollen nodes around. It often has aerial roots around the nodes. 14 species were totally found (Pattanavibool *et al.,* 2001) of which two species including Pai Sang (*D. strictus*), Sang nuan (*D. membranaceus*) and Sang mon (*Dendrocalamus* sp.). Were used to study tissue culture for bamboo propagation.

2.2 Sang nuan (*D. membranaceus*)

Its origin is in Uttaradit province. It has a very beautiful light green culm, with 25-40 cm long internodes. The no-thorn stem is 8-20 m tall, and 6-16 cm in diameter. Its shoots have brown color stained with orange. A one-year-old stem is thoroughly covered with white powder-like flour and used for instruction. In small manufacturers, it is commonly cut into strips, and weaved in various kinds of baskets, while large manufacturers produce food skewers, chopsticks, and incense sticks. However, they

are rarely consumed due to the more demand to use the culms in furniture production (Phungchik, 2010). Sang nuan is popularly propagated using rhizome separation, node cuttings, and seeding (Pattanavibool *et al.,* 2001).

2.3 Sang mon (*D. sericeus*)

Its origin is in the Chiang Dao District, Chiang Mai Province. This kind of bamboo can grow well in relatively high areas. The clumps are not as thick as wild bamboo. It has no thorns. Young stems are covered with white powder-like flour that covers the entire plant. Mature stems are green; when fully grown, they are 10–25 m tall and about 10–12 cm in diameter. Its leaves are green and have a small linear shape. Its nodes are obtrusive, and its internodes are approximately 30 cm long. The flesh at the bamboo base is very thick, about 4-6 cm. The stem is straight, beautiful, and very strong. Due to its good quality with sticky texture, it is suitable for making furniture (Phungchik, 2010). Sang mon is in demand in the market and can be sold at a good price. It is regarded as a significant economic bamboo in the country (Dongmanee, 2006).

2.4 Kab dang (*C. pergracile*)

This kind of bamboo can be found in the mixed forests of foreign countries, including Myanmar, East India, and Nepal. It is the bamboo of the rhizome type, 5–15 m tall, with a straight stem of which curved ends compacted into clumps. Its diameter is 2–8 cm, with 20–50 cm long internodes. It has a thin-walled hollow culm that is grayish-green when matured and covered with white droplets on the skin. Many small branches sprout around the nodes with lanceolate-shaped leaves. A culm leaf- sheath, which is long-lasting, is yellowish-brown or reddish-brown. It is thick, hard but easy to break and covered with black hair. The top leaf- sheath is in a triangular shape with a spiny apex. Its auricles are in a distinctive lobe, pleated or wavy. A ligule is a narrow strip with white hairs along its rims. It is useful for making wicker and household utensils. Its stems are used for construction, making wickerwork, and Khao Lam (a bamboo sticky rice dessert) because it is easy to burn and peel. Although young shoots can be consumed, they are not popular because of their bitter taste. (Pattanavibool *et al.,* 2001).

2.5 Propagation of bamboo

There are two ways of bamboo propagation: sexual propagation and asexual propagation, which includes plant tissue culture. For sexual reproduction, or seeding, only some bamboo species can be planted due to a long flowering period of about 30–100 years (Austin *et al.,* 1983) and the flowering period of each bamboo species is uncertain. In addition, the seeds had low germination (Anantachote, 1991) and the obtained seedlings took a long time to develop into a complete bamboo clump with a typical stem size (Suwannapinunt, 1983). Asexual propagation can be done in several ways, such as propagating by separating culms associated with rhizomes. This method is time-consuming and labor-intensive, with either a low survival rate of production or no rhizome development. A bamboo plant can be propagated by layering and cuttings, but these propagations cannot rapidly increase the number of plants because the varieties have a low survival rate as well. (Forestry Department, Bureau of Forest Economics and Product Research, 2004). From the mentioned problems, tissue culture techniques were introduced in order to produce large quantities of seedlings in a short period of time with an unchanging size and disease-free condition. The appropriate method depends on the species and their growth patterns. There are five commonly practiced propagation methods (Pattanavibool *et al.,* 2011).

2.5.1 Propagation by using seeds

Bamboo seeds of all kinds will be extremely matured in February to April. Keeping seeds in natural forest conditions should be done while the seeds are still attached to the tree as in some areas, the bamboos grow up on the steep area with uneven soil. This makes it impossible to collect seeds. Besides, bamboo seeds are the food of many kinds of animals. Due to their thin husks, it is easy to be damaged by diseases and insects. Collecting seeds after they had fallen onto the ground will then get poor-quality seeds with a low germination percentage. When the seeds fall into the ground, they should be collected immediately before the animals and insects destroy or damage the seeds. For collecting seeds when they are still attached to the plants, always keep checking the seed maturity by cutting and investigating some branches to see the seed maturity. The seeds may be collected from plants or maybe

shaken to let them fall. After checking the seed maturity, put the plastic covering the area where the seeds will fall down. Then cut down bamboo culms and try to position the branches producing seeds to fall on plastic. Harvest bamboo seeds by this method requires the collector's experience as harvesting during the too old seeds, cutting the culms down may cause the seeds to fall out. After cutting the culms down, cut the branches with the seeds available and pack them in the sacks, let the seeds be exposed to the sunlight for approximately 2-3 days to make the seeds fall out and reduce the seeds moisture. It is the way to prevent diseases and insects that will do harm them, then clean the seeds by using the blowers or human workforce. In general, bamboo seeds provide a very high percentage of germination up to 70% if cultivated after being immediately collected. If the seeds are need to be stored or distributed, they should be packed in plastic bags beforehand along with an attached label showing the bamboo species, their sources, and the date/month/ year of collecting seeds. Harvesting seeds and storing them at temperature of 2-4 ℃ will allow the seeds to be kept alive for 2-3 years. Keeping seeds at room temperature makes them lose viability within 6-7 months (Ramyarangsi, 1990). The advantages of this propagation method are there may be a process of natural breeds improvement at the beginning of the cross-fertilization. This makes it possible to select bamboo species with good mutations resulting in producing a lot of seedlings each time and convenient to transfer them. However, this bamboo propagation method is unpopular for commerce because of the difficulty in storing seeds, too much cost expenses. In addition, the seedlings take a long time to grow up and give slow productivity, which takes at least 10 years or more to get the bamboo clumps in the normal size (Suwannaphinan, 1985). Moreover, there are also many obstacles. For example, bamboo cannot produce seeds because of sterile flowers, or most seeds are incomplete. This causes a low germination percentage; the flowering period is unstable; sometimes it may be found in a recent planted period for only 2-3 years. However, it sometimes may be expected to wait for flowering about 30 – 40 years, bamboo seeds have an unstable germination rate (10-80%) (Anantachote, 1984; 1987) depending on the time period of seed harvest as well as the condition of the area and weather while falling seeds and devastation

from diseases and insects, some types of bamboo seeds are rare because it was immensely damaged by animals, insects, forest fire and diseases. There are studies of fungi, attached to bamboo seeds, on 5 bamboo species. all fungi up to 48 species were found. Most fungi were the same species as those on rice kernel which is in the same family plant (Pongpanich, 1985). Some fungi only made the seeds spotted, dirty, and deteriorated faster than normal but some resulted in damaging seedlings (Pattanavibool *et al.,* 2001)

2.5.2 Propagation of bamboos by rhizome cutting (rhizome or clump separating)

This method is effective for all types of bamboos, especially bamboos having a rather thick culm such as Pai Ruak, Pai Lang, Pai Hang chang, etc. The rhizomes age that can well produce new shoots is 1-2 years (Laohasiri, 1984) because the bud of the rhizome older than 2 years is often weak, not strong. Cutting should let a stump left 50-80 cm and then dig the rhizome from the parent clump by being aware of not damaging the rhizome because this bud will sprout a new shoot. Due to the propagation of this method using the parent's rhizome which has accumulated a lot of food, it leads to high survival rate, and the shoots will be strong and grow faster than by using branch cutting or culm cutting for propagation. It's also the method of getting straight culms most according to the original species. However, it is not popular due to the costly expenditure, difficulty in transportation, waste of time, and workforce. In addition, they cannot be propagated in large quantities in the short term because digging too many clumps may cause the original clump to be harmed.

2.5.3 Propagation of bamboos by culm cutting using 1 node and 2 nodes

By this method, it is commonly used for bamboo species that rarely produce seeds and have quite large culms such as Pai Pa, Pai Si suk, Pai Hok, Pai Sang kham, etc. By selecting the saplings aged approximately 1-2 years. When getting desired culms, bring them to cut into segments having 1-2 nodes and carry out the following procedures;

For 1 node explant, the propagation can be done by cutting in the middle of the bamboo culms keeping the node in their centers (cut them far from the nodes about 1 palm span). Then, take them to the branch cutting in a nursery bed turning

the buds to face up and being careful to damage them. For cutting, the node should be at the soil level and tilt about 45 degrees in order that the new shoots will stand straight. Then fill the remaining culm above the soil with water or the nursery materials.

For 2 nodes explant, after cutting the bamboo culms having 2 nodes, drill the hole in the middle of the culm for casting water. Then, rip the branches at the node out by cutting them out and letting only 2-3 inches left. Be careful not to crack the buds and place them in the nursery bed by embedding them into the soil or plant material about ½ of culm and cover the nodes with soil leaving only the area for casting water. Culm cutting can be cultivated in nursery beds. The soil should be adjusted in case of highland where a flood cannot reach. For the lower areas, the grooves should be made for good drainage. After that, the soil should be shredded and let be exposed to sunlight for about 1-2 weeks or more. Next, make a roof using coconut leaves to protect it from the sun. When the cutting is finished, the culms should be watered immediately and regularly taken care of it by watering it every day or every other day. After about 2-4 weeks, the shoots and roots will be sprouted (Laohasiri, 1984). At this stage, to prevent insects or aphids harming the young shoots, fungicides and pesticides will be sprayed. Then, continuously raise young shoots for keeping the shoots and roots extremely strong for about 1-2 months before sawing old culms out, and then move the cuttings into 5 × 8 inches plastic bags for the convenience of moving them. When the seedlings grow up completely, the covering roof will be gradually opened until they can grow well outdoors for about 6-8 months before transplanting. However, this method is not very popular because it takes a long time and high cost more than using branch cutting. Moreover, the 1- year culm which is the culm parent should be raised for new shoots in the following year. In addition, it's common to store culms to sell for other benefits as well (Pattanavibool *et al.,* 2001).

2.5.4 Propagation of bamboo by branch cutting

The branch is a part of the tree that sprouts from the bud on the node area, this propagation method is popular because it is convenient and quick and provides the branch cuttings so much that farmers do it as an occupation such as bamboo seedlings of Pai Tong sold in Prachinburi. The success of cuttings using branches

depends on bamboo species. If it is a bamboo species that has air roots at the base of the branches such as Pai Tong, the success will be highly achieved. It also depends on the selection of branches and the attention to branches in the nursery beds as well. The appropriate season for the branches cutting is the end of the rainy season (September - October) until February which is the period of a lot of branches are available. Selecting branches should consider the branches having a diameter of 1-1.5 inches, having air roots in brown or yellowish-brown, the pulmule already unfolded and the leaf sheath completely falling out branches aged at least 4-6 months or over 1 year which will be even better. When choosing the branch as required, cut off its end to be 80-100 cm long (having 3-4 nodes) and should use rice straw or gunny bags soaked in water to cover the branch for bud curing and stimulating the buds on rhizomes and buds on the base of the branches. After 2-3 days of the bud curing, the white button on the base of the branches can be observed. The mentioned buttons will grow and become to be rooted. In some branches, by curing bud, the extended terminal bud may be seen, and it is ready to sprout, this results in the selection of branches in good quality. Therefore, branch cutting is an effective propagation method that develops strong branches and has high survival percentage. The procedures take about 8 months (Pattanavibool *et al.,* 2001).

2.5.5 Propagation of bamboo by tissue culture

At present, the plant tissue culture technique has been used to extensively benefit agriculture and forestry because it is a method conducted in sterile conditions which makes it convenient to look after and maintain breeds in a large number in a short time and use in the cryopreservation and breeding, etc.

2.6 Propagation by tissue culture

According to the bamboo propagation by tissue culture technique, the researcher studied its culture by using different explants, including embryos, shoots, nodes, anthers, seeds, and inflorescences, as follows:

2.6.1 Embryo culture

The embryo of Sans mon was cultured on a medium of White (White, 1963) and Nitsch Formula (Nitsch, 1969) with 12–16 hrs of light per day. The embryo took

3- 5 days to germinate and germinated well on a medium with a 2% sucrose concentration (Alexander and Rao, 1968). Then, a white-formula medium was used to culture *B. arundinaceae* and *D. strictus* (Ravikumar *et al.,* 1998). After that, Murashige and Skoog (MS) medium formula (Murashige and Skoog, 1962) was used in the cultivation of *B. arundinacea* (Joshi and Nadgauda, 1997) and *B. tulda* (Saxena, 1990). In addition, half-concentrated MS (½ MS) medium was also used in the *B. arundinaceae* culture (Joshi and Nadgauda, 1997).

2.6.2 shoot culture

Kentri (1999) has studied the propagation of Pai Tong grown in a sterile condition. It was cultured on MS media supplemented with 0-5 mg/L BA for 15 days. The medium containing 4 mg/L BA was found the most suitable for shoot multiplication. Shoots were taken to make root induction on MS media supplemented with 0-1 mg/L BA and 0-3 mg/L NAA for 1 month. The maximum root induction percentage was obtained on medium supplemented with 2 mg/L NAA. Then, the bottles containing bamboo plantlets were kept at 30°C for 7 days and were transplanted into mixed soil and covered with a plastic bag for another 30 days. The survival rate of bamboo was found at 99.3%.

Saxena (1990) found that the propagation of *B. tulda Roxb* was carried out using the apical tip of the 3-years-old plant to be cultured on Murashige and Skoog (1962) liquid medium, supplemented with 6-benzylaminopurine (BA) at al concentration of 8×10^{-6} molar (1.8 mg/L) and KIN at a concentration of 4×10^{-6} molar (0.9 mg/L). This technique produced the bamboo shoots 4- 5 times every 3 weeks, and 90% of root induction was obtained on a modified MS medium supplemented with IAA at a concentration of 1×10^{-5}molar (0.02 mg/L) and coumarin at a concentration of 6.8×10^{-5} molar (4. 5 mg /L). More than 80% of the transplants into the planting plot were successful.

2.6.3 in vitro culture of node

Chanthanurak (1991) did experiments, using nodes from seedlings to be cultured on an MS medium containing BA associated with NAA. The best shoot multiplication was achieved on MS medium enriched with at 1 mg/L BA, associated

with at 0.5 mg/L NAA, in *D. strictus* and *T. siamensis* Gamble at 88.9%, and 77.8% respectively.

Prutpongse and Gavinlertvatana (1992) experimented by propagating 54 species of bamboo from 15 genera in vitro using nodes of stems and cultured them on an MS medium, adding BA at a concentration of 22 µmol (5 mg/L). 43 shoots emerged within 30 days in *D.* asper nodes and were rooted on an MS medium supplemented with NAA at concentrations of 2.7–5.4 µmol (0.5–1 mg/L). In addition, the shoots of many bamboo species were potentially stored in ½ MS formula at room temperature for more than 15 months without changing the medium.

Rout and Das (1944) cultured tissues from the nodes of *B. vulgaris*, *D. giganteus*, and *D. strictus* on an MS medium supplemented with 0.5 mg/L KIN, 2 mg/L with 2,4-D, 10 mg/L adenine sulphate, and 3% of sucrose. Somatic embryos were formed and developed into plantlets 95–98% of the time. In addition, 95% of them were able to be successfully transplanted.

Pattanavibool and Ramyarangsi (1997) studied the tissue culture of young buds on a node of *B. nana* Roxb, it was found that the bamboo bud in medium and large size has a sprouting rate up to 100% when cultured on MS medium supplemented with 4.5 mg/L BA with the outshoots of 8.7-17.5 shoot/clumps. For root formation, it can be achieved up to 90% on MS medium supplemented with 1.9 mg/L NAA and 2% sugar for 25 days.

Godbole *et al.* (2002) took the young shoots from the nodes of *D. hamiltonii* to induce callus on an MS medium containing BA at a concentration of 1 mg/L and 2,4-D at a concentration of 1 mg/L. Later, they were brought to culture on an MS medium supplemented with 2.5 mg/L BA concentration. Somatic embryos were induced and cultured on an MS medium without growth accelerators. Within 21 days, 80% of seedlings developed into plantlets. When they were removed from the nursery to be planted in the ground, 78% of them survived.

Lin *et al.* (2004) tissues from bamboo nodes explant on an MS medium containing 0.046 µm TDZ (0.01 mg/L) supplemented with 2,4-D, 13.6 µm (3 mg/L), and 3% sucrose. Embryos were formed and subsequently cultured on an MS medium

containing 0.455 µm TDZ (0.1 mg/L). 80% of them potentially developed into plantlets.

Songsoem *et al.* (2018) has studied the effects of BA and NAA on shoot induction of Sang mon by taking nodal explants for surface sterilization with 10% Clorox for 10 min; it was found that it is the most suitable survival percentage of 66.67%. Then, take sterilized nodal explants to be cultured on MS medium supplemented with 1 - 3 mg/L BA and 0 - 0.5 mg/L NAA for 4 weeks. The results showed the highest number of regenerated shoots of 3.31 ± 0.85 shoot

Deelom (2019) experimented with shoot induction of *Dendrocalamus* sp. It was found that the modified MS formula solid medium containing 0.6 mg/L TDZ and the modified MS formula with 2.5 mg/L BA, associated with 0.5 mg/L TDZ, induced the maximum average shoots at 4.7 and 4.9, with a length of 4.0 and 3.6 cm from each node, respectively.

Choudhary *et al.* (2022) studied the effects of different concentrations of cytokinins on shoot number and shoot length of *B. tulda* Roxb and *D. stocksii* and found that *B. tulda*, cultured on an MS + 2.5 mg/L BA +0.1 mg/L NAA produced the most induced shoots (5.9±0.64 shoots). When cultured on an MS + 1 mg/L BA + 0.1 mg/L NAA, the highest shoot was 5.5±0.63 cm. *D. stocksii*, cultured on an MS + 0.25 mg/L TDZ + 0.1 mg/L NAA + Additives. Produced the most induced shoots, while cultured on an MS + 0.1 mg/L TDZ + 0.1 mg/L NAA + Additives, the highest shoot was 7.93±0.24 cm.

2.6.4 Anther culture

Tsay *et al.* (1990) induced callus by culturing anthers of Taiwan giant bamboo *Sinocalamus latiflora* Munro McClure on the medium formula of Chu *et al.* (1975), which contained 1 mg/L 2,4-D, 1 mg/L BA, 2 mg/L charcoal, 0.8% agar, and 9% sucrose. They found that binucleate in the mid-uninucleate to early stage had a good response to induced callus to develop into somatic embryos and subsequent rooting. The counted number of chromosomes from the apical cells of bamboo roots, originating from anthers was half (n = 36) of all chromosomes

2.6.5 Seed culture

Yeh and Chang (1987) found that the plant regeneration process from callus

obtained from the zygote culture of Taiwan giant bamboo (*Sinocalamus latiflora* Munro McClure)was successful by inducing callus on an MS formula medium comprising 6 mg/L 2,4-D and at 3 mg/L KIN, at 250 mg/L polyvinylpyrrolidone and 5% sucrose. In the same formula, callus was subsequently cultured on an MS medium containing at concentration of 3 mg/L 2,4-D and at a concentration of 2 mg/L KIN, developing into seedlings and intact plants.

Chanthanurak (1991) propagated seeds of *D. strictus* and *T. siamensis* and found 2,4-D at a concentration of 3 mg/L induced the seeds and seedlings of *D. strictus*, *B. arundinaceae*, and *T. siamensis* to produce callus. In the same formula, callus could be developed to be somatic embryos and seedlings after being cultured on a medium without 2,4-D.

Woods *et al.* (1992) separated embryos from Mexican Weeping bamboo seeds (*Otatea acuminate* spp. Aztecorum, Yushania aztecorum) and cultured them on an MS medium and the B5 formula of Gamborg *et al.* (1968) in the dark and light, they developed into seedlings. However, when cultured in the dark on an MS medium containing 2,4-D at a concentration of 3 mg/L BA at a concentration of 0.5 mg/L , and 2% sucrose, more than 95% of the embryos developed into shoots and roots. When they were transplanted into planting plots, 85% survived.

Ravikumar *et al.* (1998) cultured the seeds of *D. strictus* on White's formula (1963) medium and multiplied the number of shoots from the lateral buds of mature plants on an MS medium containing 0.5 mg/L BA and 0.5 mg/L KIN. 34–35 shoots sprouted within 24–25 days, inducing roots on an MS medium supplemented with IBA at a concentration of 0.25 mg/L.

Saxena and Dhawan (1999) cultured the seeds of *D. asper* Bakcer on an MS medium containing 3×10^{-5} molar concentrations of 2,4-D (6.6 mg/L). They developed into calluses. When transplanted to be cultured on an MS medium containing 3×10^{-5} molar concentrations of 2,4-D (6.6 mg/L), 3×10^{-6} molar concentrations of KIN (0.7 mg/L), 2×10^{-6}molar concentrations of IBA (0.5 mg/L), and polyvinylpyrrolidone (PVP) 250 mg/L, inducing plantlets 2–5 times every 5 weeks. The roots were induced in the dark on an MS medium supplemented with NAA at a concentration of 3×10^{-6} molar (0.7 mg/L) and

IBA at a concentration of 2×10^{-6} molar (0.5 mg/L). They were 80% alive when removed to be transplanted.

Kentri *et al. (*1999)cultured *D. asper* Bakcer seeds on MS medium supplemented with BA at a concentration of 2 mg/L. The seedlings were brought to increase the number of shoots on the MS formula with a BA concentration of 3 mg/L, inducing a large number of shoots, which were divided into 3 shoots each. They were cultured on an MS medium containing a concentration of 4 mg/L BA. It was found that the highest number of shoots was 9.1 shoots in 15 days. For root induction, when cultured on MS medium supplemented with solely NAA at a concentration of 3 mg/L, using 3 shoots per cluster had the best root induction at 90%. After in vitro acclimatization at 30°C for 7 days, the seedlings were transplanted and covered with plastic bags for 30 days. The average survival percentage of bamboo plants was 99.3%.

Suda (2021) cultured the seeds of *D. brandisii* Munro Kurz. The experimental results showed that seed germination induction on an MS medium supplemented with BA at a concentration of 2.0 mg/L in combination with 0.5 mg/L of NAA had the highest tendency at 91.67%. For growth, an MS medium supplemented with 2.0 mg/L BA induced the optimal number of *D. brandisii* Munro Kurz shoots (4.46 shoots) and maximum root length when cultured on an MS medium supplemented with 0.5 mg/L NAA. The developed shoots had the maximum stem height, root length, leaf length, and leaf width when they were cultured on an MS medium without the growth regulators.

2.6.6 Inflorescence culture

Lin *et al.* (2003) experimented to take the inflorescence of *B. edulis* and *B. odashimae* to induce shoots on MS medium supplemented with 0.1 mg/L TDZ and 3% sucrose and developed until flowering. From the experiment, it was found that cytokinin can induce flowering while NAA affects root induction and does not affect the flower size. After flowering, the bamboo can survive and grow normally.

2.7 Factors affecting bamboo node tissue culture

Although all plants normally need the same macronutrients, they need them in different quantities or concentrations. Especially, in the case of plant growth

regulators, the demand is highly different, therefore, selecting medium for plant tissues culture should be considered;

2.7.1 Species and cultivars

Different plants and different species most often need nutrients that are not the same (Kaveeta, 1998). For example, Songsoem (2018) reported that the shoots of Sang mon could be induced on MS medium supplemented with 2 mg/L BA. In Pai Hok and Pai Tong could increase the number of shoots well on the MS medium supplemented with 2.5 mg/L BA (Godbole *et al.,* 2002).

2.7.2 Age and stage of development

Even the same plant, if the age and stage of development are different, they may require different nutrients (Kaveeta, 1998).

2.7.3 Explant materials

The plant in the same species or even in the same stem but using different explant materials such as using the shoot for tissue culture, it requires a medium formula that is different from the medium used to culture roots or leaves (Kaveeta, 1998).

2.7.4 The physiological condition of the mother plant

The age of plant and environment in which the plant grows affect the plant to respond to tissue culture. The plant should completely grow, be strong, and have non-disease traits. The weak plant naturally desperately responds to tissue culture. The plant which has received a large quantity of chemical substances for pesticide protection, when bringing to tissue culture, it is often unsuccessful to develop to be induced plant (Rotwiboon, 2010).

2.7.5 Light and temperature

Although the culms are green, they do not rely on photosynthesis to produce their own food. As plant growth as heterotroph, medium all comes from medium for culturing. Light is necessary only to morphogenic process. The intensity of light used for culturing 1,000–5,000 lux is sufficient (Hussey, 1980). Light intensity is optimal for the increase in the number of Gerbera and many types of other annual crops. Light intensity about 1,000 lux is optimal. If the light intensity increases to 3,000 lux, it will

inhibit the plant. ON the other hand, if the light intensity is little, the stems will be weak and the stems will stretch more than normal. The duration of giving light is 16 hr during the day and 8 hr at night is the most suitable period (Murashig, 1977). Culture has been kept in regular temperature of about 25 ± 2 ℃. Rooting in vitro s is the stage before it will be taken for planting. Therefore, in this period, the plant must adjust itself to withstand the impact during transplantation. Reducing the concentration of sugar (about 1%) in a medium for rooting and increasing the light intensity (1,000-3,000 Lux) in the period of rooting will help it change from heterotropic to be autotrophic to develop the food for itself by the photosynthesis process. The light intensity greatly stimulates root growth and strengthens plants and helps them withstand moisture and diseases. Under the very high light intensity, the plants may exhibit slow growth with a little chlorosis. But when planted, the survival is better than those with green color and stretches under low light intensity (Murashig, 1977).

2.7.6 The composition of culture medium

The success of tissue culture depends on one significant factor referring to choosing the suitable medium which contains nutrients the plant can apply effectively (Tantasawat and Waranyuwat, 2008). For medium formula for node culture, there are studies and experiments for medium used for tissue culture reported as MS (Murashige and Skoog, 1962). These basic mediums are adapted by adding various substances varied to both type and concentration such as plant growth regulators, types of carbon sources, amino acid, vitamins, and others which include coconut juice (Rotwiboon, 2010).

2.7.7 Hypersensitivity reaction

Hard or old plants contain tannins and substances in the Hydroxy phenol group as cell constituents. They often release phenolic oxidation, causing the culture medium to change color because the phenol oxidase or tyrosinase enzymes react with air or substances such as auxins or cytokinin causing browning or blackening, resulting in the tissue not growing. When some kinds of plants are wounded, they form compounds that react with air and cause tissue toxins. Methods to prevent browning or blackening are 1) Remove phenolic substances from tissues by first soaking or washing them with

clean water or adding activated charcoal or carbon into the medium to absorb toxins. 2) Reduce the chance of reacting with air by using chemicals called antioxidants such as ascorbic acid, citric, L- cysteine hydrochloride, dithiothreitol, glutathione, mercaptoethanol, etc. Before being cultured, it can help reduce symptoms of toxicity by soaking the explants in the mentioned solution for a while. 3) Use a liquid medium in the early stages of culture to expose the explants to a diluted phenolic substance group solution. After that, transplant them to culture in a solid medium. 4) Alter the medium as frequently as possible during the early stages to reduce the accumulated substances in the phenolic group. 5) Use the medium supplemented with substances in the polyamides group such as polyvinylpyrrolidone (PVP) at a concentration of 0.01-2.00% to absorb the phenolic compounds. 6) Use a medium supplemented with antioxidants such as vitamin C and citric acid to inhibit oxidation, which is the fundamental reaction causing browning. 7) Reduce the potassium, sucrose, and growth regulators in the tissue culture medium. 8) Reduce the light intensity to which the explants are exposed in the early stage of culture, as it can cause oxidation. Among the wholly mentioned methods, preventing Brown disease may require more than one method for effective results.

2.8 Morphogenesis in Plant Tissue Culture

The two types of Morphogenesis include embryogenesis, the process by which the body cell changes its shape to a structure with both shoots and roots, developed in the same growth stage as the embryo formed by the merger of gamete cells, called a somatic embryo or embryoid, or adventive embryo. The somatic embryo can occur in two ways: direct embryogenesis, which is the formation of a somatic embryo directly from the cell, or group of cells without a callus, and indirect embryogenesis, a somatic embryo from a callus, also known as an embryogenic callus, cultured on callus-inducing medium. When callus is formed. It is then transplanted to culture in a medium supplemented with low concentrations of auxin or without auxins. This method is more common than that in the first procedure (direct embryogenesis). Another type is organogenesis, the process of forming organs from groups of cells whose characteristics are like meristem tissues. They are small in size with little vacuole but dense cytoplasm.

A large starch seed is capable of cell division, called a meristemoid or nodule. From this point, it can develop into a single type of organ. In this process, the regeneration can take two forms: direct organogenesis, which is the direct regeneration of an organ from tissues, apical or lateral buds without callus formation. Indirect organogenesis is the regeneration of an organ from a callus obtained from explant culture. An embryogenic cell is a cell developed either directly into a somatic embryo, or indirectly through a callus with characteristics like a meristemoid cell, the origin of an organ, as shown in Figure 2.1 (Nawapanich, 2006).

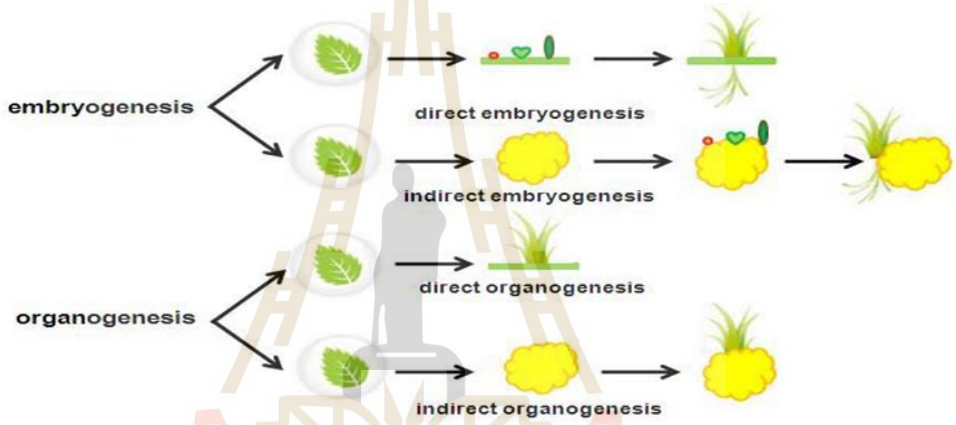


Figure 2.1 Morphogenesis in plants when cultured in a sterile condition
Source: http://encrypted-tbn1.gstatic.com

2.9 Plant tissue culture

Plant tissue culture is the use of cells, tissues, or organs that are plant meristematic tissues to be disinfected and cultured in a sterile synthetic medium under suitably controlled environmental conditions such as temperature, light, humidity, etc. (Kaveeta, 2002). These explants can grow and develop into many forms, such as organs, calluses, or embryoids. However, they can induce a large number of plants, etc. (Sahawatcharin, 1999). Because they have a property called totipotency, which is the ability of plant cells to grow into an intact plant (Chamrasphan, 2003). This technique is necessary to cultivate the explants in the right medium for each plant species. Each formula has a different composition (Phutalun, 2008). The formula of tissue culture medium generally contains the same nutrient components and substances that are

sources of carbon, amino acids, and vitamins but in different quantities, while the types and concentrations of growth regulators supplemented with the medium vary according to the plant species, age, and explants, tissue culture goals, and medium condition (Kaveeta, 2002) . Plant tissue culture consists of four steps: explant disinfection; shoot induction and shoot multiple; root induction; and transplanting of plantlets with roots. Each step has important principles and methods as follows:

2.9.1 Explant Disinfection

The main principle of plant tissue culture is sterilization. The clean explants are cut and cultured in sterilized vitro containing the medium that has been autoclaved (Sahawatcharin, 1999). The cultured plant tissues should be washed with soapy water or detergent several times depending on the explant species. The microorganisms are then disinfected with Clorox solution, the most commonly used disinfectant for tissue culture, at a concentration of 5- 30% In addition, a drop of liquid soap such as polyoxymethylene sorbitan monolaurate (Tween 20) should be applied to disinfection to reduce the surface tension between water and plant tissue. Then, rinse it with distilled water several times to remove any disinfectant attached to the tissue. The damaged tissue, often at the apical tissue, should be removed, only the intact tissues were cultured on the medium (Kitwijan, 2004). The disinfection of various types of explants including seeds and nodes has been reported. Chanthanurak (1987) took the seeds of *D. strictus* and peeled off their seed coats. The seeds were then soaked in 3-4% H_2O_2 with 2-3 drops of Tween 20 in a tightly sealed container for 15 min, then rinsed 3 times with sterilized distilled water Kentri *et al.,* (1999) took the seeds of *D. asper* and peeled them off the seed coats, and placed them in running water for 15 min. The seeds were then immersed in 0. 62% tetracycline mixed with 0. 22% benlate fungicide. The bottle of the solution was placed on the shaking machine at 120 rpm for 3 hr. After that, their surfaces were sterilized with 10% Haiter, supplemented with 2 drops of wetting agents for 15 min and 5% of Haiter supplemented with 2 drops of wetting agents for 10 min, and then washed 3 times with autoclaved distilled water. Songsoem (2018) reported the sterilization of node tissues explant with Clorox at a concentration of 10% for 10 min and found that the percentage of survival rate was

optimal. The sterilization method regarding duration, concentration, and disinfectant type showed the following results.

Table 2.1 Various disinfectants used for sterilizing plant tissue surface.

Disinfectant	Concentration	Duration (min)	Effectiveness
Calcium hypochlorite	9-10%	5-30	Very good
Sodium hypochlorite	2%	5-30	Very good
Hydrogen peroxide	10-12%	5-15	Good
Bromide water	1-2%	2-10	Very good
Silver nitrate	1%	5-30	Good
Mercuric chloride	0.1-1%	2-10	fair
Antibiotics	4-50 mg/l	30-60	Rather good

Source: (Kitwijan, 1997)

Table 2.2 Sterilization of various bamboo types.

Bamboo Species	Scientific Name	Explants	Disinfectants	Concentration (v/v)	Duration (min)	Ref.
Pai Tong	*D. aspe*	node	$HgCl_2$	0.2%	5	Arya *et al.* (1999)
Pai Sang	*D. strictus*	seed	$HgCl_2$	0.1%	5	Reddy (2006)
Pai Ruak	*T. siamensis*	node	NaOCl	10.0%	10	Dongmanee (2006)
Pai Sang	*D. strictus*	node	$HgCl_2$	0.1%	5	Goyal *et al.* (2010b)
Pai Hok	*D. hamiltonii*	seed	NaOCl	2.5%	30	Zhang *et al.* (2010a)
Pai Poah	*D. giganteus*	seed	NaOCl	20.0%	20	Devi *et al.* (2012)
Pai Hok	*D. hamiltonii*	apical tip	NaOCl	1.0%	15	Zang *et al.* (2016)

Table 2.2 reports the sterilization methods used in bamboo tissue culture of various types of bamboo explants, including nodes, seeds, and apical parts. The disinfectants used were $HgCl_2$ at a concentration of 0.1–0.3 for 5–10 min and NaOCl at a concentration of 10– 20% for 10– 20 min. In the case of a sensitive part such as the apical parts, it would be used at a concentration of 1% or a low NaOCl concentration

(2.5%) in *D. Hamiltonii*, but with a sterilization time of up to 30 min. In addition to the study on concentration and sterilization time in different bamboo species, there was also a report about how the season affects disinfection. Dongmanee (2006) disinfected the nodes of *T. siamensis* with a 10% NaOCl concentration for 10 min. It was found that the nodes were most sterile in March, April, and May at 82.00, 76.10, and 78.70%, respectively, while disinfection efficiency decreased in the range of 32.50 - 46.60% from June- October due to the high humidity during the rainy season. As a result, microorganisms are attached to the hairs and grow along the leaf axillary of the node explants, resulting in a higher percentage of contamination and difficulty for sterilization. Another reason for not being sterile is weevils drilling into shoots and aphids sucking the sap of shoots and young shoots along the nodes used for tissue culture (Choldumrongkul and Atirattanapunya, 1993). Thus, the nodes cannot be sterile made when their nodal surfaces are disinfected. The sterilization of their tissues begins with Clorox in different concentrations and times. This method is successful in bamboo species such as *B. edulis* (Lin and Chang, 1998), *D. strictus* (Lin *et al.*, 2007), and *D. hamiltonii* (Godbole *et al.*, 2002).

2.9.2 Shoot induction and shoot multiple

Shoot induction and shoot multiple need explants to be cultured on a suitable medium for propagation. Generally, plants need the same essential nutrients but in different amounts of concentration. In particular, the growth regulators that the plants need are much different from each other based on their species (Kaveeta, 2002). Which are very important in plant tissue culture. Most plants develop into organs if they receive two hormones: auxin and cytokinin, depending on their species, growth stage, and the proportional level of both types of hormones used in the medium formula. The properties of the two groups of growth regulators are as follows:

Cytokinin: Sources of cytokinin synthesis in plants are in young organs such as seeds, fruits, young leaves, and root tips (Charassamrit, 1994). BA is a widespread growth regulator due to its relatively inexpensive. The concentrations of cytokinins used are in the range of 0.5-30 mg/L but are generally used at only 1-2 mg/L. When using high concentrations, they stimulate adventitious bud formation, which is formed from any

part other than the leaf axillary or apical tips and possibly forms a callus (Kitwijan, 1997). Cytokinin has an effect on the apical dominance of lateral shoots and shoot proliferation. They help stimulate cell division in the process of cell differentiation, retard the aging of leaves, expand the size of cells and synthesis potential of pigment (Kaveeta, 1997; Kanchanapoom, 1999), in addition to stimulating lateral bud growth (Charassamrit, 1994; Kitwijan, 1997) The most popular cytokinins used are listed as follows: (6-Benzylaminopurrine; BA), (6-furfuryl aminopurine; KIN), (N-phenyl-N'-1,2,3-thidiazol-5-yl urea; TDZ), and (N-isopentenyl aminopurine; 2iP).

The substances in the cytokinin group used in this study are as follows:

1) BA is a synthetic cytokinin. It is very active and is not found in plants (Charassamrit, 1994). It is commonly used in combination with NAA to induce embryoid formation. BA is usually used alone to induce shoots (Chuichai, 2010) and reduce the apical dominance of lateral buds (Thongampai, 1986) at a concentration of 0–10 mg/L (Chamrasphan, 2003).

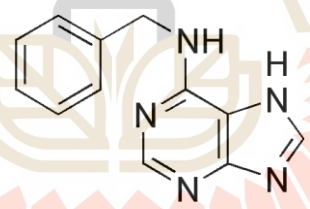


Figure 2.2 BA structure

Source: https://en.wikipedia.org/wiki/6-Benzylaminopurine

2) Kinetin is another kind of cytokinin. It is commonly used in plant tissue culture. However, it is less commonly used than BA, but similarly activates (Chuichai, 2010). It can stimulate cell division. Its chemical structure is 6- furfuryl–aminopurine but it is easily destroyed by light (at a wavelength of 300 – 800 nm). The concentration used was in the range of 0 – 10 mg/L (Chamrasphan, 2003).

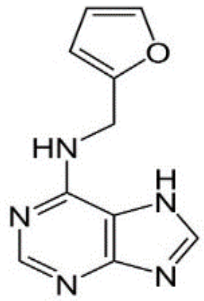


Figure 2.3 KIN structure

Source: http://www.cropasia.com/cytokinin/18-kinetin.html

Bamboo tissue culture for shoot multiple according to Songsoem (2018) found that culturing tissues from the nodes of Sang mon on an MS solid medium (Murashige and Skoog) supplemented with BA at a concentration of 1-3 mg/L, associated with NAA at a concentration of 0-0.5 mg/L for 4 weeks to induce shoots. It was found that a solid medium at a concentration of 2 mg/L developed maximum shoots of 3.31 ± 0.85 and a shoot length of 2.49 ± 0.61 cm. Four shoots per clump of *Bambusa multiplex* were taken to be cultured on MS medium supplemented with BA at a concentration of 4 mg/L in combination with IBA concentrations of 0, 0.1, 0.3, or 0.7 mg/L for three weeks to multiply the shoots. The shoots, developed on an MS medium supplemented with 4 mg/L BA concentration and 0.3 mg/L IBA, were likely to increase the maximum shoots equal to 2.8 shoots (Jirakiattikul, 2010). In addition, the node of *Bambusa multiplex* were cultured on an MS medium containing 35 µM BA (8.0 mg/L) for 3-5 weeks, regenerating 7-8 shoots, inducing 3-5 shoots per group, and multiplying shoots on an MS medium containing BA at a concentration of 10 µM (2.1 mg/L) at an expansion rate of 8–9 times every 3 weeks. After that, 3–4 shoots per group were taken for root induction on an MS medium containing 100 µM (20.0 mg/L) of IBA, which produced shoots at 93.93% and 9.7 roots with a length of 7.5 cm (Arya *et al.,* 2012). Regarding the nodes, they were cultured on an MS medium containing 3.0 µM TDZ (0.66 mg/L) and 56.0 µM ascorbic acid to induce shoots from bud nodes and multiply them on an MS medium containing TDZ at 1.5 µM (0.33 mg/L) and ascorbic acid at 56.0 µM. They produced 44.90 shoots, with a growth rate of 5.61 times. Singh *et al.* (2012) found that

root induction on MS medium with IBA at 250 µM (0.66 mg/L) and choline chloride at 36 µM resulted in 89% rooting. The nodes were then cultured on an MS medium containing BA at a concentration of 2.0 mg/L. The three regenerated shoots per group were moved and cultured on an MS solid medium containing BA at a concentration of 4.0 mg/L and adenine sulphate at a concentration of 15.0 mg/L, with a 3-fold growth rate. Root induction on an MS medium containing 5.0 mg/L IBA resulted in only 20% root proliferation (Pandey and Singh, 2012). Later, it was found that the MS medium containing 5.0 mg/L BA concentration induced 5.2 multiplied shoots with a length of 5.87 cm, or the MS medium containing BA at a concentration of 4.0 mg/L and TDZ at a concentration of 0.25 mg/L induced 6.12 multiplied shoots with a length of 6.87 cm (Kapruwan *et al.,* 2014).

Auxin: this group of hormones is naturally involved in the extension of the culms and internodes, tropism, apical dominance inhibition, the falling of leaves, flowers, and fruits, root regeneration, etc. For plant tissue cultures, auxins are used for inducing, dividing cells, and rooting. The auxins commonly used in tissue culture include Indole-3 acetic acid (IAA), Indole-3-butyric acid (IBA), 2-Phenylacetic acid (PAA), 2,4-Dichlorophenoxyacetic acid (2,4-D), 1-Naphthaleneacetic acid (NAA), and p-Chlorophenoxyacetic acid (pCPA).

However, the most widely used auxins are IAA, IBA, and NAA for root activation and in combination with cytokinin for stem growth, while 2,4-D has a good effect on stimulating callus growth. Generally, auxin is dissolved with ethanol or sodium hydroxyl zyme (NaOH), which is solvent diluted (Kitwichan, 2001). For tissue culture, low concentrations of IAA and NAA were used because high-concentrated auxin often causes toxicity to plants, such as leaf fall and growth stasis, which possibly makes them die. The optimum concentration depends on the plant species (Phongchawee *et al.,* 1999). As for the use of 2,4-D, which is highly effective in inducing embryogenesis, 2,4-D can inhibit photosynthesis and help mutated cells multiply rapidly. The use of 2,4-D must be more cautious than other auxins. Using a small amount of auxin will help with growth, but if its amount is too high, growth will be inhibited. The activation of auxins depends on various stimuli such as light, temperature, and gravity, through

which the auxin is transported within plants in a directional way. The sources of auxin synthesis and generation are the apical meristem, root apical meristem, young leaves, inflorescences, seeds, embryos, and fruits. The most commonly used auxin in tissue culture is IAA because of its less detrimental effect on organogenesis than other types (Kaveeta, 2002). Charassamrit (1994) stated that the balanced ratio of cytokinin and auxin affects organ formation and is necessary for the growth and development of cultured cells, such as callus, root, or shoot formation (Kaveeta, 2002). Cells will divide and develop into shoots, stems, and leaves if the ratio of cytokinin is higher than auxin, but if the ratio of cytokinin is lower than auxin, root regeneration will be better.

In the stage of shoot induction and multiplication, in addition to growth regulators, which affect growth development and shoot multiple, the state of the culture medium is another important factor at this stage. The medium state can be divided into liquid and solid mediums. A liquid medium is not supplemented with agar; plant tissues are constantly immersed in the medium in which air ventilation requires shaking to help dissolve oxygen. This beneficial effect on the fast growth of plant tissue also leads to tissue or cells growing rapidly (Sawacharin and Nukulkan 1983; Sukwana and Passorn, 2016). For solid medium, 0.6% agar is commonly added or other substances such as agarose and gelatin. The semi-solid medium tends to let the plant grow more slowly than liquid medium due to less surface area in contact with the medium and metabolic wastes released from cells where tissue parts come into contact with it. This may affect the growth of plant cells (Sawacharin and Nukulkan 1983; Sukwana and Passorn, 2016). However, the suitable culture medium to multiply the shoots is based on each plant species. In quince leaves (Aegle marmelos Correa.), leaf cultures were observed on an MS solid medium supplemented with 2 mg/L zeatin and at a concentration of 0.5 mg/L NAA for 8 weeks, resulting in the maximum shoots at 93.18% (Hanchana *et al.,* 2014), while culturing vanilla (Vanilla planifolia 'Andrews') on an MS liquid medium supplemented with BA at 2.15 mg/L concentration for 4 weeks, the number of shoots increased to 18.6 shoots per explant (Lee *et al.,* 2008). In addition, the results of culturing tissues of *T. siamensis* (Dongmanee, 2006) and *D. Giganteus* (Ramanayake and Yakandawala, 1997) showed that both types of bamboo shoots could grow and multiply well in a liquid medium.

2.9.3 Root induction

After multiplying the plants in sterile conditions, the next step is root induction. The main factor affecting the induced roots in sterilization is the concentration of tissue culture medium because its use often results in a ½ - ¼ time lower nutrient strength.

Another factor is growth regulators in the auxin group. Although some plants do not need them for root regeneration, for many plants, the medium culture must be supplemented with growth regulators to induce roots. According to the mentioned properties of auxin, it should be used at a low concentration in root induction in a sterile condition, or else the root length will be inhibited in the case of too high concentrations. In addition, it depends on the hormonal balance within the plant (Charassamrit, 1994) whether or not auxin has a root-stimulating effect. There have been studies reporting the root induction of various bamboo species in sterilization. The auxins used for inducing root in bamboos were NAA at a concentration of 0.5 – 3 mg/L, IBA at a concentration of 0.25 – 2 mg/L, or IBA in combination with NAA. In addition, IAA was used together with coumarin in *B. tulda* Roxb (Saxena, 1990) or TDZ in combination with 2,4-D in *B. blumeana* (Lin and Chang, 1998). Dongmanee (2006) reported on root induction in *T. siamensis* by taking three to 5 shoots to be cultured on an MS medium supplemented with NAA at 5 mg/L concentration for 3 weeks and then transplanted onto MS medium without growth regulator for 1 week. It was found that shoots were able to induce root formation at a maximum of 80%, with 2.8 roots per shoot group. Arya *et al.* (1999) showed that 3 bamboo shoots of *D. asper*, cultured on MS medium enriched with IBA concentration 10 mg/L or NAA at a concentration of 3 mg/L alone, were able to induce 10-15 roots at 4 weeks, while *Bambusa multiplex* cultured on an MS medium containing 4 mg/L BA associated with 15 mg/L NAA, the number of developed roots was 81%, and the maximum root length 3.1 and 2.36 cm respectively after 8 weeks of culture (Jirakiattikul, 2010) and *B. tulda* Roxb cultured on MS1/2 medium supplemented with IBA at a concentration of 3 mg/L, associated with BA at a concentration of 0.05 mg/L, and coumarin at a concentration of 10 mg/L, were able to induce roots 81.67%.

Table 2.3 Medium formula used for root induction of different bamboo varieties.

Species	Formula	References
Pai Bong dam	MS + 0.02 mg/L + IAA + 4.5 mg/L coumarin	Saxena (1990)
Pai Sang and Pai Ruak	MS + 2 mg/L IBA หรือ NAA	Chanlhanuraksa (1991)
Pai Tong	MS + 3 mg/L NAA	Kanyarat and Pranom (1992)
Pai Tong	MS + 0.5 – 1 mg/L NAA	Prutpongse and Gavinlertvatana (1992)
Pai Sang	MS + 0.25 mg/L IBA	Ravikumar *et al.* (1998)
Pai Si suk and *B. edulis*	MS + 0.1 mg/L TDZ + 0.5 mg/L 2,4-D	Lin and Chang (1998)
Pai Sang	MS + 0.7 mg/L NAA + 0.5 mg/L IBA (Cultured in the dark)	Saxena and Dhawan (1999)
Pai Tong	MS + 10 mg/L IBA or 3 mg/L NAA	Arya *et al.* (1999)
Pai Ruak	MS + 5 mg/L NAA for three weeks transferred on an MS for a week	Dongmanee (2006)
Pai Liang	MS +4 mg/L BA + 15 mg/L NAA	Jirakiattikul (2010)
B. tulda	$^{1/2}$MS +3 mg/L IBA + 0.05 m/L BA+10 mg/L coumarin	Waikhom and Louis (2014)

2.9.4 Transplanting of plantlets

Transfer of bamboo plantlets produced by tissue culture method into the greenhouse is a step that is more important than the production process in the laboratory. The plantlets from tissue culture method is more delicate than the normal plantlets. Therefore, moving the cutting is quite difficult. The failure to transfer the cuttings can be considered as tissue culture as well. Differences in the environment in the laboratory and nursery are the reasons that seedlings have to adapt themselves for survival and growth, The environment condition is the most important factor affecting survival and plantlet formation. Especially, in transferring the plantlet, practitioners must understand their basic needs for the right conditions to adapt to the environment. Importantly, paying attention to those plantlets must be more careful than ordinary plantlet. Environment factors within the nursery such as temperature, humidity, lighting, and nursery materials are important for the physiological processes of the plantlet, if these processes lack balance, plantlet will die or cannot stand upright. The plantlet twill leaves the laboratory with the open-closed stomata which do not function properly. The studies have shown that the plantlet moved out of the laboratory to the nursery had more water loss rate than normal seedlings due to their little epicuticular wax and there is an abnormality in the opening-closing of stomata.

The plantlet obtained from tissue culture will continue opening their stomata for 2-3 days after the transplant. While the stomata of normal plantlets will open and close regularly based on external environmental factors (Kyte, 1990; Macdonal, 1990). Selection of nursery materials that have the ability to firmly hold the bamboo breeds as well as porosity suitable for draining water and ventilating air soundly. It is suitable for growth and root development (Longman, 1993; Bonga and Aderkas, 1990; Kyte, 1990; Macdonal, 1990). In general, Pai Tong grows well in almost every soil type. It does, however, grow well in gritty soil with adequate drainage. According to Thai study, Pai Tong may grow effectively in sandy loam soil and in slightly acidic circumstances with a pH of 4.5-5.5. (Dransfield and Widjaya, 1995; Lao-siri, 1984). From the study, the suitable materials in transplanting each species of bamboo plantlet species are different. There have been studies reporting that bamboo can be cultured in sterile conditions and successfully cultivated in its natural condition. For example, *D. hamiltonii* obtained by floret tissue culture was transplanted into the mixture of peat, vermiculite, and perlite in a ratio of 1: 1: 1 by volume and placed in the greenhouse. It was found to have 100% survival (Zang *et al.,* 2016). Likewise (Goyal *et al.,*2010) used *D. strictus* obtained by tissue culture and washed agar under running water, and subsequently planted it in a mixture of perlite, soil, and manure in a ratio of 1: 1: 1 by volume and placed it in the greenhouse. After 30 days of transplanting, it survived 70% and was able to be transplanted into a planting plot. In addition, Devi *et al.* (2012) found that after root induction and transplantation into a mixture of sandy soil, soil, and manure, *D. giganteus* was able to survive as high as 80–90%, while for *D. asper*, after root induction and transplantation into rice husk ash, its survival rate was up to 90% with healthy and strong plantlet. Coconut coir is a planting material providing the lowest survivability and also gives incomplete seedlings. However, the use of rice husk ash mixed with coconut coir at a ratio of 1:1 increased the survival rate of bamboo seedlings to a maximum of 100% (Semsuntud and Ponoy, 2000). In the case of bamboo, there have been reports of instantaneous acclimation of in vitro-raised plantlets under greenhouse conditions, without a hardening phase (Jiménez et al., 2006; Lin and Chang 1998). Healthy rooted plantlets, on the other hand, are transferred to plantlet trays or polybags containing various types of potting mix such as soil, sand, soil rite, perlite,

vermiculite, compost, or farmyard manure either alone or in various ratios and kept humid before being transferred to the greenhouse. In some cases, applying lower MS minerals to plantlets has been shown to be crucial for enhanced acclimatization (Mishra et al., 2008; Singh et al., 2012a). Lin et al. (2007) also reported that axillary shoot proliferated plants were taller and produced more, and eleven media with different chemical and physical properties, including soil, fine sand, rice husk ash, coconut husk, soil:fine sand (1:1), soil:rice husk ash (1:1), soil:coconut husk (1:1), fine sand:rice husk ash (1:1), fine sand:rice. The plantlet survival rate was examined for very significant alterations. Except for rice husk ash, which resulted in 90% survival with vigorous plantlets, using only one material as a media resulted in lower plantlet survival than using a mixed medium. Coconut husk found to be an insufficient medium, resulting in minimal yields.

2.10 Utilization of tissue culture plants

Bamboo tissue culture is directly beneficial in terms of propagating large quantities of plants in a short period of time by using a suitable synthetic medium formula for each plant species for the multiplied number of plants. For example, if a single plant with transferred tissues once a month can increase to 10 plants, in just 6 months, the number of plants can produce 1,000,000 plants. Many new plants, obtained from this increasing method, are stable, so it is commercially beneficial. Plant propagation by means of culturing tissues will obtain the mother plant cells to be fed to new plants. Therefore, the good characteristics, such as leaf characteristics and plant strength of the parent plant's species can be controlled. This will help reduce mutations, control stable production, and potentially produce disease-free crops. One of the major problems in crop production is the occurrence of diseases, especially those caused by fungi, bacteria, viruses, and mycoplasma, attached to seeds or propagated explants. If the plants with pathogens do not show their symptoms and they can only be known when they appear on the grown plants, it is difficult to recover from or prevent the disease, except by eradicating or destroying those plants. Although the use of chemicals mixed with seeds or propagated explants prior to planting can reduce the number of diseases, they are probably attached to the surface of the plant material. Thus, it may

not be effective in the case of contaminated disease within the plant cells, especially from fungi and bacteria. However, due to their immediate contamination in both medium and fast growth, it is possible to eradicate them. In the case of viruses, which are very small microorganisms and can survive in plant cells, they often show no signs of contamination, even by plant tissue culture. In practice, to be certain that they are free from viruses, tissues must be screened and examined prior to culture. The most virus-free explant is the apical meristem and embryonic tissue in seeds because these tissues do not have vascular tissues, including the xylem and phloem, connecting to other parts of the plant, letting the viruses move to contaminate them.

Tissue culture does not provide the only advantages. The disadvantages of propagation using tissue culture techniques are the difficult and costly procedures as well as methods. Due to various equipment and tools whose prices are quite high, it is not suitable for small manufacturers or farmers. In addition, the large quantity of new plants with the same genetic characteristics easily causes the spread of diseases and pests. Moreover, somatic variation may occur owing to culturing on a synthetic medium with high nutrients and hormones (Jongkid, 2005). However, some variations, such as mutations of ornamental plants, have a beneficial effect on exotic species, for instance, dwarf orchids, etc.

CHAPTER III

RESEARCH METHODOLOGY

This research was divided into 2 parts:

Experiment 1 – Experiment 5: Studied bamboo tissue culture using node explants.

This research aimed to develop tissue culture techniques suitable for two Dendrocalamus species, namely Sang nuan and Sang mon by choosing the branch node explants for tissue culture, studying the appropriate sterilization methods, the comparison of shoot induction using nodes in the greenhouse, and outside the greenhouse, the suitable formula for shoot induction, shoot multiplication, and root induction.

Experiment 6 – Experiment 9: Studied Bamboo tissue culture using seed explants.

This research aimed to develop tissue culture techniques suitable for the seeds of Kab dang by selecting the seeds of bamboo for tissue culture, studying the proper sterilization method, and studying the proper medium formula for shoot induction, and root induction developing into an intact sprout as well as studying the technique of transplanting seedlings using different planting materials.

3.1 Sources of bamboo species used in the experiment

This research chose a good bamboo cultivar, 3-year-seed culture, which was obtained and introduced as well as selected according to the research of Assoc. Prof. Dr. Thanpisit Phuangchik, Department of Agricultural Technology, Faculty of Science and Technology, Thammasat University Rangsit Campus and he provided the Kab dang seed.

3.2 The Cultivation of Bamboo Cultivars Used in Experiment

Sang nuan and Sang mon were cultured in the environmental controlled greenhouse at the Science and Technology Instruments Center, Building 14, Suranaree

University of Technology (SUT), using bamboo cultivars selected from the company's good bamboo varieties. (bamboo cultivars that match the species selected by the researchers from Thammasat University, Rangsit). The selected grew fast, had beautiful clumps, and large culms responded well to water and fertilizers and yielded perennial and dense shoots. For planting the various bamboo cultivars, the trenches were raised at a distance of about 1 m, a hole dug of about 30×30×30 cm and filled at its bottom with about 500 g of manure, homogeneously mixed with the soil. After that, the hole was covered with leaf fragments to keep moisture in for the rhizome buds to grow and develop. Afterward, the plant nutrient solution was given according to the period of the plant's age.

3.3 Preparation of Bamboo Explants for Tissue Culture

Pruning shears were used to selectively excise the bamboo branches of Sang nuan and Sang mon that both have been planted for about 1-2 years with a diameter of about 0.5-0.8 cm before removing their sheaths and cutting them into pieces of about 2-4 cm long each. After preliminary cleaning, they were bleached to disinfect their surfaces before being cut at both ends into pieces approximately 1-2 cm in length and cultured on a medium.

For Kab dang, the seeds were wrapped in a thin white cloth and washed for disinfection before thoroughly washed with sterilized distilled water. After that, they were taken to be cultured on a medium.

3.4 Culture Media and Conditions

The culture medium used in this study was the MS formula (Murashige & Skoog). The growth regulator type and its concentration depended on the experiment. All formulas were supplemented with granulated sugar at 30 g/L. In a solid medium, 6 g/L of agar was added, while it was not added in a liquid medium. For root induction, 2 g/L of agar was added to the semi-solid medium, and the pH was adjusted to 5.6–5.7.8 by 1 N. HCL and 1 N. NaOH. The medium was sterilized in a vapor pressure autoclave at 121°C for 15 min, and the cultures were under 16 hr of light and 8 hr of darkness under 35-40 mol of light intensity at an m^{-2} s^{-1} temperature of 25 ± 2 °C for the required period of experimental time.

3.5 Details of the Research Experiment

3.5.1 Experiment 1: studied the sterilization of noed explants.

A completely randomized design (CRD) experiment was planned for 5 treatments, 3 replicates each (30 bottles, 1 node per 1 replicate) by taking bamboo branches of Sang nuan and Sang mon with approximately 0.5-0.8 cm in diameter and removing the bamboo sheaths before cutting them into pieces, approximately 2-4 cm long. After preliminary cleaning, they were bleached to disinfect their surfaces. After that, both ends were cut into pieces approximately 1-2 cm in length and cultured on medium for 4 weeks as detailed below.

Treatment 1: The bamboo node explant was washed and thoroughly rinsed with soap and immersed in 70% alcohol for 2 min, in 10% sodium hypochlorite (NaOCl) for 15 min, in 5% NaOCl for 10 min, and in fungicide at a concentration of 1.5 g/L for 1 hr, respectively. At all concentrations, 1-2 drops of NaOCl were added to Tween20. The bamboo node was then washed with distilled water that was autoclaved three times for a total of 3 min each.

Treatment 2: The bamboo node explant was taken to be soaked in 70% alcohol for 1 min, then in 10% sodium hypochlorite (H_2O_2) for 15 min, and was subsequently washed with distilled water that was autoclaved 3 times for 3 min each.

Treatment 3: The bamboo node explant was taken to be soaked in 70% alcohol for 1 min, then in 30% H_2O_2 for 15 min. After that, it was washed with distilled water that was autoclaved 3 times for 3 min each.

Treatment 4: The bamboo node explant was taken to be soaked in 70% alcohol for 1 min, in 30% H_2O_2 for 15 min, in 10% NaOCl for 10 min, and in 5% NaOCl for 5 min. After that, it was washed with distilled water that was autoclaved 3 times for 3 min each.

Treatment 5: The bamboo node explant was taken to be soaked in 30% H_2O_2 for 15 min. After that, it was washed with distilled water that was autoclaved 3 times for 3 min each.

Recording of experimental results: The results of the experiments were recorded weekly after the culture (for 1 month). The recorded data included 1) the percentage of contamination of the branch nodal explant sterilized by different

formulations; 2) the number of shoots per node and the experimental results were statistically analyzed by Duncan's new multiple range test at a 95% confidence level.

3.5.2 Experiment 2: studied the comparison of shoot induction by using nodes in the greenhouse and outside the greenhouse

The shoots from the nodes of Sang nuan and Sang mon planted in greenhouses and outside greenhouses were taken before cutting their nodes and sterilizing them according to Experiment 1. the experiment of Factorial in Complete Randomized Design was planned with 3 replicates consisting of 2 factors including

Factors 1: Conditions of growing bamboo 2 varieties are outside greenhouses and greenhouses

Factors 2: Medium for 3 culture media formulas: MS+BA2 (mg/L), MS+BA4 (mg/L), and MS+BA6 (mg/L)

Recording of experimental results: Experimental results were recorded weekly (for a 2-week duration). The collected data included 1) the number of shoots per node, 2) the shoot length, and 3) the survival percentage. The results were statistically analyzed by Duncan's new multiple range test at a 95% confidence level.

3.5.3 Experiment 3: studied the appropriate formula for shoot induction

The CRD experiment was planned for 10 treatments, 3 replicates each (1 node per 1 replicate). The Sang nuan and Sang mon node explants were bleached and sterilized according to the appropriate method from the latest experiment. They were cultured on MS solid medium with 3 growth regulator types, namely BA (6-benzyl amino purine), NAA (naphthalene acetic acid), and KIN (kinetin), at 10 concentrations as shown in Table 3.1, and placed in the nursery for 4 weeks.

Recording of experimental results: Experimental results were recorded weekly (for 1 month). The collected data included 1) the number of shoots per node, 2) the shoot length, and 3) the survival percentage. The results were statistically analyzed by Duncan's new multiple range test at a 95% confidence level.

Table 3.1 Concentrations of BA, KIN, and NAA supplemented with culture medium for shoot induction from the nodes of Sang nuan and Sang mon.

Treatments	Basal Media	Hormone Concentration Level		
		BA (mg/L)	KIN (mg/L)	NAA (mg/L)
1	MS	-	-	-
2	MS	2	-	-
3	MS	4	-	-
4	MS	6	-	-
5	MS	2	1	-
6	MS	4	1	-
7	MS	6	1	-
8	MS	2	-	1
9	MS	4	-	1
10	MS	6	-	1

3.5.4 Experiment 4: studied the optimal formula for multiple shoots induction

The shoots from the nodes of Sang nuan and Sang mon obtained from Experiment 3 with about 1.5–2.5 cm in length were taken, and the shoots were excised and cultured on the formula in groups of 3 shoots each for 4 weeks. A Factorial in a Complete Randomized Design with 3 replicates was planned, consisting of 2 factors:

The first factor was the media state, i.e., 1.) solid medium state, 2.) liquid medium state.

The second factor was the tissue culture media formulas, including 1.) MS 2.) MS+BA2 (mg/L) 3.) MS+BA4 (mg/L) 4.) MS+BA3+NAA0.5 (mg/L)

Recording of experimental results: The results were recorded weekly (for 2 weeks). The collected data included 1) the number of shoots developed per clump, 2) the shoot length, and 3) the percentage of shoot multiplication. The results were statistically analyzed by Duncan's new multiple range test at a 95% confidence level.

3.5.5 Experiment 5: studied the suitable medium formula for root induction

The experimental design of CRD was planned for 4 treatments, 3 replicates each, and 15 bottles (1 clump per 1 replicate). By taking approximately 3 shoots Sang nuan and Sang mon from Experiment 4 to be cultured on MS medium, supplemented with growth regulators according to Table 3.2.

Recording of experimental results: The results were recorded weekly (for 1 month). The collected data included 1) the percentage of root formation from the shoot group, 2) the root length from the shoot group, and 3) the growth characteristics of the roots. The characteristics of the roots were recorded, and the results were statistically analyzed by Duncan's new multiple range test at a 95% confidence level.

Table 3.2 Concentrations of NAA supplemented with culture medium for root induction.

Treatments	Basal Media	Hormone Concentration Level
		NAA (mg/L)
1	MS	-
2	MS	4
3	MS	5
4	MS	6

3.5.6 Experiment 6: studied the disinfection from bamboo seeds

The seeds of Kab dang were obtained from Assoc. Prof.Dr. Thanapisit Phuangjik, Thammasat University. Rangsit Campus, and online purchase, namely Sang nuan and Sang mon. Then, they were bleached and sterilized in each sterilization formula. Factorial in Complete Randomized Design was planned, with three replicates, using 20 bottles per treatment, comprising 3 factors:

Factor 1: Three varieties of bamboo seeds, namely Kab dang, Sang nuan and Sang mon.

Factor 2: The cultures on 3 modified MS formulations as follows: Formula 1 MS, Formula 2 MS+BA2 (mg/L), and Formula 3 MS+BA3 (mg/L).

Factor 3: Two different methods of disinfection were:

Method 1: The seeds of Kab dang, obtained from Assoc. Prof. Dr. Thanapisit Phuangchik, Thammasat University Rangsit Campus, and the seeds of Sang nuan and Sang mon, bought online, were wrapped inside a sterilized cloth filter before soaking in 70% alcohol for 2 min and bleaching with 2% NaOCl for 8 min. They were subsequently washed with distilled water that was autoclaved 3 times for 3 min each and cultured on a modified MS medium for 1 month.

Method 2: The Kab dang seeds obtained from Assoc. Prof. Dr. Thanapisit Phuangchik, Department of Agricultural Technology, Faculty of Science and Technology Thammasat University Rangsit Campus, and bamboo seeds bought online, Sang nuan and Sang mon, were bleached by removing the seed coats and wrapped in a sterilized cloth filter. After that, the seeds were soaked in 70% alcohol for 1 min, sterilized with 10% NaOCl for 30 min, and 5% for 20 min, and subsequently washed with distilled water that was autoclaved 3 times for 3 min each and cultured on a modified MS medium for 1 month.

Recording of experimental results: The experimental results were recorded weekly (for a 2-week duration). The collected data included 1) the survival percentage and the experimental results were statistically analyzed by Duncan's new multiple range test at a 95% confidence level.

3.5.7 Experiment 7: studied shoot induction from the seed of Kab dang

The experimental (CRD) design was planned for 3 treatments, 3 replicates each (1 seed per 1 replicate). The seed explants of Kab dang were bleached and sterilized according to the appropriate method from experiment 6 and cultured for 4 weeks on MS medium with the addition of growth regulators according to Table 3.3.

Recording of experimental results: Results were recorded weekly (for a 4-week duration). The collected data was as follows: 1) the number of shoots 2) the shoot length 3) the number of leaves 4) the number of roots 5) the root length 6) the survival percentage. The results were statistically analyzed by Duncan's new multiple range test at a 95% confidence level.

Table 3.3 Concentrations of BA supplemented with culture medium for shoot induction from the seeds of Kab dang.

Treatments	Basal Media	Hormone Concentration Level
		BA (mg/L)
1	MS	-
2	MS	2
3	MS	3

3.5.8 Experiment 8: studied root induction from the seeds Kab dang

The experimental design of (CRD) was planned for 4 treatments, with 3 replicates (20 bottles, 1 seed per 1 replicate). For 3 weeks, using seedling was cultured on MS medium supplemented with growth regulators according to Table 3.4.

Recording of experimental results: The results were recorded weekly (for a 3-week duration). The collected data included 1) the number of roots 2) the root length 3) the number of leaves 4) the bamboo length, and 5) the survival percentage. The results were statistically analyzed by Duncan's new multiple range test at a 95% confidence level.

Table 3.4 Concentrations of NAA supplemented with culture medium for root induction from the seeds of Kab dang.

Treatments	Basal Media	Hormone Concentration Level
		NAA (mg/L)
1	MS	-
2	MS	1
3	MS	2
4	MS	3

3.5.9 Experiment 9: studied the suitable method for transferring bamboo seedlings from the seeds of Kab dang from the laboratory to the nursery

The seedlings of Kab dang with intact roots, obtained from the culture in Experiment 8, were taken to clean in order to remove the sugar and agar residues used in the culture with distilled water 2-3 times, then they were soaked in water containing 1 teaspoon of benlate fungicide for about 10 min. With the peach moss material available for transplanting, they were then transferred into 22-ounce plastic cups. The cultivation period was approximately 14 days. After that, the plastic cover was gradually opened on the 15th day (for 1 day) before transplanting them into the pot of each experimental method according to Table 3.5. The experimental design (CRD) was planned, comprising 4 treatments with 3 replicates each, for 10 plants (1 plant per 1 replicate). They have been cultured for 2 months.

Recording of experimental results: After transplantation, the results were recorded every 1 week for 2 months. The collected data included 1) the number of shoots, 2) the shoot length, 3) the number of leaves, and 4) the survival percentage. The results were statistically analyzed by Duncan's new multiple range test at a 95% confidence level.

Figure 3.1 Transplantation of seedlings of Kab dang by the seed tissue culture from the laboratory.

Table 3.5 Planting materials for transferring Kab dang seedlings by the seed tissue culture from the laboratory.

Treatments	Planting materials	Planting materials ratio
1	sand: rice husk: filter cake	2:2:1
2	rice husks: coconut husks	1:1
3	soil: sand: vermiculite	1:1:1
4	SUT planting soil	-

3.6 Location of experiment

Facility building 14 (F14) of the Science and Technology Center the Suranaree University of Technology.

CHAPTER IV
RESULTS AND DISCUSSION

4.1 Experiment 1: studied the node explant sterilization

The nodes from the branches of Sang nuan and Sang mon, disinfection with various solutions, and the time it takes to disinfect. Culture them in MS solid medium without the addition of growth regulators for 4 weeks. The results revealed that the preparation of sterilized nodal explants in five formulas for contamination was a statistically significant difference ($P<0.01$; Table 4.1). Sang nuan formula 4 had the minimum contamination (18.01%), followed by Formula 1 (30.39%), and Formula 3 (37.14%), respectively. In addition, Formula 4 had the highest average shoot number, equal to 2 shoots. When considering the characteristics of bamboo nodes after testing for aseptic bleaching treatment, the tissue color at the ends of bamboo nodes on both sides changed to a paler green than the original. However, the buds could develop into shoots, both in the formulas 1 to 5. Sang mon, the results in reducing contamination were statistically significantly different ($P<0.01$; Table 4.1). In Sang mon, Formula 1 and Formula 4 caused the least amount of contamination at 30.39 and 37.14%, with the highest average shoot number of 1 shoot (Table 4.1). The outcomes showed that varieties, disinfectants, and sterilization duration resulted in different percentages of contamination and a tendency for surviving nodes.

However, the efficacy of these disinfectants depends on their responses to the duration and amount of substances used. Commonly, the efficiency will be higher with the increase in time and concentration. On the other hand, overuse can harm the viability of plant cells, tissues, or organs. Therefore, they must be initially tested to find the proper amount. The general method of bleaching and eliminating infection on the skin, in general, is shown in Table 2.1 (Kaveeta, 2002).

The disinfectant concentration and the bamboo varieties require a suitable amount. Chlorox solution at concentrations of 5–30% is the disinfectant most

commonly used for tissue culture. In addition, for disinfection, a drop of liquid soap such as polyoxyethylene sorbitan monolaurate (Tween 20) should be used to reduce the surface tension between water and plant tissue. Then, rinse it with distilled water several times to remove any disinfectant attached to the tissue. The damaged tissue, often at the end of the tissue, should be removed, while the complete tissues are those cultured in medium (Kitwijai, 2004). There have been some reports on the various explants of other bamboo varirties. Songsoem (2018) took the nodal explant to sterilize with a 10% concentration of Chlorox solution for 10 minutes and found that the percentage of survival was optimal, with the highest survival of 66.67%. This can be seen that the sterilization method, the duration, the concentration, and the variety, as well as the season, affected the disinfection. For the r season, Dongmanee (2006) has sterilized bamboo nodes of Pai Ruak with 10% concentration of NaOCl for 10 min, it was found that the nodal explants were most disinfected during March, April, and May, at 82.00, 76.10 and 78.70%, respectively.

However, from the months of June to October 2006, the disinfection efficiency decreased in the range of 32.50 - 46.60% due to the high humidity during the rainy season. As a result, microorganisms are attached to the hairs and grow well along the leaf axils of the nodal explants causing the sterilization difficulty and resulting in a higher percentage of contamination. Likewise, sterilizing bamboo nodes of Pai Poh (Giant bamboo) with a 0.3% concentration of $HgCl_2$ for 10 minutes from April 1994–October 1996, showed that the contamination percentage was up to 90% in August 1994 (Ramanayake and Yakandawala, 1997). The disinfectant most commonly used for initial tissue surface is NaOCl (Kaveeta, 2002). Several types of bamboos used NaOCl sterilization at different concentrations as follows: *Bambusa edulis* (Lin and Chang, 1998), *D. strictus* (Singh *et al.*, 2000), *Sinocalamus latiflora* (Yeh and Channg, 1987), *D. hamiltonii* (*Godbole et al,* 2002), and the sterilization method with mercuric chloride ($HgCl_2$) solution at different concentrations in *Thamnocalamus spathiflorus* (Niladri *et al.,* 2000), *Phyllostachys viridis* (Hassan and Debergh, 1987), and *B. tuida* (Saxena, 1990). For the use of H_2O_2 at a concentration of 10–12% in 5–10 min, the disinfection efficiency was good (Kaveeta, 2002), but still based on the tissues, environment, and selected

explants, etc., This resulted from the response time and the substances' concentration used. The disinfection on bamboo explants, whether nodes, seeds, or inflorescences, etc., if using H_2O_2, was hardly disinfected. NaOCl has been mostly used at concentrations of 10-14% (Kaveeta, 2002) because it is generally available on the market. Ketudat-Cairns (2021) reported that NaOCl and H_2O_2 were used to disinfect the leaves. These two disinfectants had distinct negative impacts on leaf explants. The greatest incidence of green explants without contamination was obtained from leaf explants treated with NaOCl (51.2%). All of the leaf explants, however, were injured. The leaf explants got dry and wilted after 3 weeks of tissue culture. They eventually turned brown and perished. NaOCl had a greater effect on contaminations and leaf explants than H_2O_2. It not only removed all of the pollutants, but it also killed the explants. The contamination of microorganisms could not be controlled by using 2.5% H_2O_2. However, greater concentrations of H_2O_2 destroyed the leaf explants in the same way as NaOCl did. As a result, 5% H_2O_2 was the best concentration to employ to eradicate pollutants while still allowing the leaf explants to survive. It yielded up to 47.0% of uncontaminated green explants.

Sterilization is essential in plant tissue culture to attain the desired outcomes. As a result, chemicals and cleaning processes must be addressed. Simple and environmentally friendly procedures were investigated in order to determine the best process for *J. curcas* tissue culture using leaf explants. In our trials, the results showed that NaOCl was ineffective at sterilizing *J. curcas* leaves. However, 5% NaOCl was found to be effective in sterilizing *Coccinia abyssinica* and *J. curcas* leaves (Khurana *et al*., 2010; Guma *et al*., 2015; Liu *et al*., 2015; 2016). When NaOCl was tested on grapevine axillary buds, the contamination rate was just 19.8% after one hour of sterilization with 1.3% NaOCl (Lazo *et al*., 2016). This disparity in results might be attributed to variances in the explant, species, or variable levels of contamination from the surroundings.

Other methods for surface disinfection of explants have been effectively used in tissue culture of other plant species but have not been widely studied for Anthurium. Because of its oxygen-oxygen single bond, H_2O_2 is a powerful oxidizing and disinfecting agent that can be used to clean the surfaces of various plant explants such as sugar

apple, water lettuce, sour cherry, and cotton (Farooq *et al*. 2002, Aasim *et al*. 2013, Mihaljevi *et al*. 2013, Barampuram *et al*. 2014). When it was used to disinfect non-seed explants (nodal explants, buds, green shoots), it resulted in 50-60% contamination-free explants (Farooq *et al.* 2002, Aasim *et al*. 2013, Mihaljevi *et al*. 2013).

This study concluded that the sterilization of the node explants of Sang nuan with the Formula 4 solution and Sang mon with Formula 1 solution provided the lowest percentage of contamination and were likely to have the highest percentage of survival, in addition to giving rise to the highest average rate of the new shoots.

Table 4.1 Effect sterilization of bamboo Sang nuan and Sang mon per sterile percentage and number of shoots.

Media sterilization	Sang nuan		Sang mon	
	Contamination (%)	Shoot number	Contamination (%)	Shoot number
70% alcohol, 2 min+10% NaOCl, 15 min+5% NaOCl, 10 min + 1.5 g/l concentration in fungicide, 1hr and wash DI water three times	30.39±6.72bc[1/]	1.35±0.28b	25.89±6.22c	1.35±0.24a
70% alcohol, 1 min+10% NaOCl, 1 min and wash DI water three times	59.65±6.72a	0.50±0.22c	72.04±5.54a	0.24±0.11c
70% alcohol, 1 min+30% H_2O_2, 15 min and wash DI water three times	37.14±6.82b	1.56±0.32b	54.03±6.49b	0.75±0.19bc
70% alcohol, 1 min+30% H_2O_2, 15 min+10% NaOCl, 10 min+5% NaOCl, 5 min and wash DI water three times	18.01±5.54c	2.44±0.31a	37.14±6.62c	1.01±0.18ab
30% H_2O_2, 15 min, and wash DI water three times	76.53±4.89a	0.21±0.13c	75.41±4.38a	0.38±0.14c
F-test	**[2/]	**	**	**
%CV	40.70	65.00	35.32	76.02

[1/]data shows mean ± SE, different characters in vertical rows mean statistically different at 0.05 level compared by Duncan's New Multiple Range Test (DMRT).

4.2 Experiment 2: studied the comparison of shoot induction by using bamboo nodes in the greenhouse and outside the greenhouse.

The node surfaces of Sang nuan and Sang mon, which had already passed the sterilization process in Experiment 1, were taken and then cultured inside and outside the greenhouse on the MS solid medium containing BA at concentrations of 2, 4, and 6 mg/L for 2 weeks. In both indoor and outdoor conditions, Sang nuan was able to induce 1-3 shoots, have the number of shoots, shoot lengths, and survival rates with no statistically significant difference ($P>0.05$; Table 4.2) in week 1 and week 2. From the observation of bamboo culms, the bamboo planted in greenhouses had higher branch endurance than those planted outside.

Sang nuan tissue culture medium in week 1 had a statistically significant difference ($P < 0.01$; Table 4.2) in the number of shoots cultured on MS+BA 4 mg/L. In addition, MS+BA 6 mg/L yielded the greatest number of shoots 2.28±0.11 and 2.00±0.23 shoots, respectively, and we found that the shoot lengths and survival rates were not statistically different ($P>0.05$; Table 4.2). In week 2, the tissue culture medium showed that the number of shoots and shoot lengths were statistically different (Table 4.2). The MS+BA 6 mg/L medium yielded the highest number of shoots 3.14± 0.24 shoots and the higher the BA, the more the shoots were induced but the lower the shoot length. The MS+BA 2 mg/L and MS+BA 4 mg/L media produced the highest shoot lengths of 4.05 ± 0.18 and 3.94 ± 0.31 cm, respectively, and their survival rates were not statistically different ($P>0.05$; Table 4.2).

Sang mon variety planted in indoor and outdoor conditions showed that the number of shoots, shoot lengths, and survival rates had statistically significant differences ($P<0.01$; Table 4.4) in both weeks. In week 2, planting in the greenhouse showed the highest shoot length and the survival percentage, which were 4.05±0.29 cm and 86.71±2.29%, respectively, while the shoots outside the greenhouse induced the most shoots (3.48 ± 0.42 shoots), which were statistically significant difference from the nodes produced from the inside greenhouse ($P<0.01$; Table 4.4). In week 1, Sang mon tissue culture medium had statistically significant differences ($P<0.01$; Table 4.4) in the shoot length and survival rates. The MS+BA 2 mg/L medium yielded the highest

shoot length 2.44±0.26 cm and the MS+BA 4 mg/L medium gave the highest 90.05% survival rate. All medium formulas were able to induce 2-3 shoots (Table 4.4). In week 2, the number of shoots and shoot lengths had statistically significant differences ($P<0.01$; Table 4.4). The MS+BA 4 mg/L produced the most of 3.36±0.54 shoots, while the MS+BA medium 2 mg/L yielded the highest shoot length of 4.28±0.34 cm, with no statistically significant difference in survival rates ($P > 0.05$; Table 4.4).

The shoot induction and multiplication entail the plant explants being cultured on the proper medium, which subsequently prompts propagation. Plants generally need the same macronutrients but in different quantities or concentrations, especially growth regulator substances due to their different needs (Kaveeta, 2002). The types of growth regulators and their concentrations depend on the kinds of plants and the explants used. Growth regulators, especially cytokinins and auxins, are very important in plant tissue culture.

Prutpongse and Gavinlertvatana (1987) also stated that bamboo tissue suitable for culture should be that from matured branches, resulting in a high rate of surviving tissues. Planting bamboo in greenhouses yielded fewer and smaller branches than planting it outdoors. The branches in the greenhouse are more endurable than those outside the greenhouse, in which planting bamboo is rare because it is a fast-growing plant family, reaching a full height of up to 40 meters in 2-4 months and 30 meters in diameter (Rao *et al*., 1998).

From the experiments on the tissue culture of each bamboo variety, most of the bamboo for the experiments was harvested from plots outside the greenhouse. Dongmanee (2006), for example, collected bamboo nodes from Silpakorn University in Nakhon Pathom Province and discovered that shoot induction from the lateral buds of Pai Ruak (*Thyrsostachys siamensis* Gamle) cultured on MS semi-solid medium with BA supplemented at a concentration of 2.5 mg/L induced the greatest number of 13.5 shoots. Deelom (2019) collected the samples of Beijing bamboo (*Dendrocalamus* sp.) and found that the modified MS formula containing 0.6 mg/L TDZ and the modified MS formula with 2.5 mg/L BA, supplemented with 0.5 mg/L TDZ was able to induce an average of 4.7 and 4.9 shoots per nodal explant with shoot lengths of 4.0 and 3.6 cm, respectively.

4.2.1 Interaction between factors affecting shoot induction using nodes of Sang nuan and Sang mon in the greenhouse and outside the greenhouse

Interaction between the two factors was growing conditions and Formula culture medium. In the results of planting conditions associated with the Sang nuan tissue culture medium, there was a statistically significant difference in shoot lengths in week 2 ($P<0.01$; Table 4.3). Planted in greenhouses and cultured on the MS medium, supplemented with BA at a concentration of 4 mg/L, yielded the highest shoot length, which was 4.46 ± 0.47 cm, and planted outside the greenhouse, cultured on the MS medium, enriched with BA at a concentration of 2 mg/L, yielded a shoot length of 4.31 ± 0.22 cm.

The results of planting conditions associated with Sang mon tissue culture medium, the shoot lengths and survival rates in week 1 were statistically significant differences ($P<0.01$; Table 4.5). Plants were planted in greenhouses and cultured on MS medium enriched with BA at a concentration of 4 mg/L yielded the highest shoot length of 2.64 ± 0.07 cm, with survival rates of 90.05% in all media, and were able to induce 2-3 shoots. In week 2, the number of shoots, the shoot lengths, and the survival rates were statistically significant differences ($P<0.01$; Table 4.5). For the cultivation outside the greenhouse, the highest number of shoots was 4.94 ± 0.45 shoot on MS medium with BA added at a concentration of 4 mg/L. The highest shoot length was 5.19 ± 0.12 cm on MS medium added with BA at a concentration of 2 mg/L, and cultures on BA-added MS medium at concentrations of 2 and 6 mg/L yielded the highest survival rate of 90.05%.

The buds are located at the bamboo nodes, whether at the young shoots, on the culms, on the branches, or at the rhizomes, where the meristem is located. The buds are capable of sprouting and rooting. When they are cultured in suitable conditions or media containing growth regulators, the sprouting of buds depends on a combination of internal and external factors, possibly depending on the different bud conditions. Large buds are more capable of inducing shoots than smaller ones due to the more nutrients inside, resulting in the power of the better splitting of the shoots. In addition, the quantity of hormones within plants, which is an important factor in

regulating the development and growth of plants, directly affects the bud shooting, while the external environment is a stimulus for sprouting. When bamboo buds are ready and get into the right environment, they are able to promptly split the shoots. Regarding the culture of bamboo buds, they are cultured on a medium consisting of the basic plant nutrients required according to the MS formula and growth regulators of the Auxin and Cytokinin groups, which function as shoot inducers by putting them in the appropriate quantity.

Table 4.2 Testing bamboo planting in greenhouses and outside the greenhouses using node explant of Sang nuan to shoot induces differences in survival rates.

Experimental factors		1 week			2 weeks		
		Shoot number	Shoot length (cm)	Survival rate (%)	Shoot number	Shoot length (cm)	Survival rate (%)
Growing conditions (A)	Greenhouse	1.98±0.14[1/]	1.85±0.04	90.05±0.00	2.83±0.21	3.61±0.25	86.71±2.29
	Outside the greenhouse	1.87±0.11	1.66±0.09	86.71±1.16	2.39±0.14	3.64±0.15	76.71±2.83
Formula medium (B)	MS+BA2 mg/L	1.50±0.13b[2/]	1.78±0.14	85.04±3.37	2.28±0.22b	4.05±0.18a	80.04±6.75
	MS+BA4 mg/L	2.28±0.11a	1.94±0.16	90.05±0.00	2.42±0.22b	3.94±0.31a	85.04±3.37
	MS+BA6 mg/L	2.00±0.23a	1.55±0.14	90.05±0.00	3.14±0.24a	2.88±0.14b	80.04±4.27
F-test	A	ns	ns	ns	ns	ns	ns
	B	**	ns	ns	*	**	ns
	A*B	ns	ns	ns	ns	*	ns
%CV		29.42	26.90	7.16	28.79	19.26	20.49

[1/]data shows mean ± SE, different characters in vertical rows mean statistically different at 0.05 level compared by Duncan's New Multiple Range Test (DMRT).

[2/] ** means a statistically significant difference at the 0.01 level, * means a statistically significant difference at a 0.05 level, ns means not statistically different.

Table 4.3 Interaction of growth conditions and formula medium on shoot length in weeks 2 of bamboo Sang nuan.

Experimental factors		2 weeks
Growing conditions (A)	Formula medium (B)	Shoot length (cm)
Greenhouse	MS+BA2 mg/L	3.78±0.26ab[1/]
	MS+BA4 mg/L	4.46±0.47a
	MS+BA6 mg/L	2.6±0.09c
Outside the greenhouse	MS+BA2 mg/L	4.31±0.22a
	MS+BA4 mg/L	3.43±0.31bc
	MS+BA6 mg/L	3.16±0.2bc
F-test		*

[1/]data shows mean ± SE, different characters in vertical rows mean statistically different at 0.05 level compared by Duncan's New Multiple Range Test (DMRT).

Table 4.4 Testing bamboo planting in greenhouses and outside the greenhouses using node explant of Sang mon to shoot induces differences in survival rates.

Experimental factors		1 week			2 weeks		
		Shoot number	Shoot length (cm)	Survival rate (%)	Shoot number	Shoot length (cm)	Survival rate (%)
Growing conditions (A)	Greenhouse	1.57±0.13b[1/]	2.51±0.19a	90.05±0.00a	1.59±0.17b	4.05±0.29a	86.71±2.29a
	Outside the greenhouse	2.69±0.26a	1.40±0.10b	80.04±3.43b	3.48±0.42a	2.42±0.21b	66.70±4.58b
Formula medium (B)	MS+BA2 mg/L	2.06±0.41	2.44±0.26a	85.04±3.37ab	2.36±0.44b	4.28±0.34a	80.04±4.27
	MS+BA4 mg/L	2.17±0.19	1.90±0.24b	90.05±0.00a	3.36±0.54a	3.11±0.42b	80.04±4.27
	MS+BA6 mg/L	2.17±0.28	1.53±0.17b	80.04±4.27b	1.89±0.36b	2.32±0.12c	70.04±6.75
F-test	A	**	**	**	**	**	**
	B	ns	**	*	**	**	ns
	A*B	ns	*	*	**	**	**
%CV		42.57	25.39	10.52	42.05	17.07	16.50

[1/]data shows mean ± SE, different characters in vertical rows mean statistically different at 0.05 level compared by Duncan's New Multiple Range Test (DMRT).

Table 4.5 Interaction of growth conditions and formula medium on shoot number, shoot length and survival rate in weeks1 and 2 of bamboo Sang mon.

Experimental factors		1 week			2 weeks		
Growing conditions (A)	Formula medium (B)	Shoot number	Shoot length (cm)	Survival rate (%)	Shoot number	Shoot length (cm)	Survival rate (%)
Greenhouse	MS+BA2 mg/L	1.22±0.14	3.13±0.30a[1/]	90.05±0.00a	1.22±0.14c	5.19±0.12a	90.05±0.00a
	MS+BA4 mg/L	1.78±0.25	2.64±0.07a	90.05±0.00a	1.78±0.25c	4.41±0.28b	80.04±6.33ab
	MS+BA6 mg/L	1.72±0.20	1.78±0.30b	90.05±0.00a	1.78±0.39c	2.56±0.19d	90.05±0.00a
Outside the greenhouse	MS+BA2 mg/L	2.89±0.67	1.75±0.16b	80.04±6.33ab	3.5±0.55b	3.37±0.41c	70.04±6.33b
	MS+BA4 mg/L	2.56±0.19	1.17±0.16b	90.05±0.00a	4.94±0.45a	1.81±0.08e	80.04±6.33ab
	MS+BA6 mg/L	2.61±0.46	1.28±0.10b	70.04±6.33b	2.00±0.63c	2.08±0.05de	50.03±6.33c
F-test		ns	*	*	**	**	**

[1/] ** means a statistically significant difference at the 0.01 level, * means a statistically significant difference at a 0.05 level, ns means not statistically different.

4.3 Experiment 3: studied the suitable medium formula for shoot induction

The bamboo nodes of Sang nuan and Sang mon of approximately 0.5 cm in diameter and 2-4 cm long that have been sterilized on the nodal surface. Both ends were cut to 2 cm long and cultured in an MS semi-solid medium containing three types of growth regulators, namely BA (benzyl aminopurine), NAA (naphthalene acetic acid), and KIN (kinetin) at 10 concentration levels: Treatment 1 without growth regulator addition, Treatment 2-4 supplemented with (BA) at concentrations of 2, 4, and 6 mg/L Treatment 5-7 supplemented with (BA) concentrations of 2, 4, and 6 mg/L, and supplemented with KIN 1mg/L, respectively. For treatments 8–10, they were supplemented with BA at concentrations of 2, 4, and 6 mg/L with NAA at 1 mg/L, respectively. After 4 weeks, it was found that the 67-80% of nodes cultured in all medium formulas were able to induce lateral buds to develop into shoots. All formulas shoot induced and shoot length: Sang nuan in week 1, with a statistically significant difference ($P<0.01$; Table 4.6,4.7). In addition, there were statistically significant differences in Week 2 and Week 4 ($P< 0.01$; Table 4.6). At week 4, in 6 mg/L BA-added formulations, the highest shoot induction was 6.73±0.94 shoots per node. Sang mon, the statistically significant differences were noted at Weeks 1, 3, and 4 ($P<0.01$; Table 4.6). Particularly, at Week 4, the medium formula, supplemented with BA at a concentration of 4 mg/L, gave rise to the highest shoot induction of 4.03±0.233 shoots per node (Table 4.6).

Sang nuan, in weeks 1, 3, and 4, there was a statistically significant difference in shoot length ($P<0.01$; Table 4.7), and at week 4, the 4 mg/L BA added medium formula yielded the highest mean shoot length of 5.04 ± 0.14 cm. Sang mon, it was found that in weeks 1-4, there was a statistically significant difference in length ($P<0.01$; Table 4.7), and at week 4, the 2 mg/L MS (Control) and BA medium at a concentration of 2 mg/L gave rise to the highest mean shoot length of 3.52±0.44 and 3.44±0.21 cm, respectively (Table 4.7). The obtained shoots with green stems and leaves showed that the lateral buds of Sang nuan and Sang mon potentially grew and developed into the shoots. Chanthanurak (1991) stated that the tissues from bamboo lateral bud explants were

able to grow and develop into shoots. Many reports have revealed the development of lateral buds into shoots in many bamboo varieties when the nodal explants are cultured, such as Pai Hok (Godbole *et al.,* 2002), Pai Tong (Kumar *et al.,* 2014), Pai Hok (Kapruwan *et al.,* 2014), Pai Ruak (Dongmanee, 2006), Pai Poh (Ramanayake and Yakandawala, 1997), and *Bambusa Vulgaris* (Kaladhar *et al.,* 2017)

For the survival percentage of Sang nuan and Sang mon, cultured in solid MS medium formula, the survival rate of both varieties at week 1 was not statistically different ($P > 0.05$; Table 4.8). Sang nuan showed a statistically significant difference in survival rates at weeks 2-4 ($P<0.01$; Table 4.8). The survival percentage at week 4 revealed that the MS+BA4 mg/L medium formula yielded the greatest percentage of survival (67.56%), followed by MS+BA2 mg/L, and MS (Control) (65.28%). Sang mon, there was a statistically significant difference in survival rates at weeks 3 –4 ($P<0.01$; Table 4.8). At week 4, the MS+BA2 mg/L and MS+BA4 mg/L medium formulas gave rise to the greatest percentage survival of 67.53% (Table 4.8). Sang nuan of which nodes were cultured in medium without any growth regulators, the number of lateral bud inductions was low when compared to those cultured in medium supplemented with the growth regulators, with a mean number of 1.82 shoots per node (Table 4.6) and a mean shoot length of 3.44 cm (Table 4.7). For Sang mon of which nodes cultured in a medium without any growth regulators, it had good lateral bud induction with an average of 1.82 shoots per node (Table 4.6). A greater number of lateral bud inductions compared to that of the node cultured in a medium with growth regulator solution was 3.32 shoots per node (Table 4.6) and the mean shoot length was 3.52 cm (Table 4.7).

The addition of BA and NAA had a beneficial effect on the increase in the number of bamboo shoots in the Sang variety. This is because BA is a growth regulator in the cytokinin group, which has properties in dividing cells, stimulating the stems and lateral bud growth. NAA is a growth regulator in the auxin group that is commonly used with cytokinins for shoot proliferation (Kaveeta, 2002), where auxins are often added in lower concentrations than cytokinins to increase the number of shoots. Excessive concentrations inhibited shoot growth (Chuichai, 2010). These results are consistent

with the culturing of many bamboo tissues using BA alone or in combination with NAA. To induce shoots and increase the number of shoots, BA is used in different concentrations. For example, the nodes of Pai Hok and Pai Tong were able to proliferate well in MS solid medium with BA at 2.5 mg/L (Godbole *et al.*, 2002) and 5 mg/L (Prutpongse and Gavinlertvatana, 1992), respectively.

KIN has a similar effect to BA, making the shoots look thin, not succulent. As a result, after increasing the concentration of BA for plumb shoots, it was used to supplement with BA. However, each plant responds differently to each cytokinin. Therefore, it is advisable to initially test the interesting plants in a medium containing different cytokinins. There have been reports on the lateral node development in many bamboo varieties, using BA supplemented with KIN when node explants were cultured, such as *M. baccifera* (Waikhom and Louis, 2014), *D. strictus* (Ravikumar *et al.,* 1998), *D. Giganeus* (Arya *et al.,* 2002a), and *B. wamin* (Arshad *et al.,* 2005).

Due to experimental observation, the addition of only BA produced more succulent shoots than supplemented with KIN and NAA. After tissue culture for more than 4 weeks, the shoots turned yellow and died. Adding KIN, however, was not able to promote shoot induction in Phai Sang nuan and Phai Sang mon. However, KIN had an effect similar to BA, making the shoots thin but not succulent. Therefore, it was used to be supplemented with BA. Actually, each plant responds differently to each cytokinin type. Kin addition depends on different factors, such as bamboo species. Different plants and different species often need different nutrients, even the same plants at different ages and at different development stages (Kaveeta, 1998). As for NAA, according to the theory of equilibrium ratio quantity of cytokinins and auxins, they affect organ formation and are necessary for the growth and development of cultured cells, such as callus, roots, or shoots (Kaveeta, 2002). If the ratio of cytokinin is higher than that of auxin, cells are divided and developed into shoots, stems, and leaves, but if the ratio of cytokinins is lower than that of auxin, rooting is more productive.

Table 4.6 Effects of BA, KIN, and NAA growth regulator concentrations in MS medium on the shoot induction of bamboo Sang nuan and Sang mon cultured for 4 weeks.

Treatments	The number of shoots Sang nuan and Sang mon							
	Sang nuan				Sang mon			
	1 week	2 weeks	3 weeks	4 weeks	1 week	2 weeks	3 weeks	4 weeks
MS (Control)	1.80±0.21abc[1/]	1.90±0.23c	1.88±0.26	1.82±0.24c	3.06±0.17ab	3.09±0.16	3.03±0.18ab	3.32±0.11abc
MS+BA2 mg/L	1.75±0.17abc	2.17±0.06bc	2.47±0.24	2.48±0.22bc	2.38±0.24bc	2.96±0.19	2.85±0.23bc	3.35±0.315abc
MS+BA4 mg/L	2.17±0.10ab	2.28±0.17bc	2.28±0.24	3.51±0.27b	3.22±0.24ab	3.55±0.35	3.73±0.22a	4.03±0.23a
MS+BA6 mg/L	2.17±0.23ab	3.39±0.37a	4.54±0.52	6.73±0.94a	3.70±0.86a	2.75±0.06	2.48±0.22bcd	2.35±0.18c
MS+BA2 mg/L+KIN1 mg/L	2.41±0.22a	2.77±0.20ab	2.37±0.12	1.87±0.05c	2.95±0.37ab	2.72±0.29	2.14±0.25cd	2.68±0.42abc
MS+BA4 mg/L+KIN1 mg/L	2.11±0.20ab	2.83±0.59ab	2.78±0.41	3.06±0.60bc	2.30±0.23bc	2.03±0.19	2.35±0.37bcd	2.85±0.53abc
MS+BA6 mg/L+KIN1 mg/L	2.22±0.10ab	2.21±0.12bc	2.93±0.21	3.56±0.52b	2.76±0.33ab	3.43±0.41	3.76±0.37a	3.81±0.66ab
MS+BA2 mg/L+NAA1 mg/L	1.29±0.44c	1.65±0.08c	1.75±0.18	1.80±0.40c	1.54±0.11c	1.57±0.16	1.71±0.17d	2.18±0.62c
MS+BA4 mg/L+NAA1 mg/L	1.88±0.07abc	1.81±0.13c	1.92±0.11	2.22±0.43bc	2.12±0.13bc	2.17±0.31	2.53±0.30bcd	3.12±0.43abc
MS+BA6 mg/L+NAA1 mg/L	1.70±0.07bc	1.59±0.08c	1.87±0.15	1.96±0.21c	2.17±0.14bc	1.81±0.15	1.95±0.22d	2.50±0.51bc
F-test	*	**	ns	**	**	ns	**	**
%CV	21.55	22.72	21.99	31.40	26.77	18.95	19.71	29.00

[1/] ** means a statistically significant difference at the 0.01 level, * means a statistically significant difference at a 0.05 level, ns means not statistically different.

Table 4.7 Effects of BA, KIN, and NAA growth regulator concentrations in MS medium on the shoot length of bamboo Sang nuan and Sang mon cultured for 4 weeks.

Treatments	The number of shoots length Sang nuan and Sang mon (cm)							
	Sang nuan				Sang mon			
	1 week	2 weeks	3 weeks	4 weeks	1 week	2 weeks	3 weeks	4 weeks
MS (Control)	1.64±0.07abc[1/]	3.34±0.24	2.82±0.26cd	3.44±0.29cde	1.11±0.03ab	2.54±0.14a	2.84±0.06a	3.52±0.44a
MS+BA2 mg/L	2.06±0.10a	4.46±0.27	5.11±0.18a	5.94±0.45a	1.20±0.11a	1.99±0.26ab	2.73±0.33a	3.44±0.21a
MS+BA4 mg/L	1.91±0.05ab	3.73±0.25	4.20±0.41ab	5.04±0.14ab	1.12±0.09ab	1.46±0.13bc	2.22±0.21ab	2.37±0.10ab
MS+BA6 mg/L	1.66±0.11abc	3.73±0.37	3.98±0.59bc	4.20±0.53bcd	0.76±0.05c	1.23±0.06bc	1.91±0.32abc	2.49±0.36ab
MS+BA2 mg/L+KIN1 mg/L	1.53±0.23bc	3.24±0.14	3.34±0.21bcd	4.39±0.23bc	0.39±0.06d	0.73±0.05bc	1.32±0.11bc	1.49±0.17bc
MS+BA4 mg/L+KIN1 mg/L	1.46±0.16bc	2.91±0.25	3.68±0.45bcd	5.39±0.21ab	0.83±0.06bc	1.66±0.23b	1.49±0.15bc	1.85±0.20bc
MS+BA6 mg/L+KIN1 mg/L	1.20±0.04cd	1.84±0.21	3.12±0.35bcd	4.27±0.34bcd	0.71±0.05c	1.83±0.18ab	2.01±0.35abc	2.45±0.20ab
MS+BA2 mg/L+NAA1 mg/L	0.85±0.35d	2.49±0.38	2.87±0.37cd	3.09±0.35de	0.75±0.13c	1.18±0.18c	1.04±0.22c	0.98±0.26c
MS+BA4 mg/L+NAA1 mg/L	1.21±0.07cd	2.22±0.32	3.56±0.35bcd	4.45±0.53bc	0.58±0.11cd	1.15±0.37bc	1.21±0.16bc	1.40±0.22bc
MS+BA6 mg/L+NAA1 mg/L	0.87±0.03d	1.95±0.27	2.61±0.33d	2.67±0.48e	0.85±0.18bc	1.75±0.53ab	2.02±0.70abc	2.62±0.94ab
F-test	**	ns	**	**	**	**	**	**
%CV	21.36	18.66	20.81	17.74	23.53	32.86	33.33	34.03

[1/] ** means a statistically significant difference at the 0.01 level, * means a statistically significant difference at a 0.05 level, ns means not statistically different.

Table 4.8 Effects of BA, KIN, and NAA growth regulators concentrations in MS medium on survival percentage of bamboo Sang nuan and Sang mon cultured for 4 weeks.

Treatments	The percentage of number survival rates Sang nuan and Sang mon (%)							
	Sang nuan				Sang mon			
	1 week	2 weeks	3 weeks	4 weeks	1 week	2 weeks	3 weeks	4 weeks
MS (Control)	90.05±0.00	78.79±4.31ab[1/]	72.04±0.00ab	65.28±2.25a	83.29±4.31	63.03±5.20abcd	63.03±5.20abcd	56.28±7.69ab
MS+BA2 mg/L	81.04±0.00	74.29±4.31abc	69.79±4.31ab	65.28±4.31a	87.79±2.25	81.04±6.37ab	74.29±7.69ab	67.53±11.33a
MS+BA4 mg/L	90.05±0.00	85.54±2.60a	83.29±4.31a	67.56±7.78a	85.54±2.60	76.54±10.72abc	72.04±9.73ab	67.53±7.80a
MS+BA6 mg/L	78.79±2.25	67.53±5.81bc	63.03±3.68bc	58.53±5.81ab	83.29±4.31	83.29±4.31a	81.04±3.68a	56.28±5.67ab
MS+BA2 mg/L+KIN1 mg/L	81.04±3.68	65.28±4.31bc	58.53±5.81bcd	51.78±6.75abc	87.79±2.25	65.28±2.25abcd	58.53±7.80abcd	49.53±9.37ab
MS+BA4 mg/L+KIN1 mg/L	60.78±2.25	40.52±4.50d	40.52±4.50d	31.52±2.60c	90.05±0.00	76.54±2.60abc	67.53±5.81abc	51.78±7.69ab
MS+BA6 mg/L+KIN1 mg/L	83.29±2.25	60.78±2.25c	49.53±5.81cd	40.52±5.81bc	83.29±4.31	72.04±3.68abcd	56.28±4.31bcd	40.52±2.60bc
MS+BA2 mg/L +NAA1 mg/L	78.79±2.25	63.03±6.36bc	58.53±10.72bcd	54.03±12.19ab	76.54±5.81	60.78±7.69bcd	45.02±11.62cd	33.77±9.99bc
MS+BA4 mg/L+NAA1 mg/L	76.54±2.60	63.03±6.37bc	60.78±7.69bc	42.77±4.31bc	76.54±2.60	54.03±3.68d	42.77±5.67d	38.27±4.31bc
MS+BA6 mg/L+NAA1 mg/L	76.54±2.60	67.53±5.81bc	60.78±7.69bc	38.26±7.69bc	78.79±11.26	58.53±10.72cd	47.27±7.69cd	24.76±6.75c
F-test	ns[2/]	**	**	**	ns	*	**	**
%CV	5.45	14.59	19.76	25.45	11.76	18.61	24.04	31.80

[1/] ** means a statistically significant difference at the 0.01 level, * means a statistically significant difference at a 0.05 level, ns means not statistically different.

Figure 4.1 Bamboo shoot development of Sang nuan after culture 4 weeks, the node was grown on MS solid medium with BA concentrations of 2-6 mg/L, KIN concentrations of 1 mg/L, and NAA concentrations of 1 mg/L. (No PGR, growth regulator was added.) (Scale bar=1 cm)

Figure 4.2 Bamboo shoot development of Sang mon after culture 4 weeks, the node was grown on MS solid medium with BA concentrations of 2-6 mg/L, KIN concentrations of 1 mg/L, and NAA concentrations of 1 mg/L. (No PGR, growth regulator was added.) (Scale bar=1 cm)

4.4 Experiment 4: studied the suitable formula for shoot multiplication

The shoots from nodes of approximately 1.5–2.5 cm in height from tissue culture on MS solid medium containing 6 mg/L BA in Sang nuan and 4 mg/L BA in Sang mon in Experiment 3 were taken to be excised into groups of 3 shoots and cultured on MS medium containing BA 2,4 mg/L, BA 3 mg/L supplemented with NAA 0.5 mg/L, and with coconut juice 8% (v/v), and controlled without the addition of hormone substances. They were cultured on different media, including liquid medium and solid medium, for 2 weeks. According to the results of the medium state experiment, Sang nuan cultured on a liquid medium produced more shoots than those cultured on a solid medium and could multiply shoots. The percentage of shoot multiplication both in week 1 and week 2 was a statistically significant difference ($P<0.01$; Table 4.9). In week 2, the shoot multiplication and the percentage of shoot multiplication produced the highest of 8.80 ± 0.33 shoots and 193.33 ± 11.15% due to being agar-free of liquid medium. Agar is an ingredient that causes medium density. While culturing in a liquid medium, the culture flasks must be placed on a 120 rpm shaker to allow more ventilation and air circulation in the medium than those in a solid medium (Somphon, 2006). This resulted in good growth and shoot multiplication. These experimental results were consistent with the first 2-4 weeks of shoot multiplication of Pai Ruak (*Thyrsostachys Siamensis* Gamble) (Dongmanee, 2006) and Pai Poh (*Dendrocalamus giganteus*) (Ramanayake and Yakandawala, 1997) that were cultured in a liquid medium as mentioned above. However, the optimal medium states were different based on the bamboo species, and the shoot lengths were not statistically different. For medium formulas, all medium formulas had a tendency to be used to develop new shoots. The development of new shoots in each culture group was not statistically significant ($P>0.05$; Table 4.9). In the shoot multiplication experiment, the number of shoots was consistent with the reports of Deelom (2019), Jirakiattikul (2010), Dongmanee (2006), and Semsuntud *et al*. (1997), which used more than 3 shoots to culture Beijing bamboo (*Dendrocalamus* sp.), Pai Liang (*Bambusa multiplex*), Pai Ruak (*Thyrsostachys siamensis* Gamble), and Pai Tong (*Dendrocalamus asper*, respectively. The tissue culture by adding BA as a growth regulator in the cytokinin group had an effect on cell division and plant stem growth

(Peeradej, 1986) as well as the lateral bud stimulation. NAA is a growth regulator in the auxin group that is commonly added to a culture medium supplemented with cytokinins for shoot induction. It must be added in lower amounts than cytokinins (Kitwijan, 2004), where the results are consistent with the study of Chanthanurak (1991), who cultured the tissue of Pai Sang and Pai Ruak. It was found that there were good multiple shoots sprouting from lateral buds from nodes. The propagation was productive when cultured on a medium with a BA concentration of 1 mg/L supplemented with an NAA concentration of 0.5 mg/L, but when the NAA concentration was increased, the number of shoots was reduced. Likewise, Nim nuan(1985), Prutpongse and Gavinlertvatana (1987) reported that bud culture from various bamboo branches on MS medium enriched with BA concentrations in the range of 1–25 mg/L.

For the group of Sang mon shoots cultured in liquid and solid media in week 1, there were no statistically significant differences in shoot multiplication, shoot multiplication percentage, and shoot length ($P>0.05$; Table 4.11). There was a tendency to multiply the shoots both week 1 and week 2, (7.15 ± 0.22 shoots, $138.33 \pm 7.22\%$) and a plant height was (2.27± 13 cm) in week 2, while the culture medium formula on MS+BA2 mg /l gave the highest shoot length of 1.54c ±0.12 cm. All medium formulas tended to develop new shoots and the new shoots cultured in groups were not statistically different ($P>0.05$; Table 4.11), which corresponds to Songsoem (2018), where the shoots of Sang mon cultured in MS liquid medium supplemented with 2 mg/L BA in combination with 8% coconut juice had the highest number of newly developed shoots, equal to 4.67. ± 0.02 shoots but not statistically different from those newly developed in MS liquid medium with a BA at a concentration of 2 mg/L and coconut juice at concentrations of 0.8 and 15% (4.00 ± 0.82 and 4.17 ± 0.63 shoots, respectively). The number of new shoots developed on MS solid medium with 2 mg/L BA added with 0. 8 and 15% coconut juice, the number of new shoots developed was 2.38 ± 0.85, 2.75 ± 1.52, and 2.46 ± 0.90, respectively (Figure 4.5 B). By measuring the length of the developed shoots, it was found that there was no statistical difference when culturing the shoot group on all medium formulas, with the shoot length of, 2.10 ± 0.36 – 2.55 ± 0.40 cm. Moreover, there were reports on other plant species, using

coconut juice to multiply the number of shoots, such as vanilla. It was found that MS solid medium containing 1 mg/L BAP and 15% coconut juice cultured for 20 days was able to increase the maximum shoot multiplication of 9.4 shoots (Kalimuthu *et al.*, 2006). and Nidaporn *et al* (2016).

From the shoot multiplication study Sang nuan and Sang mon, MS formulas with BA at concentrations of 2, 4, and 6 mg/L and MS without hormones were studied. The formula MS+ BA 6 mg/L was unable to multiply shoots due to its impact, causing yellowish stems and death. Referring to the theory that BA is a growth regulator in the cytokinin group that affects cell division and stimulates plant stem growth (Peeradej, 1986) as well as lateral bud stimulation, while NAA is a growth regulator substance in the auxin group which is commonly added into culture medium supplemented with cytokinins to induce shoots, an additional formula was invented to multiply the shoots, but it must be added in lower amounts than cytokinins (Kitwijan, 2004). This formula was chosen due to the similar concentration levels.

4.4.1 Interaction of factors shoot multiplication

Considering the interaction between the two factors, including medium conditions and formula medium, it was found that culturing Sang nuan in the liquid medium was better than culturing in a solid medium with a statistical difference (Table 4.10). In particular, when cultured on MS medium without hormone additives, MS supplemented with 2 and 4 mg/L BA and MS supplemented with 3 mg/L BA and 0.5 mg/L NAA, the shoot multiplication, and the multiplication percentages were found to be maximum of about 5-6 shoots and 94%. These results were consistent with the culture for shoot multiplication of Pai Ruak in 2-4 weeks. In week 1, It was able to multiply more shoots in a liquid medium than in a semi-solid medium (Dongmanee, 2006). This is also consistent with research reports that the agar added to solid medium binding to water and growth regulators results in the reduction of plants' ability to absorb nutrients. In addition, when time passed, in a solid medium, it resulted in the accumulation of phenolic compounds at the shoot base or plant base, increasing its toxicity, causing the reduction of plant growth, the shoot development, and the appearance of yellow leaves and fall faster than usual. The liquid medium had the

absorption of nutrients and less phenolic accumulation, resulting in better plant growth and characteristics (Arshad *et al.,* 2005). Using with zeatin at a concentration of 2 mg/L in combination with NAA at a concentration of 0.5 mg/L for 8 weeks, developed the highest number of shoots at 93.18% (Hanchana *et al*., 2014). For vanilla culture (*Vanilla planifolia* 'Andrews'), it was found that MS liquid medium added with BA at a concentration of 2.15 mg/L for 4 weeks was able to increase the number of shoots by 18.6 shoots per explant (Lee-Espinosa *et al*., 2008). This could be because cultivating in a liquid medium provided the plant tissues with a larger contact surface area than cultivating in a solid medium (Nittaya and Supaporn, 2016). From tissue culture of Pai Ruak (Dongmanee, 2006) and Pai Poh (Ramanayake and Yakandawala, 1997), it was found that both types of bamboo shoots were able to grow and multiply well in a liquid medium.

Table 4.9 Effect of plant growth regulators on multiple shoots formation of Sang nuan.

Experimental factors		1 week			2 weeks		
		Shoot number multiplications	Shoot length (cm)	Multiplication percentage	Shoot number multiplications	Shoot length (cm)	Multiplication percentage
Medium status (A)	Liquid medium	5.60±0.16a[1/]	1.64±0.08	86.67±5.18a	8.80±0.33a	3.13±0.22	193.33±11.15a
	Solid medium	4.55±0.13b	1.65±0.11	51.67±4.30b	6.75±0.26b	2.79±0.14	125.00±8.75b
Formula medium (B)	Control	4.80±0.22	1.45±0.12	60.00±7.49	7.30±0.45	2.70±0.25	143.33±14.93
	MS+BA2 mg/L	5.10±0.28	1.60±0.11	70.00±9.33	7.80±0.55	3.16±0.22	160.00±18.35
	MS+BA4 mg/L	5.20±0.25	1.65±0.16	73.33±8.24	7.90±0.55	2.56±0.20	163.33±18.24
	MS+BA3mg/L+NAA 0.5mg/L	5.20±0.17	1.89±0.13	73.33±5.72	8.10±0.38	3.42±0.32	170.00±12.54
F-test	A	**	ns	**	**	ns	**
	B	ns	ns	ns	ns	ns	ns
	A*B	*	ns	*	ns	ns	ns
%CV		17.00	35.60	41.58	23.87	37.41	38.87

[1/] ** means a statistically significant difference at the 0.01 level, * means a statistically significant difference at a 0.05 level, ns means not statistically different.

Table 4.10 Interaction of medium conditions and formula medium on shoot number multiplications and multiplication percentage in weeks 1 of bamboo Sang nuan.

Experimental factors		1 week	
Medium status (A)	**Formula medium (B)**	**Shoot number multiplications**	**Multiplication percentage**
Liquid medium	control	5.60±0.16a	86.67±5.44a
	MS+BA2 mg/L	5.80±0.44a	93.33±14.74a
	MS+BA4 mg/L	5.80±0.39a	93.33±12.96a
	MS+BA3mg/L+NAA0.5mg/L	5.20±0.13ab	73.33±4.44ab
Solid medium	control	4.00±0.21c	33.33±7.03c
	MS+BA2 mg/L	4.40±0.16bc	46.67±5.44bc
	MS+BA4 mg/L	4.60±0.16bc	53.33±5.44bc
	MS+BA3mg/L+NAA0.5mg/L	5.20±0.33ab	73.33±10.89ab
F-test	A*B	*	*

[1/] ** means a statistically significant difference at the 0.01 level, * means a statistically significant difference at a 0.05 level, ns means not statistically different.

Table 4.11 Effect of plant growth regulators on multiple shoots formation of Sang mon.

Experimental factors		1 week			2 weeks		
		Shoot number multiplications	Shoot length (cm)	Multiplication percentage	Shoot number multiplications	Shoot length (cm)	Multiplication percentage
Medium status (A)	Liquid medium	5.00±0.13[1/]	1.07±0.08	66.67±4.47	7.15±0.22	2.27±0.13	138.33±7.22
	Solid medium	4.65±0.18	1.22±0.12	55.00±5.91	6.50±0.33	2.35±0.16	116.67±11.13
Formula medium (B)	control	4.60±0.21	1.01±0.15b	53.33±7.01	6.60±0.37	2.18±0.24	120.00±12.43
	MS+BA2 mg/L	4.90±0.22	1.54±0.12a	63.33±7.21	6.90±0.43	2.08±0.12	130.00±14.29
	MS+BA4 mg/L	4.80±0.17	0.86±0.07b	60.00±5.72	6.90±0.28	2.27±0.21	130.00±9.33
	MS+BA3mg/L+NAA0.5mg/L	5.00±0.29	1.17±0.17ab	66.67±9.67	6.90±0.52	2.69±0.22	130.00±17.25
F-test	A	ns	ns	ns	ns	ns	ns
	B	ns	**	ns	ns	ns	ns
	A*B	ns	ns	ns	ns	ns	ns
%CV		20.50	51.41	54.18	26.48	38.72	47.25

[1/] Data shows mean ± SE, different characters in vertical rows mean statistically different at 0.05 level compared by Duncan's New Multiple Range Test (DMRT).

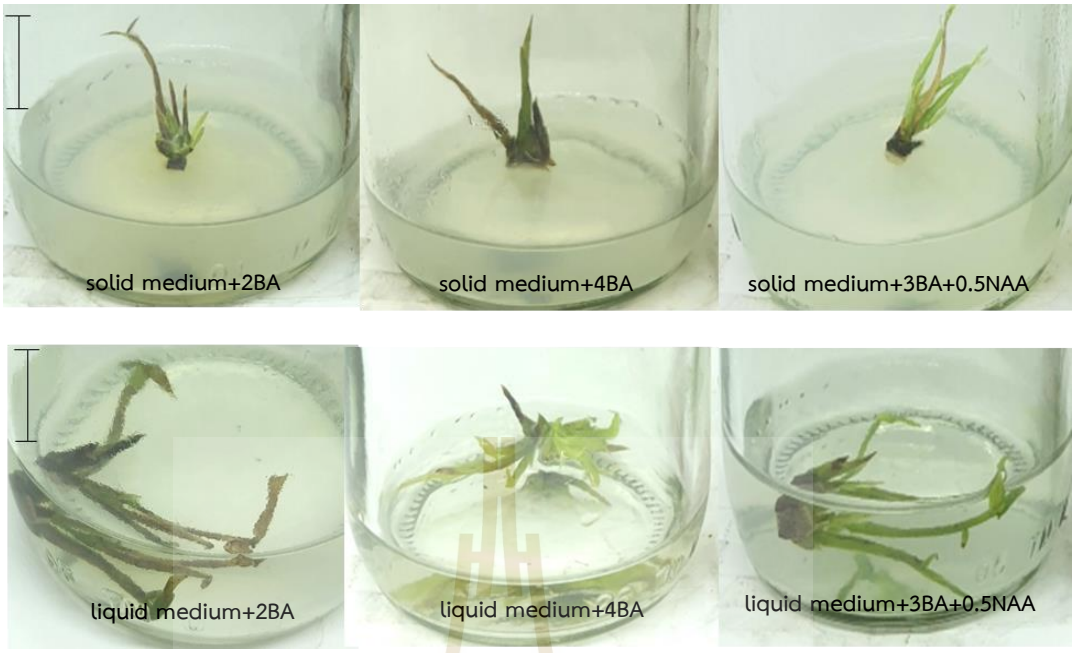


Figure 4.3 Bamboo shoot multiplication development of Sang nuan after culture 2 weeks, the group of shoots are cultured on solid medium (top row) and in liquid medium (bottom row). (Scale bar=1 cm)

Figure 4.4 Bamboo shoot multiplication development of Sang mon after culture 2 weeks, the group of shoots are cultured on solid medium (top row) and in liquid medium (bottom row). (Scale bar=1 cm)

4.5 Experiment 5: studied the suitable formula for root induction

The shoot multiplication induced 3 shoots per group with a height of about 1.5–2.5 cm., cultured on a semi-solid medium with non-hormonal MS and MS containing 4-6 mg/L of NAA, it was found that Sang nuan and Sang mon of which the shoot group was cultured on MS semi-solid medium containing 4 and 5 mg/L NAA were able to form root tubercles and some bottles have a few fabulous roots about 1-2 cm long. The roots were fleshy, short, and white. After being cultured for 12 days, the bamboo had a yellowish-brown appearance and eventually died, when cultured on MS semi-solid medium without hormonal additives and MS containing 6 mg/L NAA, there was no root formation with the brown substances around the shoots. Later, the shoots turned yellow-brown and eventually died.

This may be due to the proper concentration of root-stimulating auxins. In some plants, auxin growth regulators may not be required, but most plants need them in a culture medium to promote root formation as the auxin properties have been mentioned earlier. To induce a plant to root in sterile conditions, low concentrations of auxin should be used because at too high a concentration, root elongation is inhibited. In addition, whether or not auxin has a root-stimulating effect depends on the hormonal balance within those plants (Charassa, 1994). There have already been reports on the shoot induction of various bamboo species in root formation in sterile conditions. The auxins used for bamboo root induction were NAA at a concentration of 0.5–3 mg/L, IBA at concentrations of 0.25–2 mg/L, or IBA, which was also used in combination with NAA. In addition, IAA was used with coumarin in Pai Bong dam (*Bambusa tulda*) (Saxena, 1990), or TDZ with 2,4-D was used in Pai Si suk (*Bambusa blumeana*) (Lin and Chang, 1998), in which Dongmanee (2006) reported on root induction in Pai Ruak (*Thyrsostachys siamensis* Gamble) by culturing three to five shoots on MS medium with NAA at a concentration of 5 mg/L for 3 weeks and transplanting them onto MS medium without growth regulator for 1 week. It was found that up to 80% of the shoots were able to induce root formation. From the experiments of Prutpongse and Gavinlertvatanana (1992), NAA at concentrations of 2.7-5.4 μm was able to induce bamboo roots at NAA concentrations based on the bamboo species.

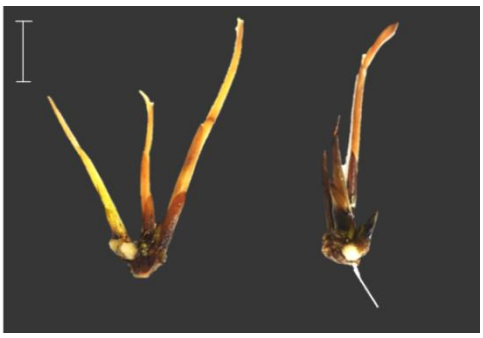


Figure 4.5 Bamboo root development culturing three shoots in MS medium supplemented with NAA at a concentration of 5 mg/L for 12 days. (Scale bar=1 cm)

4.6 Experiment 6: studied the disinfection of bamboo seeds.

The seeds of Kab dang, Sang nuan and Sang mon were taken, bleached, and sterilized in each disinfection formula, and cultured on MS medium without the addition of growth regulators and on MS containing 2-3 mg/L BA concentration for 4 weeks, showing that the Sang nuan variety had a statistically significant difference in the percentage of clean culture ($P>0.01$; Table 4.12) with the highest percentage of (61.53±5.45%), while the Kab dang variety and Sang mon variety had the lowest percentages of clean culture (49.53±5.83% and 43.52±5.86), respectively, with no seed germination rate and eventually died because of seed quality deterioration. In other words, seeds that have passed the physiological maturity stage will begin to deteriorate due to a lack of accumulated medium. At the same time, the seeds continue to breathe; the metabolism of stored food causes deterioration, the rate of which is different for each seed unit. Therefore, the percentage of germination was different. Deteriorated seeds when taken to cultivate cause the seeds not to germinate or germinate into abnormal seedlings (Duangpatra, 1986). Studies on bamboo seed harvesting indices are difficult to conduct because of bamboo's uncertain flowering, different amounts of seed production, and uncertain seed germination rate (10–80 percent) (Anantachote, 1985). The germination rate of Kab dang is about 30–40%. For the seed bleaching formula, both treatments had a tendency to bleach and disinfect seeds, while Treatment 2 had a greater percentage of clean culture than that of

Treatment 1. The highest percentage of clean culture was in week 4 at 57.03 ±4.60%. For the tissue culture medium, no significant difference was observed in all 4 weeks (P>0.05; Table 4.12). All factors had no interaction with each other. Most seed cultures were found to be contaminated with microorganisms, both fungi, and bacteria because the bamboo flowers were formed in groups at the nodes with a lot of sheaths. The sterilization on the surface of the initial tissue uses chloroform at different concentrations and times. It is a method that has been successful in various bamboo species, such as *D. strictus* (Reddy, 2006), *D. hamiltonii* (Zhang *et al.,* 2010a), and *D. giganteus* (Devi *et al.,* 2012).

Table 4.12 Using seeds explant, a method for disinfecting the surface of tissues is developed. Culture begins per survivor percentage.

Factors of experimentation		The percentage of number survival rates			
		1 week	2 weeks	3 weeks	4 weeks
Variety (A)	Kab-dang	55.53±5.70b[1/]	52.53±5.78b	49.53±5.83b	49.53±5.83ab
	Sang-nuan	73.54±4.54a	69.04±4.96a	69.04±4.96a	61.53±5.45a
	Sang-mon	55.53±5.70b	49.53±5.83b	48.03±5.85b	43.52±5.86b
Method of disinfectant	Treatment 1	56.03±4.63	51.03±4.73	50.03±4.74	46.03±4.77
bleaching (B)	Treatment 2	67.04±4.16	63.04±4.37	61.03±4.46	57.03±4.60
Formula medium (C)	control	61.53±5.45	61.53±5.45	58.53±5.59	54.03±5.74
	MS+BA2 mg/L	58.53±5.59	52.53±5.78	52.53±5.78	49.53±5.83
	MS+BA3 mg/L	64.54±5.28	57.03±5.65	55.53±5.70	51.03±5.81
F-test	A	*	*	*	*
	B	ns	ns	ns	ns
	C	ns	ns	ns	ns
	A*B	ns	ns	ns	ns
	A*C	ns	ns	ns	ns
	B*C	ns	ns	ns	ns
	A*B*C	ns	ns	ns	ns
CV%		68.51	71.71	79.47	88.24

[1/]data shows mean ± SE, different characters in vertical rows mean statistically different at 0.05 level compared by Duncan's New Multiple Range Test (DMRT).

4.7 Experiment 7: studied shoot induction from the seeds of Kab dang

The seeds of Kab dang were taken to be disinfected on their surfaces in Experiment 6 and cultured on MS semi-solid medium without the addition of growth regulators and on MS semi-solid medium containing BA at a concentration of 2 mg/L and 3 mg/L for 4 weeks. It showed that all culture media tended to induce shoots. In the adventitious areas, the cultured seeds produced new tiny shoots of 2.55 ± 1.47 (Table 4.13) in green, with tall stems and green leaves, and could break up into clumps.

There was no statistically significant difference in height ($P>0.05$; Table 4.13) in the range of 7.04 ± 4.28 cm. In week 3, seeds cultured on all media could induce rooting.

The root characteristics are primary root or tap root, white, and elongated. Its stem base is broad and gradually tapers to the tip, functioning as the main support for the stability of other parts. The medium for inducing roots was in the range of 3.61 ± 2.35 roots with a root length in the range of 4.41 ± 3.17 cm at week 3 (Table 4.13). For leaf counts, there was no statistically significant difference in all cultured media ($P>0.05$; Table 4.13). For bamboo seeds cultured on MS medium with no growth regulator added and MS solid medium containing BA at a concentration of 2 mg/L and 3 mg/L, the plant explants need to be cultivated on the appropriate medium for fast reproduction. Generally, plants require the same macronutrients but different concentrations, especially plant growth regulators, which cause considerable differences in the needs of different plants (Kaveeta, 2002), indicating that BA had an effect on shoot formation. This is because BA is a growth regulator in the cytokinin group, which affects cell division and the transition to an adventitious shoot from cultured organs. As the increase in tRNA synthesis is involved in the synthesis of proteins, their importance in cell division influences shoots induction (Sachs T., 1991).

The success of tissue culture depends on one of the most important factors: the selection of the appropriate medium, containing nutrients that plants can use effectively (Tantasawat and Waranyuwat, 2008). For seeding, the MS formula is sufficient because sowing seeds can germinate into plants due to an accumulated medium inside as well as various factors, such as water, temperature, light, and nutrients. Therefore, in tissue culture, the required nutrients similar to natural provision must be provided. However, because of the diverse factors in plant types, species, and explants to be cultured, the need for each factor is in different quantities and proportions.

Table 4.13 BA concentration applied to culture media to stimulate shoot formation from Kab dang bamboo seeds explant.

Shoot induced formula		1 week	2 weeks	3 weeks
Shoot numbers	MS	0.85±0.08[1/]	1.75±0.28	2.60±0.39
	MS+BA 2 mg/L	0.90±0.07	2.30±0.25	3.35±0.31
	MS+BA 3 mg/L	0.90±0.07	1.90±0.25	2.95±0.34
F-test		ns	ns	ns
%CV		37.20	58.89	52.50
Shoot length	MS	3.75±0.52	5.00±0.82	5.10±0.82
	MS+BA 2 mg/L	3.75±0.39	6.20±0.53	6.45±0.59
	MS+BA 3 mg/L	3.90±0.49	5.65±0.74	5.85±0.74
F-test		ns	ns	ns
%CV		55.14	56.24	55.86
Root numbers	MS	2.25±0.24	2.10±0.33	2.80±0.45
	MS+BA 2 mg/l	2.40±0.21	2.75±0.22	3.10±0.35
	MS+BA 3 mg/l	2.40±0.21	2.40±0.29	3.20±0.41
F-test		ns	ns	ns
%CV		41.93	52.67	59.59
Root length	MS	3.38±0.46	2.62±0.56	3.25±0.65
	MS+BA 2 mg/l	3.36±0.35	4.17±0.47	3.78±0.47
	MS+BA 3 mg/l	3.58±0.42	3.23±0.56	3.79±0.62
F-test		ns	ns	ns
%CV		53.30	71.11	72.56
Leaf numbers	MS	1.70±0.22	2.10±0.33	2.25±0.35
	MS+BA 2 mg/l	2.05±0.21	3.10±0.28	3.20±0.30
	MS+BA 3 mg/l	1.80±0.20	2.35±0.29	2.60±0.32
F-test		ns	ns	ns
%CV		50.79	53.71	54.47

[1/] data shows mean ± SE, different characters in vertical rows mean statistically different at 0.05 level compared by Duncan's New Multiple Range Test (DMRT).

4.8 Experiment 8: studied root induction from the seeds of Kab dang

The seeds of Kab dang cultured in Experiment 7 using seedlings from MS formula without growth regulator for 2 weeks, and then they were transferred to be cultured on MS semi-solid medium without growth regulator added and on MS semi-solid medium containing 1-3 mg/L NAA concentration for 3 weeks. It was found that in week 3, the number of roots and leaves were significantly different ($P<0.01$; Table 4.14), and root length and plant height were statistically different ($P>0.01$; Table 4.14). It was found that MS formulation without the addition of growth regulators could induce roots. The highest number of roots and root length was 4.00±0.21 roots and 5.71±0.52 cm., followed by the MS+NAA3 mg/L formula, which provided 3.80±0.27 roots and 4.97±0.49 cm. The MS+NAA3 mg/L formula had the highest leaf number and plant height, 3.80±0.17 leaves and 7.60±0.27 cm., followed by MS without growth regulator, which provided 3.60±0.11 leaves and 7.50±0.38 cm. In some plants, the addition of auxin growth regulators may not be required for root formation, but in most plants, it needs to be added to the culture medium to induce root formation. The properties of auxin are an invasive growth regulator in plants that regulate cell elongation and help the cells in different explants grow longer and stimulate the formation of more cell walls (Chuichai, 2010). A small amount of auxin helps in growth, but if the amount is too high, it inhibits growth. The action of auxins is dependent on stimuli such as light, temperature, and gravity by transporting auxins within plants in a direction. Charassamrit (1994) stated that the equilibrium ratio of cytokinin and auxin affects organ formation and is necessary for the growth and development of cultured cells, such as callus, root, or shoot formation (Kaveeta, 2002). If the ratio of cytokinin is higher than that of auxin cells, plants will divide and develop into shoots, stems, and leaves, but if the ratio of cytokinins is lower than that of auxins, the root formation is improved. This is done at a low concentration because too high a concentration inhibited root elongation. In addition, whether auxin has a root-stimulating effect or not depends on hormonal balance within that plant as well (Charassamrit, 1994).

Table 4.14 NAA concentrations were applied to the culture medium to induce root formation in Kab dang bamboo seeds explants.

	Root induced formula	1 week	2 weeks	3 weeks
Root numbers	MS	3.00±0.00a[1/]	3.20±0.09a	4.00±0.21a
	MS+NAA 1 mg/L	2.40±0.11c	2.80±0.09ab	3.6.0±0.18a
	MS+NAA 2 mg/L	2.80±0.09ab	2.40±0.28b	2.60±0.31b
	MS+NAA 3 mg/L	2.60±0.11bc	3.00±0.00a	3.80±0.27a
	F-test	**	**	**
	%CV	15.18	23.88	31.57
Root length (cm)	MS	4.43±0.28	4.78±0.30	5.71±0.52a
	MS+NAA 1 mg/L	3.93±0.39	4.10±0.58	3.57±0.26b
	MS+NAA 2 mg/L	4.17±0.21	4.37±0.61	4.37±0.53ab
	MS+NAA 3 mg/L	3.62±0.29	4.71±0.33	4.97±0.49a
	F-test	ns	ns	*
	%CV	33.24	47.64	44.46
Leaf number	MS	2.20±0.17b	2.80±0.17b	3.60±0.11a
	MS+NAA 1 mg/L	1.80±0.09c	3.00±0.00b	3.00±0.00b
	MS+NAA 2 mg/L	2.80±0.09a	2.60±0.31b	2.80±0.34b
	MS+NAA 3 mg/L	1.80±0.09c	3.60±0.18a	3.80±0.17a
	F-test	**	**	**
	%CV	24.35	29.81	26.75
Shoot length (cm)	MS	5.20±0.44a	6.50±0.39a	7.50±0.38a
	MS+NAA 1 mg/L	3.40±0.11c	6.90±0.29a	7.20±0.28ab
	MS+NAA 2 mg/L	4.70±0.22ab	5.10±0.59b	6.10±0.72b
	MS+NAA 3 mg/L	4.10±0.36bc	7.20±0.28a	7.60±0.27a
	F-test	**	**	*
	%CV	32.08	28.38	28.39

[1/] ** means a statistically significant difference at the 0.01 level, * means a statistically significant difference at a 0.05 level, ns means not statistically different.

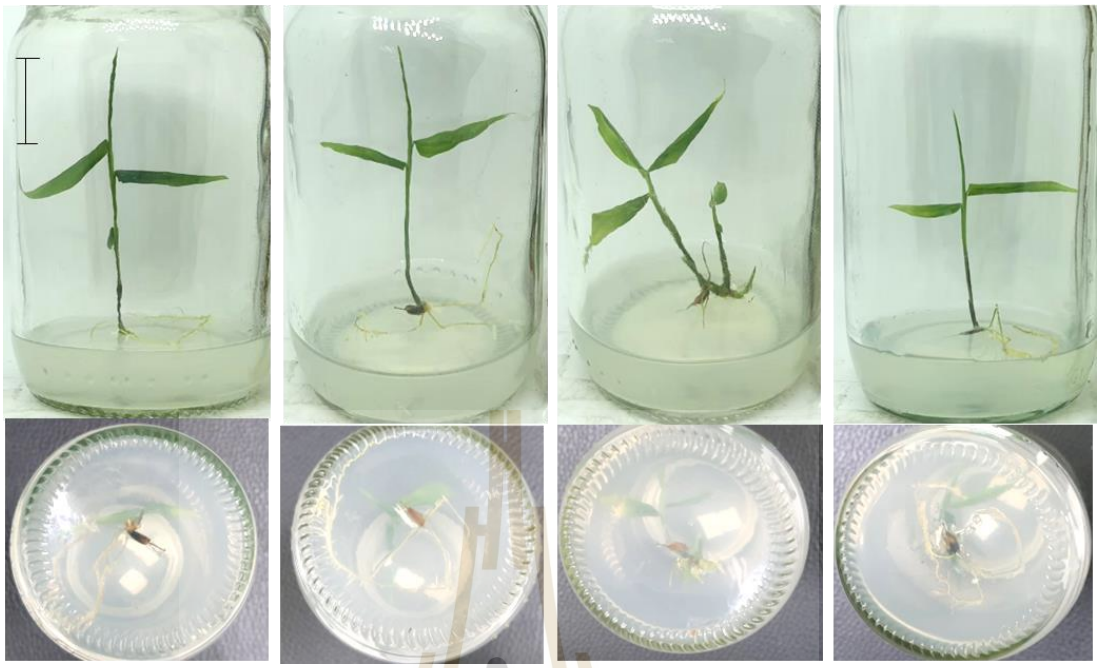


Figure 4.6 Bamboo root development of Kab dang from seeds explant after culture 3 weeks. (Scale bar=2 cm)

4. 9 Experiment 9: examined the suitable method for transplanting bamboo seedlings from the seeds of Kab dang from the laboratory to the nursery

The seedlings of Kab dang with intact roots were taken to be cultured in Experiment 8 and washed to remove the sugar and agar residues in the culture by rinsing with distilled water 2–3 times. Then they were soaked in the water mixed with Benlate fungicide (1 teaspoon per 1 liter of water) for about 10 min. With peat moss material ready for transplanting, the seedlings were then transplanted into a 22-oz plastic cup to be cultured for 14 days. On the 15th day, the plastic cover was gradually opened, and the seedlings were transplanted into the pot for each experimental method. After 2month- of culture, the culture materials for each treatment at weeks 4 and 8 were able to germinate with all treatments with no statistical difference ($P>0.05$; Table 4.15). The most prone to sprouting was SUT planting soil (3.00±0.68 shoots), with the highest survival rate (87.03±1.92%) and the medium least sprouting including rice husk: coconut husk (1.50±0.22 shoots) showed the lowest percentage of survival

(75.2±6.00%) when compared to cultivation materials of other treatments. This is inconsistent with Semsuntud (2000), which reported that the seedlings of Pai Tong (*Dendrocalamus asper*) transplanted into rice husk ash and coconut husks at a rate of 1:1 had the highest survival rate (100%) and the lowest leaf count (4.50±0.41 leaves). In week 8, the plant height was statistically significant differences ($P<0.01$; Table 4.16). The SUT planting soil was the highest (14.60±1.47 cm.), followed by soil: sand: vermiculite and sand: rice husk: future cake (12.04±0.90 and 11.88±1.25 cm). Rice husk: coconut husk was found at the lowest plant height (9.46±0.97 cm.), and the planting material for all 4 treatments was statistically acidic-alkali different ($P>0.01$; Table 4.15 and 4.16). Most cultivation materials were relatively neutral at (5.26-6.30). The chemical and physical properties of the cultivation materials play a very important role in plant growth and development. The chemistry, which is the acid-pH of the cultivation materials, plays a direct role in the number of nutrients that plants can use. Likewise, the functioning of microorganisms makes plants grow well when planted in soil or soil with an appropriate pH between 5.5-7.5 (Davidson and Mecklenburg, 1981). There are two main roles in physiotherapy: seed retention and controlling water and air supply to the roots. However, when mixing materials, the water-holding properties should be taken into account as well. Therefore, the substrate should be capable of retaining moisture sufficient to meet the plant's needs (Bunt, 1987; Davidson and Mecklenburg, 1981). Transplanting plants into their natural state is the final stage of plant tissue culture for propagation. As tissue cultured plants are grown in a controlled laboratory environment, they do not contain cutin, a fatty coating on the leaves and stem surfaces, and the stomata that are always open. Because the relative humidity in the culture flask was up to 90–100% when planting, plants would lose water quickly and wither easily. To reduce such problems, place the seedling juice that comes out of the bottle in an area with high humidity, such as in a misting tank or covered with a plastic bag. Then the humidity is gradually reduced until it is equal to the normal atmosphere in natural conditions (Chamratpan, 2003). Moreover, it should also be exposed to sunlight and natural temperature, and then transplanted into planting material (Bhojwani and Razdan, 1996). Bamboo cultured in sterile conditions has been reported and

successfully transplanted in natural conditions, such as the transplantation of Pai Hok (*Dendrocalamus hamiltonii*) obtained from tissue culture into a mixture of peat, vermiculite, and perlite, in a ratio of 1: 1: 1 by volume before placing it in the nursery. It showed a 100% survival rate (Zang *et al*., 2016). Likewise, Goyal *et al*. (2010) took Pai Sang (*Dendrocalamus strictus*) obtained from tissue culture, removed the agar away by washing it under running water, and planted it in a mixture of perlite, soil, and manure at a ratio of 1: 1: 1 by volume before putting it in the nursery. After 30 days of transplanting, it was found that 70% survived and was able to be transplanted into a planting plot. In addition, for Pai Poh (*Dendrocalamus giganteus*) explants, after rooting induction and transplanting them into a sandy soil mixture (sandy soil: soil: manure), they survived up to 80 - 90% (Devi *et al*., 2012).

Table 4.15 Comparison of planting material to seedling transfer in Kab dang bamboo cultivars at 4 weeks.

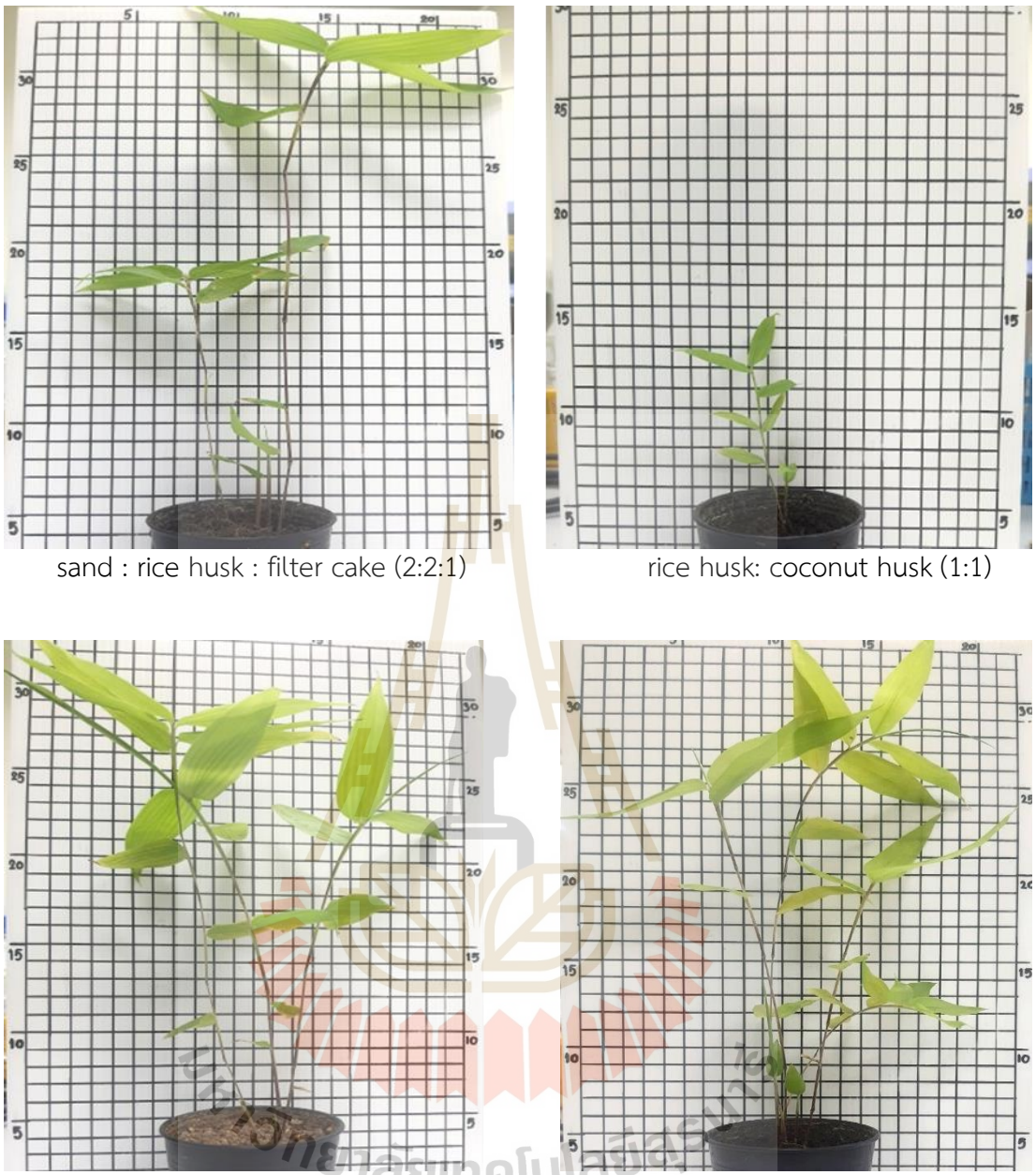
Cultivation material (Material ratio)	Leaf numbers	shoot length (cm)	survival rates	Shoots number
sand : rice husk : filter cake (2:2:1)	4.86±0.56[1/]	9.45±0.98ab	90.05±0.00a	1.67±0.42
rice husk: coconut husk (1:1)	4.17±0.53	8.29±1.25b	77.65±4.99b	0.33±0.21
soil: sand: vermiculite (1:1:1)	5.66±0.34	9.34±0.75ab	87.89±2.15a	1.17±0.31
SUT planting soil	5.35±0.26	11.63±0.89a	90.05±0.00a	1.67±0.49
F-test	ns	ns	**	ns
%CV	20.45	24.96	7.75	75.94

[1/]data shows mean ± SE, different characters in vertical rows mean statistically different at 0.05 level compared byDuncan's New Multiple Range Test (DMRT).

Table 4.16 Comparison of planting material to seedling transfer in Kab dang bamboo cultivars at 8 weeks.

Cultivation material (Material ratio)	Leaf numbers	shoot length (cm)	survival rates	Shoots number
sand : rice husk : filter cake (2:2:1)	5.99±0.62[1/]	11.88±1.25a	84.61±3.48	2.33±0.49
rice husk: coconut husk (1:1)	4.50±0.41	9.46±0.97b	75.2±6.00	1.50±0.22
soil: sand: vermiculite (1:1:1)	6.58±0.72	12.04±0.90a	83.02±7.03	2.00±0.58
SUT planting soil	6.21±0.47	14.60±1.47a	87.03±1.92	3.00±0.68
F-test	ns	**	ns	ns
%CV	24.05	25.51	14.85	58.03

[1/] data shows mean ± SE, different characters in vertical rows mean statistically different at 0.05 level compared byDuncan's New Multiple Range Test (DMRT).



sand : rice husk : filter cake (2:2:1)

rice husk: coconut husk (1:1)

soil: sand: vermiculite (1:1:1)

SUT planting soil

Figure 4.7 Bamboo seeding development of Kab dang after transplanted into the field for 8 weeks. (Scale bar=1 cm

CHAPTER V

CONCLUSION AND RECOMMENDATION

After passing the disinfection on the nodes explant surfaces of Sang nuan with a 70% alcohol for 1 min, their nodes were immersed in a solution of 30% H_2O_2 for 15 min, in 10% NaOCl for 10 min, and in 5% NaOCl for 5 min. After that, they were washed with sterile distilled water 3 times for 3 minutes each, resulting in the least contamination at 18.01%. The Sang nuan species was able to induce 2.39 shoots with nondifference when cultured on MS+BA medium, 6 mg/L, yielding the highest number of shoots of 3.14±0.24 shoots. It was found that the higher the BA, the more shoots were induced but the lower the shoot length. It was also found that MS+BA 2 mg/L and MS+BA 4 mg/L yielded the highest shoot lengths of 4.05 ± 0.18 and 3.94 ± 0.31 cm, respectively. In week 2, the optimal formula for shoot induction showed that MS supplemented with BA at a concentration of 6 mg/L developed the highest number of 6.73±0.94 shoots, and the shoot length was 4.20±0.53 cm. All formulas were suitable for shoot multiplication and the liquid state was selected for tissue culture. When the medium status was co-influenced with the tissue culture medium, all formulas cultured on a liquid medium induced a maximum of about 6 shoots. For the optimal formula for root induction, it was found that shoots cultured on MS semi-solid medium containing NAA at 4 and 5 mg/L were able to develop root tubercles, and some bottles had a few fabulous roots, about 1-2 cm long, fleshy, short, and white. After being cultured for 12 days, the bamboo stems became yellowish-brown and eventually died.

For Sang mon species, the disinfection was performed by bleaching and sterilizing the surface, the branch nodes were washed with soap, and rinsed thoroughly. After that, they were immersed in 70% alcohol for 2 min, in 10% NaOCl for 15 min, and 5% NaOCl for 10 min, and soaked fungicide at a concentration of 1.5 g/L for 1 hr, respectively. Soaking in sodium hypochlorite in all concentrations, 1- 2 drops of Tween20 were added. The nodes were then washed with sterile distilled water three

times, for 3 minutes each, resulting in the least contaminant effect at 25.89%. For the comparison of shoot induction from nodes in the greenhouse and outside the greenhouse. the Sang mon species cultured outside the green was able to induce the highest of 3.48 ± 0.42 shoots, on MS supplemented with BA at a concentration of 4 mg/L medium. The optimum formula for shoot induction showed that the MS medium supplemented with 4mg/L BA developed the highest number of 4.03±0.233 shoots and the shoot length of 2. 37±0. 10 cm. The suitable medium formula for shoot multiplication found that using liquid or solid medium and all medium formulas induced shoots with no differences. Regarding root induction, the shoots cultured on MS semi-solid medium containing NAA 4 and 5 mg/L could form root tubercles, and some vials had slightly fabulous roots, about 1-2 cm long, fleshy, short, and white. After being cultured for 12 days, the bamboo stem turned a yellowish-brown and eventually died.

For Kab dang species using bamboo seeds, the disinfection solution was used by removing the seed coat and wrapping them in a sterile cloth filter. After that, the seeds were soaked in 70% alcohol for 1 min, sterilized with 10% NaOCl for 30 min and 5% for 20 min, and washed with sterile distilled water 3 times for 3 minutes each. They were then cultured on MS medium without the addition of growth regulators, resulting in the most sterile results (54.03±5.74%). Sang nuan and Sang mon bamboo seeds had no seed germination rate. However, the shoot induction from Kab dang bamboo seeds found that all cultured medium formulas tended to be able to induce shoots. The medium that was able to induce the most shoots was MS formula containing BA at a concentration of 2 mg/L, which yielded 3.35±0.31 shoots, and all medium formulas cultured from seeds could also be rooted. An MS formula containing BA at a concentration of 3 mg/L (3.20±0.41 roots). Root induction from Kab dang bamboo seeds found that the culture medium on MS semi-solid medium with no growth regulator added and MS semi-solid medium containing 3 mg/L NAA, cultured for 3 weeks, could induce root, with maximum shoot lengths, leaf counts, and plant heights equal to (4.00±0.21 roots and 5.71±0.52 cm. 3.60±0.11 leaf counts 7.50±0.38 cm. and 3.80±0.27 roots, 4.97±0.49 cm., 3.80±0.17 leaf counts and 7.60±0.27 cm.), respectively.

Then, the Kab dang seedlings with intact roots were then transferred from the laboratory to the greenhouse in different planting materials. The highest sprouting was found in SUT planting soil, equal to (3. 00±0. 68 shoots) with a survival rate. (87.03±1.92%) and a plant height (14.60±1.47 cm).

Recommendations

1. The percentage of survival and the appropriate time for disinfection of nodal explants of Sang nuan and Sang mon should be studied not only at the specified time but throughout the year.
2. For shoot induction from nodes, only one growth regulator should be first studied, such as KIN, NAA, etc., and bring them together, such as KIN, NAA, and IBA for promoting multiple shoot induction.
3. For multiplying the shoots of Sang nuan and Sang mon, the optimal formula should be studied, such as adding TDZ instead of using BA, and the combinations to promote more shoot multiplication, such as MS+BA+KIN, MS+TDZ+KIN, and MS+BA+NAA, etc., as well as the culture system such as bioreactor.
4. Due to the inappropriate level of growth regulators, root induction was not as successful as expected. Growth accelerators in the cytokinin group and various types of auxins should be studied in co-inducing root formation associated with dark culture.
5. Seeds to be bought should be reliable with information about storage date and method. and seed germination should be tested.

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BIOGRAPHY

Miss Janyaphat Rattanakom was born in Wang Sa, Surat Thani, on January 17, 1994. She attended and graduated from Loei Pittayakhom High School in Mueang, Loei Province, from 2006 to 2011. She enrolled in the school of crop production technology at the Suranaree University of Technology in 2013 to pursue a bachelor's degree in crop production technology. In 2016, I earned a Bachelor of Science in crop production technology. She completed the cooperative education program at Green World Genetics (Thailand) Co., Ltd. as an assistant breeder, and in 2017, she enrolled imaster's degree program School of crop Production Technology, Institute of Agricultural Technology at Suranaree University of Technology, as well as working as a teaching assistant in the field of crop production technology.