ENHANCING PRODUCTION OF PLANTING MATERIALS THROUGH *IN VITRO* CULTURE TECHNIQUE IN THE DEPARTMENT OF AGRICULTURE SABAH

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Abstract
The Department of Agriculture (DOA) Sabah has been involved in producing tissue culture planting materials since 1996 as part of the Seventh Malaysia Plan (7th MP, 1996-2000). The objective of this paper is to highlight the growth and achievements of DOA Sabah in the production of tissue culture planting materials over the past 27 years. This paper will explore the application and benefits of tissue culture, the varieties of plants and culture mediums utilized, the evaluation of clones in both laboratory and field settings, implementation strategies, encountered challenges, identified opportunities, and the future prospects of implementing tissue culture techniques within DOA Sabah. A total of 10 formulations have been developed between 1996 and 2023 to produce more than 205,000 tissue culture planting materials including banana, pineapple, orchid, oil palm, potato, and ginger, which have been distributed throughout the region. The use of tissue culture has significantly improved the production of planting materials for large-scale and commercial purposes within DOA Sabah.

***Keywords*:** Plant tissue culture, *in vitro* plant culture.

1. INTRODUCTION

Agriculture forms the backbone of global food systems and plays a critical role in the economy of many nations. In recent years, the demand for high-quality planting materials in agriculture has been on the rise due to factors such as increasing population, climate change, and the need for sustainable farming practices. A significant factor influencing crop yields is the quality of planting materials. Nowadays, tissue culture is adopted as a viable horticultural propagation method which has revolutionized the horticultural industry. Tissue culture, also known as micropropagation or a method of vegetative propagation based on biotechnology, offers a viable solution to these challenges by enabling mass production of disease-free and genetically uniform planting materials in a controlled environment (Bachraz, 1998; Chatenet et al*.,* 2001; Altpeter, F. et al., 2016). This technique involves the growth of plant cells, tissues, or organs in a nutrient-rich medium under sterile conditions (Thorpe, 2007; Espinosa-Leal et al., 2018). Tissue culture is also useful in the regeneration of genetically modified cells into whole plants as well as in embryo rescue techniques (BioVision, 2008). By using tissue culture, farmers can rapidly propagate plants with desirable traits, leading to increased yields, improved crop quality, and reduced dependency on traditional methods of propagation.

As the Department of Agriculture (DOA) Sabah strives to support farmers and promote agricultural development, the need for reliable sources of planting materials remains a critical concern. Traditional methods of propagation often result in variability in crop quality and yield, posing challenges to farmers. Therefore, DOA Sabah has initiated tissue culture planting material production since 1996 during the 7th MP (1996-2000) with a vision to enhance research projects, conservation efforts, and commercial production. The utilization of *in vitro* culture techniques revolutionized the production of planting materials, allowing DOA Sabah to scale its operations and cater to various agricultural needs. Tissue culture, with its ability to mass-produce disease-free and genetically uniform planting materials, offers a viable solution to address these challenges. By incorporating tissue culture techniques into agricultural programs, the Department can enhance the availability of high-quality planting materials, thereby supporting farmers in achieving better yields and improved crop resilience. This paper aims to showcase the evolution and achievements of DOA Sabah in tissue culture planting material production over the past 27 years.

2. APPLICATIONS AND BENEFITS OF TISSUE CULTURE IN DOA SABAH

Tissue culture in agriculture has numerous applications and benefits, including micropropagation for the rapid mass production of genetically identical plant clones, particularly advantageous for high-value crops like fruits and ornamentals. The *in vitro* culture techniques provide key advantages, including efficient production of high-quality planting materials, genetic uniformity for consistent crop characteristics, space efficiency through multi-generations cultivation in lab environments, and year-round propagation. These benefits have significantly enhanced agricultural productivity and efficiency in DOA Sabah's research and extension initiatives. As reported by Chadha and Choudhary (2010), this technique ensures the availability of disease-free planting materials through the use of meristematic tissues, which enhances crop health and productivity by improving disease resistance. Additionally, it plays a crucial role in conserving rare species, maintaining genetic diversity, and facilitating the reintroduction of endangered plants into their natural habitats. Tissue culture also underpins plant genetic engineering, enabling the introduction of desirable traits such as pest resistance and drought tolerance (Suman, 2017).

2.1 TYPES OF EXPLANTS AND MEDIA COMPOSITION USED

In the principles of *in vitro* culture, plant cells, tissues, or organs are grown in a sterile environment on a nutrient medium (Thorpe, 2007). The technique depends mainly on the concept of totipotentiality of plant cells which refers to the ability of a single cell to express the full genome by cell division (Haberlandt, 1902). Regeneration in tissue culture can occur through somatic embryogenesis, where embryos from somatic cells (Suman & Kumar, 2016), or through organogenesis (Espinosa-Leal et al., 2018), leading to the development of roots, shoots, or other organs from tissue cultures. The key aspects to consider include explant selection, which involves using suitable plant parts like seeds, leaves, or stems that can regenerate into whole plants (Hussain et al., 2012; Espinosa-Leal et al., 2018). The choice of explant material also determines if the plantlets developed via tissue culture are haploid or diploid. Also, the risk of microbial contamination is increased with inappropriate explants (Suman, 2017).

Sterile conditions are also crucial to prevent contamination and promote healthy growth through aseptic techniques. The nutrient medium is essential as it provides the necessary nutrients, hormones and growth regulators for cellular growth, differentiation, and development (Murashige & Skoog, 1962). In DOA Sabah, the choice of explants, types of tissue culture and types of media culture used are shown in Table 1.

Table 1: Types of plants, explants, method of culture and types of media culture used.

Types of plants	Types of explants	Method of culture	Types of media culture
Banana	Sucker and male inflorescence	Meristem culture	MS (1962) + PGRs
Pineapple	Sucker and crown	Meristem culture	MS (1962) + PGRs
Orchid	Seed and inflorescence nodal segments	Seed culture and bud culture	VW (1949) + PGRs, MS (1962) + PGRs
Potato	Sprouting buds and shoot tip	Bud culture and meristem culture	MS (1962) + PGRs
Ginger	Sprouting buds and shoot tip	Bud culture and meristem culture	MS (1962) + PGRs
Oil Palm	Ortet and root tip	Meristem culture and callus culture	MS (1962) + PGRs

MS- Murashige and Skoog; VW- Vacin and Went; PGRs- Plant Growth Regulators.

2.2 ASSESSMENT OF CLONES IN LABORATORY AND FIELD TRIALS

The careful selection of healthy and selected explants from germplasm collections at various Agriculture Research Centres in Sabah (Agriculture Research Centre (ARC) Ulu Dusun Sandakan, ARC Quoin Hill, Tawau, ARC Lagud Sebrang, Tenom, ARC Tuaran) followed by cultivation through *in vitro* culture technique, showcases the meticulous approach taken by DOA Sabah in ensuring the quality of the planting materials. It is crucial to evaluate these planting materials in the laboratory, during the hardening stage and in the field to ensure optimal growth performance before distribution. Figure 1 illustrates the *in vitro* culture process and assessment of pineapple tissue culture as it progresses from the laboratory to the field. To ensure a high survival rate, tissue culture plants aged four to six months were provided for field planting or distribution.

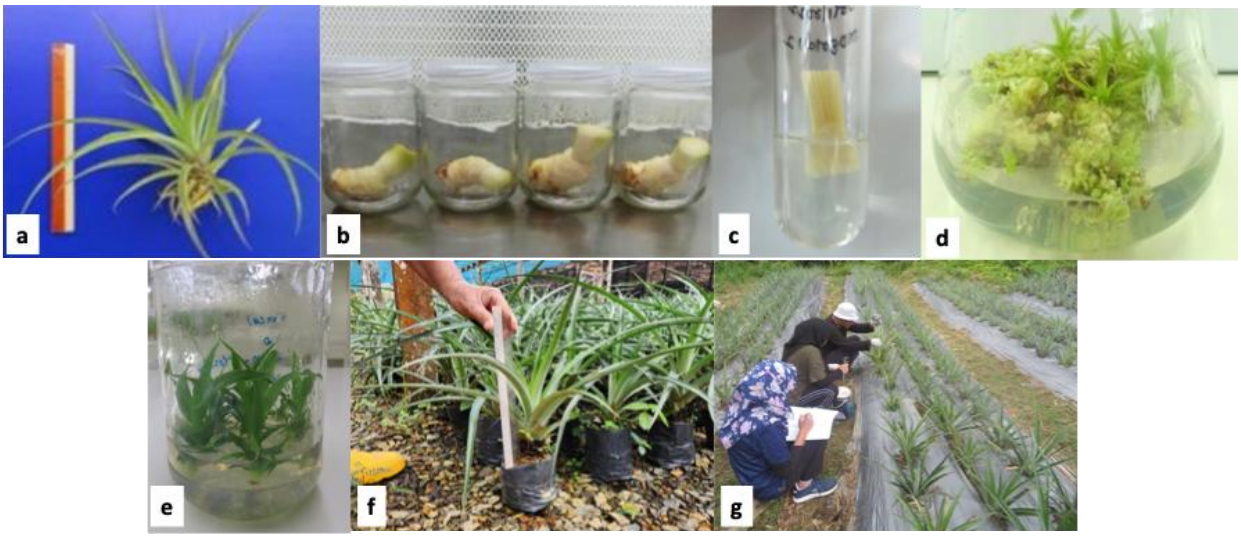

Figure 1. a) Selected pineapple sucker; b) Sterilized explant sucker; c) Explant inoculated in nutrient growth media; d) Embryogenic and shoot formation; e) Plantlets in rooting media; f-g) Evaluation of plants in the nursery (hardening stage) and field.

3. ACHIEVEMENT AND IMPACT ON DOA SABAH PRODUCTIVITY

Since 1996 to 2023, DOA Sabah has achieved significant milestones in productivity development. This included the successful development of the formulation of 10 different plant species as shown in Table 2. Additionally, DOA Sabah is able to produce and distribute tissue culture planting materials for various crops such as banana, pineapple, orchid, oil palm, potato, and ginger. Through Department efforts, over 205,000 tissue culture planting materials were produced (Jabatan Pertanian Sabah, 1997-2024) and sent out across Sabah, making a notable impact on agricultural practices in the region as shown in Figure 2.

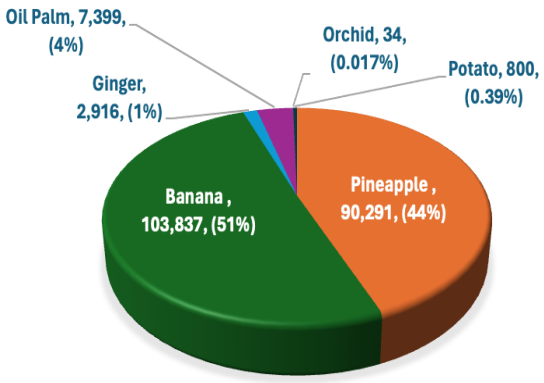

Figure 2. Total production of tissue culture planting materials by the DOA Sabah from 1996 to 2023.

Table 2: Development of 10 *in vitro* culture formulations of different plant species.

No.	Achievements	References
1.	Development of *In Vitro* Culture of Banana and Pineapple Formulation for Production of Planting Materials.	Jabatan Pertanian Sabah (1996)
2.	Launching of Berangan Banana (*Musa acuminata* cv. Berangan) Clone on Mei 27th, 1997.	Jabatan Pertanian Sabah (1997)
3.	Launching of Sekaki Banana (*Musa paradisiaca* cv. Sekaki) Breeding Technology Through *In Vitro* Tissue Culture Techniques on July 10th, 2012.	Jabatan Pertanian Sabah (2012)
4.	Launching of Ginger (*Zingiber officinale* Roscoe) Breeding Protocol Through *In Vitro* Tissue Culture Technique on November 22nd, 2012.	Jabatan Pertanian Sabah (2012); Johnny, D., Rolland, J and Felix, E. (2023).
5.	*In Vitro* Propagation of Sabah Orchid Species Formulation.	Johnny, D. (2013)
6.	Evaluation of Culture Media for Micropropagation of Potato Plantlets (*Solanum tuberosum* L.) and Mini Tubers.	Johnny, D. (2013)
7.	*In Vitro* Culture Formulation of Oil Palm (*Elaeis guineensis*) Using Root Tips Explant as an Explant.	Johnny, D. (2020)
8.	Launching of Pineapple Variety Sabah Volcanic Sweet (SVS) Breeding Technology Through *In Vitro* Tissue Culture Techniques on September 24th, 2022.	Jabatan Pertanian Sabah (2022)
9.	Micropropagation of *Musa* spp. var "Berangan" and "Saba" Using Male Flower.	Rolland, J. and Johnny, D. (2023)
10.	*In Vitro* Propagation and Multiplication of "Saba Banana" (*Musa* spp.).	Rolland, J. and Johnny, D. (2024)

The utilization of tissue culture techniques has significantly impacted the agricultural landscape in Sabah, particularly in the production of high-quality planting materials. The consistent efforts of DOA Sabah in the development of tissue culture formulation, plant assessment, and distribution have facilitated advancements in agricultural research, conservation initiatives, and commercial production. Moreover, the management of tissue culture nurseries and rigorous field trials have been instrumental in ensuring the success and viability of tissue culture plants in various environments.

4. IMPLEMENTATION STRATEGIES WITHIN DOA SABAH

To successfully implement tissue culture techniques within the DOA Sabah, a holistic approach has been adopted, encompassing strategies such as capacity building through training programs, infrastructure development for specialized laboratories, collaboration with other agencies and private companies to leverage technology and expertise e.g. Malaysian Palm Oil Board (MPOB) and Sawit Kinabalu Sdn. Bhd. (SKSB), provision of extension services to educate practical students and farmers on benefits and technical support, allocating budgets in the 12th MP and for future planning in 13th MP to ensure sustainable integration and success**.**

5. CHALLENGES AND OPPORTUNITIES

The adoption of tissue culture techniques within the DOA Sabah presents both challenges and opportunities. Challenges include the initial investment costs for setting up facilities, the need for skilled manpower trained in tissue culture techniques, ensuring rigorous quality control measures to prevent contamination, and scaling up production to meet the demands of a large-scale agricultural system. However, these challenges also present opportunities for economic growth, increased efficiency, improved crop quality, and sustainable agricultural practices once successfully navigated with strategic action plan and resource allocation.

6. FUTURE DIRECTIONS

Future directions in tissue culture can be focused on optimizing protocols for different plant species to improve efficiency e.g. coconut, integrating tissue culture with genetic technologies like CRISPR for developing crops with enhanced traits, adopting sustainable practices to reduce waste and environmental impact, such as application of *Malaysia Good Agricultural Practices* (MyGAP) in nursery and field management, and providing capacity building through training programs for students, farmers and agricultural extension workers to enhance their understanding and application of tissue culture techniques, fostering innovation and sustainability in agriculture.

7. CONCLUSION

The DOA Sabah's dedication to tissue culture planting material production since 1996 has yielded commendable results, benefiting internal research projects, conservation programs, and

commercial agricultural activities. The journey of DOA Sabah in advancing tissue culture techniques underscores the organization's commitment to innovation and sustainability in agriculture. Moving forward, the continued focus on formulation development, plant assessment, and effective management practices will further strengthen DOA Sabah's position as a team player in tissue culture technology for sustainable agriculture in Sabah.

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REFERENCES

Altpeter, F., Springer, N. M., Bartley, L. E., Blechl, A. E., Brutnell, T. P., Citovsky, V., ... & Stewart Jr, C. N. (2016). Advancing crop transformation in the era of genome editing. *The Plant Cell*, *28*(7), 1510-1520.

Bachraz, D. Y. (1996). The role of tissue culture in agricultural Diversification. In *ANNUAL MEETING OF AGRICULTURAL SCIENTISTS* (p. 96).

BioVision (2008). Modern tissue culture lab offer hope to farmers. Newsletter. 10. available online at www.biovisioneastafrica.com.

Chadha, K. L. & Choudhary, M. L. (2010). Horticulture, plantation crops and organic farming for the XI five-year plan (2007- 2012). Planning commission, Government of India.

Chatenet, M., Delage, C., Ripolles, M., Irey, M., Lockhart, B. E. L., & Rott, P. (2001). Detection of Sugarcane yellow leaf virus in quarantine and production of virus-free sugarcane by apical meristem culture. *Plant Disease*, *85*(11), 1177-1180.

Espinosa-Leal, C. A., Puente-Garza, C. A., & García-Lara, S. (2018). *In vitro* plant tissue culture: means for production of biological active compounds. *Planta*, *248*, 1-18.

Haberlandt, G. (1902). Kulturversuche mit isolierten Pflanzenzellen. Sitzungsber. Akad. Wiss. Wien. Math. - Naturwiss. Kl. *Abt. J.* 111, 69-92.

Husain, M. K., & Anis, M. (2009). Rapid in vitro multiplication of Melia azedarach L. (a multipurpose woody tree). *Acta Physiologiae Plantarum*, *31*, 765-772.

Jabatan Pertanian Sabah (1996). Laporan Mesyuarat Tahunan Jabatan Pertanian Sabah 1995.

Jabatan Pertanian Sabah (1997). Laporan Mesyuarat Tahunan Jabatan Pertanian Sabah 1996.

Jabatan Pertanian Sabah (2001). Laporan Mesyuarat Tahunan Jabatan Pertanian Sabah 2000.

Jabatan Pertanian Sabah (2006). Laporan Mesyuarat Tahunan Jabatan Pertanian Sabah 2005.

Jabatan Pertanian Sabah (2011). Laporan Mesyuarat Tahunan Jabatan Pertanian Sabah 2010.

Jabatan Pertanian Sabah (2012). Ulang Tahun Ke-50 “Jubli Emas” Stesen Penyelidikan Pertanian Ulu Dusun, Sandakan, Jabatan Pertanian Sabah. 22 November 2012.

Jabatan Pertanian Sabah (2016). Laporan Mesyuarat Tahunan Jabatan Pertanian Sabah 2015.

Jabatan Pertanian Sabah (2021). Laporan Mesyuarat Tahunan Jabatan Pertanian Sabah 2020.
Jabatan Pertanian Sabah (2022). Laporan Mesyuarat Tahunan Jabatan Pertanian Sabah 2021.
Jabatan Pertanian Sabah (2022). Ulang Tahun Ke-60 "Jubli Intan" Stesen Penyelidikan Pertanian Ulu Dusun, Sandakan, Jabatan Pertanian Sabah. 24 September 2022.
Jabatan Pertanian Sabah (2023). Laporan Mesyuarat Tahunan Jabatan Pertanian Sabah 2022.
Jabatan Pertanian Sabah (2024). Laporan Mesyuarat Tahunan Jabatan Pertanian Sabah 2023.
Johnny, D. (2013). *In Vitro* Propagation of Sabah Orchid Species. Sabah Agricultural Research Journal 3, 63-70. Department of Agriculture Sabah.
Johnny, D. (2013). Evaluation of Culture Media for Micropropagation of Potato Plantlets (*Solanum tuberosum* L.) and Mini Tubers. Sabah Agricultural Research Journal 3, 53-62. Department of Agriculture Sabah.
Johnny, D. (2020). Terminal Report: *In Vitro* Culture Formulation of Oil Palm (*Elaeis guineensis*) Using Root Tips Explant as an Explant. Annual Research Technical Meeting 2020.
Johnny, D., Rolland, J & Felix, E. (2023). Micropropagation of Ginger (*Zingiber officinale* Roscoe) Through Tissue Culture Using Rhizome Sprouted Buds. Presented at 33rd Malaysian Society of Plants Physiology Conference (MSPPC) 2023, WaterFront Hotel, Kuching Sarawak on 18-20 September 2023.
Kumar, A., Sharma, D., Tiwari, A., Jaiswal, J. P., Singh, N. K., & Sood, S. (2016). Genotyping-by-sequencing analysis for determining population structure of finger millet germplasm of diverse origins. *The plant genome*, *9*(2), plantgenome 2015-07.
Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia plantarum*, *15*(3).
Rolland, J. & Johnny, D. (2023). Micropropagation of *Musa* spp. var "Berangan" and "Saba" Using Male Flower. Presented at 17th Asian Food Conference (AFC), Borneo Convention Centre Kuching, Sarawak on 23-28 Oktober 2023.
Rolland, J. and Johnny, D. (2024). *In Vitro* Micropropagation and Multiplication of Sabah Banana (*Musa acuminata x balbisiana*) Using Pseudostem. Presented at 34th Malaysian Society Plant Physiology Conference 2024, Primula Beach Hotel Kuala Terengganu, Terengganu. 27-29 August 2024.
Skoog, F. (1957). Chemical regulation of growth and organ formation in plant tissue cultured *in vitro*. In *Symp. Soc. Exp. Biol. 11*, 118-131.
Suman, S. (2017). Plant tissue culture: A promising tool of quality material production with special reference to micropropagation of banana. *Biochemical & Cellular Archives*, *17*(1).
Thorpe, T. A. (2007). History of plant tissue culture. *Molecular biotechnology*, *37*, 169-180.