

URBAN DEVELOPMENT
AND INFRASTRUCTURE

Wayan Suparta, PhD
Editor

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WAYAN SUPARTA

EDITOR



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As Professor at the Universitas Pembangunan Jaya, with daily activities in lecturing, doing research, as well as water resources development planning, I really praise the Nova Science Publishers for publishing selected papers from “2020 International Conference on Urban Sustainability, Environment, and Engineering (CUSME 2020)”. Hence, this publication would be useful for professionals, reseachers, scholar, policymakers, and NGO. I believe that currently, many professionals would like to give more attention on development of sustainable urban. In addition, this publication could be used as reference for City authorities to make appropriate policy choices to protect the provision of equitable housing, health, and transportation services.

Prof. Ir. Frederik Josep Putuhena M.Sc., Ph.D
Center for Urban Studies – Universitas Pembangunan Jaya



Urban Development and Lifestyle are trend issues for the cities around the world. Learning from experiences is the most effective way to support the cities to be sustainable developed. This book offers the knowledge sharing among countries which covers variety of cities' issues. It also provides the great lessons for researchers, officers and policy makers on coping with several urban problems.

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PREFACE

One of the issues of urban development and urban lifestyle, which can be studied from the sea to space, has posed important challenges for humanities, environmental management of cities and urban areas, and the economy. This field is one of the pillars of sustainable development from urban studies towards sustainability welfare. Research and development (R & D) in this part plays a crucial role where urban problems are always alive and increasing every year because of changing customer preferences and needs. City authorities must make appropriate policy choices to protect the provision of equitable housing, health, and transportation services in the future. The megatrends 2030 triggered by the Industrial Revolution 4.0 estimates urbanization will increase sharply, massive move from rural to urban areas, and the land is getting narrower, especially in Asia. New directions and developments in this field and discussion of future priorities must be well anticipated, meticulous, dignified, and innovative.

This book highlights the latest views and solutions to technological innovations adapted to achieve prosperity in urban sustainability. For instance, adapting new buildings for urban needs with low-cost and modern design materials, the housing environment and the layout of city space, weather changes to disaster, and smart transportation systems are also taken into account. It also involves electricity, environmental management, and ways to use agricultural land to increase income. The ease of technology produced will change the business model.

This contributed volume presents solicited selected papers of the 2020 International Conference on Urban Sustainability, Environment, and Engineering (*CUSME 2020*) with the theme “Urban Life and Technology”. The book covers the point of view in urban architectures with green technology, sustainable environmental, management, agrotechnology, and smart transportation systems. The impact of urban development such as psychological and cultural influences, communication and social complexity, information systems and technology is also discussed with various solutions offered. The outcomes of the conference will certainly support government policy, stakeholders, policymakers, scientists, and engineers by bringing together their latest findings towards achieving a sustainable economy, improved quality of life, and protecting the environment. The findings of this study will create opportunities for further collaboration and are expected to improve the welfare of humanity.

The conference committee and all our contributors wish to pleasantly thank for their efforts and cooperation in finalizing this volume. We wish to acknowledge and gratitude

Nova Science Publishers Team for supporting our book proposal and for granting the opportunity to publish these conference proceedings and for their cooperation and support.

Wayan Suparta

Chairperson of CUSME 2020

The Editor-in-Chief

Chapter 25

**PROPAGATION OF ARROWROOT PLANTS
IN VITRO FOR THE AGROFORESTRY
ENVIRONMENT DEVELOPMENT**

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ABSTRACT

Arrowroot (*Maranta arundinaceae* L) is a type of tuber forest plants with the potential to be developed in an agroforestry environmental pattern because it is able to adapt to environmental conditions and to grow on marginal land or under the stands of forest plants. The difficulty of getting superior varieties and seeds in relatively large numbers and uniformity can be overcome by *in vitro* technique. The research objective was to determine the effect of giving 2.4 D and BA on the growth of arrowroot explants by *in vitro*. The research was carried out in a laboratory using the 2-factor Randomized Complete Design Method. The first factor is the 2.4 D concentration which consists of 3 levels: 0.5 mg, 1, and 1.5 mg/L. The second factor is the concentration of BA consisting of 3 levels, namely 1, 2, and 3 mg/L. The observational data were analyzed with Variance analysis at a 5% real level and continued with Duncan Multiple Range Test (DMRT). The results showed that 2.4 D 1 mg/L produced a greater percentage of life. The combination treatment 2.4 D 1 mg/L and BA 2 mg/L will accelerate the callus and root growths.

Keywords: arrowroot, *in vitro*, agroforestry

INTRODUCTION

Agroforestry is the management and integration of trees, crops, and/or livestock on the same plot of land and can be an integral component of productive agriculture. It may include

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existing native forests and that of established by landholders. It is a flexible concept, involving both small and large-sized land holdings [1]. Agroforestry is one of the effective means for equity and stages for overcoming poverty in the forest village community environment, moreover, it can increase income and food production [2]. The development of agroforestry has quite good prospects in its contribution to increasing farmers' income, so that it facilitates access to food, in addition to maintaining security and preservation of the forest with the community or farmers around the forest [3]. The selection of arrowroot plants (*Maranta arundinacea* L) as an alternative food type in the development of plantations, especially community forests is certainly very potential to be developed. These plants can live among forest stand plants and have high adaptability.

The taxonomy of arrowroot plants is as follows: Kingdom: Plantae (Plants); Subkingdom: Tracheobionta (Vascular Plants); Super Division: Spermatophyta (Producing seeds) Division: Magnoliophyta (flowering plants); Class: Liliopsida (one-piece/monocotyledon); Zingiberales; Family: Marantaceae; Genus: *Maranta*, and Species: *Maranta arundinacea* L. [4].

Arrowroot plants are upright and clumped plants with plant height of 0.5 - 1.5 m. The stem of the plant is green with a height of 75-90 cm with a pseudo type of stem, which is round in shape and forms like a leafy rhizome and has a branching spreading. Long-stemmed leaves have a midrib and thickened with oval-shaped or oval-leaf strands with a pointed tip. Flower arrowroot compound plants are at the end of the stem, zygomorphic with white flowers. Arrowroot fruits are oval-shaped, bald to dark red hair. Arrowroot tubers are white, 10-30 cm long [4].

Ecologically arrowroot plants require hot climates and wet conditions. Arrowroot can grow at an altitude of 0 to 900 m dpl, but it is best at an altitude of 60 to 90 m dpl with sandy soil. The land must be cultivated and plowed properly and fertilized first. Arrowroot plants can live and thrive in the shades.

Arrowroot (*Maranta arundinacea* L.) is a potential food source substitute for wheat flour. Arrowroot has the potential of an international market in St. Vincent (Central America). This plant has been commercially cultivated, and around 95% of the world's needs are supplied from Central America. Arrowroot exporting country in the Southeast Asian region is the Philippines. The flour produced in this country is high-quality, fine-sized, and expensive flour.

Arrowroot tuber yields ranged from 9-12 ton/ha with starch content of 1.92-2.56 ton/ha. Arrowroot tubers can be used as raw materials for the food processing industry, namely starch and arrowroot chips, while the pulp can be used as animal feed. Arrowroot tubers are beneficial for health as a source of food fiber and have a lower glycemic index than other tubers. Arrowroot starch can substitute the use of flour in various food products with a substitution rate of 50-100 [5]. Many studies suggest that tubers have a functional value because they contain resistant starch, inulin, anthocyanin, glucomannan, and low glycemic index [6]. A glycemic index is a number that shows the state of a person's blood sugar after consuming food.

Arrowroot plants in Indonesia have not been cultivated intensively, therefore, it is necessary to have proper cultivation techniques and plant materials/varieties that are in accordance with the conditions of the growing environment [7]. So far, the conventional

propagation of plants is carried out through seed tubers with 2-3 buds. Other constraints faced include the limited arrowroot seeds because farmers generally use seedlings (stolons) as seeds. Propagation by tuber cuttings has limitations because it is difficult to determine the length of tuber dormancy and slow-growing cycles [8].

Planting with tuber cuttings requires organic fertilizer from the results of Setyowati's study [9], it was shown that the use of organic fertilizer (manure and compost) can improve the growth of arrowroot seedlings derived from harvest residual extraction. The highest growth was seen in the use of goat manure (P2), namely plant height 87.70, leaf number 78.80 statistically significantly different from other treatments, while the number of tillers 5.80 was not significantly different but tended to be the highest from other treatments. Therefore, it can be recommended the use of goat manure fertilizers for the addition of fertilizer in arrowroot propagation that uses seedlings from the harvest extraction. Therefore, it is necessary to do alternative propagation with tissue culture technique.

Arrowroot plant tissue culture is an effective and efficient technique in producing plant seedlings quickly, uniformly, and identically with the parent, and in unlimited and continuous quantities. The use of tissue culture technique in the supply of quality and disease-free seeds is needed for seed production, conservation, and plant breeding.

Propagation of arrowroot plants *in vitro* with a small explant of the results of research that has been done. As annual crop, arrowroot has a period of dormancy. When dormancy of the stems and leaves of the plant withers and dies, only tubers or seeds can be used as a source of explants. Seeds do not always exist because they have to enter the generative phase. The original habitat is far from the laboratory and because of the glucomannan content, so tuber culture is done as a source of explants.

In tissue culture technique, an important aspect that must be considered in the composition of a medium is the need for growth regulators, specifically, the combination and concentration of growth regulators used [10]. Growth regulators in tissue culture function to stimulate plant growth, such as root growth, shoots, germination, and so on. Growth regulators commonly used in tissue culture are auxin and cytokinin groups because they play a role in growth and organogenesis. Auxin groups that are often used are 2,4-D, IAA, NAA, while the cytokinin groups are Kinetin, Benzyl Adenin (BA), and BAP.

Auxin has a dual role depending on the chemical structure, concentration, and tissue of the plant being treated. In general, auxin is used to induce callus formation, suspension culture, and roots, namely by encouraging cell elongation and division in cambium tissue [11]. To stimulate the formation of embryogenic callus and somatic embryonic structure, often, auxin is needed in relatively high concentrations.

Auxin has a role in cell growth, apical dominance, and callus formation. The range of auxin concentration commonly used is 0.01-10 ppm. The effect of auxin on cell development shows that auxin can increase protein synthesis. With the increase in protein synthesis, it can be used as a source of energy in growth [12]. Pierik [11] states that natural auxin (IAA) is commonly used at concentrations of 0.01-10 mg/L, and for synthetic auxins (IBA, NAA, and 2,4-D) which are relatively more active, it is used at concentrations of 0.001-10 mg/L.

The first cytokinin found was 6-furfurylamino purine or commonly called kinetin. Cytokinins are derivatives of adenine whose function is to encourage cell division, morphogenesis, and slow the degradation of chlorophyll [13]. According to George and

Sherrington [14], the main influence of cytokines is to stimulate cell division and enlargement and bud formation. Cytokines that are often used in tissue culture include BA (Benzyl Adenin) and Kinetin.

A comparison of the concentration of auxin with cytokines will affect the morphology of the roots and shoots. The ratio of auxin concentration with high cytokinin will encourage root formation, conversely, if the ratio of auxin and cytokinin is low, it will encourage bud formation [13].

The results of Masruri et al. [15] showed that at medium MS + 1 mg/L 2.4 D + 1 mg/L, BAP can stimulate callus induction time of 13 days, average callus biomass, mean expansion of explants with compact and colorful callus textures white on *iles-iles* plants. The results of Mahardi *et al.* [16] study showed that BAP administration of 1.5 mg/L and BA 2 mg/L increased 100% growth, leaf primordial appeared 14 hst, root appeared 20 hst, and mean root number was 5 [15].

Besides the multiplication of arrowroot bulb plants (*Maranta arundinaceae*) in tissue culture to date, there are still few recommendations for the type of use of culture media, composition, and growth regulators to initiate and multiply explants. To get the culture media and the concentration of growth-regulating substances, research is needed.

METHODOLOGY

Arrowroot tissue culture research was carried out in the biotechnology laboratory of the Faculty of Agriculture of UPN "Veteran" Yogyakarta from September to November 2019. Laboratory Experiments were in Completely Randomized Design (CRD) 2 factors. The first factor is the concentration of 2.4 D and the second factor is the concentration of BA. 2,4 D auxin treatment for root multiplication is carried out with a concentration of 0.5 mg/L (D1), 1 mg/L (D2) and 1.5 mg/L (D3). On the other hand, to stimulate the formation of shoots, BA is given a concentration of 1 mg/L (B1), 2 mg/L (B2), and 3 mg/L (B3). Each treatment was repeated 3 times (bottles) and each test contained 5 tubers.

The explants used were 9-month-old tubers of arrowroot from Bantul. Bulbs with buds were peeled and cleaned from the sheath and then washed thoroughly with a detergent. Furthermore, soaking in fungicide and bactericide 2 mg/L for 30 minutes, followed by 30% chlorok for approximately 10 minutes, then rinsed with distilled water for 3 times, followed by physical sterilization by burning the explants after being dipped in spiritus (Metil alcohol). Subsequently, planting is carried out in the Laminar Air Flow Cabinet.

The basic media used were Murashige and Skoog (MS) for each treatment of 200 ml. To 50 ml of distilled water, 100 ml of macronutrient stock solution, 0.8 ml of micronutrient stock solution, 1 ml of iodine stock solution, 1 ml of vitamin stock solution, 0.25 ml of EDTA stock solution, 1 ml of iron stock solution, 80 mg myoinositol, 6 g sucrose are added. Adjust the pH to 5.85 with the addition of 1N NaOH or 1N HCL. MS media were added with 2.4 D and BA with the appropriate concentration of treatment.

Furthermore, the explants were maintained in an incubation chamber at a temperature of 25-26°C with continuous lighting. Maintenance of explants includes rack sterilization by

spraying 70% alcohol every three days to avoid bacteria and fungi. Observations are carried out every week from week 0 to 8 weeks, for growth parameters which were observed were the percentage of life, days of callus appearance, and days of root appearance.

RESULTS AND DISCUSSIONS

The results of the analysis of the variety of live callus percentage that the treatment concentration of 2.4 D showed a significant effect, but the treatment of BA concentration showed no significant effect. There was no interaction between the two treatments. The mean percentage of callus life at 8 mst can be seen in Table 1.

Table 1 shows that the treatment of 2.4 D concentration of 1 mg/L produced the largest percentage of life compared to other treatments. This is because 2.4 D in MS media can induce the process of embryogenesis, as well as the formation of callus, which is then induced to form plantlets. Administration of 2.4 D at concentrations of 10⁻⁷ - 10⁻⁵ M without cytokinins is very effective for the induction of callus proliferation in most cultures [17]. In the treatment of various concentrations of BA, no significant difference between treatments was shown, meaning that all plants show the same growth response for life.

From the results of the analysis of various days of the callus appearance, the treatment of 2.4 D concentration had a significant effect, but the treatment of BA concentration had no significant effect. There is an interaction between the two treatments. The average value of the days of callus appearance can be seen in Table 2.

Table 2 shows that the combination of 2.4 D treatment with a concentration of 1 mg/L and BA 2 mg/L (D1B2) was significantly faster in forming callus but not significantly different from the D1B1 and D2B1 treatments. The appearance of callus in explants is marked by swelling or the appearance of clear white tissue such as water/mucus dots on explant slices and incisions on the explant surface which then develop into clear small dots and callus aggregates. Giving 2, 4-D and BA to the media had a significant interaction effect on callus initiation.

Table 1. The mean live percentage of arrowroot callus growth at the age of 8 mst (%)

Concentration ZPT	BA 1 mg/L (B1)	BA 2 mg/L (B2)	BA 3 mg/L (B3)	average
2.4 D 0.5 mg/L (D1)	73,333	80,000	86,667	80,000 c
2.4 D 1.0 mg/L (D2)	93,333	93,333	80,000	88,889 a
2.4 D 1.5 mg/L (D3)	93,333	80,000	86,667	86,667 b
average	86,667 p	84,444 p	84,444 p	(-)

Note the average treatment followed by the same letter shows no significant difference in Duncan's multiple range test (DMRT) with a real level of 5%. Sign (-) indicates there is no interaction.

Table 2. Average days of callus appearance (days)

Concentration ZPT	BA 1 mg/L (B1)	BA 2 mg/L (B2)	BA 3 mg/L (B3)	average
2.4 D 0.5 mg/L (D1)	15,333 ab	17,667 de	16,333 bc	16,444
2.4 D 1.0 mg/L (D2)	15,333 ab	14,000 a	16,667 cd	15,333
2.4 D 1.5 mg/L (D3)	17,667 de	19,000 e	18,667 e	18,444
average	16.111	16,889	17,222	(+)

Note the average treatment followed by the same letter shows no significant difference in Duncan's multiple range test (DMRT) with a real level of 5%. Sign (+) indicates there is an interaction.

This shows that balancing the ratio of auxin and cytokinin can accelerate callus induction. Cytokines are compounds that can increase cell division in plant tissue and regulate plant growth and development, and so can the BA. The role of auxins and cytokines is very evident in the regulation of cell division, cell lengthening, cell differentiation, and organ formation [18].

The results of the analysis of the variety of days of the emergence of roots showed that the treatments of 2,4 D and BA concentrations significantly affected. There is an interaction between the two treatments. The average value of the days of root appearance can be seen in Table 3. The table shows that the combination of 2.4 D treatment with a concentration of 1 mg/L and BA 2 mg/L (D1B2) was significantly faster in forming roots, but not significantly different from the D1B1 treatment. This shows that balancing the ratio of auxin and cytokinin can accelerate root induction. The provision of 2,4 D and BA interacts with other chemical compounds and is influenced by environmental factors such as temperature and light. An auxin is a group of compounds whose function is to stimulate the elongation of shoot cells whose spectrum of activity resembles IAA.

Pierik [11] states that in general auxin increases cell lengthening, cell division, and adventitious root formation. Low auxin concentrations will increase adventitious root formation, while high concentrations of auxin will stimulate callus formation and suppress morphogenesis. Giving auxin and cytokinin in a balanced way to induce certain morphogenesis patterns, including root formation [18]. In-plant tissue exogenous auxin administration is sufficiently high, so that the process of root formation is formed, offset by the administration of BA which can stimulate bud formation. The callus and root formed can be seen in Figure 1.

Table 3. Results of various analysis of the emergence of arrowroot days

Concentration ZPT	BA 1 mg/L (B1)	BA 2 mg/L (B2)	BA 3 mg/L (B3)	Average
2.4 D 0.5 mg/L (D1)	34,667 ab	41,000 cd	35,667 b	37,111
2.4 D 1.0 mg/L (D2)	38,000 bc	30,333 a	44,000 d	37,444
2.4 D 1.5 mg/L (D3)	42,000 cd	38,000 bc	42,333 d	40,778
average	38,222	36,444	40,667	(+)

Note the average treatment followed by the same letter shows no significant difference in Duncan's multiple range test (DMRT) with a real level of 5%. Sign (+) indicates there is an interaction.

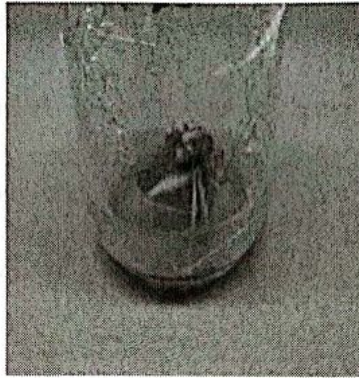


Figure 1. Arrowroot of root on MS media added 2,4 D 1 mg/L and BA 2 mg/L at 45 d.a.

CONCLUSION

From the result obtained, it can be summarized that the treatment of 2.4 D concentration of 1 mg/L produces a greater percentage of life than other treatments. The combination treatment 2.4 D and BA 2 mg/L will accelerate the callus and root growth.

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