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In vitro germination of lemon cui (*Citrus microcarpa* Bunge)

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ABSTRACT

Lemon cui (*Citrus microcarpa* Bunge) is widely utilized by the people of Gorontalo as a cooking ingredient, traditional medicine, cleaning agent, and cosmetic ingredient. This diversity of uses indicates the potential for further development of lemon cui. The initial stage of development that can be undertaken is the mass propagation of high-quality lemon cui seedlings. This study aims to produce lemon cui seedlings using in vitro culture techniques through seed germination.The experimental design used in this study is a Completely Randomized Design (CRD) with four treatments, namely: A: MS0 (without the addition of plant growth regulators and young coconut water); B: MS + 50 mL young coconut water + 0.5 ppm 2,4-D; C: MS + 50 mL young coconut water + 1 ppm 2,4-D; and D: MS + 50 mL young coconut water + 1.5 ppm 2,4-D. The culture medium used was Murashige and Skoog (MS) supplemented with young coconut water and 2,4-dichlorophenoxyacetic acid (2,4-D). The study's results showed that using young coconut water and 2,4-D significantly accelerated the germination of lemon cui seeds. The best result was observed in treatment D, where the citrus seeds exhibited a maximum germination rate of 100%. In addition, the combination of young coconut water and 2,4-D is capable of inducing callus formation during the germination process. The combination of 50 mL of young coconut water and 1.5 ppm 2,4-D is the most effective concentration for inducing germination of lemon cui seeds and callus formation.

Keywords: Gorontalo, in vitro germination, lemon cui, multiplication technique

INTRODUCTION

In Gorontalo, Kalamansi oranges (*Citrus microcarpa* Bunge) are lemon cui, and other names are calamondin, calamandarin, and kasturi lime. Lemon cui is one of the native citrus plants found in Southeast Asia (Palma et al., 2019). Lemon cui contains sugar, essential oils, polyphenols, vitamins, and minerals that are beneficial for health (Husni et al., 2021; Xin et al., 2022). The essential oil of Lemon cui acts as a natural antiseptic and antibacterial agent (Chandra et al., 2022; Palogan et al., 2023). The important oil content in the flowers and leaves of Lemon cui are 1.1% and 0.8%, respectively. The flower oil is dominated by monoterpenes, with limonene (74.5%) as the main component. The predominant element in the leaf oil of Lemon cui is sesquiterpenes (82%), with elemol (37.5%) as the main component (Thao Nguyen et al., 2018).

The utilization of lemon cui in the community is highly diverse. Lemon cui can be used to make juice and food seasoning (Mahadi et al., 2015). In Gorontalo, lemon cui is widely used as an additional spice in cooking to enhance the flavor, remedy cough, and to combat dandruff (Ilahi et al., 2016). Additionally, the local people use lemon cui to eliminate the fishy odour of fish (Dika et al., 2021). The chemical compounds in lemon cui, such as flavonoids, alkaloids, polyphenols, and monoterpenoid hydrocarbons like limonene, sabinene, citronellal, linalool, and hedycaryol, possess bioactivities as antimicrobial, antioxidant, antibacterial, and antifeedant agents (Chen et al., 2017; Kindangen et al., 2018; Othman et al., 2016; Ragasa et al., 2006; Wulandari et al., 2013). Therefore, lemon cui is also widely utilized in the field of pharmacology.

Lemon cui holds significant potential for development due to its numerous benefits. Increasing the production of lemon cui needs to be supported by the availability of high-quality lemon cui plant seeds. One technique used to produce a large quantity of lemon cui seedlings in a short time is through in vitro culture techniques. One of these methods involves in vitro seed germination using an organic compound, such as young coconut water, as a substitute for synthetic growth regulators.

Coconut water has been known as a source of active substances necessary for embryo growth. In coconut water, there is an interaction between cytokinins and other phytohormones which play a role in the embryo development process (Wattimena, 1987). Gunawan (1988) found that growth was better when coconut water was combined with auxin in the media. Certain combinations of auxin and coconut water may show synergistic effects. Auxin 2,4-D is a strong synthetic auxin that stimulates callus formation, cell elongation, root initiation, and induction of somatic embryogenesis (Damayanti et al., 2005). In research by Katuuk (2000), giving 250 ml/l of coconut water accelerated the germination of tiger orchid seeds (*Grammatohyllum scriptum*). Research conducted by Permatasari et al. (2022) demonstrated that the combination of 10% coconut water and 1 ppm IAA resulted in the highest seed growth percentage, reaching 38.75%, which was significantly higher than the control treatment at 24.21%. Based on the findings from the use of coconut water and 2,4-D in several studies that have been conducted, this study uses a combination of coconut water and 2,4-D to promote germination in lemon cui seeds.

METHOD

This research was conducted in the Genetics and Tissue Culture Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Gorontalo. The explants in this study are seeds from fresh lemon cui fruits. The seeds were cultured in a medium containing young coconut water and 2,4-dichlorophenoxyacetic Acid. In 1 litre of the medium, there was a mixture of (4.43 g of synthetic MS, 30 g of sugar, 8 g of agar-agar, and 50 mL of young coconut water), with the addition of 2,4-D according to each treatment: 0.5 ppm (B), 1 ppm (C), and 1.5 ppm (D) per liter of medium. The pH of the medium was maintained between 5,6 - 5,8. The seed explants were sterilized using a 10% sodium hypochlorite solution (Bayclin-scJohnson Indonesia) for 10 minutes, followed by sterilization with 70% alcohol (OneMed, Indonesia) for 10 minutes. Rinsing was done using sterile distilled water. Each culture bottle contained 4 lemon cui seeds. Observations were conducted every week for one month. The parameters observed included seed and seedling morphology, as well as the percentage of germination. Quantitative data in the form of germination percentages were processed using Ms. Excel.

RESULTS AND DISCUSSION

Morphology of *Citrus microcarpa* seeds and seedlings

The study successfully demonstrated the potential of *Citrus microcarpa* seeds for in vitro germination and callus induction, utilizing fresh fruits obtained from local orchards in Talumopatu Village, Tapa District, Bone Bolango Regency, Gorontalo (Figure 1A). Prior to sterilization, seeds were carefully separated from the fruit pulp (Figure 1B), and only those with large diameters, firm textures, and non-flat surfaces were selected for germination.

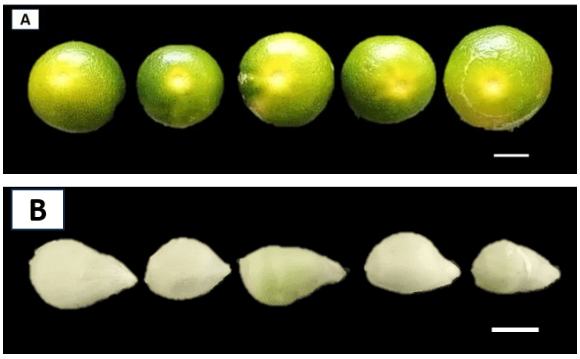


Figure 1. Morphology of fresh fruit and seeds of lemon cui, A) Bar 10 mm; B) Bar 5 mm.

This selection process was crucial, as previous studies, such as Silva et al. (2019) and Souza et al. (2020), underscore the importance of seed morphology in germination tests to assess seed viability and classify abnormalities. Despite the limited research on the germination of Citrus genus seeds (Ragagnin et al., 2022), this study provides valuable insights.

The lemon cui seeds displayed hypogeal germination, characterized by cotyledons that remained beneath the soil surface. Hypogeal germination also forms a hypocotyl. In hypogeal germination, the hypocotyl usually remains short and does not elongate significantly, causing the cotyledons to stay below the soil surface.

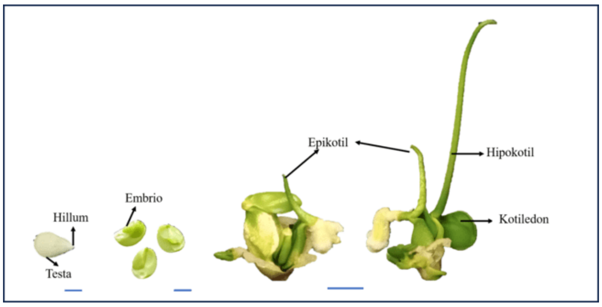
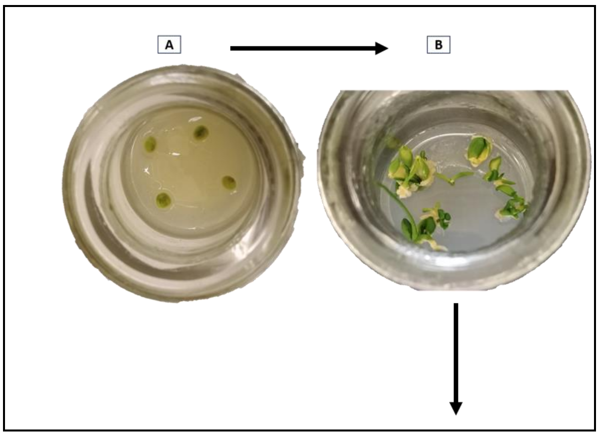


Figure 2. Morphological aspects of lemon cui seeds and seedlings (Bar 5 mm).

As depicted in Figure 2, the seeds are polyembryonic and capable of producing multiple shoots or potential new plants. In various citrus cultivars, polyembryony is a common occurrence. Seeds develop through an apomictic mechanism that produces multiple maternal embryos from the nucellar tissue surrounding the sexually derived zygotic embryo (Koltunow et al. 1996).

Observations at 21 days after planting (DAP) (Figure 3B) revealed that each seed produced more than one shoot (Figure 3C), confirming the high regenerative capacity of the seeds.



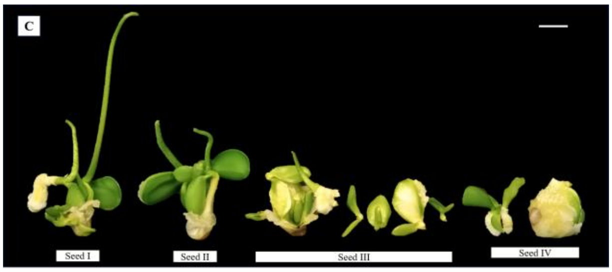


Figure 3. The growth of lemon cui seedlings. A) Seeds at 0 days after planting (DAP); B) Seeds at 21 DAP; C) The seedlings growing from each seed (Bar 5 mm)

The percentage of germination

In this experiment, the MS medium enriched with young coconut water and various concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) induced seed germination. During the germination process, callus formation was also observed (Figure 4). This was due to the presence of the auxin compound 2,4-D in the culture medium. This finding is consistent with the statement of Damayanti et al. (2005), who stated that 2,4-D is a potent synthetic auxin that stimulates callus formation, cell elongation, root initiation, and the induction of somatic embryogenesis. The highest germination percentage was obtained in treatment D, which reached 100%. These results demonstrate that combining these components accelerates the germination process and induces early callus development during germination. The role of 2,4-D in callus induction has been well-documented, and numerous studies support its effectiveness across various plant species. For example, Hasan et al. (2019) reported that MS medium enriched with 3 mg/L 2,4-D resulted in the highest callus induction rate, achieving 86.7% ± 3.4% in *Citrus sinensis*. Syahid et al. (2010) reported that 0.3 mg/L 2,4-D combined with 0.1 mg/L Benzyl Adenine (BA) produced optimal friable callus in Dutch teak, while Yelnititis (2012) and Rasud (2012) highlighted the role of 2,4-D in inducing callus formation in Ramin and clove leaves, respectively. These findings are consistent with the present study, where 2,4-D promoted callus formation as early as the third week after planting.

The addition of young coconut water further enhanced germination rates and supported callus formation. As reported by Kristina & Syahid (2012) and Sholikhah et al. (2022), young coconut water contains endogenous cytokinins, including kinetin and zeatin, which promote rapid germination. The presence of auxins, combined with 2,4-D, contributed significantly to the callus formation observed in this study. The use of coconut water as a supplement for in vitro culture has also been supported by Junairiah et al. (2020), who found that a combination of coconut water and 2,4-D facilitated callus induction in *Piper betle.*

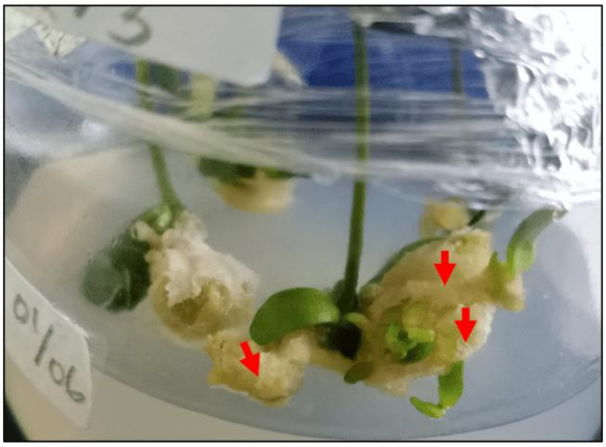


Figure 4. Callus that develops during the germination of lemon cui seeds (3 WAP) treatment D (50 mL young coconut water + 1.5 ppm 2,4-D).

Throughout the experiment, the average germination rate of lemon cui seeds ranged from 50-100%, depending on the medium and growth regulators used (Table 1).

Table 1. The percentage of in vitro germination of lemon cui seeds.

Treatment	4 WAP			Average	Percentage (%)
	U1.1	U1.2	U1.3		
A	2	2	2	2.0	50.00
B	3	2	3	2.6	66.75
C	3	4	3	3.3	83.25
D	4	4	4	4.0	100.00

Note:
WAP : Weeks after planting
A : MS0 (without plant growth regulators and young coconut water)
B : MS + 50 mL young coconut water + 0.5 ppm 2,4-D
C : MS + 50 mL young coconut water + 1 ppm 2,4-D
D : MS + 50 mL young coconut water + 1.5 ppm 2,4-D

Treatment A, which lacked both plant growth regulators (PGR) and young coconut water, achieved a 100% germination rate after 6 DAP, though it required more time to complete germination compared to treatments with PGR and coconut water. These findings highlight the role of 2,4-D and young coconut water in improving both the speed and quality of germination and callus formation, confirming the utility of these components in optimizing in vitro culture conditions for lemon cui.

CONCLUSION

The combination of 50 mL of young coconut water and 1.5 ppm 2,4-D (treatment D) is the most effective concentration for in vitro germination and callus formation in lemon cui seeds. Lemon cui seedlings at 3 days after planting (DAP) are recommended as explants for callus induction to regenerate a large number of lemon cui plants.

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