AgriHealth: Journal of Agri-food, Nutrition and Public Health. 5(2), 85-91, 2024
URL: https://jurnal.uns.ac.id/agrihealth/article/view/85776
DOI: http://dx.doi.org/10.20961/agrihealth.v5i2.85776
ISSN 2722-0656 (Print) 2722-0648 (Online)

A Study of Acclimatization Media on Strawberry (*Fragaria x ananassa* Duch.) Plantlets Produced from Meristem Culture

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Received: March 29, 2024; **Accepted**: June 16, 2024

Abstract

Acclimatization is the final stage of propagation by *in vitro* culture. This phase is crucial in micropropagation, as it will determine the survival of plantlets outside culture jars. This phase's efforts should ensure optimal conditions, including the media type. This study sought the best media for acclimating strawberry plantlets produced under meristem culture. The research employed the randomized block design, utilizing 7 various media treatments, which include husk charcoal; perlite; cocopeat; perlite + husk charcoal; soil + sand; husk charcoal + cocopeat; and perlite + cocopeat. The media mix was set at 1:1 a ratio (weight/weight), with 9 replicates. The chemical properties of the media were then analyzed, including several parameters such as organic C, total N, and water content. Several parameters were observed for plantlet's growth parameters, including plant survival rates, number of leaves per plant, average leaf area, and root fresh weight. The results found that cocopeat was the best medium for acclimating strawberry plantlets. In cocopeat media, the plant survival rate reached 96.68%, with the leaves number of 7.67 plant^{-1}, an average leaf area of 120.92 cm^2 plant^{-1}, and root fresh weight of 4.30 g plant^{-1}. These results indicate that cocopeat is a medium derived from coconut fiber powder, a natural resource that can be renewed sustainably and produces better plant plantlet growth.

Keywords: acclimatization; cocopeat; meristem culture; rooting; survival rate

INTRODUCTION

Strawberry (*Fragaria x ananassa* Duch.) is a source of vitamin C, folate acid, K, Mn, riboflavin, and anthocyanin. In addition, it provides bioactive compounds to reduce cardiovascular incidents and anti-cancer benefits (Harnaningsih, 2010; Neri et al., 2022). The strawberry plant is native to subtropical countries, but it has been widely planted in tropical countries, especially in the plateaus of Indonesia. Virus infection in the field is a crucial issue faced by strawberry farmers in many parts of the world, including in Pancasari, Bali, Indonesia. The meristem culture method is a technology that produces virus-free products (Taskin et al., 2013; Park, 2021). This research is a follow-up of a previous investigation by Dwiyani et al. (2020), who successfully obtained strawberry plantlets from meristem (shoot-tip) culture from strawberry cultivation. A study of media in the acclimatization phase is reported in this research to generate a complete protocol to produce healthy, ready-to-plant strawberry plantlets.

Acclimatization is the last phase of *in vitro* culture propagation. Polivanova and Bedarev

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Cite this as: Dwiyani, R., Fitriani, Y., Ana, F. G. S., & Bimantara, P. O. (2024). A Study of Acclimatization Media on Strawberry (*Fragaria x ananassa* Duch.) Plantlets Produced from Meristem Culture. *AgriHealth: Journal of Agri-food, Nutrition and Public Health*, *5*(2), 85-91. doi: http://dx.doi.org/10.20961/agrihealth.v5i2.85776

(2022) described that the environmental conditions for *ex vitro* growth are far different from those for *in vitro* cultivation. *In vitro* conditions include relatively low light intensity, constant temperature, high humidity level, low gas exchange rate, and a high level of carbohydrate and phytohormone supplementation in the growth media, hence inducing abnormalities and death throughout the acclimatization process (Boliani et al., 2019). According to Hazarika et al. (2006), such conditions produce plantlets with abnormalities in morphology, anatomy, and physiology. Hoang et al. (2020) mentioned that plants that are kept high in nutrients (sugar and vitamins) and considered to have undertaken prerequisite sterilization under *in vitro* conditions are usually flimsy. Such conditions give rise to the plantlets' need for an adaptation process called acclimatization before transfer to the field. The issue of how crucial this acclimatization phase is in plant propagation by *in vitro* culture has also been raised in the same study by Ehirim et al. (2014). The products from this tissue culture technique are expected to have genetically superior and better characteristics, including resistance to pests and diseases and drought stress.

Studies of acclimatization media needed by plantlets from *in vitro* culture have been reported for several species, including *Fagraea fragrans* (Roxb.) Miq. (Purmadewi et al., 2019) and *Dendrobium sylvanum* (Hariyanto et al., 2019). Acclimatization of strawberry plantlets from meristem culture itself was once reported by Jofre-Garfias et al. (2006), but this study used different media. This acclimatization stage will ensure optimal survival and growth of the plant in the external environment and develop a root system to absorb water and nutrients in the planting medium used. This research aimed to obtain the best medium for strawberry plantlet growth during the acclimatization phase.

MATERIALS AND METHOD

Experimental area condition

This study was conducted at the Experimental Station of the Faculty of Agriculture, Universitas Udayana, located in Denpasar with an altitude of 300 meters above sea level (m asl) and a greenhouse located in Pancasari, Tabanan with an altitude of 1,200 m asl. Research materials were 4-month-old strawberry plantlets in culture that was derived from meristem culture (Figure 1).

Figure 1. Plantlets used for acclimatization

Experimental design and analysis

The experiment was set under the randomized block design with 7 media treatments: husk charcoal; perlite; cocopeat; perlite + husk charcoal; soil + sand; husk charcoal + cocopeat; and perlite + cocopeat. The media mixtures were made at a ratio of 1:1 (soil:treatment material). Each treatment combination was repeated 9 times. Every unit consisted of 1 strawberry plantlet. The strawberry plantlets were carefully removed from the culture jars and then rinsed with clean water to remove the agar. The plantlets were then soaked in fungicide (2 g l^{-1}) for 30 minutes, drained on paper for 15 minutes, and planted in acclimatization media in plastic cups. The plantlets were covered with plastic boxes and room-stored at 24 °C for 6 weeks. Plantlets were sprayed with water daily and an atonic spray at 1 g l^{-1} weekly. Afterward, the strawberry plantlets were taken from Denpasar (300 m asl) to Pancasari (1,200 m asl) to condition the microclimate requirement at acclimatization. In Pancasari, plantlets were uncovered for adaptation under field environmental conditions. The plantlets were placed in a mist-blower-equipped greenhouse. The observation was carried out 8 weeks after uncovering. The variables observed include the percentage of plant survival (%), number of leaves per plant, average leaf size (mm^2), and root fresh weight per plant (g).

Media chemical analysis was performed for several parameters, namely organic C (the Walkley-Black chromic acid wet oxidation method), total N (the Kjeldahl method), and water content (the gravimetric soil water content method). The media were placed in polybags with drainage holes and wetted (watered) with water in uniform volumes until water drops appeared. The media analysis was conducted 3 days later and was measured before planting under the field capacity conditions.

Data analysis

The data were analyzed with an analysis of variance (ANOVA) to determine whether different media treatments influence strawberry plant yield parameters. If the *p*-value from the ANOVA test is significant between the treatments, it continued with the least significant difference (LSD) test at a 5% level. LSD test results with different letters mean they have significantly different effects between treatments.

RESULTS AND DISCUSSION

Chemical properties of media

Table 1 shows data on soil analysis from 7 different treatments. Cocopeat media shows the highest organic material and water content, at 38% and 14.18%, respectively. Perlite media, conversely, has the lowest organic content (0.39%), total N (0.02%), and water content (0.38%) of any media—the combination of perlite and cocopeat results in superior chemical characteristics than using single perlite media alone.

Table 1. The chemical properties of various media combinations used in this experiment

Media	Organic C (%)	Total N (%)	Water content (%)
Husk charcoal	1.64	0.21	5.17
Perlite	0.39	0.02	0.38
Cocopeat	38.01	0.31	14.18
Perlite + husk charcoal	1.22	0.11	4.12
Soil + sand	0.40	0.03	3.46
Husk charcoal + cocopeat	26.69	0.36	8.41
Perlite + cocopeat	3.38	0.33	8.40

The soil's organic C content plays a vital role in the function and ecosystem of the soil (Lehmann et al., 2020). Organic C significantly improves soil physical conditions and biochemical properties (Zhang et al., 2017; Wu et al., 2019; Xue et al., 2020). It was found that the cocopeat medium and mixed media containing cocopeat had relatively higher water content, organic C, and total N (Table 1). Results of the research by Hariyanto et al. (2019) revealed that the growth of the Dendrobium orchid within the acclimatization phase was optimal in media with higher moist contents and nutrients. Medium water content and nutrients are essential to plantlet growth during acclimatization. In this research, organic C, total N, and water content in media were 3 key factors influencing strawberry plantlet growth. Besides, medium selection for acclimatization requires highly porous and compact materials that provide water retention, mechanical support, and water and air absorption by roots (Díaz et al., 2010; Ab Rahman et al., 2020), all of which were found in cocopeat.

The growth of strawberry plantlets

Table 2 shows observation data after the plantlets were removed from the culture jars in the 14th week and uncovered in the 8th week. It was found that the cocopeat medium yielded the best results for all variables—number of leaves, average area per leaf, and root fresh weight, showing statistically significant differences compared to other treatments (Table 2). These findings were closely related to the media analysis results (Table 1), which showed that the cocopeat medium had high organic C, total N, and water content.

Table 2 demonstrates that strawberry plants grown in cocopeat media have the highest average plant survival rate (96.68%), average leaf area (120.92 cm^2 plant^{-1}), and root fresh weight (4.30 g plant^{-1}). Perlite has the lowest value for the variables mean plant survival rate (63.50%), average leaf area (60.5 cm^2 plant^{-1}), and root fresh weight (2.30 g plant^{-1}), followed by husk charcoal (2.26 g plant^{-1}).

The plant growth is supported by the number of leaves, average leaf area, and root fresh weight. The phenotypes of the strawberry plantlets can be seen in Figure 2. Plantlets growing in the cocopeat medium had more robust stands. This was supported by the previous experiment, which

Table 2. Growth results of strawberry plantlets in media treatment

Treatment	Plant survival rate (%)	Leaves number per plant	Average leaf area (cm^2 $plant^{-1}$)	Root fresh weight (g $plant^{-1}$)
Husk charcoal	81.16[b]	4.22[bc]	57.12[b]	2.26[d]
Perlite	63.50[d]	3.78[c]	60.50[b]	2.30[d]
Cocopeat	96.68[a]	7.67[a]	120.92[a]	4.30[a]
Perlite + husk charcoal	73.34[c]	4.33[bc]	65.25[b]	2.88[c]
Soil + sand	86.69[b]	5.56[bc]	63.88[b]	2.91[c]
Husk charcoal + cocopeat	87.12[b]	6.00[b]	82.88[b]	3.93[ab]
Perlite + cocopeat	73.34[c]	5.56[bc]	64.75[b]	3.67[b]
LSD at 5%	6.37	1.93	27.16	0.39

Note: Numbers followed by different letter notations mean values indicate no significant difference between the treatments based on the LSD at the 5% probability level

indicated heavier root masses, allowing the plantlets to stand taller than others in other media.

Da Silva et al. (2017) stated that the success of functional rooting system establishment in plantlets from *in vitro* culture was the main factor in plant survival after acclimatization. This finding was also presented in this research, where the cocopeat medium, which yielded the highest root fresh weight, also yielded the highest plant survival rate (96.68%). In addition, the results showed that root fresh weight positively correlates with number of leaves and average leaf area (Table 2). It indicated that good root growth supports upper-part organ development. These results were also found in previous research by Dwiyani et al. (2022) and Tamyiz et al. (2022). In addition, plant propagation through tissue culture, promoting root growth, is important because those with good growth absorb nutrients from the culture media to stimulate the development of leaves, shoots, and overall plantlets. Root absorbs nutrients from the culture media to grow upper plant organs (Dwiyani et al., 2022). The ability of plants to survive post-acclimatization is one of the indicators of acclimatization process success in plants derived from *in vitro* culture. This finding mirrored that of Sriskanda et al. (2021), who found that the cocopeat medium gave a maximum survival rate in the acclimatization of *Ficus carica*. Prabhuling and Huchesh (2018) also found that cocopeat gave 100% live plants to acclimate Brown Turkey Figs.

Strong plant rooting is interlinked with the water and nutrient contents in the medium. The water and nutrient contents in the medium render better root growth, in turn, allows for maximum absorption of water and nutrients from the medium. In contrast to the cocopeat medium, which resulted in the highest plant survival rate, the unmixed perlite medium yielded the lowest (63.5%). Table 1 shows that the perlite medium had the lowest organic C, total N, and water content of all the media used in this research. Water content was highly influential from the 3 components analyzed because pure perlite had a shallow water content (0.38%). Water deficiency is critical in acclimatization since plants from *in vitro* culture are highly vulnerable to dehydration after contact with outdoor environmental conditions (Jorfe-Garfias et al., 2006). Therefore, media with the ability to retain water are highly needed. Although watering was conducted every other day, the perlite media could not retain water, hence incapable of providing good growth to the strawberry plantlets during acclimatization.

The mixed husk charcoal and cocopeat medium gave the second-best plant survival rate (87.12%). Still, it was not significantly different from the soil and sand mixture treatment (86.69%) and unmixed husk charcoal treatment (81.16%). Other researchers have also previously performed media mixing for acclimatization. Shatnawi et al. (2019) reported that an 80% survival rate was obtained by uniformly mixing soil, perlite, and peat during *F. carica* acclimatization. Mixing cocopeat and perlite at 1:1 resulted in an 85% survival rate in ginger plants under greenhouse conditions (Miri, 2020). In this research, the same mixture, cocopeat and perlite, yielded a plant survival rate of 71.34%. The presence of cocopeat in this media mixture was able to increase perlite

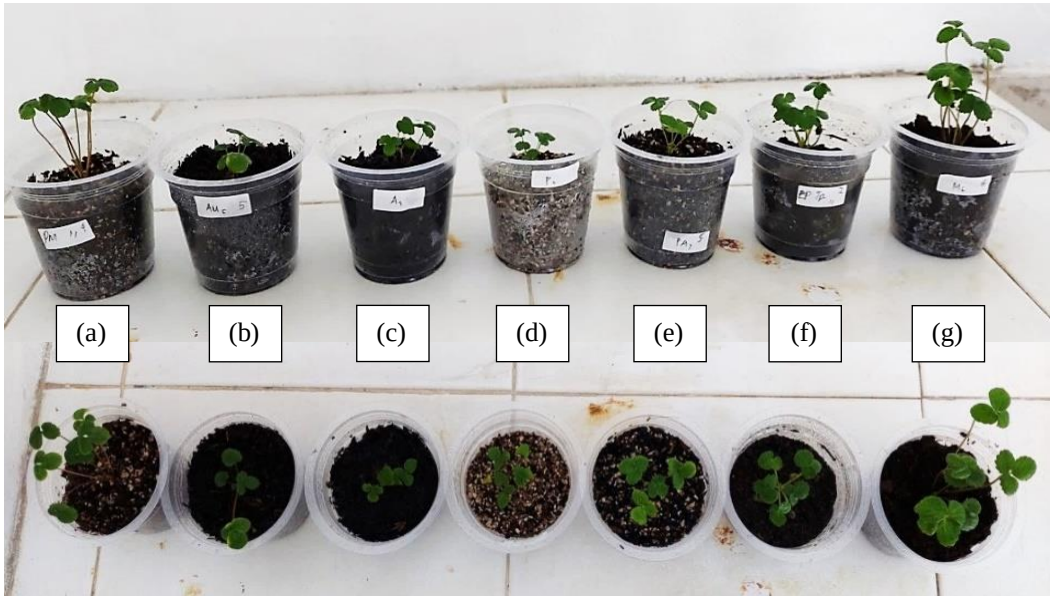

Figure 2. Phenotypes of the strawberry plantlets in various media; (a) perlite + cocopeat; (b) husk charcoal + cocopeat; (c) husk charcoal; (d) perlite; (e) perlite + husk charcoal; (f) soil + sand; (g) cocopeat

water retention capacity, leading to the mixed cocopeat and perlite medium having 8.40% water content, far higher than the water content of pure, unmixed perlite (0.38%). This indicates that the role of cocopeat in acclimatization media is critical.

Cocopeat cuts coconut husk fibers into pieces (Sriskanda et al., 2021). Cocopeat has several advantages as a planting medium, including neutral pH (Awang et al., 2009), micronutrient contents such as Fe, K, Mn, Cu, and Zn in high amounts (Khan et al., 2019), capability of facilitating root growth and distribution, high degree of aeration, high porosity, and high level of water holding capacity (Bharati et al., 2018). Another advantage of using cocopeat as planting media is reducing agriculture waste. This research showed that the cocopeat medium exhibited the most significant acclimatization medium for strawberry plantlets without mixing with other media.

CONCLUSIONS

The results found that the best medium for acclimatizing strawberry plantlets from meristem culture was cocopeat, while the least suitable as an acclimatization medium was perlite. The implication of the findings is the potential for developing new technology, where cocopeat is a medium derived from coconut fiber powder. This natural resource can be renewed sustainably and produces better plant plantlet growth.

ACKNOWLEDGEMENTS

This research was funded by the Universitas Udayana PNBP Fund of 2021, for which the authors would like to thank Universitas Udayana and the Research and Community Service Institute of Universitas Udayana.

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