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Research Article
In vitro Culture Optimization of Pomelo Seeds (*Citrus maxima* (Burm.) Merr.): A South Sulawesi Orange

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Abstract

Background and Objective: Indonesia boasts a variety of delicious tropical fruits, including pomelo, mainly grown in Pangkep Regency, South Sulawesi Province. However, in this region, some challenges hinder such as inadequate care, aging trees and limited seed supply hinder productivity in this region. *In vitro* culture methods present a solution by rapidly producing high quality, disease-free pomelo seeds. This study aims to determine the optimal concentration of the BAP added to the culture medium to induce shoots from pomelo seeds. **Materials and Methods:** The seeds were planted on MS media with the addition of BAP hormone (0.5, 1, 1.5, 2 and 2.5 ppm) and 0 ppm as the control. The experimental units were arranged in a CRD and analyzed using SPSS 20.0 software, employing the Shapiro-Wilk normality test and Levene's Statistic for homogeneity. If the data met the normality and homogeneity assumptions, ANOVA was applied, followed by the DMRT for a parametric test. Otherwise, a non-parametric test namely the Kruskal-Wallis was conducted and differences were further analyzed using the Mann-Whitney test at a 5% significance level. **Results:** The application of the BAP accelerated shoot emergence, with the most rapid development occurring on the 10th day after planting (DAP), at a BAP concentration of 2.5 ppm for red pomelo. For white pomelo and sweet pomelo, shoots appeared on the 19th and 20th days, respectively at a 2 ppm BAP. Interestingly, root development was fastest between the 4th and 6th DAP in 0 ppm BAP (control). **Conclusion:** The addition of the BAP at a concentration of 1.5 ppm in the culture medium promotes faster shoot emergence and has a significant impact on the number of shoots in red pomelo.

Key words: Cytokinin, local pomelo oranges, micropropagation, regeneration, shoot induction

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Indonesia is a tropical fruit-producing country renowned for the diversity and superior flavor of its produce compared to tropical fruit from other producing countries. Among these fruits, oranges are a particularly promising product for development. They offer high nutritional content and an appealing taste, making them popular among consumers[1]. Orange cultivation is widespread across 14 Indonesian provinces, including South Sulawesi, where the heart of the industry lies in the Pangkep Regency. This region is also recognized as the largest producer of *Citrus maxima* (Burm.) Merr., commonly known as pomelo in Eastern Indonesia with some varieties such as red pomelo, white pomelo and sweet pomelo.

Previous research indicates that pomelo production in the Pangkep Regency remains low due to insufficient maintenance by farmers. This lack of care, stemming from budget constraints, affects important aspects such as irrigation, pruning, fertilization and orchard sanitation. Inadequate maintenance has negative effects on both the productivity and quality of pomelo fruit[2]. Another contributing factor to declining productivity is the presence of pomelo trees aged over 20 years[3]. Traditional seed propagation methods also face limitations in providing a large-scale supply of seedlings. Aging pomelo trees are inherently unable to bear fruit optimally, necessitating a focus on plant regeneration[4]. The implementation of *in vitro* culture offers a viable solution to the challenges faced in the Pangkep Regency.

Plant propagation through *in vitro* culture provides many advantages, including the regeneration of mature pomelo plants with identical characteristics to their parent trees[5]. It also accelerates the production of a substantial quantity of superior, disease-free pomelo seeds[6,7]. *In vitro*, seed culture is a fitting method for the propagation of endemic plant species, particularly for conservation purposes aimed at preserving genetic diversity[8,9]. One pivotal factor influencing the success of *in vitro* seed culture is the use of plant growth regulators. This study, focuses on optimizing the media through the addition of the BAP (6-benzylaminopurine) plant growth regulator to induce shoots from superior South Sulawesi pomelo seeds. This research yields a method and procedure that can be repeatedly applied for the production of high-quality pomelo seedlings through shoot induction *in vitro* seed culture.

MATERIALS AND METHODS

Study site and sample collection: This research was conducted from July to December, 2022. The plant material consisted of 162 pomelo seeds from *Citrus maxima* (Burm.) Merr., including red pomelo, white pomelo and sweet pomelo varieties, obtained from the pomelo plantations in Pangkep Regency. The research was carried out in the Plant Tissue Culture Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences and the Biotechnology and Tree Breeding Laboratory, Faculty of Forestry, Universitas Hasanuddin.

Equipment sterilization: All equipment used for planting must always be in a sterile condition. Glass and metal equipment were thoroughly cleaned with liquid soap and hypochlorite, then dried and sterilized in an oven at 121°C, 17.5 psi pressure for 15 min.

Media preparation: The medium used was Murashige and Skoog medium with vitamins (MSP09-50LT, Caisson Labs). To prepare 1 L of media, 4.43 g was needed, mixed with 30 g L^{-1} of sucrose and 7 g L^{-1} of agar (phytagel, Sigma). Then, 1 L of distilled water was added and the pH was adjusted to 5.8. Next, it was dissolved in a chemical glass, stirred with a stirring rod and heated until it became homogeneous and the solution turned clear. The medium was then distributed into culture bottles and sealed with aluminum foil before being sterilized with an autoclave.

Plant material sterilization: The sterilization of plant materials was performed by thoroughly washing red pomelo, white pomelo and sweet pomelo seeds (Fig. 1) with running water. They were then washed with sunlight liquid soap (commercial liquid soap, Unilever Indonesia) for 5 min, rinsed until the foam disappeared and the outer peel was peeled. The pomelo seeds were then soaked in a tween 80 solution for 15 min and rinsed with sterile distilled water three times. The seeds were soaked in a 20% NaOCl solution for 15 min, rinsed with sterile distilled water three times and soaked in 90% alcohol for 15 min, followed by rinsing with sterile distilled water[9,10].

Culturing seeds on media with various BAP concentrations: The sterilized seeds were planted on media with five different concentrations of BAP (6-Benzylaminopurine) plant growth regulator, which were 0.5, 1, 1.5, 2 and 2.5 ppm and one control medium with 0 ppm. Three seed explants were planted in each culture bottle and the culture bottles were stored on culture racks. Each treatment consisted of three replicates with five culture bottles per replicate. The cultures were kept at a temperature of 23±2°C with a lighting schedule of 16 hrs of light and 8 hrs of darkness.

Fig. 1(a-d): Morphology of the pomelo fruit *Citrus maxima* (Burm.) Merr., (a) White pomelo, (b) Red pomelo, (c) Sweet pomelo and (d) Seed
Scale bar = 0.5 cm

Observation and data analysis: The experimental units were arranged in a completely randomized design. Observations of shoot induction from seed cultures were conducted for 8 weeks after planting (WAP). Data was analyzed using Statistical Package for Social Science (SPSS) 20.0 software with tests for normality (Shapiro-Wilk) and homogeneity (Levene Statistic)[11]. If the data met the assumptions of normality and homogeneity, an Analysis of Variance (ANOVA) test was performed and if there was an effect or difference, Duncan's Multiple Range Test (parametric test) was conducted. If the data was not normal and not homogenous or one of them, a non-parametric test Kruskal-Wallis was carried out and if differences were found, a Mann-Whitney test was performed at a 5% significance level[12-14].

RESULTS AND DISCUSSION

Shoot emergence time: Observations of shoot emergence time and root emergence were counted from the day after the planting (DAP) of explants. Data from each treatment was then calculated and the values from each replicate were averaged as presented in Table 1. The emergence of shoots is an indicator in tissue culture studies that shows that plants can grow with the given treatment[15]. The fastest shoot emergence time was observed in treatment 5 (MS+BAP 2 ppm) for white and sweet pomelos, which occurred on day 20 and day 19 after planting, respectively. In red pomelos, it was observed in treatment 6 (MS+BAP 2.5 ppm) on day 10. This may be attributed to the higher endogenous hormone content in red pomelos compared to white and sweet pomelos. The endogenous hormone content in each explant varies, so the addition of exogenous cytokinins to the culture medium will result in varying responses. The addition of the BAP in the medium stimulates faster shoot growth because one of BAP's functions is to induce shoot formation in plants. However, it should be noted that excessive use of cytokinin-class growth regulators, such as BAP in concentrations exceeding 2 ppm, can be toxic to the plant. This corresponded to the findings of a previous study which indicated that if the BAP level exceeds the optimum limit of 2 ppm, it can be toxic to plant tissues. Cytokinins like BAP can reduce the dominance of apical meristems and induce the formation of axillary and adventitious shoots in plants[16,17].

Root emergence time: The fastest root emergence time was observed in treatment 1 (control) for all varieties of red, white and sweet pomelos, with each of them appearing on day 6, 5 and 4 after planting, respectively. This is because the medium without the addition of the growth regulator BAP more quickly stimulates root growth, as pomelo plants generally already have higher levels of endogenous auxin hormone. This aligns with the statement by Mahadi *et al.*[18], that in pomelo plants grown in a medium without the addition of cytokinin hormones, the formation of roots is better than in media containing cytokinins. This is also supported by Márquez *et al.*[19], who stated that cytokinin hormones have been proven to inhibit root growth in pomelo plants.

Table 1: Days when the roots and shoots of pomelo oranges emergence after planting

Variety	Treatments	Shoot emergence time (DAP)	Root emergence time (DAP)
Red pomelo	1 (MS+0)	16.00	6.00
	2 (MS+0.5)	21.67	8.00
	3 (MS+1)	30.67	8.33
	4 (MS+1.5)	21.67	8.00
	5 (MS+2)	32.00	8.33
	6 (MS+2.5)	10.00	8.33
White pomelo	1 (MS+0)	25.00	5.00
	2 (MS+0.5)	25.00	6.00
	3 (MS+1)	25.00	9.00
	4 (MS+1.5)	25.67	11.00
	5 (MS+2)	20.67	12.00
	6 (MS+2.5)	24.67	13.00
Sweet pomelo	1 (MS+0)	31.67	4.00
	2 (MS+0.5)	24.67	4.67
	3 (MS+1)	27.67	8.00
	4 (MS+1.5)	20.00	8.00
	5 (MS+2)	19.67	8.67
	6 (MS+2.5)	23.33	10.33

1: MS+BAP 0 ppm, 2: MS+BAP 0.5 ppm, 3: MS+BAP 1 ppm, 4: MS+BAP 1.5 ppm, 5: MS+BAP 2 ppm and 6: MS+BAP 2.5 ppm

Influence of BAP on the number of shoots, leaves and roots: Observations of the number of shoots, leaves and roots in pomelo fruit were conducted in the final week of observation, which was 8 weeks after planting (WAP). The data was then analyzed using a normality test to determine whether the observed data were normally distributed or not. Subsequently, a homogeneity test was conducted to identify significant treatment differences[12,14]. In the normality test, if the obtained significance value is greater than 0.05, the data is considered normally distributed. Conversely, if the significance value is less than 0.05, the data is not normally distributed[20]. The normality test used in this study is the Shapiro-Wilk test, which is suitable for small-scale samples[21,22]. Furthermore, Levene's test was performed to assess the equality of population variances in the research[23]. In the homogeneity test, if the significance value is greater than 0.05, the data is considered homogeneous. Conversely, if the significance value is less than 0.05, the data is not homogeneous[24]. If the normality and homogeneity test results do not meet the parametric test requirements (normal and homogeneous), a non-parametric Kruskal-Wallis test is performed[25,26]. If the Kruskal-Wallis test results in a significance value less than 0.05, it indicates an effect of treatment and a further Mann-Whitney test is conducted.

The results of the normality test for the effect of BAP on the number of shoots, leaves and roots in pomelo fruit were presented in Table 2. Based on the normality test results, the significance values were less than 0.05 for the number of shoots, leaves and roots of red, white and sweet pomelo fruit, indicating that the data was not normally distributed. In the homogeneity test presented in Table 3, the significance values were less than 0.05 for the number of shoots and roots in red and white pomelo fruit, indicating that the data was not homogeneous. However, for the number of leaves in red and sweet pomelo fruit, the significance values were greater than 0.05, indicating homogeneous data. The significance value for white pomelo was 0.05, indicating homogeneous data. Based on the results of the normality and homogeneity tests for the number of shoots, leaves and roots in red, white and sweet pomelo fruit, none of the data met the requirements for parametric tests (normal and homogeneous). Therefore, a non-parametric Kruskal-Wallis test was performed.

Based on the Kruskal-Wallis test results presented in Table 4, significant results were obtained for red pomelo, with significance values of 0.016 for shoots and 0.011 for roots, both less than 0.05. This indicates an effect of the concentration of the BAP on the number of roots and shoots. Therefore, a Mann-Whitney test was conducted as a follow-up. For the number of leaves, a significance value of 0.082 was obtained, which is greater than 0.05, so no further test was conducted. For white pomelo, the Kruskal-Wallis test resulted in significance values of 0.106, 0.0472 and 0.053 for the number of shoots, leaves and roots, respectively. These values are greater than 0.05, indicating no significant effect of the addition of the BAP plant growth regulator on the number of shoots, leaves and roots in white pomelo, so no further test was conducted. For sweet pomelo, the number of shoots had a significance value of 0.066 and the number of roots had a significance value of 0.284, both of which are greater than 0.05, so no further test was conducted. However, the number of leaves had a significance value of 0.018, which is less than 0.05, indicating an effect of the application of the BAP on the number of leaves in sweet pomelo (Fig. 2). Therefore, a Mann-Whitney test was conducted to determine significant differences between treatments.

Table 2: Normality test results of the effect of BAP on the number of shoots, number of leaves and number of roots of pomelo oranges

		Red pomelo				White pomelo				Sweet pomelo			
Parameter	Treatment	Statistic	df	Sig.	Decision	Statistic	df	Sig.	Decision	Statistic	df	Sig.	Decision
Number of shoots	1	0.75	3	0.305	Non-normally distributed	0.75	3	0	Non-normally distributed	0.75		0	Non-normally distributed
	2	0.75	3	0	Non-normally distributed		3		Non-normally distributed				Non-normally distributed
	3		3		Non-normally distributed	0.75	3	0	Non-normally distributed				Non-normally distributed
	4	0.75	3	0	Non-normally distributed		3		Non-normally distributed				Non-normally distributed
	5	0.75	3	0	Non-normally distributed	0.75	3	0	Non-normally distributed	0.87		0.295	Normally distributed
	6	0.75	3	0	Non-normally distributed	0.75	3	0	Non-normally distributed	1		1	Normally distributed
Number of leaves	1	0.873	3	0.305	Normally distributed	0.866	3	0.283	Normally distributed	0.893		0.363	Normally distributed
	2	0.964	3	0.637	Normally distributed	0.75	3	0	Non-normally distributed				Non-normally distributed
	3	0.75	3	0	Non-normally distributed	0.75	3	0	Non-normally distributed	0.923		0.463	Normally distributed
	4	1	3	0.984	Normally distributed	1	3	0.992	Normally distributed	1		1	Normally distributed
	5	0.922	3	0.459	Normally distributed	0.75	3	0	Non-normally distributed	1		1	Normally distributed
	6	0.75	3	0	Non-normally distributed	0.75	3	0	Non-normally distributed	0.99		0.808	Normally distributed
Number of roots	1	0.873	3	0.305	Normally distributed	0.994	3	0.848	Normally distributed	0.964		0.637	Normally distributed
	2	0.75	3	0	Non-normally distributed	0.75	3	0	Non-normally distributed	0.963		0.63	Normally distributed
	3	0.75	3	0	Non-normally distributed	0.871	3	0.298	Normally distributed	0.75		0	Non-normally distributed
	4	0.75	3	0	Non-normally distributed	0.75	3	0	Non-normally distributed				Non-normally distributed
	5	0.75	3	0	Non-normally distributed	0.75	3	0	Non-normally distributed				Non-normally distributed
	6	0.75	3	0	Non-normally distributed	0.75	3	0	Non-normally distributed				Non-normally distributed

Table 3: Homogeneity test results of the effect of BAP on the number of shoots, number of leaves and number of roots of pomelo oranges

	Red pomelo					White pomelo					Sweet pomelo				
Parameter	Levene statistic	Dfl	Df2	Sig.	Decision	Levene statistic	Dfl	Df2	Sig.	Decision	Levene statistic	Dfl	Df2	Sig.	Decision
Number of shoots	3.202	5	12	0.046	Non-homogenous	8.694	5	12	0.001	Non-homogenous	5.720		12	0.006	Non-homogenous
	0.200	5	12	0.956		0.543	5	12	0.740		1.196		12	0.368	
	0.200	5	9.994	0.955		0.543	5	4.177	0.741		1.196		5.080	0.423	
	2.522	5	12	0.088		6.848	5	12	0.003		5.195		12	0.009	
Number of leaves	2.442	5	12	0.095		3.100	5	12	0.050	Homogenous	5.720		12	0.006	
	0.428	5	12	0.821		0.496	5	12	0.773		1.196		12	0.368	Homogenous
	0.428	5	6.806	0.816	Homogenous	0.496	5	6.170	0.771		1.196		5.080	0.423	
	2.179	5	12	0.125		2.743	5	12	0.071		5.195		12	0.009	
Number of roots	6.833	5	12	0.003	Non-homogenous	5.842	5	12	0.006	Non-homogenous	9.574		12	0.001	Non-homogenous
	0.830	5	12	0.552		0.517	5	12	0.759		1.471		12	0.270	
	0.830	5	3.685	0.592		0.517	5	4.288	0.757		1.471		2.296	0.433	
	5.819	5	12	0.006		4.846	5	12	0.012		8.429		12	0.001	

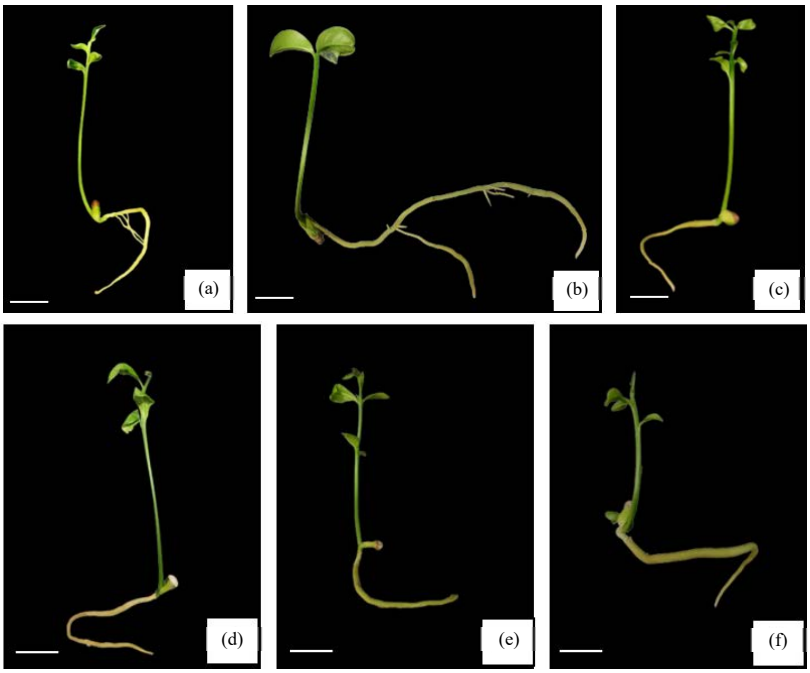

Fig. 2(a-f): Comparison of the number of sweet pomelo leaves, (a) MS+0, (b) MS+0.5, (c) MS+1, (d) MS+1.5, (e) MS+2 and (f) MS+2.5

Table 4: Kruskal-Wallis test results of the effect of BAP on the number of shoots, number of leaves and number of roots of pomelo oranges

	Red Pomelo			White pomelo			Sweet pomelo		
	Number of shoots	Number of leaves	Number of roots	Number of shoots	Number of leaves	Number of roots	Number of shoots	Number of leaves	Number of roots
Kruskal-Wallis	13.971	9.757	14.959	9.071	4.558	10.909	10.362	13.712	6.235
Df	5	5	5	5	5	5	5	5	5
Asymp. Sig.	0.016	0.082	0.011	0.106	0.472	0.053	0.066	0.018	0.284

The results of the Mann-Whitney test were presented in Table 5. For red pomelo, there were significant differences in the number of roots in treatment 6:1 with a value of 0.018, which is less than 0.05. As for the number of shoots, there were significant differences in treatment 6:4 with a value of 0.036, which is less than 0.05. However, none of the other treatments showed significant differences in the growth of red pomelo. In the case of sweet pomelo, all treatments had significance values exceeding 0.05, indicating that none of the treatments significantly affected the number of leaves in sweet pomelo.

The addition of the BAP plant growth regulator affected the number of shoots in red pomelo in treatment 4 (MS+BAP 1.5 ppm) and there were no shoots in treatment 6 (MS+BAP 2.5 ppm). Media without the addition of BAP (control) had an effect on the number of roots in red pomelo, with the fewest roots in treatment 6 (MS+BAP 2.5 ppm) (Fig. 3). Cytokinin hormone regulates cell division and increases cell expansion during proliferation, lateral shoot growth and leaf cell development[27,28]. The BAP as a plant growth regulator with various concentrations can affect plant growth, especially in red pomelo[29,30]. Some studies have shown that the optimal concentration of the plant growth regulator BAP for the growth of pomelo is between 1-2 ppm[13,31].

For red pomelo, the optimal concentration for the number of roots was found in treatment 1 (control) with 2.11 roots. This is because media without the addition of cytokinin hormone (BAP) stimulates endogenous auxin hormone to work optimally, promoting root growth. If the content of endogenous auxin hormone is higher, it will result in more roots[32]. Even though endogenous hormones are synthesized in small amounts by plants, they are highly active physiologically[33]. Shoot and root formation is regulated by the

Fig. 3(a-b): Comparison of the number of red pomelo roots, (a) MS+0 and (b) MS+2.5
Scale bar = 0.5 cm

Table 5: Mann-Whitney test results of the effect of BAP on the number of shoots, number of leaves and number of roots of red pomelo and sweet pomelo oranges

	Red Pomelo		Sweet Pomelo	
Treatment	Number of roots	Number of shoots	Treatment	Number of leaves
6:3	1.000	0.129	1:2	1.000
6:5	1.000	1.000	1:3	1.000
6:4	0.921	0.036	1:6	0.323
6:2	0.108	0.491	1:5	0.087
6:1	0.018	0.491	1:4	0.054
3:5	1.000	0.730	2:3	1.000
3:4	1.000	1.000	2:6	1.000
3:2	1.000	1.000	2:5	0.578
3:1	0.291	1.000	2:4	0.394
5:4	1.000	0.258	3:6	1.000
5:2	1.000	1.000	3:5	1.000
5:1	91	1.000	3:4	1.000
4:2	1.000	1.000	6:5	1.000
4:1	1.000	1.000	6:4	1.000
2:1	1.000	1.000	5:4	1.000

balance between auxin and cytokinin, with high auxin and low cytokinin promoting root formation, low auxin and high cytokinin promoting shoot formation and a balance between auxin and cytokinin promoting callus formation. In treatment 6 (MS+BAP 2.5 ppm), the fewest roots were produced among all the treatments, with only 0.11 roots. This was because the culture medium had a high concentration of BAP at 2.5 ppm, resulting in a higher level of cytokinin hormones compared to auxin hormones, which inhibited root growth. This aligns with the statement by Kurepa and Smalle[34] that the function of cytokinin hormones is to promote shoots and inhibit root growth, while auxin does the opposite by promoting root growth and inhibiting shoot growth. This is also supported by Khan *et al.*[35], stated that, BAP concentrations higher than 2 ppm can inhibit the extension of adventitious meristems and their transformation into complete plants. Some studies have also indicated that media with low cytokinin concentrations, i.e., below 1 ppm BAP are suitable for plant root growth[36].

The number of shoots is an important indicator in determining the potential of tissues regulated by genetic factors and growth hormones[15]. Based on the data obtained for the number of shoots in red pomelo, the optimal concentration was found in treatment 4 (MS+BAP 1.5 ppm) with 2.33 shoots. This was because one of BAP's functions is to stimulate shoot growth in plants. This aligned with Devsharmma *et al.*[37] statement that media supplemented with the cytokinin BAP can produce many shoots. This was also supported by Pereira *et al.*[38] stated that BAP significantly stimulates the growth of axillary shoots, adventitious shoots

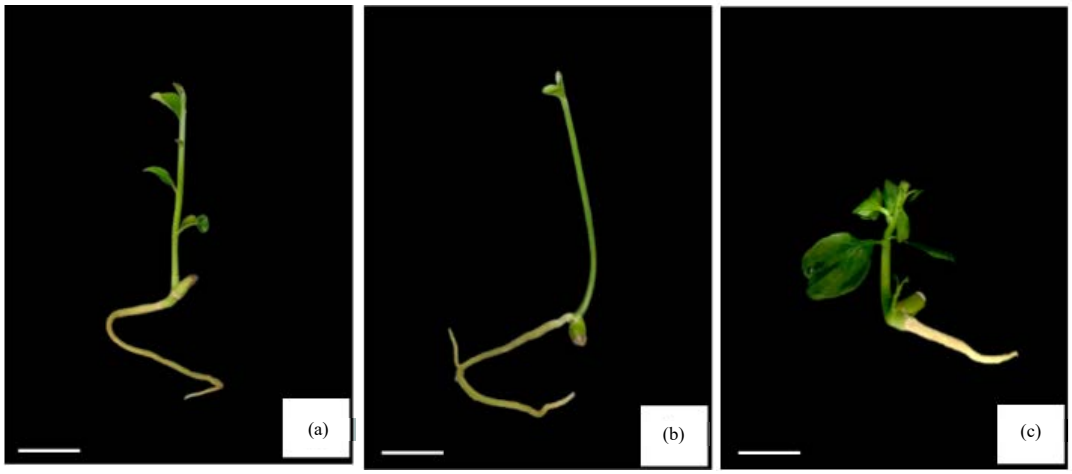

Fig. 4(a-c): Comparison of the number of red pomelo leaves, (a) MS+1.5, (b) MS+0 and (c) MS+2.5
Scale bar = 0.5 cm

and leaves. The BAP is a cytokinin hormone that plays a role in cell division and when combined with auxin, it can also promote cell expansion[39]. However, if the concentration of BAP exceeds the optimum level, it can be toxic to plants. The BAP plays a role in cell division and plant regeneration by stimulating seeds to differentiate into shoots, but if it exceeds the optimum level, it can be toxic to plant tissues[16]. This was evident in treatment 6 (MS+BAP 2.5 ppm), as shown in Fig. 4, where red pomelo growing in media with a concentration of 2.5 ppm resulted in abnormal plants with many leaves but dwarfed growth. This aligned with the study by da Silva *et al.*[40] stating that higher BAP concentrations lead to explants forming shoots without the development of new shoots, resulting in dwarfed and abnormal shoots.

Future recommendations are drawn from the significant implications across various domains found in this study. They offer promising avenues for agricultural advancement by employing *in vitro* cultivation techniques for pomelo seeds. These methods facilitate accelerated and superior plant growth, potentially elevating fruit yield. Moreover, this research underscores the importance of genetic conservation by employing *in vitro* culture to safeguard rare or indigenous pomelo varieties. Additionally, the study highlights its role in driving technological innovation, particularly in adopting *in vitro* technology for propagating region-specific fruit plants, paving the way for future advancements in this field. The outcomes of this study offer practical applications in promoting sustainable agriculture. *In vitro* techniques showcased here hold immense potential for widespread adoption in agriculture, expediting plant reproduction and bolstering overall productivity. Additionally, these methods can significantly contribute to regional progress. Specifically, implementing *in vitro* methodologies in pomelo cultivation across South Sulawesi stands to invigorate and strengthen the local economy. Based on this research, it is recommended to explore and expand the application of *in vitro* methods in pomelo cultivation through further investigation. Additionally, fostering a deeper understanding of *in vitro* techniques in agricultural practices necessitates training and educational initiatives for farmers. However, it's crucial to note limitations in *in vitro* culture research, particularly regarding costs and accessibility. The considerable investment of resources and the challenge of scaling production hinder its widespread adoption. Moreover, reliance solely on *in vitro* technology could pose obstacles to its practical implementation in the field.

CONCLUSION

The results indicate that the BAP treatment stimulates the faster emergence of shoots and has a significant effect on the number of shoots in red pomelo (*Citrus maxima* (Burm.) Merr.) at a concentration of 1.5 ppm. Understanding this precise concentration can serve as a cornerstone for refining agricultural methods, ultimately enhancing output. Furthermore, leveraging BAP in *in vitro* plant propagation forms the foundation for pioneering technological advancements in pomelo orange plant multiplication endeavors.

SIGNIFICANCE STATEMENT

This study proposes a method and process for propagating pomelo plants through *in vitro* seed culture. The research will assist researchers in determining seed surface sterilization procedures and in establishing the optimal concentration of the cytokinin hormone, especially BAP for faster shoot growth. The results of this research make a fundamental contribution to the field of crop cultivation and plant breeding, as it allows for the production of high-quality pomelo plants through plant tissue culture technology, thereby supporting the continuous availability of plant seedlings.

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