The growing of taro Colocasia esculenta (L.) Schott var. antiquorum plantlet in several media during acclimatization stage

M Tuwo and E Tambaru

Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Hasanuddin, Makassar, South Sulawesi, 90245

E-mail: mustikatuwo@gmail.com

Abstract. Japanese taro or satoimo Colocasia esculenta (L.) Schott var. antiquorum, is widely consumed in Japan thus increasing exports. Plant seedlings can be obtained in large quantities and in a relatively short time through the propagation method of plant tissue culture. Final stage of plant tissue culture is acclimatization. Growing media and planting techniques are important factors in the acclimatization process. The ideal planting medium can be obtained from a combination of organic and inorganic materials. This study aims to obtain optimal acclimatization media for the growth of taro plants. The planting material used 24 weeks old taro plantlets. The plantlets were washed with tap water and soaked in distilled water + fungicide and washed with sterile water. The plantlets were planting in plastic cups with the different substrate mixture namely Soil: Manure: Rice Husks (1:1:1); Soil: Manure: Cocopeat (1:1:2); Rice Husk: Sand (1:1); Nutrient Film Technique (NFT) Hydroponics System. They were watered daily and fertilized. Survival percentage, plantlet height and number of leaves were recorded for 12 weeks. Data were analyzed using ANOVA and if there is a significant effect of treatment, a further test of the least significant difference (LSD) is carried out. The optimal acclimatization medium for acclimatization was obtained in (P1) Soil: Manure: Rice Husks (1:1:1) treatment with a percentage of survival of 62% with an average plant height and number of leaves 13.3 cm and 4.6 respectively.

1. Introduction
Taro plant Colocasia esculenta (L.) Schott belongs to the Araceae family, is a food plant that has edible tubers and leaves, and is a staple food plant in many regions in Asia Pacific, Africa, the Caribbean and several regions in Indonesia. In optimal conditions taro productivity can reach 30 tons per hectare [1,2]. Taro plants are a staple food source in several regions of Indonesia. There are two commonly cultivated variants of Colocasia esculenta, which of them is C. esculenta (L.) Schott var. antiquorum, which has a small globular central corm with several relatively large cormels arising from the main corm, this variety is referred to as the ‘eddoe’ type of taro. The other variant is C. esculenta (L.) Schott var. esculenta, which possesses a large cylindrical central corm with only few cormels classified as the ‘dasheen’ type of taro [3]. Japanese taro or satoimo Colocasia esculenta (L.) Schott var. antiquorum, is widely consumed in Japan so that there are opportunities for export to that country [4,5]. Plant tissue culture is well known as a method of plant propagation to obtain plant seedling in large quantities and in a relatively short time. Therefore, propagation by plant tissue culture has the potential to be developed.
The final stage of plant propagation by plant tissue culture is acclimatization. The resulting plantlets still need serious attention for further growth through the acclimatization process in greenhouses [6].

Acclimatization is carried out by moving the plantlets to acclimatization media [7] with low light intensity and high relative humidity, then gradually the humidity is lowered and the light intensity is increased [8]. This stage is a critical stage because the climate in the greenhouse and in the field is very different from the conditions in the culture bottle and is a stage that determines the success of plant propagation using tissue culture techniques. Growing media and planting techniques are important factors in the acclimatization process. Media is one of the environmental factors that functions to provide nutrients and water for plantlet growth. A mixture of two or more types of media can improve the deficiencies of each of these media, one of which is the provision of plant nutrients, and the ability to maintain media humidity [9]. The requirements for acclimatization media in general are not to be a source of disease for plants, have good circulation and availability of air (aeration), be able to control excess water (drainage) is good, and easily bind water [10]. The ideal planting medium can be obtained from a combination of organic and inorganic materials. Organic materials that can be used for acclimatization are rice husk and cocopeat [11] and inorganic materials in the form of soil or sand. This research aims to obtain optimal acclimatization media for the growth of taro plants.

2. Materials and methods
The planting materials used were 24 weeks old taro Colocasia esculenta (L.) Schott var. antiquorum plantlets. The plantlets were washed with tap water to remove the media residues. In addition, the plantlets roots were soaked in distilled water + fungicide 1g/L for 2 minutes and washed with sterile water. Furthermore, the plantlets were drained on paper and then planted in each treatment. After all of these steps, the plantlets were cultured in plastic cups with the different substrate mixture. They were watered daily and fertilized with Gandasil D. The acclimatized plants were observed regularly. In the hydroponic system, plantlets are planted in plastic cups whose bottom has been perforated and contains rice husk media. Furthermore, the plastic cups containing the plantlets is placed in the pipe hole. Maintenance is carried out by checking the nutrients in storage tub periodically.

Four types substrate mixture namely (P1): Soil: Manure: Rice Husks (1:1:1); (P2) Soil: Manure: Cocopeat (1:1:2); (P3) Rice Husk: Sand (1:1); (P4) Nutrient Film Technique (NFT) Hydroponics System were used at acclimatization stage. Survival percentage, plantlet height and number of leaves were recorded 12 weeks after plantlets transferring into the acclimatization medium. Data were analyzed using ANOVA and if there is a significant effect of treatment, a further test of the least significant difference (LSD) is carried out.

3. Results and discussion
All in vitro plantlets were transferred to external environment (figure 1) and planted on plastic cups with different substrate mixture during acclimatization. In vitro plantlets are obtained in a high humidity and heterotrophs condition so that they cannot be directly transferred to the field or greenhouse but must go through the acclimatization stage, namely the stage of adjustment to a new environment. In vitro plantlets generally have unfavorable properties such as the cuticle does not develop properly due to high humidity in the bottle, the leaves are thin, soft and have few palisade cells, the vessel tissue from root to shoot is not well developed, and stomata are not functioning [12].

The acclimatization stage is an important stage in tissue culture propagation. Changes in growing environmental conditions change gradually from an artificial environment to a natural environment. Ensure that the plantlet conditions are optimal in order to survive in an ex vitro environment, so the acclimatization method must be ensured according to the type of plant [13]. Plantlets generally require high humidity, which is around 65-80%. Moisture around the roots must also be maintained because high humidity can cause plant roots to rot. During the research, the temperature during the day reached 35°C so that the plants experienced a high transpiration process and caused the planting medium to easily dry out. High temperatures cause dehydration, thus inhibiting plantlet growth. Acclimatization trains plants previously grown in culture bottles with a complete supply of media to be able to live
independently and photosynthesize under external conditions. The success of acclimatization is not only influenced by plant root factors, but also the ability to control environmental conditions and growing media in greenhouses [14].

Data showed (figure 2) that the highest surviving percentage was at (P1) Soil + Manure + Rice Husks (62%). One of the good growing media is rice husk because it is light (specific gravity 0.2 kg / l), lots of pores, high water holding capacity, and black color so it can absorb sunlight effectively. This is because rice husks, both raw and roasted, have sufficient aeration to absorb water and nutrients needed for plant growth [15]. Rice husks that are burned into husk charcoal have been widely used for commercial hydroponic media. In P2 treatment obtained a percentage of survival of 46%. Basically, cocopeat media has a high water holding capacity. Cocopeat media has micro pores which can inhibit the movement of larger water, causing higher water availability. Cocopeat growing media can hold water up to 73% [16,17]. In P4 treatment obtained a percentage of survival of 38%. The continuity of circulation of nutrient solutions plays an important role in the NFT hydroponic system. If the flow stops, the consequences are fatal. When the pump is off, there is indeed water stuck in the pipe. However, this condition is very difficult to estimate the durability of plants in the network during power failure. During the study, there was a power outage for several hours which affected the flow of nutrients. On the other, P3 treatment recorded the lowest surviving percentage (16%). After the acclimatization process the plantlets were ± 7 days old, the plantlets died and experienced decay. Plantlets grown in vitro might be
easily impaired by sudden changes in environmental conditions after ex vitro transfer. They usually need several weeks under shade and gradually decreasing air humidity to acclimate to the new conditions [18]. According to [19], plantlet mortality is caused by the deterioration of the plantlet organ function, high temperature and media humidity. The plantlets are also subject to decay by the fungus that appears at the base of the stem touching the media. Fungi attack plantlets grown on media containing organic material. This is because organic materials can store water and cause moisture to support the growth of microorganisms, especially fungi [20,21].

![Graph: Percentage of Survival (%)](image)

**Figure 2.** Effect of acclimatization substrate mixtures on taro plantlets survival after 12 weeks.

In P1 treatment (figure 3), the highest plant height and number of leaves were obtained, followed by P4 and P2 treatments. The same thing was obtained by [22] where the highest number of leaves was obtained in the treatment of soil media: husk charcoal: manure (1:1:1) on the growth of micro-cuttings of potato *Solanum tuberosum* L. of granola variety. The type of planting medium (soil, sand, manure, roasted husk, raw husk, and cocopeat) showed a significant effect on the plant height parameters in the Kepok banana plantlet of the unti Sayang variety [23]. The type of soil used is top soil. Top soil has nutrients needed by plants [24]. The second best treatment was obtained in treatment P4. Nutrient absorption is an important component in NFT hydroponic system. In the NFT system, plant roots grow in a shallow and circulating flow of nutrients, so that plants can get enough water, nutrients and oxygen. The water containing the nutrient solution circulates continuously with the aid of a pump. Not much different from P2 treatment. Cocopeat growth media contains essential nutrients, such as calcium (Ca), magnesium (Mg), potassium (K) and phosphorus (P) [16].
Figure 3. Effect of acclimatization substrate mixtures on taro plantlets height (cm) and number of leaves after 12 weeks.

4. Conclusion
The optimal acclimatization medium for acclimatization of taro plantlets was obtained in Soil: Manure: Rice Husks (1:1:1) treatment with a percentage of survival of 62% with an average plant height and number of leaves 13.3 cm and 4.6 respectively.

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