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Development of an acclimatization kit as a learning medium for plant tissue culture course

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ABSTRACT

Plant tissue culture is an important biotechnology technique that requires both conceptual understanding and practical skills. However, learning activities related to plant tissue culture in higher education are often constrained by limited laboratory facilities, resulting in learning processes that rely mainly on theoretical explanations. One critical stage in plant tissue culture is acclimatization, which determines the survival of plantlets during the transition from in vitro to ex vitro conditions. This study aimed to develop an acclimatization kit as a learning medium for the Plant Tissue Culture course in the Biology Education Program at UIN Sunan Kudus. The research employed a Research and Development approach using the 4D model, implemented up to the develop stage. The acclimatization kit was designed using orchid plantlets and simple planting media to support hands-on learning activities. The results indicated that the acclimatization kit was highly feasible in terms of learning aspects, design, and usability. Students responded positively, showing increased motivation, engagement, and understanding of the acclimatization process. In conclusion, the developed acclimatization kit provides an effective and practical learning medium that supports experiential learning in plant tissue culture courses and offers a solution to overcome laboratory limitations in higher education institutions.

INTRODUCTION

Plant tissue culture is a technique for isolating cells, tissues, organs, and plantlets, developed and grown under aseptic conditions and in a controlled environment (Bhojwani, 2013). This tissue culture technique has been widely used both domestically and internationally. It is highly beneficial for conservation and medicine. One advantage of tissue culture is that propagation is not dependent on seasons and can be carried out at any time. Furthermore, the planting time is relatively short compared to the number of plants produced, making this technique often chosen for conservation activities and the cultivation of secondary metabolites for later use in medicine (Anis, 2016). The benefit of this in vitro cultivation method is that manufacturing is

done in a controlled, sterile environment, thus it is unaffected by weather conditions (Kadapi, 2024).

Plant tissue culture begins to be studied at the high school level in the Biotechnology chapter, but it is still limited to knowledge, not yet fully developed into practical applications. Furthermore, universities offer separate courses, allowing for a deeper understanding of plant tissue culture techniques, both theoretically and practically. However, not all universities have laboratories that allow for practical plant tissue culture, including UIN Sunan Kudus. This makes tissue culture courses require learning media that can provide students with a concrete picture of plant tissue culture techniques, as these techniques are essential to learn and offer numerous benefits.

Despite the numerous benefits of plant tissue culture techniques, many people are still unfamiliar with them. While some are aware of these techniques, they are unable to practice them due to limited funding and equipment. Therefore, to optimize the public's understanding of these tissue culture techniques, it is essential to introduce or teach them in the curriculum, particularly the MBKM curriculum.

The MBKM curriculum provides students with opportunities to study according to their interests related to their study program. This allows them to acquire additional skills to prepare them for entering the workforce or even for entrepreneurship. The MBKM curriculum includes an elective course in Plant Tissue Culture to provide students with the opportunity to optimally conserve plants, especially rare and difficult-to-propagate plants. In this course, the learning process still faces limitations, including the fact that learning is still limited to presentations and videos, and there are no laboratory facilities specifically for plant tissue culture, making practical work not fully

The development of learning media aims to help students optimize the theory they learn and hone their skills in applying plant tissue culture. Based on the steps of tissue culture: sterilization, media preparation, planting, and acclimatization, acclimatization is the crucial stage for successful plant tissue culture, as it represents the transition from in vitro to in vivo. This stage is widely practiced by the community. Even without a dedicated laboratory, the community can utilize the culture results through acclimatization, as the acclimatization process does not require a sterile room. The process simply involves transferring the cultured plantlets to a suitable medium and placing them indoors before being placed in the wild.

The results of tissue culture techniques are now commercialized, both in the form of plantlets and plant seeds as aseptic explants. Plantlets are also starting to be used as souvenirs or gifts, but few include instructions on how to care for the cultured products. For example, the Creative Economy Product Bazaar organized by the Dayak Women's Institute in Palangka Raya is one form of women's economic empowerment based on creativity and local wisdom. This bazaar features a variety of traditional handicrafts, woven goods, beads, and locally sourced processed food products, opening up marketing opportunities for women-owned micro, small, and medium-sized enterprises (MSMEs). In addition to craft products, this event also showcased plant seedlings produced thru tissue culture technology, such as plantain bananas, as well as various other horticultural seedlings, demonstrating the utilization of biotechnology innovations in supporting the development of agricultural-based creative economic enterprises. The presence of tissue culture products at the bazaar reflects the integration of technology, creativity, and

entrepreneurship as an effort to enhance the competitiveness and economic independence of women. (Nitra, 2025)

The results of plant tissue culture provide benefits such as the availability of same seeds high-quality seedlings that are disease-free and available in large quantities, thus supporting plant cultivation and commercialization needs. The success of using tissue culture seedlings is highly dependent on the acclimatization stage, making it necessary to have practical and integrated media and support equipment, such as an acclimatization kit, to improve the survival rate of seedlings when transferred from in vitro conditions to the field environment. The application of this technology has been proven to increase crop productivity while also opening up opportunities for agricultural-based business development and the creative economy (Danial, 2020).

Therefore, a complete medium is needed to facilitate acclimatization, one of which is the KIT (integrated instrument components). KIT is a medium produced and packaged in a box containing practical equipment for specific materials. The use of KIT can help students apply the theory learned through reading materials to real things that can be directly observed in the working process. Students can be directly involved in conducting experiments, thus being motivated to learn and gain their own experience in building their knowledge. This will make learning more enjoyable and more effective. This will make learning more enjoyable and memorable because students are directly involved in the learning process (M.S.Novembli, 2015). Research by (Widayanti, 2019) indicates that the use of KIT learning media can improve students' cognitive learning achievement by 38.03%. Similar research by (Mandhu, 2015) indicates that the use of KIT can increase the average student score by 81.67 compared to 72.25 for students who do not use KIT.

This research has only been conducted at the school level, mostly in science subjects. There are no learning KITs used at the university level, making it crucial to assist lecturers and students who lack adequate laboratory facilities, such as at UIN Sunan Kudus. To facilitate the acclimatization phase, an Acclimatization KIT (Integrated Instrument Component) is needed as a Learning Medium for Plant Tissue Culture.

METHOD

This research is a development research with the resulting product being an acclimatization KIT. The approach used is qualitative because it only reaches the product development stage. The stages of development research consist of four stages or often referred to as 4D. The first stage is Define (needs analysis stage), the second stage is Design (preparing the conceptual framework of the model and learning tools), the third stage is Develop (development involves validation testing or assessing the feasibility of the media) and the fourth and final stage is Disseminate, (implementation on the actual target, namely the research subjects) (Thiagarajan, 1974). In this study only reached the 3D stage as preliminary research, because the research is gradual. The define stage was conducted to analyze the need for developing an acclimatization kit as a learning medium for the Plant Tissue Culture course, including an analysis of student characteristics, learning problems in plant tissue culture, and course learning outcomes. The design stage involved the conceptual design of the acclimatization kit, including tool specifications, learning objectives, planting media used, and supporting instructional materials. The develop stage consisted of producing the product based on the proposed design, followed by validation by

subject matter experts and learning media experts, as well as feasibility testing to assess the validity and practicality of the media. The disseminate stage was not conducted in this study.

RESULTS AND DISCUSSION

The development of the acclimatization kit in this study focused on providing a practical learning medium that enables students to understand the acclimatization stage of plant tissue culture comprehensively. One of the first steps in the development process was the selection of orchid plantlets as learning objects. Orchids were chosen because they are among the most commonly propagated plants using tissue culture techniques due to their extremely small seeds and the absence of endosperm, which makes conventional propagation difficult (Hartmann, 1990); (Zulkarnain, 2009). In addition, orchids have high economic and ornamental value, making them relevant examples for students to study both scientifically and practically.

The first stage is "Define," which involves identifying and analyzing existing needs. This is done through classroom observations. The results revealed that students desperately need appropriate learning media to understand the plant tissue culture course. This is because the laboratory at UIN Sunan Kudus is not yet adequate for optimal plant tissue culture practice. Biology students at UIN Sunan Kudus only saw videos and images about plant tissue culture during lectures, while the learning outcomes of the plant tissue culture course were to develop skills in implementing plant tissue culture. Therefore, a medium that can train students' skills is needed, which is in accordance with the Course Learning Outcomes (CPL) for the subject, namely: Comprehensively applying relevant skills in the fields of biology and applied technology in resource management, entrepreneurship, and self-development efforts with an Islamic perspective. In addition, the Course Learning Outcomes (CPMK) for the Plant Tissue Culture subject state that students are able to explain and apply plantlet acclimatization.

The define stage in this study focused on identifying students' learning difficulties and instructional needs in the plant tissue culture course. The needs analysis revealed that students experienced difficulties in understanding tissue culture procedures due to limited laboratory facilities and the lack of practical learning media. These findings are consistent with the study conducted by (Huda, 2017), which reported that biology students at the State University of Medan faced significant difficulties in learning tissue culture, particularly in applying theoretical concepts to practical procedures.

The second stage, Design an acclimatization kit. By connecting theoretical ideas with hands-on practical exercises, learning kits as a practicum tool improve students' comprehension, abilities, and involvement in the educational process. The kits' integrated, useful, and user-friendly design makes learning more efficient, contextual, and supportive of meeting practice-based course learning objectives. (Prapaskah, 2020).

The development of the acclimatization kit design began with the selection of plantlets, specifically orchids, due to their ease of cultivation, large size, and aesthetic appeal, which could be a key factor in attracting students. Orchids are also frequently used for plant tissue culture due to their high economic value and their difficult natural propagation due to their very small seeds and almost no endosperm. Orchids are high-value ornamental plants with stable market demand and high selling prices, especially in genera such as Dendrobium, Phalaenopsis, Cattleya, and Vanda. The utilization of tissue culture technology, including the liquid culture system (Temporary Immersion Liquid Culture System/TILCS), enables the mass, uniform, and high-quality production

of orchid seedlings, thereby accelerating the commercialization process. Successful acclimatization results of tissue culture orchid seedlings support the development of nurseries and increase income thru the sale of flowering orchids at competitive prices (Dewanti, 2023). Therefore, they are highly suitable for cultivation and propagation using plant tissue culture techniques.

The next step is plantlet subculturing, which is performed to separate the attached plantlets for optimal growth and development. The medium used is VW, a specialized orchid culture medium, ensuring optimal nutrient requirements. Besides VW medium, MS medium can also be used, as in (Melisa A. O., 2018) research, which used MS medium with the addition of growth regulators in the in vitro culture of *Grammatophyllum scriptum* orchids. VW medium was chosen because it has a higher success rate because it is a specialized medium for orchids. Vacin and Went (VW) medium has been shown to be effective for in vitro orchid culture, particularly during seed germination and protocorm development, by supporting advanced protocorm formation and early seedling growth. This effectiveness is associated with the availability of essential nutrients, especially phosphorus, which meets the physiological requirements of orchids at early developmental stages (Utami, 2019).

In the review article, Pasternak emphasizes that the success of in vitro plant tissue culture is highly dependent on various factors, including nutrient availability, regulation of endogenous hormones (especially auxins), organic compounds, and culture environmental conditions. Through a review of the literature and long-term practical experience, Pasternak presents data-driven physiological recommendations to improve the effectiveness and reproducibility of tissue culture techniques in research and industrial applications (Pasternak, 2024)

Figure 1. Orchid plantlets that have been subcultured for 1 month

Figure 1 showed the subculture are growing well. It can be concluded that sterilization was successful, eliminating contamination of the equipment, media, and explants. As explained by (Pangestika, 2015), the success of plant tissue culture is influenced by several factors, including sterilization, explant selection, environmental factors such as pH, light, and temperature, and the content of plant growth regulators (PGRs) in the culture medium.

Figure 2. The culture medium and explant sources are stored in a fume hood to prevent contamination.

Subculture plantlets results are stored in a fume hood with lighting resulted in well-developed plantlets. In theory, the storage space should be sterile, and the lights should remain on and air-conditioned to maintain humidity (Figure 2.). Light serves as a substitute for sunlight, which is essential for photosynthesis and stomata formation. Without light, stomata formation will not occur, and photosynthesis will not be optimal. The subculture results demonstrate good growth, suggesting that even when stored in a pre-sterilized fume hood, plantlets can still grow optimally. This is due to proper explant sterilization during planting. Lighting relies solely on fume hood and LED lights, but the leaves are dark green, indicating proper photosynthesis.

Room temperature and humidity are also maintained. The room temperature should not fall below 25°C, as lower temperatures can create an environment conducive to fungal growth. Fungi are common contaminants in tissue culture, and occur when the environment is too dry and hot. Bacterial contamination can occur if the environment is too humid and the sterilization process is not carried out properly. Good subculture results are supported by 24-hour air conditioning to maintain humidity.

The successful growth of orchid plantlets during the subculture stage indicates that the selected culture medium and environmental conditions adequately supported physiological development. Recent studies have emphasized that nutrient balance and environmental stability during in vitro culture significantly influence plantlet vigor and subsequent acclimatization success. Optimal macro- and micronutrient composition in orchid culture media plays a crucial role in maintaining cellular metabolism, chlorophyll synthesis, and root initiation, which are essential for survival during ex vitro transfer (George, 2008).

The next step is selecting the growing medium for acclimatization. The acclimatization growing medium was selected through preliminary research, using six different combinations of spaghno moss, chopped fern, and activated charcoal. The preliminary research determined that the best growing medium was the combination of these three, with activated charcoal at the bottom, followed by chopped fern, and spaghno moss at the top. This is because spaghno moss maintains humidity, allowing the plantlets to adapt well. The humidity in the culture bottle is high, while the humidity decreases during acclimatization. If planted directly using charcoal or fern, the plants will quickly die due to the low humidity.

The acclimatization stage represents a critical phase in plant tissue culture, as plantlets must transition from controlled in vitro conditions to ex vitro environments that expose them to

fluctuating humidity, light intensity, and microbial presence. Failure at this stage often results in high mortality rates, even when in vitro propagation has been successfully achieved. Acclimatization is considered a major bottleneck in micropropagation because plantlets developed in vitro generally possess thin cuticles, non-functional stomata, and limited photosynthetic capacity, making them highly susceptible to environmental stress (Hazarika, 2003).

The selection of growing media plays a crucial role in determining acclimatization success. The combination of sphagnum moss, chopped fern roots, and activated charcoal used in this study proved effective in maintaining humidity while providing adequate aeration for root development. Sphagnum moss-based media significantly improve survival rates of orchid plantlets during acclimatization due to their high water-holding capacity, which reduces water stress during early adaptation stages (Zakiah, 2023). Additionally, the inclusion of activated charcoal has been shown to enhance root respiration and adsorb potentially toxic compounds, thereby supporting healthier root systems. Activated charcoal has also been reported to improve acclimatization outcomes by adsorbing toxic substances and enhancing root respiration.

The selection of acclimatization media is also based on Melisa's research which states that media with a composition of spagno moss, charcoal and fern can be used in the acclimatization of the *Vanda limbata x Vanda tricolor* hybrid orchid (Melisa A. O., 2019). Environmental factors such as temperature, humidity, and light intensity also strongly influence plantlet survival during acclimatization. Maintaining moderate temperatures and high humidity during the early stages of acclimatization helps minimize transpiration stress and promotes plantlet recovery (Irsyadi, 2021).

The design of Acclimatization Kit consists of a 17.2 x 14 x 13.5 cm plastic box labeled "Acclimatization Kit" and a picture of a subcultured plantlet on the front of the box. The box contains acclimatization tools and materials: subcultured plantlets, tweezers, pots, growing media (spaghetti moss, chopped fern, and activated charcoal), fungicide, vitamin B1, a spray bottle, a flacon bottle, and an acclimatization kit guide (Figure 3).

Figure 3. Kit acclimatization

Student responses fell into the acceptable category, with an average score of 78.7%, with average scores for each aspect ranging from 70% to 83%. This demonstrates that students responded well and showed high interest in the acclimatization kit. This was evident in the students' enthusiasm when given the acclimatization kit and their numerous questions about it.

This was evidenced by the students' enthusiasm when given the acclimatization kit and their numerous questions related to the kit (Figure 4.). In the study by Kamal et al., the student response showed that 93% of students stated the smart trainer kit helped them understand network

concepts more easily. 87% of students stated they were more motivated to participate in practical work. 84% of students were able to configure networks independently after using the trainer kit. Thus, learning media in the form of kits have proven effective for use as a modern learning medium and also serve as a solution to the limitations of learning facilities (Kamal, 2025).

Figure 4. Classroom conditions during the small-scale trial

The appropriateness rating, according to media experts, reached 96%, with 89.5% for design and 87.5% for media. Based on these scores, it can be categorized as very suitable for use as a learning medium in the acclimatization course on plant tissue culture. Learning media is crucial in the learning process. Media serve as a bridge between lecturers and students, facilitating student understanding of the material, in this case, the acclimatization course in the plant tissue culture course. Interesting, attractive, and interactive media can enhance students' five senses, thereby increasing their understanding. Media can also motivate and engage students, thereby enhancing their retention. Appropriate learning media will enable students to better understand and comprehend the material presented by lecturers. For example, research conducted by teachers on the use of media kits in science lessons at SMP Negeri 4 Singkawang City found that the use of media kits at SMP Negeri 4 Singkawang increased student learning activities, increased student interest in science, created a fun and enjoyable learning environment, and developed students' ability to collaborate and participate in group activities (Ismail, 2016). This study demonstrated that the acclimatization kit was suitable for use as a learning medium. The media kit consists of several components that constitute a system and requires sufficient preparation before use in learning activities.

The results of this study indicate that the acclimatization kit developed as a learning medium was well received and demonstrated high feasibility based on expert validation and student responses. The feasibility assessment conducted by media experts and material experts showed high validation scores, indicating that the acclimatization kit met instructional, technical, and content-related criteria. These findings suggest that the kit was appropriately designed to support learning objectives in the plant tissue culture course, particularly in understanding the acclimatization stage. This is consistent with the research by Prapaskah that the pneumatic trainer kit is very suitable for use as a learning medium and for strengthening competencies, particularly psychomotor skills (Prapaskah, 2020).

Student responses further strengthened these findings. The percentage of positive student responses, which fell within the good to very good category, reflects high levels of interest, motivation, and engagement during learning activities using the acclimatization kit. Students demonstrated enthusiasm when interacting with the kit and actively participated in discussions related to acclimatization procedures. This result indicates that hands-on learning media can effectively enhance students' understanding of complex biological processes by allowing them to directly observe and practice real phenomena.

These findings are consistent with the results reported by Musfiroh, who found that interactive learning media for plant tissue material received very high feasibility scores from experts and positive responses from students (Musfiroh, 2024). Musfiroh also reported a significant improvement in student learning outcomes, as indicated by increased post-test scores compared to pre-test scores. Although the present study employed a physical learning kit rather than digital media, both studies demonstrate that well-designed instructional media can significantly improve student engagement and comprehension.

The alignment between expert validation results and student responses in this study indicates that the acclimatization kit effectively bridges theoretical knowledge and practical application. This finding supports the notion that instructional media designed through a systematic development process can function as effective alternatives to conventional laboratory practices, particularly in institutions with limited laboratory facilities. Overall, the high feasibility scores and positive student responses obtained in this study confirm that the acclimatization kit is an effective learning medium. The results reinforce previous research suggesting that instructional media—both digital and hands-on—play a crucial role in improving learning quality and outcomes in biology education (Musfiroh, 2024).

The findings of this study are consistent with the results reported by Restiani, who demonstrated that basic plant tissue culture activities significantly improved students' knowledge and practical skills (Restiani, 2024). Their study emphasized that hands-on learning experiences allow students to better understand complex biological processes through direct involvement in laboratory-based activities. Similarly, the acclimatization kit developed in the present study provides students with opportunities to observe and practice real acclimatization procedures, thereby strengthening their conceptual understanding and technical skills.

Previous research has indicated that experiential learning effectively enhances student motivation and classroom engagement in higher education, as active participation and practical experience enable learners to connect theoretical knowledge with real academic tasks. This aligns with the positive student responses obtained in the present study, where learners showed high enthusiasm and interest when using the acclimatization kit. Such findings indicate that instructional media designed to support experiential learning can enhance students' ability to connect abstract concepts with real biological phenomena (Kong, 2021).

In the study conducted by Budiastra et al., the use of natural sciences kits was shown to significantly support the implementation of practical activities in higher education, particularly in undergraduate teacher education programs, even when conducted through distance learning. The kits enabled students to perform laboratory practices independently and to achieve practical learning objectives that would otherwise be difficult to attain due to limitations in laboratory facilities (Budiastra, 2024). The study by Permana also found that the development of home-based laboratory kits distributed to students improved their attitudes, practical skills, and learning

achievement, as students gained hands-on experience in conducting experiments even though the learning process was carried out through distance learning (Permana, 2023).

In the context of this study, the acclimatization kit functions as an alternative learning medium that enables students to acquire essential competencies in plant tissue culture, particularly during the acclimatization stage. This includes removing the plantlets from the bottles, cleaning off the remaining medium, planting in porous media, and gradually adjusting humidity and light until the plants can grow normally and are ready for cultivation or commercialization. Therefore, the integration of practical learning media, as demonstrated by both studies, plays a crucial role in improving the quality of biology education.

CONCLUSION

Plant tissue culture is a course that requires learning media to aid student comprehension, as it still presents a relatively high level of learning difficulty. One of the causes of learning difficulties in plant tissue culture is the learning media. Therefore, innovative, engaging, and creative learning media are needed to help students understand the material in plant tissue culture. The most crucial stage in tissue culture is the acclimatization phase. The success of plant tissue culture can be determined by the success of the acclimatization phase. This acclimatization phase is ideal for using a plant tissue culture kit (KIT) because it can be practiced anywhere and anytime, as the tools and materials are already provided in the acclimatization kit. The design of the acclimatization kit consists of a plastic box measuring 17.2 x 14 x 13.5 cm with the words "Acclimatization Kit" and a picture of the subcultured plantlets on the front of the box. The box contains acclimatization tools and materials: subcultured plantlets, tweezers, pots, growing media (spaghetti moss, chopped fern, and activated charcoal), fungicide, vitamin B1, a spray bottle, a flacon bottle, and an acclimatization kit guide. The feasibility assessment, according to the lecturer, reached 96%, with 89.5% for design and 87.5% for media, resulting in an average of 91%. The average student response rate was 78.7%, with average scores for each aspect ranging from 70% to 83%. Based on these scores, the kit can be categorized as highly suitable for use as a learning medium in the acclimatization course on plant tissue culture.

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