Application of kinetin for the proliferation of suren (*Toona sureni* merr.) in technique in vitro

S H Larekeng[1], Junardi[2], Mirza Arsiaty Arsyad[3], Fitri Datu Panannangan[4] and M Restu[1]*
[1]Biotechnology and Tree Breeding Laboratory, Faculty of Forestry, Universitas Hasanuddin, Makassar
[2]Student of Biotechnology and Tree Breeding Laboratory, Faculty of Forestry, Universitas Hasanuddin, Makassar
[3]Faculty of Agrotechnology, Halu Oleo University, Kendari, Indonesia
[4]2nd Regional of Forest Seed/Seedling Center (BPTH), Sulawesi, Indonesia

Abstract-Suren (*Toona sureni* Merr.) is a tree that belongs to the Meliaceae family. The propagation of suren is still difficult to do both generatively and vegetatively conventional methods such as cutting or grafting. The obstacle of suren propagation is slightly low seed productivity. Moreover, the In-vitro technique is an efficient and effective mass propagation technique for plants that can be continuously carried out to produce superior, equal, and many plants in a short time. For the suren growth, it is important to support effective tissue culture media with the addition of growth regulators of kinetin. Then, the data were analyzed by Microsoft Excel (Microsoft Corporation) software. The results showed that M2 media (1/2 MS + Kinetin 1 ppm) was the best media in tissue culture plant toward the germination time, number of shoots, number of leaves, plant height, length of leaf, and percentage of live explants.

Keywords: Toona sureni Merr., in vitro, kinetin, MS medium, WPM medium

Introduction

Suren is a type of tree that cannot grow under the shadow[1]. This species is classified as a fast-growingplant with straight stem shape, open-canopy, deep-rooted, and many-branched roots[2]. This plant has been received special attention from farmers and researchers due to it is easy to grow and use in agroforestry[3].

The supply of the suren seeds in large quantitiesis still challenging to do through generative and vegetative propagations, such as conventional cutting or grafting. The use of fresh suren seeds for seedlings must be immediately plantedbecause they are recalcitrant. Then, the suren seeds storage in the cold room only lasts for three months. Time and seed quality degradation of storage can affect the quality and quantity of seeds [4]. Thus, the propagation techniques that can be done to overcome these problems is through in vitro technique.

Tissue culture plant is one of the effective and efficient mass plant propagation that can be continuously carried out because it produces sterile culture (*mother stock*) so that it can be used as the material for further propagation[5] and conservation [6]. Moreover, the application form of tissue culture techniques that has a goal in plant propagation is induction. The induction stage is the initial stage of tissueculture, which is aimed to get thefree microorganisms explantand the initiation of new growth [7].

The success of plant propagation by tissue culture method is strongly influenced by several factors, such as the type of explants, sterilization, growth regulators, and growing media. In addition, factors that influence the success of tissue culture are the growth regulators. One of the growth regulators that is widely used in tissue culture is cytokinins [8]. Cytokinins are compounds that can increase cell division in plant tissues and regulate plant growth and development. The types of cytokines include AdSO4 (*adenine sulfate*), BAP (*6-benzyl amino purine*), kinetin (*6-furfurylaminopurine*), and thidiazuron (*N-Phenyl-N 'N-Phenyl-N') 1,2.3-thi-5-phenyl urea*)[9]. Moreover, natural,synthetic cytokines are divided into two:BAP and Kinetin. Kinetin is the growth regulator, which is classified as a synthetic cytokinin. Besides, its use is influenced by other growth regulators. Cytokinins affect various physiological processes in plants. The main activity of cytokines is cytokinesis or cell division [10].

A research conducted by Harahap (2011) toward the induction of mangosteen Shoots (*Garciniamangostana* L.) showed that the highest number of shoots on mangosteen was obtained on MS media with the addition of 5 mg/l kinetin (K). Hidayat (2007) [11]states that kinetin concentrations of 1.0 to 10.0 mg/l induced the formation of *Eusideroxylonzwageri*'s endosperm buds. Furthermore, the research by Kosmiatin et al. (2006)[12] focused on the comparison of MS and WPM media concentrations in germination and propagation of agarwood (*Aquilaria malaccensis* Lank.) showed different explant responses in each media treatment. The highest germination (66.67) was obtained from cultured seeds on MS media without the addition of vitamins. The specific media for woody plants also provide fairly high germination. This germination is better than the use of MS media with the addition of vitamins and ½ MS. Based on this problem, it is important to conduct this research in order to

DOI: 10.5373/JARDCS/V12I6/S20201111
**Corresponding Author: S H Larekeng ,Email: sitih5h.82@gmail.com*
Article History: Received: Mar 21, 2020, Accepted: June 22, 2020

produce a good quality of suren seeds through tissue culture with a variety of kinetin growth regulators that are suitable for producing good tissue culture seedlings.

Research methodology

The plant material used in this study was the suren seed. It wasobtained from the UPTD 2^{nd}Regional of Forest Seed/Seedling Center (BPTH), Makassar, Sulawesi Selatan. The media used in this study wereMurashige and Skoog (MS) and Woody Plant Medium (WPM). The growth regulatorwas kinetin. The sterilizers usedwere sterile distilled water, 96% alcohol, 70% alcohol, 30%, and 20% commercial bleach solutions.

Figure 1. Suren Seed

The research procedure

The media construction with plant growth regulators (PGR) can be done by adding the PGRbased on the treatment and concentration. It was used in the dissolved media before putting into prepared media bottles. The type of ZPT used in this study was cytokinin in the form of kinetin and its combination of both MS and WPM media with concentrations of 1 ppm and 2 ppm (parts per million). PPM is a unit of dissolved concentration expressed in units of mg/L. Then, the ½ MS and ½ WPM media use half the material from MS and WPM media.

Table 1. The types of media used in the Initiation of Suren (*Toona Sureni* Merr.) through in vitro technique in various treatments of kinetin growth regulator.

Treatment	Media Type
M1	½ MS
M2	½ MS + Kinetin 1 ppm
M3	½ MS+ Kinetin 2 ppm
M4	MS
M5	MS + Kinetin 1 ppm
M6	MS+ Kinetin 2 ppm
M7	½ WPM
M8	½ WPM + Kinetin 1 ppm
M9	½ WPM + Kinetin 2 ppm
M10	WPM
M11	WPM + Kinetin 1 ppm

M12	WPM + Kinetin 2 ppm

The total number of media treatmentsusedwas 12 media and repeatedsix times. The number of observation unit was 72 bottles by planting in each bottle and unit by three explants. Hence, the total number of observation unit was 216 units.

The Sterilized explants
The explant sourceis takenfrom the suren seedexplant of thegood quality plant that is free from bacterial infection of pests and diseases.
The stages of explant sterilization were carried out with the following steps:

The sterilizers outside the LAFC include:
1. The seedswere released from the wing lining.
2. The seeds were soaked in warm distilled water for 24 hours at a temperature of 50 ° C.

The sterilization in LAFC includes:
1. The seeds were soaked in a 30% commercial bleach solution for 5 minutes.
2. Rinsed the seed explants with sterile distilled water three times.
3. The seeds were soaked in a 20% commercial bleach solution for 5 minutes.
4. Rinsed the seed explants with sterile distilled water three times.
5. Seeds were soaked in 70% alcohol solution for 5 minutes.
6. Rinsed the seed explants with sterile distilled water three times.
7. After drying, the seed explants are ready to be planted on the media based on thetreatment.

Theobservedvariable
The observations were made after planting for 90 days. The observed variables include:

Germination timeof the explant (days after planting / DAP), it wasobserved by counting the days from the first shoots appeared in each treatment. The observations were carried out once every two days.

The percentage of live explants (%), contaminant explants (%), and browning explants were calculated using the following formula:

$$\frac{\Sigma \text{Live explants}}{\Sigma \text{Total explants planted}} \text{ x } 100\%$$

$$\frac{\Sigma \text{Contaminated explants}}{\Sigma \text{Total explants planted}} \text{ x } 100\%$$

$$\frac{\Sigma \text{Browning explants}}{\Sigma \text{Total explants planted}} \text{ x } 100\%$$

The number of shoots (fruit)was calculated based on the number of shoots that appeared in shoot explants each observation time.

Plant height (cm), the observations made in the final week of observation, and calculated based on the number of shoots appeared.

The number of leaves (strands)calculated based on the number of leaves on each explant planted and counted at the end of the observation.

*Leaf length (cm)*measured based on the length of the leaf that appeared at each explant planted and carried out at the end of the observation.

DOI: 10.5373/JARDCS/V12I6/S20201111
**Corresponding Author: S H Larekeng ,Email: sitih5h.82@gmail.com*
Article History: Received: Mar 21, 2020, Accepted: June 22, 2020

The Data analysis
The data analysis used in this study is the descriptive analysis by observing and identifying each explant growth observation parameters using Microsoft Excel (Microsoft Corporation).

Results and discussion

3.1. Germination time of the explant
The germination time began to occur in the 26th week on several treatment media marked by the split explants and the appearance of the sprouts (Figure 2). The observations were carried out twice a week. The number of germinated explantsgrew more and moreuntil the end of the observation. The results showed that the effect of the media and the addition of kinetin growth regulators influenced the speed of explant germination time. Then, the germination time of Suren seed explants in each treatment can be seen in Figure 3.

Figure 2. The Suren seed germination explants

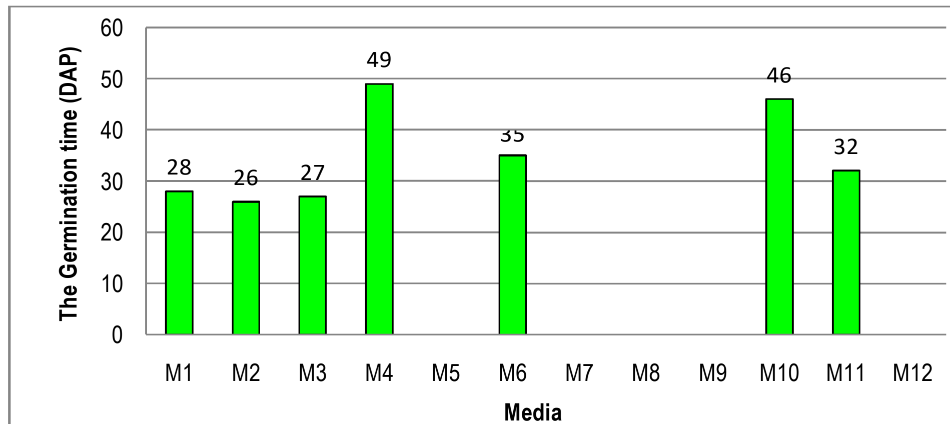

Figure 3. The germination time of Suren seed on each treatment

Figure 3 depicts the germination time, which was influenced by the media comparison and the addition of kinetin. M1 (1/2 MS), M2 (1/2 MS + Kinetin 1 ppm), and M3 (1/2 MS + Kinetin 2ppm) had the fastest response media, namely 26DAP. These results indicated that the ½ MS medium was able to stimulate the emergence of shoots with different concentrations. This was in accordance with the research of Islam et al., (2003), which stated that the medium 1/2 MS proved capable of inducing the growth of orchid buds.M5 (MS + Kinetin 1 ppm), M7 (1/2 WPM), M8 (1/2 WPM + Kinetin 1 ppm), M9 (1/2 WPM + Kinetin 2 ppm), and M12 (WPM + Kinetin 2 ppm) wereunresponsivemedia for plants due to high percentage of contamination and browning.This wasdue to the explant size and addition of kinetin growth regulators. Zulkarnain (2009) states that the size can affect the survival response of the plant so that it will affect the success of in vitro culture. Another problemisthe variation in the genetic ability of explants to plant growth regulators[13]. The ability of plant metabolism is very dependent on the genetic of plants (endogenous factors) and some unresponsive plants of the given growth regulators (exogenous factors)[14]. The growth is also influenced by the balance between the interaction of endogenous and exogenous factors [11].Study genetic of plants is very important after tissue culture studies [14] case for endemic and endangered plants [15][16].

3.2. The Percentage of Live, Contaminated, and Browning Explants
The percentage of live explants reached 83% in the M2 (1/2 MS + Kinetin 1 ppm). The live explants were characterized by fresh brown to pale brown seeds. The highest percentage of contaminated explants observed in

M6 (1/2 WPM) and M7 (1/2 WPM + Kinetin 1 ppm) were 67%,with 33% live explants. The contaminated explants were characterized by the appearance of fungi and bacteria in explants and media. The most common type of contamination is from bacteria, which is characterized by the media becoming more fluid and turbid. In addition, some factors that cause contamination in explants arethe sterilization processes, equipment used, stages, and ways of working. The contamination can also be found by types of explants from a variety of mother stock obtained. The average percentages of explants that were alive and contaminated are presented in Figure 4.

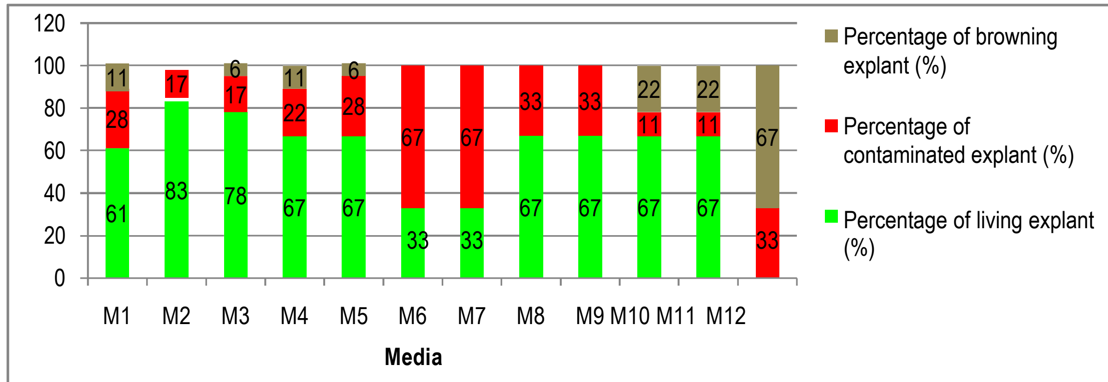

Figure 4. The percentages of living, contaminated and browning of suren seeds.

The contamination is a major problem that often arises in tissue culture. Bacterial contamination is marked by the presence of mucus on wet lump media [17]. Contamination can be removed by using stable equipment, room temperature, and support lighting.

The highest percentage of browning explants was on M12 (WPM + kinetin two ppm), 67%. Santoso and Nursandi (2003) [9] stated that browning is a natural occurrence that usually occurs in biological systems. An adaptive process of plant parts due to physical or biochemical influences, such as bruising, stripping, disease attacks, or abnormal conditions. Generally, woody plants produce phenolic compounds, and the best media in woody plants is WPM media. The treatment media that experienced browning and contamination can be seen in Figure 5.

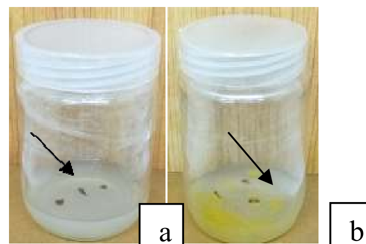

Figure 5. The media of suren.a. Browning, and b. Contamination.

3.3. The Number of Shoots

The number of shoots induced by explants is the most critical factor in plant growth. It is calculated on all shoots that arise from the elongation of the Shoots. Then, the number of shoots formed can be multiplied by a culture so that it produces new shoots in relatively large quantities.

DOI: 10.5373/JARDCS/V12I6/S20201111

**Corresponding Author: S H Larekeng ,Email: sitih5h.82@gmail.com*

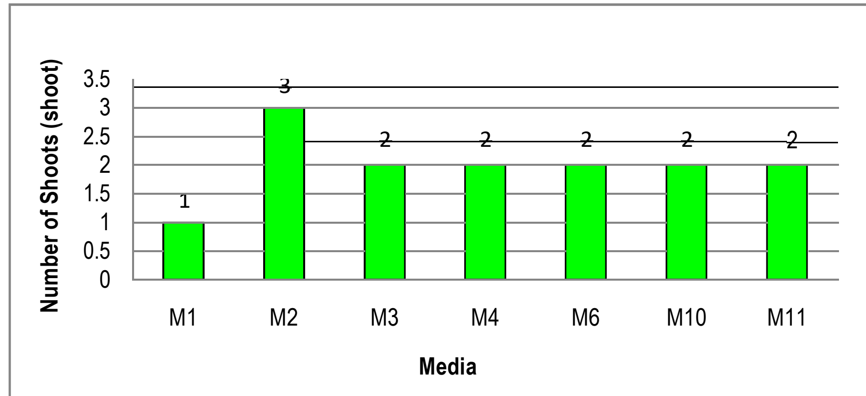

Figure 6. The number of suren shootson each treatment for 12 weeks.

Figure 6 shows that M2 (½ MS + Kinetin 1 ppm) obtained the highest number of shoots(3 shoots), and M1 (1/2 MS) was the treatment media with the lowest number of shoots (1 shoot). Furthermore, M2 was the best media in increasing the number of shoots. Then, the research conducted by Winarsih et al. (1998) [18]suggested that media within adding the cytokinins (kinetin) can stimulate shoot growth even if the availability of cytokinins (kinetin) in culture media is very limited. As a result, the cell division in cultured tissue will be delayed.

3.4. The Plant height

The measurement of the increase in plant height in this study was only done once at the end of the observation. Plant height measurements were not carried out during the observation process due to conditions that made it impossible to retrieve height data. There was a bias when measuring outside the culture bottle. In addition, the position of plants is also difficult to do the measurements.

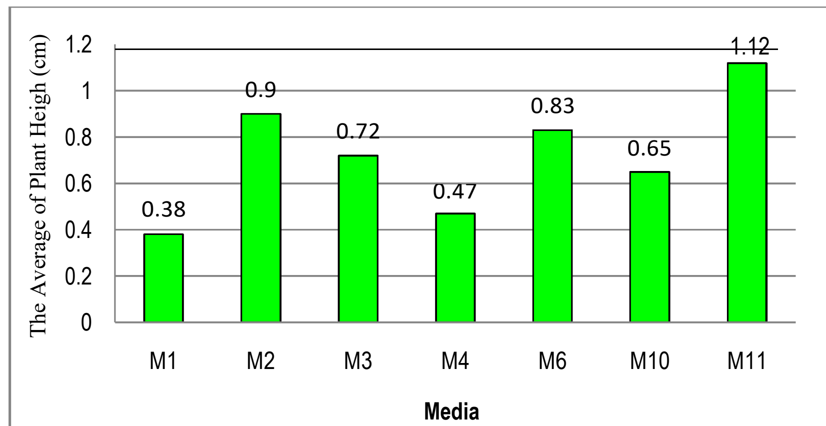

Figure 7. The average height of the shoot on the media.

FM11 (WPM + Kinetin 1 ppm) obtained the highest average value of 1.12 cm,and M1 (1/2 MS) had the lowest average value of 0.38cm. Thesewere in accordance with the research of Joni et al. (2014)[19], tissue culture of *Garcinia mangostana* L. using WPM media achieved good results on seed explants.

The addition of kinetin growth regulators to the media can stimulate cell division (cytokinesis), which causes the increasing number of cleaved cells and is able to absorb more water so that there is an enlargement/elongation of cells. It is increased in high shoot growth, but the use of higher kinetin concentrations inhibits plant growth [20]

3.5. The Number of Leaves

The observation of leaves number isconducted by counting the number of leaves formed in each treatment. Leaf formation started at seven weeks after planting with the number of leaves formed in each treatment in different culture bottles. The growth of suren leaves number can be seen in Figure 8.

Figure 8. The growth of suren leaves on Week 12.

The observations showed that the leaves appeared at the 7th week after planting, yet, not all treatment media were overgrown with leaves. This was due to the influence of the media and the addition of growth regulators, and also some explants sprouted but experiencedthe contamination. The number of leaf parameters can be seen in Figure 9.

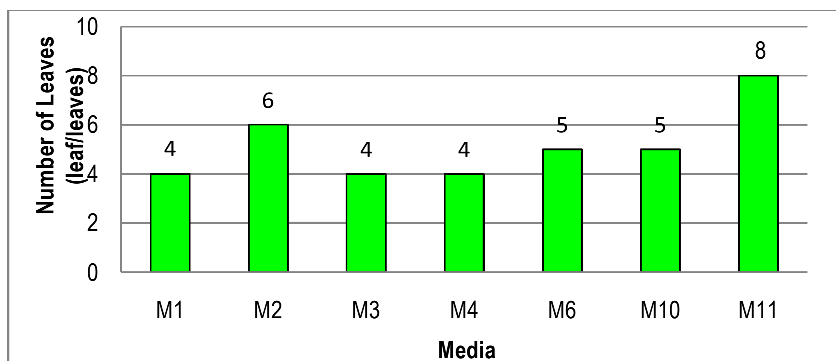

Figure 9. The number of Suren leaves on each treatment media on the 12th week.

Figure 9 shows that the M11 (WPM + Kinetin 1 ppm) obtained the highest average number of leaves, eightleaves. M1 (1/2 MS), M3 (1/2 MS + Kinetin 2 ppm), and M4 (MS) obtained the average value of a small number of leaves, fourleaves. Rohma's research (2012)[21]stated that the media, with the addition of cytokinins, could encourage leaf cells so that they are meristematic to be able to divide and develop into Shoots. The Shoots then form the intact plant organs through organogenesis.

3.6. The Leaf Length

The measurement of raising the leaf length treat onceonly at the end of the observation because it was not possible due to data collection was in a culture bottle. Leaf lengths in several treatments had different responses. The average leaf length is presented in Figure 10.

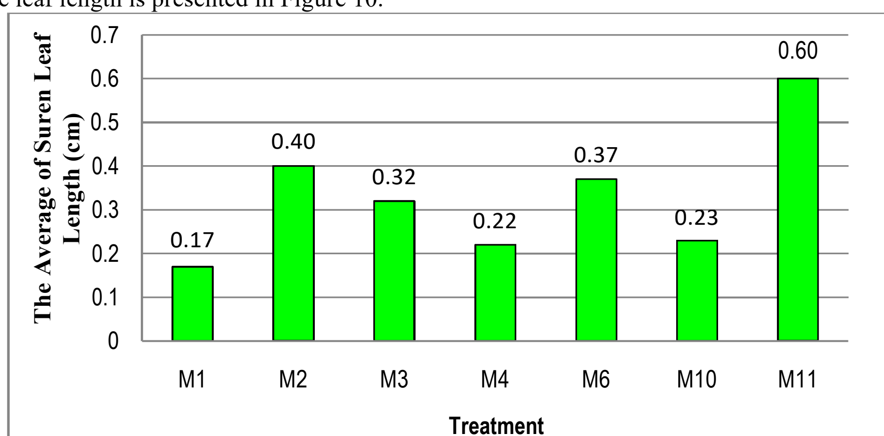

Figure 10. The average of Leaf lengthon Suren on each treatment

Figure 10 shows M11 (WPM + Kinetin 1 ppm) had an average leaf length value of 0.60 cm, while the lowest average leaf length value was observed on M1 (1/2 MS), 0.17 cm. This was in line with Rohma (2012)[21], leaf length has a relationship with the number of leaf growth; a good number of leaf growth produces the largest average leaf length. In Olive research want to know to establish a micropropagation system. The

DOI: 10.5373/JARDCS/V12I6/S20201111
**Corresponding Author: S H Larekeng ,Email: sitih5h.82@gmail.com*

elongated shoots were rooted containing 160 mg/L Putrescine, 1.5 mg/L naphthaleneacetic acid (NAA), 30 g/L mannitol, and solidified with 0.65% (w/v) Agar-Agar. All plantlets olive were successfully acclimatized in a climate chamber, and the plants were transferred to greenhouse conditions [22].

Conclusions

M2 (1/2 MS + Kinetin 1 ppm) was the best media in tissue culture against the parameters of observing the time of germination, number of shoots, number of leaves, plant height, leaf length, and percentage of live Suren explants.

References

[1] Siahaan A A J and Setiawan A 2015 Densitas Pohon Dewasa Dan Permudaan Pulai (Alstonia Scholaris) Dan Suren (Toona Sureni) Dalam Blok Koleksi Tumbuhan Di Taman Hutan Raya Wan Abdul Rachman *J. Sylva Lestari***3** 91–102

[2] Departemen Kehutanan 2002 *Pedoman Pembuatan dan Pengukuran Petak UkurPermanen (PUP) untuk Pemantauan Pertumbuhan dan Riap Hutan Alam Tanah Kering Bekas Tebangan.Badan Penelitian dan pengembangan Kehutanan* (Jakarta, Indonesia: Departemen Kehutanan)

[3] Sandabunga R M, Umar A, Millang S, Bachtiar B, Paembonan S, Restu M and Larekeng S H 2019 Land compliance of agroforestry compiler components evaluation in Pangli sub-district Desean district , North Toraja regency *IOP Conf. Ser. Earth Environ. Sci. 343 012053***343** 1–11

[4] Dirr M A 1987 *The reference manual of woody plant propagation: from seed to tissue culture; a practical working guide to the propagation of over 1100 species, varieties and cultivars*

[5] R B, Larekeng S H, Arsyad M A, Gusmiaty G and Restu M 2020 In vitro growth response on three provenances of Jabon Merah based on auxin and cytokinin combinations *IOP Conf. Ser. Earth Environ. Sci. 486 012088***486** 1–16

[6] Werden L K, Sugii N C, Weisenberger L, Keir M J, Koob G and Zahawi R A 2020 Ex-situ conservation of threatened plant species in island biodiversity hotspots: A case study from Hawai'i *Biol. Conserv.***243** 108435

[7] Nursyamsi 2010 *Teknik Kultur Jaringan Sebagai Alternatif Perbanyakan Tanaman Untuk Mendukung Rehabilitasi Lahan* (Makassar: Balai Penelitian Kehutanan Makassar)

[8] Gunawan 1992 *Teknik Kultur Jaringan In Vitro dalam Hortikultura* (Jakarta, Indonesia: Penebar Swadaya)

[9] Santoso U and Nursandi 2003 *Kultur Jaringan Tanaman* (Malang: Penerbit Universitas Muhammadiyah Malang)

[10] Widiastoety D 2016 Pengaruh auksin dan sitokinin terhadap pertumbuhan planlet anggrek Mokara *J. Hortik.***24** 230–8

[11] Hidayat 2007 Induksi Pertumbuhan Eksplan Endosperm Ulin dengan IAA dan Kinetin *Agritrop.***26** 147–52

[12] Kosmiatin M, Husni A and Mariska I 2016 Perkecambahan dan perbanyakan gaharu secara in vitro *J. AgroBiogen***1** 62–7

[13] Bakhshipour M, Mafakheri M, Kordrostami M, Zakir A, Rahimi N, Feizi F and Mohseni M 2019 In vitro multiplication, genetic fidelity and phytochemical potentials of Vaccinium Arctostaphylos L.: An endangered medicinal plant *Ind. Crops Prod.***141** 111812

[14] Jogam P, Sandhya D, Shekhawat M S, Alok A, M M, Abbagani S and Allini V R 2020 Genetic stability analysis using DNA barcoding and molecular markers and foliar micro-morphological analysis of in vitro regenerated and in vivo grown plants of Artemisia vulgaris L. *Ind. Crops Prod.***151** 112476

[15] Larekeng S H, Restu M, Arif A, Cahyaningsih Y F and Mukti J 2019 A genetic approach to study mating system on Jabon Merah (*Anthocephalus macrophyllus* Roxb.) from three different provenances in South Sulawesi *IOP Conf. Ser. Earth Environ. Sci.***235** 012049

[16] Shi S, Larekeng S H, Lv P, Nie Y, Restu M, Shi S, Halimah S, Lv P, Nie Y, Restu M and Yang H 2020 The complete chloroplast genome of Neolamarckia macrophylla (Rubiaceae) *Mitochondrial DNA Part B***5** 1611–2

[17] Nisa C and Rodinah R 2018 Kultur Jaringan beberapa Kultivar Buah Pisang (Musa paradisiaca L.) dengan Pemberian Campuran NAA dan Kinetin *BIOSCIENTIAE***2**

[18] Winarsih S 1998 Pengaruh Zat Pengatur Tumbuh terhadap Perbanyakan Kerk Lily secara In-vitro *J. Hortik.***8** 1145-1152.

[19] Joni Y Z, Efendi D and Tambunan I R 2016 Morfogenesis eksplan keping biji dari tiga klon manggis (Garcinia mangostana L.) pada tiga jenis media dasar *J. Hortik.***24** 94–101

[20] Hartoyo R D, Sulichantini E D and Eliyani 2018 Pengaruh Konsentrasi Kinetin Terhadap Pertumbuhan Stek Mikro Eucalyptus pellita F. Muell secara In Vitro *J. Agroekoteknologi Trop. lembab***1** 4–37

[21] Rohma 2012 *Pertumbuhan Tunas Apikal dan Aksilar Kultur In-Vitro Ubi Kayu (Mannihot esculenta*

Crantz) Genotipe. Fakultas Ilmu Pengetahuan Alam (Makassar: Universitas Indonesia)
[22] Bayraktar M, Hayta-Smedley S, Unal S, Varol N and Gurel A 2020 Micropropagation and prevention of hyperhydricity in olive (Olea europaea L.) cultivar 'Gemlik' *South African J. Bot.* **128** 264–73

DOI: 10.5373/JARDCS/V12I6/S20201111
**Corresponding Author: S H Larekeng ,Email: sitih5h.82@gmail.com*
Article History: Received: Mar 21, 2020, Accepted: June 22, 2020