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MICROPROPAGATION OF TEMBESU (*FAGRAEA FRAGRANS* ROXB.) FROM SHOOT TIP AND NODAL EXPLANTS

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SUMMARY

The tembesu (*Fagraea fragrans*) has the highest ecological and economic values; however, its seedlings rarely exist in the field. The reason is the constraints of natural seed germination and an infrequent vegetative propagation of shoots. Therefore, artificial propagation efforts are essential, including through tissue culture propagation. The following study examined the use of 6-benzylaminopurine (BAP) for shoot proliferation from shoot tip and nodal explants and indole-3-butyric acid (IBA) for root induction from in vitro shoots of tembesu. The study comprised two stages—shoot multiplication and root induction. The stimulation of shoot multiplication consisted of five treatments, i.e., 0, 0.5, 1, 2, and 4 mg/L BAP. Root induction treatment used ½ MS media with the addition of IBA, consisting of four concentrations (0, 0.5, 1, and 2 mg/L IBA). Results showed the BAP treatment considerably enhanced the percentage of shoot formation and the number of shoots. The highest number of shoots surfaced in explants treated with 1 mg/L BAP, with 32 and 35 shoots from shoot tip and nodal explants, respectively. The best root induction occurred from in vitro shoot explants of tembesu on ½ MS medium containing 1 mg/L IBA, with an observed 100% root percentage and 7.6 root numbers.

Keywords: BAP (6-Benzylaminopurine), IBA (Indole-3-butyric acid), root induction, shoot tip and nodal explants, shoot proliferation, tembesu (*F. fragrans*)

Key findings: In tembesu (*F. fragrans*), shoot tips and nodal explants were explants with high shoot regeneration potential through 1 mg/L BAP treatment. The best root formation emerged using 1 mg/L IBA treatment in ½ MS media.

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INTRODUCTION

Tembesu (*Fagraea fragrans*) is a plant with high ecological and economic value, which can be effective for vegetation rehabilitation, erosion control, and carbon sequestration (Azmi and Mohidin, 2020; Ghani *et al*., 2023). Tembesu wood has a supreme selling price for use in heavy construction, such as bridge beams, house pillars, and furniture (Asmaliyah *et al*., 2012; Mindawati, 2014). Tembesu leaves and bark are also beneficial as medicines, especially for treating malaria (Abuga *et al*., 2022; Pillai and Young, 2022), anemia, hemorrhoids, asthma, and coughs (Rattanaburi *et al*., 2022). Tembesu grows in various regions in Indonesia, including in agroforestry at Jambi (Ratna and Lestari, 2021) and at various locations in South Sumatra (Djam'an *et al*., 2016). Tembesu is also prevalent in multiple forests at Riau, along the Tesso Nilo and the Sultan Syarif Hasyim National Forest Parks (Indrayati *et al*., 2015). The higher harvesting of tembesu timber compared with its planting resulted in diminishing tembesu stands (Djam'an *et al*., 2016). Therefore, it is necessary to plant tembesu in various areas, which requires numerous young seedlings.

Generally, tembesu seedlings rarely exist in the field, thus, requiring artificial propagation should be progressive. Seed propagation has been ongoing, including stem cuttings forming roots 60% to 76% (Payung and Susilawati, 2014, Istomo *et al*., 2014). However, seed propagation showed a germination percentage of 75% (Azahra and Suharto, 2022). In tembesu plant propagation, another alternative is using tissue culture (in vitro culture) technique, which allows the production of numerous seedlings from a few plant parts.

Stages via in vitro culture include shoot induction, multiplication, root induction, and acclimatization. Induction of tembesu shoots from apical or lateral shoot explants from tembesu seedlings grown on MS medium showed the highest percentage of live explants (33.33%). The highest number of shoots (11.86 shoots) came from a modified MS medium ([NO_3 - :NH_4 +, 3:1, total N 80 µM] + 1.5 mg/L BAP) (Ardiansyah *et al.*, 2015). Shoot induction emerged from leaves of 10-month-old tembesu seedlings, as grown on MS media. The best results occurred in the treatment of 1.5 mg/Lł BAP, with a percentage of shoot formation of 40% and formed 14 shoots (Sianturi *et al.*, 2017). Based on the shoot induction research, the percentage of live and shoots formed from shoot and leaf explants from tembesu seedlings is still low. Therefore, other alternative explants are necessary to increase the percentage of shoot formation, including using tembesu seed explants. Shoot induction from tembesu seed explants has continued on MS medium with sucrose and honey treatment. The results showed the best treatment was on MS medium by adding 1 mg/L BAP + 12 ml/L honey, with a shoot formation percentage of 93.33% creating 40 shoots (Fatonah and Isda, 2018).

The stage of micropropagation after shoot induction was multiplying shoots. In vitro, shoots formed from shoot induction then incur multiplication to obtain more shoots. Potential parts of in vitro shoots used as explants for shoot multiplication include shoot tips and nodal explants. Shoot tips and nodal explants have the potential to form axillary shoots. Adventitious shoots can emerge directly from leaves, stems, and callus. Adventitious buds with regeneration can produce more shoots. Past studies have shown shoot tips and nodal explants regenerate into many shoots with BAP treatment, either individually and in combination with NAA (Sarma and Tanti, 2017; Mohamed *et al*., 2019; Asmono, 2020).

The next step after shoot multiplication is in vitro root induction. The best in vitro root formation of *Garcinia mangostana* manifested on MS ½ N + 3 mg/L IBA medium (Harahap *et al*., 2014). The encouraging root formation from adventitious shoot explants of *Argania spinosa* materialized from 1.5 mg/L IBA treatment on MS media (Amghar *et al*., 2021). Based on the above discussion, the presented study aimed to determine the effect of BAP on shoot multiplication from shoot tips and nodal explants, as well as, the effect of IBA on root induction in tembesu.

MATERIALS AND METHODS

Shoot multiplication

This study had a randomized group design. The treatment with BAP growth regulator on MS media (Murashige and Skoog, 1962) to stimulate shoot multiplication consisted of five levels: control (without growth regulator), 0.5, 1, 2, and 4 mg/L BAP, with each treatment repeated five times. The explants used were in vitro shoots from the collection of the Integrated Biology Laboratory, Department of Biology, Faculty of Mathematics and Sciences, Universitas Riau, as results of shoot induction from seed explants from previous research (Fatonah and Isda, 2018). In vitro, shoots placed in a Petri dish had the apical part of the shoot and nodal with leaves cut about 0.75 cm, then planted on culture media for shoot multiplication. Each culture bottle sustained filling with shoot tips and nodal explants. The culture, grown in an incubation room, had temperatures of 23 °C–25 °C under fluorescent lighting. Observations ensued after the culture was 32 weeks old. The variables observed were the percentage of shoot regeneration, percent callus formation, shoot number, shoot length, and leaf number.

Root induction

The root induction study of tembesu was in a randomized group design layout. The culture medium for root induction used was ½ MS medium with IBA treatment comprising four concentrations of 0, 0.5, 1, and 2 mg/L, repeating each treatment 10 times. The explants used were in vitro shoots from the Integrated Biology Laboratory collection. The apical part of in vitro shoots, cut about 1 cm, reached growth on culture media for root induction, with each culture bottle filled with one shoot. Observations proceeded until the culture was 17 weeks old. The variables observed were the percentage of root formation, number of roots, root length, number of shoots, shoot length, and number of leaves. All recorded data underwent the analysis of variance. After obtaining a significant effect, the Tukey test (HSD - Honestly Significant Difference) followed.

RESULTS AND DISCUSSION

Shoot multiplication

Shoot regeneration response was notable through shoot formation percentage and the number of shoots in tembesu. The result showed the percentage of shoot regeneration from the shoot tips explants was 20% to 80%, while from nodal explants, it was 0% to 80% (Table 1). However, the highest percentage (80%) of shoot regeneration came from shoot tips explants grown on MS medium with 2 mg/L BAP and nodal explants grown on MS medium with 4 mg/L BAP. Shoot tips provided the topmost shoot-forming ability against the nodal, except for nodal explants treated with 4 mg/L BAP. Shoot tips and nodal explants unable to form shoots died, as indicated by all explants in brown (browning) and white. Browning also causes explant death, with the initial symptoms being all brown and black

Table 1. Effect of BAP on shoot regeneration and callus formation from shoot tips and nodal explants of tembesu after 32 weeks of culture.

BAP concentrations (mg/L)	Percentage of shoots regeneration (%)		Percentage of callus formation (%)		Number of shoots	
	Shoot tips	Nodal	Shoot tips	Nodal	Shoot tips	Nodal
0	60	0	0	0	2.67	0.00
0.5	40	20	20	20	16.00	8.00
1	20	20	60	20	32.00	45.00
2	80	40	80	40	28.75	33.00
4	20	80	40	100	25.00	16.00

explant tissues (Martin *et al.,* 2007). In the case of tembesu shoots, the brown shoots changed to white after a few weeks. Browning is one of the problems from in vitro culture. The main factor causing browning of explants is enzymatic browning, which occurs when specific enzymes (mainly polyphenol oxidase-PPO), phenolic compounds, and oxygen come into contact. The mechanism of action of the PPO enzyme is through the oxidation of phenolic compounds, as triggered by the injury. In the explant tissue undergoing injury, plastids rupture, vacuoles rupture, and phenolic compounds come out, and then, PPO reacts with phenolic compounds. Factors increasing the rate of browning include the concentration of phenolic compounds, specific activity of PPO, temperature, and pH (Permadi *et al.,* 2024). Reducing browning can proceed by giving anti-browning materials as antioxidants, such as citric acid, ascorbic acid, and polyvinylpyrrolidone (EL-Gioushy *et al*., 2020; Gemechu and Amante, 2021).

Explants from in vitro tembesu shoots showed a lower percentage of shoot formation (80%) than tembesu seed explants, with 2 mg/L BAP treatment for shoot tip explants and 4 mg/L BAP treatment for nodal explants. Tembesu seed explants treated with 1 mg/L BAP + 12 ml/L honey produced a percentage of shoot formation of 93.33% (Fatonah and Isda, 2018). This is related to the type of seed explants that do not suffer from wounding. In addition, honey may be an alternative antioxidant to reduce browning. Honey is high in antioxidants (Samsul *et al.,* 2022).

In some explants, the shoot regeneration may not occur because of the smaller size of explants, at around 0.75 cm with two to three leaves. The cutting results in various parts of the explants being damaged, and only a few parts of the explants were likely to grow. Most nodal explants revealed a lower response on the shoot regeneration because the more the cuts (two side cuts) resulted in more wounds and damage to the explant part, as well as less viable meristematic tissue. The shoot regeneration and growth response of tembesu explants was generally quite slow, and shoots formed after 12 weeks of culture. Some new explants experienced swelling and produced callus, and the number of shoots appeared with small sizes. Shoot regeneration from in vitro shoot explants from shoot tips and nodal explants was slower than shoot regeneration from seed explants. Shoot regeneration from seed explants occurs after two weeks of culture in tembesu (Nurnilawati *et al*., 2016).

Another factor influencing the success of explants in creating shoots is with the concentration of exogenous growth regulators. In this study, it was the BAP, which belonged in the synthetic cytokinins group. The BAP treatment tended to increase the shoot regeneration from shoot tip and nodal explants. In shoot tip explants, the elevated shoot regeneration occurred in those which received 2 mg/L BAP treatment, with a shoot percentage of 80%. Node explants treated with BAP raised the percentage of shoot regeneration, with the highest percentage (80%) obtained with the 4 mg/L BAP treatment. The BAP treatment (4 mg/L) resulted in swelling of the leaves attached to the nodes after 12 weeks of culture. Shoot formation can begin with a swelling process in the explant, wherein research on swelling in leaves attached to nodes caused a process of cell division in the leaf mesophyll tissue (Deswiniyanti and Lestari, 2020). BAP is a growth regulator of the cytokinin group, stimulating division and differentiation to form shoots (Ashraf *et al*., 2014; Raspor *et al*., 2021).

Callus developed from shoot tip and nodal explants grown on MS medium containing BAP. The topmost percentage (80%) of callus formation appeared from shoot tip explants treated with 1 and 2 mg/L BAP and nodal explants (100%) treated with BAP (4 mg/L). The results indicated callus formation occurs in explants that produce the shoots. Callus surfaced in explants before and after shoot formation; however, callus formation encourages more shoots to form. The BAP treatment (1 mg/L) can stimulate the callus formation from nodal explants before shoots formation, and then regenerate into many shoots (Figure 1). The results further revealed shoots developed from callus previously created from explants grown on MS medium

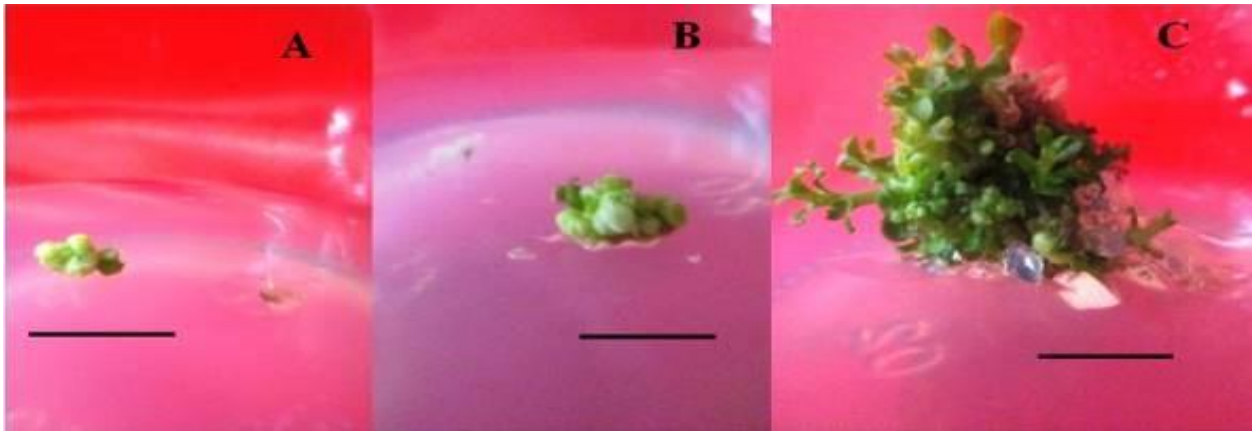

Figure 1. Growth of nodal explants grown on MS medium treated with BAP (1 mg/L): a) Callus and nodal explant growth after 12 weeks of culture, b) Callus growth increased after 13 weeks of culture, and c) Subsequently regenerated into many shoots after 32 weeks of culture. (Scale bar = 0.5 cm).

with the BAP treatment. The growth regulator BAP stimulates the callus development from shoot tip and nodal explants, and later, the callus regenerates to boost the shoot construction. Regeneration of shoots from callus allows numerous shoots to form because the callus has many cells with the potential to produce shoots in apple shoot culture (Caboni *et al*., 2000). Organogenesis of explants to create shoots can occur through callus formation with cytokinins treatment (Kumari *et al*., 2017; Zhang *et al*., 2017). The callus formed in this study was compact callus (Figure 1) (Ikeuchi *et al*., 2013). This callus formation is a response to the wounding of the shoot explants. The process of shoot regeneration from callus involves division, dedifferentiation, and trans-differentiation. Cells re-enter the cell cycle, multiply, and regenerate tissues and organs (Ikeuchi *et al*., 2013; Bidabadi and Jain, 2020; Shin *et al*., 2020).

Shoot regeneration also manifested by the number of shoots formed. The BAP treatment (0.5 to 4 mg/L) stimulated shoot regeneration, producing 16 to 32 shoots from shoot tip explants, and eight to 45 shoots from node explants. The most number of shoots came from explants grown in MS medium containing 1 mg/L BAP, obtaining 32 and 45 shoots from shoot tip and nodal explants, respectively (Figure 2). Results indicated the shoot tip and nodal explants can regenerate shoots over a long period of time (32 weeks of culture). Several species also exhibited the ultimate shoot regeneration ability from shoot tip and nodal explants. The number of shoots formed from the shoot tip and nodal explants of *Smilax zeylanica* with BP (4 mg/L) was lower in the shoot tip (4.3 shoots) and nodal (6.5 shoots) explants, respectively (Azad *et al.,* 2022). Numerous shoots (16.20) appeared from shoot tip explants of *Cryptocoryne wendtii* grown on MS medium treated with BAP (3 mg/L) after 60 days of culture (Klaocheed *et al*., 2020).

Other species showed the number of their shoots formed was few in the shoot tips, nodal, and other explants. Lower shoot formation was visible in the nodal explants of *Stevia rebaudiana* Bertoni grown on MS medium treated with BAP (1.5 mg/L) (Yesmin, 2019). Fewer shoots (4.48 shoots) occurred from shoot tips of Sesame (*Sesamum indicum* L.) explants grown on MS medium treated with BAP (0.5 mg/L) (Asad *et al*., 2020). Their results further disclosed the shoot tip and nodal explants from in vitro shoots can regenerate into shoots; however, their level of regeneration depends upon the type of plant, the concentration of the growth regulator, and the duration of culture.

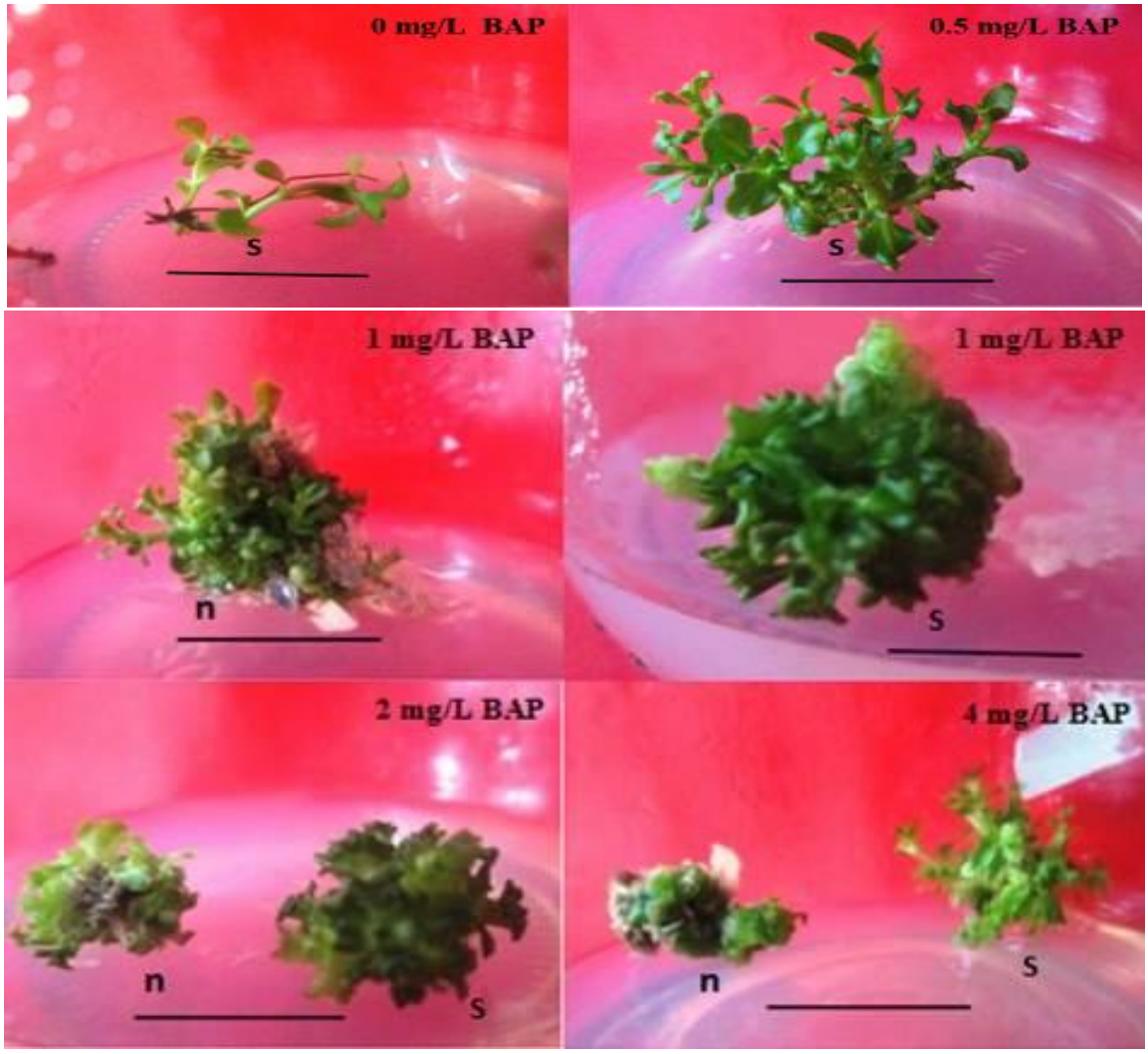

Figure 2. Regeneration of shoots from shoot tip (s) and nodal explants (n) with BAP concentrations after 32 weeks of culture. (Scale bar = 1 cm).

Shoot growth recording depended on shoot length and the number of leaves (Table 2). The longest shoot length (2.5 cm) came from apical shoot explants on MS medium supplemented with BAP (4 mg/L). However, most explants on MS medium supplemented with BAP showed shorter shoots. This is because cytokinins stimulate shoot formation more than cell elongation in shoots (Gu *et al*., 2018). Shoots growing from shoot tip explants have obtained 1.2 to 12 leaves, while shoots emerging from nodal explants form 4 to 14 leaves. The most number of leaves (12 leaves) from shoot tip explants appeared on MS media supplemented with BAP (4 mg/L). In nodal explants, the topmost number of leaves (14 leaves) occurred in explants grown with MS media treated with BAP (0.5 mg/L). The leaves produced from shoot tip explants on MS media without BAP were larger. Shoots developed from shoot tip and nodal explants displayed smaller leaves, with some shoots unable to shape the leaves yet. Small shoot generally occurs in explants producing many shoots. This happens due to competition between in vitro shoots to obtain water, nutrients, and sucrose in the culture medium (Yildiz, 2011; Aycan *et al*., 2014).

Table 2. Effect of BAP on shoot growth from shoot tips and nodal explants of tembesu after 32 weeks of culture.

BAP concentrations (mg/L)	Shoot length (cm)		Number of leaves	
	Shoot tips	Nodal	Shoot tips	Nodal
0	1.60	0.00	8.67	0.00
0.5	1.65	1.20	1.20	14.00
1	0.50	1.70	3.00	6.00
2	0.83	0.60	3.75	6.50
4	2.50	0.70	12.00	4.00

This study used the basic MS medium for in vitro culture and did not use WPM (Wood Plant Medium) media. The reason is some research results showing the MS medium is also a commonly used media in woody plants, providing success in shoot formation. *Myrtus communis* woody plants grown on MS and WPM media exhibited developing shoots from apical buds (Şimşek *et al.,* 2017). *Psidium guajava* node explants grown on ½ MS, WPM, and ½ DKW (Driver and Kuniyuki walnut) media were able to produce shoots, with the highest number of shoots on ½ MS media (Elezaby and Abd Allatif, 2017). MS, WPM, and DKW are the basic media in many woody plant species (Phillips and Garda, 2019; Jayusman *et al.*, 2022).

The promising results displayed numerous shoots appearing in shoot tip and nodal explants grown on the MS medium with BAP (1 mg/L), at 32 and 45 shoots, respectively. However, the shoot regeneration percentage of the nodal explants was still low (20%) compared with the shoot regeneration from shoot tip explants (60%). The low percentage of shoot regeneration correlated with explants' living ability and tissue damage. Therefore, to enhance the percentage of shoot regeneration, the size of explants requires increasing, and then, reducing the damaged tissue and raising the proportion of live tissue. The size of explants affects the success of shoot regeneration. El-Boullani *et al.'s* (2017) findings stated the size of the axillary shoot explants of globe artichoke (*Cynara cardunculus* var. Scolymus L.) influenced the percentage of survival and number of shoots.

Root induction

The analysis of variance showed considerable effects of the IBA growth regulator on the number of roots, root and shoot length, but a nonsignificant effect on the percentage of rooting and number of leaves in tembesu. The average root formation and shoot growth of in vitro shoots with various IBA concentrations after 17 weeks of culturing are available in Tables 3 and 4. Likewise, the response of root formation and shoot growth appears in Figure 3. In vitro rooting of tembesu ranged from 70% to 100%. The greatest rooting efficiency was 100% on ½ MS media supplied with IBA (1 mg/L), followed by IBA (0.5 and 0 mg/L), with 90% and 80%, respectively. The lowest rooting percentage of 70% resulted from the medium supplemented with IBA (2 mg/L) (Table 3). The outcomes indicated the IBA can increase the percentage of root induction; however, the increased IBA concentration (2 mg/L) decreased the rooting percentage in tembesu. Several research results detailed the optimal concentration of IBA in raising the percentage of root formation depends on the type of plant. These include 3 mg/L IBA + 4 mg/L NAA for *Garcinia mangostana* shoots (Harahap *et al.,* 2014), 5 mM IBA for *Casuarina cunninghamiana* (Shen *et al.,* 2010), and 2 mg/L IBA for *Pyrus* (Song *et al*., 2024).

The IBA application significantly increased the number of roots from 7.2 to 12. However, the maximum number of roots (12) emerged on ½ MS media with IBA (2 mg/L), while the least roots (2.3) on ½ MS media with the control treatment. Therefore, it was

Table 3. Effect of IBA concentration on root growth from in vitro shoots of tembesu after 17 weeks of culture.

IBA concentrations (mg/L)	Percentage of root formation (%)	Number of roots	Root length (cm)
0	80	1.3[a]	7.18[c]
0.5	90	7.2[b]	3.52[b]
1	100	7.6[b]	3,72[b]
2	70	12.0[c]	1.32[a]

Note: Mean values within a column followed by the same letters are not significantly different at $P < 0.05$ according to the *Honestly Significant Difference* Test.

Table 4. Effect of IBA concentration on shoot growth from in vitro shoots of tembesu after 17 weeks of culture.

IBA concentrations (mg/L)	Number of shoots	Shoot length (cm)	Number of leaves
0	1.7[a]	1.81[a]	11.5
0.5	2.2[ab]	2.76[ab]	12.0
1	2.5[b]	3.07[b]	11.1
2	1.5[a]	2.55[a]	10.5

Note: Mean values within a column followed by the same letters are not significantly different at $P < 0.05$ according to the *Honestly Significant Difference* Test.

Figure 3. The shoot growth response of in vitro shoot explants on ½ MS medium with IBA concentrations. (Scale bar = 0.5 cm).

evident that as the concentration of IBA rises in the media, the number of roots also rises. IBA is a group of auxin growth hormones involved in root formation. The process of root development contains three phases: root induction, root initiation, and elongation of roots. Auxin is one of the crucial plant hormones playing a vital role in regulating the root development (Kou *et al*., 2022).

The longest root length (7.12 cm) resulted in ½ MS media without IBA, while the shortest root length (1.32 cm) came from the ½ MS media supplemented with IBA (2 mg/L). This difference in root length refers to the number of roots formed. The ½ MS media without IBA treatment (control) with fewer roots allows for higher root elongation because the proportion of water and nutrients absorbed by the roots is greater than ½ MS media containing IBA (2 mg/L) with many roots. More water and nutrient absorption causes root growth, thereby increasing the root elongation (Takahashi and Kinoshita, 2016). The in vitro shoot rooting response to various IBA concentrations is available in Figure 3. The control treatment displayed only one root with a significant length. The IBA (1 mg/L) treatment exhibited thicker roots, while the IBA (2 mg/L) showed numerous roots with a smaller size. This phenomenon also occurs via in vitro cultures of other plants, for example, the 4 mg/L IBA treatment on sugarcane shoots formed the most number of roots (11.01) but with the shortest roots (0.21 cm) (Rahman *et al*., 2018). However, some studies disclosed shoots with many roots also show long roots. For example, the treatment of 50 µM IBA + 100 µM IAA in *Juglans nigra* L shoots gave the highest number of roots and the longest roots (Stevens and Pijut, 2018). The treatment of 0.5 mg/L IBA on *Lippia dulcis* shoots indicated the topmost number of roots and root length (Tomaszewska-Sowa, 2020). This difference is parallel with the type of plant, culture conditions, and duration of culture.

The number of shoots formed ranged from 1.7 to 2.5 shoots. The IBA (0.5 and 1 mg/L) doses increased the number of shoots and shoot length; however, IBA (2 mg/L) treatment did not increase the number of shoots and shoot length. The ultimate number of shoots (2.5 shoots) and enhanced shoot length (3.07 cm) were visible on ½ MS media supplemented with IBA (1 mg/L). This phenomenon also linked to the higher role of auxin in root growth and more inhibition of shoot growth. IBA treatment with higher concentration (2 mg/L) promotes a superior auxin-cytokinin ratio, which promotes root formation more than the shoot. The lowering of auxin-cytokinin ratio can proceed by administering auxin together with cytokinin, and this may inhibit root formation. For example, 1 mg/L NAA + 1 mg/L BAP treatment on medium for rooting *Musa* sp hinders root development (Ngomuo *et al*., 2013). Treatment of auxin combined with cytokinin (0.2 mg/L NAA + 2.25 mg/L BAP) provided a lower root production than single auxin (2 mg/L IBA) in garlic (*Allium sativum* L.) shoots (Ngomuo *et al*., 2013).

The most number of shoots resulting from root induction with IBA treatment after 17 weeks of culture was only 2.5 shoots because IBA is an auxin growth regulator, playing a remarkable role in stimulating root induction and shoot elongation. Growth regulators vital in shoot propagation are cytokinins, like BAP. The in vitro rooting stage aims to form roots and elongate the shoots, preparing plantlets ready for use in acclimatization. The IBA treatment on culture media also increased the shoot length. The highest shoot elongation occurred on MS media with IBA (1 mg/L) treatment. The effectiveness of the growth regulator auxin in stimulating root formation and shoot elongation based upon the species, IBA concentration, and the culture medium. The topmost roots came from *Artocarpus altilis* shoot explants treated with IBA (0.50 mg/L) on MS media in vitro after six weeks of culture (Noorrohmah and Imelda, 2015). Root formation reached 60.44% and three roots from in vitro shoots of *Santalum album* grown on MS media treated with a combination of IBA (20 mg/L), IAA (0.15 mg/L), and kinetin (0.15 mg/L) after six months of culture (Putri and Herawan, 2018).

The findings revealed the best root induction from in vitro shoots was the medium ½ MS containing IBA (1 mg/L), resulting in the maximum root percentage (100%), root

number (7.6 root), and root length (3.72 cm). These results gained fortification by the larger root, the most number of shoots (2.5 shoots), and the enhanced shoot length (3.07 cm). The shoots formed from these root induced results were fewer, however, have longer shoots with more broader leaves (10.5 to 12 leaves), and the leaf size was bigger than the leaves formed on the shoots from the shoot multiplication stage. IBA is a class of auxin growth regulators that generally increases the percentage of root formation and the number of roots created. Although, the optimal concentration can be different in various plant species. In this study, the optimal IBA in promoting in vitro rooting of tembesu shoots was 1 mg/L on ½ MS medium. The treatment of 1 mg/L IBA on ½ MS medium also gave the best results in boosting in vitro rooting in another plant species, Prunus 'Garnem' (Ak *et al*., 2021). Other plants indicate IBA treatment at lower concentrations is more optimal in spurring in vitro root induction. For example, 0.4 and 0.5 mg/L IBA is more optimal in increasing in vitro rooting of *Berlin poplar* (*Populus x berolinensis* K. Koch) shoots (Pavlichenko and Protopopova, 2023). Other species showed optimal IBA treatment at higher concentrations, for example, 5 mg/L IBA treatment was most optimal in promoting in vitro rooting of *Tectona grandis* shoots (Nor-Aini *et al.,* 2009).

CONCLUSIONS

The results revealed that BAP treatment increased the percentage of shoot formation and the number of shoots from shoot tips and nodal explants of tembesu. The highest number of shoots resulted in explants with BAP (1 mg/L) treatment from shoot tip and nodal explants. The best in vitro root induction of tembesu was evident on ½ MS medium with BAP (1 mg/L), indicated by the maximum root percentage and the number of roots. Therefore, it is necessary to optimize the shoot propagation to increase the shoot regeneration by using increased size of shoot tip and nodal explants on MS media with the addition of BAP.

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REFERENCES

Abuga I, Sulaiman SF, Wahab RA, Ooi KL, Rasad MS (2022). Phytochemical constituents and antibacterial activities of 45 Malay traditional medicinal plants. *J. Herb. Med*. 32: 100496.

Ak BE, Kıyar PK, Hatipoglu IH, Dikmetaş B (2021). Effects of different BA and IBA concentrations on proliferation and rooting of 'GARNEM' rootstock in vitro propagation. *Int. J. Agric. Environ. Food Sci.* 5(4): 470-476.

Amghar I, Ibriz M, Ibrahimi M, Boudra A, Gaboun F, Meziani R, Iraqi D, Mazri MA, Diria G, Abdelwahd R (2021). In vitro root induction from argan (*Argania spinosa* [L.] Skeels) adventitious shoots: Influence of ammonium nitrate, auxins, silver nitrate and putrescine, and evaluation of plantlet acclimatization. *Plants.* 10(6): 1062.

Ardiansyah R, Supriyanto S, Wulandari AS, Subandy B, Fitriani Y (2015). Teknik Sterilisasi Eksplan dan Induksi Tunas dalam Mikropropagasi Tembesu (*Fagraea fragrans* ROXB). *J. Silvikultur Trop*. 5(3): 167-173.

Asad M, Ahmed N, Sohail A, Burni T, Hadi F, Ali R, Khan MN, Aziz A, Muhammad A (2020). In vitro shoots multiplication from nodal explants of sesame (*Sesamum indicum* L.). *Pure Appl. Biol.* 9(1): 303-308.

Ashraf MF, Aziz MA, Kemat N, Ismail I (2014). Effect of cytokinin types, concentrations and their interactions on in vitro shoot regeneration of *Chlorophytum borivilianum* Sant. & Fernandez. *Electron. J. Biotechnol.* 17(6):275-9.

Asmaliyah A, Imanullah A, Darwiati W (2012). Identifikasi dan potensi kerusakan rayap pada tanaman Tembesu (*Fagraea fragrans*) di kebun percobaan Way Hanakau, Lampung Utara. *J. Pen. Hutan Tan.* (4): 187-194.

Asmono SL (2020). In vitro regeneration of *Stevia rebaudiana* Bertoni from internode and leaf explants using different concentrations of BAP (*6-Benzyl Amino Purine*). *IOP Conf. Ser.*: *Earth and Environ. Sci.* 411(1): 012004.

Aycan M, Kayan M, Yildiz M (2014). The effect of 'in vitro' spacing competition on shoot regeneration from cotyledon node explants of '*Lathyrus chrysanthus*' Boiss. *Aust. J. Crop Sci*. 8(7):1019-1023.

Azad MA, Adhikari M, Sarker MN, Hasan AM, Amin MN (2022). In vitro shoot regeneration from nodal and shoot tip explants of a threatened medicinal plant-Smilax zeylanica L. *J. Biosci.* 30(1):69-78.

Azahra T, Suharto E (2022). Pengaruh lama perendaman H_2SO_4 dan ukuran biji terhadap perkecambahan BIJI tembesu (*Fagraea fragrans* Roxb.). *J. Glob. For. Environ. Sci*. 2(3): 11-21.

Azmi NK, Mohidin FS (2020). Rehabilitation of sandy coastal areas in Kuala Rompin, Pahang. *IOP Conf. Ser. Earth and Environ. Sci.* 549(1): 012055.

Bidabadi SS, Jain SM (2020). Cellular, molecular, and physiological aspects of in vitro plant regeneration. *Plants.* 9(6):702.

Caboni E, Lauri P, D'Angeli S (2000). In vitro plant regeneration from callus of shoot apices in apple shoot culture. *Plant Cell Rep.* 19: 755-760.

Deswiniyanti NW, Lestari NK (2020). In vitro propagation of *Lilium longiflorum* bulbs using NAA and BAP plant growth regulator treatment. *KnE Life Sciences. IC-BIOLIS. The 2019 International Conference on Biotechnology and Life Sciences,* Volume 2020: 32-45.

Djam'an DF, Putri KP, Rustam E, Pramono AA (2016). Potential of fruit production tembesu (*Fagraea fragrans* Roxb.) of Ogan Ilir (Oi) and Ogan Komering Ilir (Oki) the South of Sumatera. *J. Silvikultur Trop*. 7(3): S22-S23.

El-Boullani R, Lagram K, El Mousadik A, Serghini MA (2017). Effect of explants density and size on the in vitro proliferation and growth of separated shoots of globe artichoke (*Cynara cardunculus* var. scolymus L.). *J. Mater. Environ. Sci*. 8(7): 2469-2473.

Elezaby AA, Abd Allatif AM (2017). In vitro propagation of guava (*Psidium guajava* L.): Effect of antioxidants, nutrient media and growth regulators. *J. Hortic. Sci. Ornamental Plants* 9(3):144-9.

EL-Gioushy SF, Liu R, Fan HK (2020). A complete protocol to reduce browning during coconut (*Cocos nucifera* L.) tissue culture through shoot tips and inflorescence explants. *Plant Arch*. 20(2):2196-2204.

Fatonah S, Isda MN (2018). In vitro shoot regeneration of tembesu (*Fagraea fragrans* Roxb.) from seed explants on different concentrations of sucrose and honey. *Biosci. Res.* 15(2): 655-662.

Gemechu EC, Amante G (2021). Control of browning in plant tissue culture: A review. *J. Sci. Innov. Res.* 10(4):89-93.

Ghani DM, Solihat RF, Wilujeng S (2023). Carbon reserve potential, carbon dioxide absorption capacity, and oxygen release in the conservation forest of Kamojang, West Java. *Greenation Int. J. Eng. Sci.* 1(2):85-91.

Gu J, Li Z, Mao Y, Struik PC, Zhang H, Liu L, Wang Z, Yang J (2018). Roles of nitrogen and cytokinin signals in root and shoot communications in maximizing of plant productivity and their agronomic applications. *Plant Sci.* 274: 320-331.

Harahap F, Poerwanto R, Suriani C, Rahayu S (2014). In vitro growth and rooting of mangosteen (*Garcinia mangostana* L.) on medium with different concentrations of plant growth regulator. *HAYATI J Biosci*. 21(4): 151-158.

Indrayati, Yoza D, Arlita T (2015). Studi pengembangan ekowisata melalui pendekatan supply dan demand di Tahura Sultan Syarif Kasim Riau. *Jomfaperta.* 2(1).

Istomo I, Subiakto A, Rahmadianto S (2014). Pengaruh asal bahan dan media stek terhadap keberhasilan stek pucuk tembesu *Fagraea fragrans* (Roxb.). *Ber. Biol*. 13(3): 275-281.

Jayusman, Hakim L, Dalimunthe A (2022). Season, basal media and plant growth regulators effect in wood plant in vitro propagation: A comprehensive review. *IOP Conf. Ser.: Earth Environ. Sci.* 1115(1): 012051.

Klaocheed S, Jehsu W, Choojun W, Thammasiri K, Prasertsongskun S, Rittirat S (2020). Induction of direct shoot organogenesis from shoot tip explants of an ornamental aquatic plant, *Cryptocoryne wendtii*. *Walailak J. Sci. Tech*. 17(4): 293-302.

Kou X, Zhao X, Wu B, Wang C, Wu C, Yang S, Zhou J, Xue Z (2022). Auxin response factors are ubiquitous in plant growth and development, and involved in crosstalk between plant hormones: A review. *Appl. Sci.* 12(3): 1-15.

Kumari A, Ray K, Sadhna S, Pandey AK, Sreelakshmi Y, Sharma R. (2017). Metabolomic homeostasis shifts after callus formation and shoot regeneration in tomato. *PloS one.* 12(5):e0176978.

Martin KP, Zhang CL, Slater A, Madassery J (2007). Control of shoot necrosis and plant death during micro-propagation of banana and plantains (*Musa* spp.). *Plant Cell Tiss Org.* 88:51-9.

Mindawati N (2014). Tembesu: Kayu Raja Andalan Sumatera. Forda Press.

Mohamed SS, Kolar AB, Malayaman V, Basha MG (2019). Rapid in vitro multiplication and plant regeneration from shoot tip explants of *Ammannia baccifera* L.: An important medicinal plant. *J. Pharmacogn. Phytochem*. 8(3): 837-841.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol. Plant*. 15(3): 473-497.

Ngomuo M, Mneney E, Ndakidemi P (2013). The effects of auxins and cytokinin on growth and development of *Musa* sp. var. "Yangambi" explants in tissue culture. *Am. J. Plant Sci*. 4(11):2174.

Noorrohmah S, Imelda M (2015). Root induction and acclimatization of in vitro plantlets of *Artocarpus altilis*. In *Proceeding Seminar Nasional Masyarakat Biodiversitas, Indonesia* 1(8): 1896-1899.

Nor-Aini AS, Goh BL, Ridzuan R (2009). The effects of different indole-3-butyric acid (IBA) concentrations, two light regimes of in vitro rooting and acclimatization of in vitro teak (*Tectona grandis* Lf) plantlets. *Afr. J. Biotechnol.* 8(22): 6158-6161.

Nurnilawati, Fatonah, Isda MN (2016). Optimization of induced tunas in vitro plant tembesu (*Fagraea fragrans* Roxb) by provision of *benzylaminopurine* (BAP) and increased sucrose concentration. National Seminar of the Association of Indonesian Glorification (PERIPI). Pekanbaru, 10-11 June 2014.

Payung D, Susilawati S (2014). Pengaruh zat pengatur tumbuh rootone-f dan sumber bahan stek terhadap pertumbuhan stek tembesu (*Fagraea fragrans*) Di PT. Jorong Barutama Greston Kalimantan Selatan. *Environ. Scienteae* 10(3): 140-149.

Permadi N, Akbari SI, Prismantoro D, Indriyani NN, Nurzaman M, Alhasnawi AN, Doni F, Julaeha E (2024). Traditional and next-generation methods for browning control in plant tissue culture: Current insights and future directions. *Curr. Plant Biol*. 38: 100339.

Phillips GC, Garda M (2019). Plant tissue culture media and practices: An overview. In Vitro Cellular & Developmental Biology-Plant. *In Vitro Cell Dev Biol Plant* . 55:242-57.

Pillai MK, Young DJ (2022). Phytochemical characterization of extracts from *Fagraea fragrans* and *Juglans regia* by GC-MS analysis. *Fine Chem. Eng*. 15: 107-120.

Putri AI, Herawan T (2018). Regenerasi perakaran plantlet in vitro dan *ex vitro* pada kultur jaringan cendana (*Santalum Album*). *J. Pem. Tan. Hutan*. 12(2):143-50.

Rahman MM, Ivy NA, Mian MA, Rasul MG, Hossain MM, Rahman MA (2018). Effect of auxin (NAA, IBA and IAA) in root regeneration through in vitro culture of sugarcane. *Int. J. Plant Biol. Res*. 6:1109.

Ratna A, Lestari TH (2021). Rubber agroforestry experiments in Jambi at the end of a 25-Year Cycle. In *E3S Web of Conferences*. 305: 03005. EDP Sciences.

Rattanaburi S, Kaikaew K, Watanapokasin R, Phongpaichit S, Mahabusarakamb W (2022). A new lignan from the stem bark of *Fagraea fragrans* Roxb. *Nat. Prod. Res*. 36(7): 1851-1856.

Samsul NR, Johannes E, Syahribulan S (2022). Analysis of antioxidant content in pollen and honey produced by bees *Trigona* spp at several locations in South Sulawesi. *Int. J. Appl. Biol.* 6(1): 26-37.

Sarma B, Tanti B (2017). In vitro regeneration of plantlets from nodal explants of *Aristolochia saccata* and *Aristolochia cathcartii*. *Eur. J. Biol. Res*. 7(3): 191-201.

Shen X, Castle WS, Gmitter FG (2010). In vitro shoot proliferation and root induction of shoot tip explants from mature male plants of *Casuarina cunninghamiana* miq. *HortSci*. 45(5): 797-800.

Shin J, Bae S, Seo PJ (2020). De novo shoot organogenesis during plant regeneration. *J. Exp. Bot.* 71(1):63-72.

Sianturi RD, Supriyanto S, Wulandari AS, Subandy B (2017). Regeneration of adventitious shoots from leaf explant of tembesu (*Fagraea fragrans* Roxb.) by tissue culture. *J. Pem. Tan. Hutan*. 14(1): 1-17.

Şimşek Ö, Biçen B, Dönmez D, Kaçar YA (2017). Effects of different media on micropropagation and rooting of Myrtle (*Myrtus communis* L.) in in vitro conditions. *Int. J. Environ. Agric. Res*. 3(10):54-9.

Song JY, Bae J, Lee YY, Han JW, Lee YJ, Nam SH, Lee HS, Kim SC, Kim SH, Yun BH (2024). High-efficiency in vitro root induction in pear microshoots (*Pyrus* spp.). *Plants* 13(14): 1904.

Stevens ME, Pijut PM (2018). Rapid in vitro shoot multiplication of the recalcitrant species *Juglans nigra* L. *In Vitro Cell Dev Biol Plant.* 54:309-17.

Takahashi K, Kinoshita T (2016). The regulation of plant cell expansion: Auxin-induced turgor-driven cell elongation. Molecular cell biology of the growth and differentiation of plant cells. CRC Press. Amazon.com156-73.

Tomaszewska-Sowa M (2020). In vitro clonal propagation and efficient acclimatization with use of hydrogel of intensively sweet medicinal plant *Lippia dulcis* Trev. *Herba Pol.* 66(4): 25-31.

Yesmin S (2019). In vitro micropropagation of *Stevia rebaudiana* Bertoni. *Plant Tiss. Cult Biotechnol*. 29:277-284.

Yildiz MU (2011). Evaluation of the effect of in vitro stress and competition on tissue culture response of flax. *Biol. Plant.* 55:541-4.

Zhang TQ, Lian H, Zhou CM, Xu L, Jiao Y, Wang JW (2017). A two-step model for de novo activation of WUSCHEL during plant shoot regeneration. *The Plant Cell* 29(5): 1073-1087.