Scientific Research Publishing

American Journal of Plant Sciences, 2022, 13, 734-743
https://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

Micropropagation of *Kaempferia angustifolia* Roscoe via Direct Regeneration

Zuraida Ab Rahman[1*], Ayu Nazreena Othman[1], Mohd Norfaizal Ghazalli[2], Nur Auni Syazalien Adlan[3]

[1]Biotechnology & Nanotechnology, Research Centre, MARDI HQ, Persiaran MARDI-UPM, Serdang Selangor, Malaysia
[2]Agrobiodiversity & Environment Research Center, MARDI HQ, Persiaran MARDI-UPM, Serdang Selangor, Malaysia
[3]Gambang Campus, Universiti Malaysia Pahang, Pahang, Malaysia
Email: *azuraida@mardi.gov.my

How to cite this paper: Rahman, Z.A., Othman, A.N., Ghazalli, M.N. and Adlan, N.A.S. (2022) Micropropagation of *Kaempferia angustifolia* Roscoe via Direct Regeneration. *American Journal of Plant Sciences*, **13**, 734-743.
https://doi.org/10.4236/ajps.2022.136049

Received: April 22, 2022
Accepted: June 14, 2022
Published: June 17, 2022

Abstract

This study has established an efficient and reproducible protocol to micropropagation *Kaempferia angustifolia* Roscoe via direct regeneration. The use of young shoots as explants showed the best results compared to rhizome shoots, where the young shoots showed a low percentage of contamination of 10% - 30% (agar 6 g/L) and 45% - 55% (agar 3 g/L), respectively, compared to the use of rhizome shoots, where the contamination rate exceeded 80%. For shoot initiation, the combination of BAP (6 Benzylaminopurine) and NAA (1-Naphthaleneacetic acid) showed higher results for the percentage of initial shoots and number of micro shoots/explants compared to BAP with Kin (Kinetin). The highest concentration of BAP (5 mg/L) combined with the lowest concentration of NAA (0.5 mg/L) resulted in 90% of initial shoots and a number of shoots/explants of 5.8. The highest number of shoots for micropropagation was in treatment with 30 g/L sucrose that was segmented with 3 mg/L BAP + 0.5 mg/L NAA. For the number of roots, the highest number of roots was 11.8 recorded at sucrose (45) with only BAP (1 mg/L) used as the plant growth regulator, while the longest length of roots was 7 - 8 cm, recorded both at sucrose with the combination of BAP and NAA.

Keywords

BAP, NAA, Micropropagation, *Kaempferia angustifolia*, Direct Regeneration

1. Introduction

The *Zingiberaceae* family of Angiosperms is the biggest ginger family, with over 1300 species that are extensively used as spices and medicinal herbs [1] [2]. *Kaempferia* is a genus of tiny rhizomatous herbs belonging to the ginger family, with

about 60 species, some of which are therapeutic rhizomes. In Java, *Kaempferia angustifolia* Roscoe is one of the genus' therapeutic rhizomes [3]. In Malaysia, *Kaempferia angustifolia* is an underutilised plant compared to other species of *Kaempferia* such as *Kaempferia rotunda* Linn and *Kaempferia galanga* Linn [2]. *Kaempferia angustifolia* is a small perennial herb producing leaves up to 20 cm long from a tuberous base, stemless, and commonly found in the forests of Indonesia's Western and Central Java, as well as some sections of Thailand [4]. K. *angustifolia* has a pleasant odour and its tiny roots and tubers contain astringent properties that are often used as masticatory by locals for medicinal purposes, such as a remedy for colds, coughs, stomach-aches, diarrhoea, fever, and dysentery [5] [6]. Besides that, the watery part of the main rhizome is considered cooling and reduces body temperature when consumed [5]. *Kaempferia angustifolia* Roscoe is an underutilised plant that has significant benefits and potential that needs to be discovered for further analysis, especially in view of its potential medicinal properties. *In vitro*, culture techniques have been used for the past year because they are considered an important step in propagation. Thus, the aim of this study was to establish an efficient micropropagation protocol for *Kaempferia angustifolia* Roscoe via direct regeneration.

2. Materials and Methods

2.1. Establishment of Initial Cultures

Two types of explants were used for the experiment, young shoots (**Figure 1(a)**) and rhizome shoots (**Figure 1(b)**). The rhizomes were cultivated in the lab to allow the sprouting of immature rhizome shoots. For young shoot explants, a 10-month-old mother plant that produces shoots measuring 4 - 10 cm was used as the source of explants. The explants were cleaned under running tap water for two hours, then washed with detergent (Decon 5%, v/v) and rinsed thoroughly with tap water. The explants were then soaked in 1.5% (v/v) fungicide for one and a half hours, and subsequently rinsed thoroughly under running tap water for 10 minutes. Subsequently, the explants were sterilised with 10% - 20% Clorox® and 5 drops of Tween-20. The explants were then washed with sterile distilled water three times. Young shoots were dissected using a sterile surgical blade to remove the outer layers of leaf sheaths beneath the laminar air floor, yielding 5 to 8 mm pieces that were subsequently grown on medium. For rhizome shoots, the burnt layer needs to be removed once cultured on the medium. The sterilised explants were inoculated onto Murashige and Skoog's (1962) basal medium, which was supplemented with different concentrations of agar (3.0 and 6.0 g/L), BAP (0, 1.0, 3.0, and 5.0 mg/L), and 3% of sucrose. Prior to autoclaving, the pH of the medium was adjusted to 5.8 (15 min, 121˚C). The culture containers were sealed with parafilm and incubated in the culture chamber at 25˚C ± 2˚C for 16 hours under white fluorescent light with a light level of 3000 lux. The cultures were checked on a weekly basis to see how they were progressing. After two months of culturing, the percentage of contamination, the number of shoots

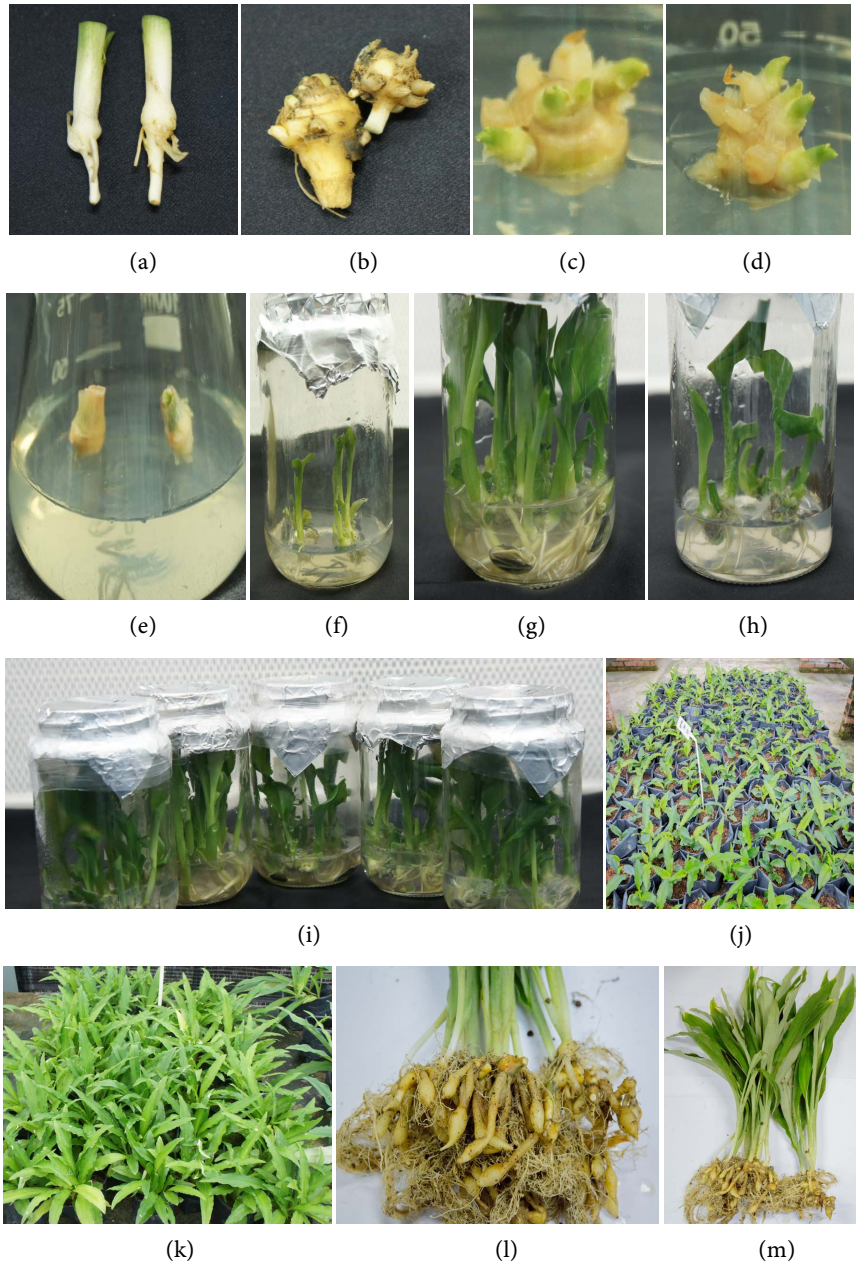

Figure 1. Micropropagation of *Kaempferia angustifolia* Roscoe via direct regeneration. Young shoots explants (a), rhizome shoots explant (b), initial shoots (c, d), no microshoots initiated (e), initial shoots after 60 days of cultured (f), shoots produced cultured on media containing 30 g/L sucrose + 3 mg/L BAP + 0.5 mg/L NAA (g, h), *in vitro* plantlets with shoots and roots (i), successfully acclimatized in glasshouse (j, k), harvested rhizomes after 8 months (l, m).

that responded, and the number of micro shoots/explants that were made were all recorded.

2.2. Proliferation of Shoots, Rooting and Acclimatation

For shoot multiplication, single micro shoots initiated from young shoot explants were transferred to MS medium supplemented with 3 g/L agar and vari-

ous concentrations of BAP (0.5, 1, 3, 5 mg/l) in combination with NAA (0.5, 1, 3, 5 mg/l) or Kinetin (0.5, 1, 3, 5 mg/l), respectively. After 60 days of culture with a monthly subculture interval, the results were expressed as a percentage of proliferation and the number of micro shoot/explants produced. In order to optimise the rooting system and enhance the proliferation rate, the obtained shoots were sub-cultured on medium containing different concentrations of sucrose; 15, 30, and 45 g/L supplemented with BAP (1, 3 mg/l) alone or in combination with NAA (0.5, 1.0 mg/l). Data was recorded at weekly intervals. After 60 days of culture, the mean number of shoots and roots, shoot length, and root length were recorded.

Rooted explants with shoots about 4 - 5 cm in length were removed from the culture bottles and the roots were washed under running tap water to remove the agar. The plantlets were individually transplanted into polybags containing organic soil and topsoil at a ratio of 1:1, maintained under controlled conditions in the glasshouse with 75% shading. To ensure its humidity, the plants were watered periodically. The survival rate of the plantlets was recorded after 4 weeks. A standard deviation of the mean was calculated for the degree of response, which is represented in the tables as ±value.

3. Statistical Analysis

The data were subjected to one way analysis of variance (ANOVA) to assess treatment differences and interaction using the SPSS version. Significance between means was tested by DMRT ($p \leq 0.05$). The experiment was conducted with 10 replications per treatment.

4. Result and Discussion

4.1. Establishment of Initial Culture

Contamination is the limiting factor in generating *in vitro* techniques. The contaminant organisms that are commonly found *in vitro* plant cultures include viruses, bacteria, yeasts, fungi, mites, and thrips. Furthermore, the study was conducted using two types of explants; young shoots and rhizome shoots. Both explants were cultured on media that were supplemented with different concentrations of BAP (0, 1, 3, and 5 mg/l) and two concentrations of agar (3 g/L and 6 g/L). Hence, the explants were measured for their contamination rate, initial shoot response, and number of micro shoots/explants. Based on **Table 1**, young shoots explants were described as the best result in terms of contamination rate percentage and shoot initiation compared to when using rhizome shoots.

Young shoots cultured on media containing agar 3 g/L and 6 g/L showed a low percentage of contamination of 10% - 30% and 45% - 55% respectively. The highest response to the initial shoot (75%) and the lowest contamination (10%) were recorded at the treatment of agar (6 g/L) combined with 5 mg/L BAP. Initiation of micro shoots/explants from young shoot explants was recorded up to 4, which was observed at 3 mg/L BAP (3 g/L agar) (**Figure 1(c)**). In addition, those

Table 1. The effect of explant type contamination rate and sprouting of shoots.

Type of explants	Agar (g/L)	BAP (mg/l)	% Contamination	Response initial shoot %	Micro shoots/ explants
Young shoots	3	0	55 ± 4.08 ^{d}	55 ± 10.17 ^{b}	0.85 ± 0.69 ^{abc}
		1	50 ± 8.16 ^{d}	62 ± 11.12 ^{bcd}	2.28 ± 0.75 ^{d}
		3	42 ± 7.55 ^{cd}	65 ± 10.00 ^{bcd}	4 ± 0.81 ^{e}
		5	50 ± 7.31 ^{d}	60 ± 10.00 ^{bc}	1.85 ± 0.89 ^{cd}
	6	0	20 ± 5.34 ^{ab}	61 ± 10.29 ^{bcd}	0.14 ± 0.37 ^{a}
		1	30 ± 5.34 ^{bc}	70 ± 8.16 ^{cd}	1 ± 0.57 ^{abc}
		3	30 ± 5.00 ^{b}	62 ± 10.35 ^{bcd}	0.57 ± 0.78 ^{ac}
		5	10 ± 6.07 ^{a}	74 ± 13.04 ^{d}	2.28 ± 0.75 ^{d}
Rhizome shoots	3	0	97 ± 3.93 ^{f}	0 ^{a}	0 ^{a}
		1	100 ^{f}	0 ^{a}	0 ^{a}
		3	100 ^{f}	0 ^{a}	0 ^{a}
		5	100 ^{f}	0 ^{a}	1.42 ± 0.53 ^{bcd}
	6	0	90 ± 6.45 ^{ef}	4 ± 0.69 ^{a}	1 ± 0.81 ^{abc}
		1	85 ± 11.18 ^{e}	4 ± 0.97 ^{a}	1 ± 0.81 ^{abc}
		3	82 ± 9.51 ^{e}	5 ± 1.00 ^{a}	0.85 ± 0.89 ^{abc}
		5	80 ± 9.57 ^{e}	3 ± 1.21 ^{a}	1 ± 0.81 ^{abc}

Data are given as means ± SD of results from 20 replicates.

treatments showed only 42% contamination and a 65% response to the initial shoot. The use of rhizome shoots as explants was observed to have the highest contamination rate (100%) and a 0% response to the initial shoot with no micro shoots initiated when using agar at 3 g/L combined with all concentrations of BAP (**Figure 1(d)**). Contamination plays a crucial part *in vitro* plant technique because it may disrupt the multiplication of micro shoots/explants. In research by [7], most likely form of contamination *in vitro* plant cultures was due to ineffective decontamination of explants, recipients, apparatus, or media, or when handling plant material.

4.2. Micropropagation of Shoot Cultures

Plant growth regulators are biosynthetic substances that influence plant physiological processes that are involved in plant growth and development. In this study, BAP, NAA, and Kin are used as plant growth regulators to boost the number of shoots and shoot growth. From **Table 2**, it shows the effect of using different types of growth regulators on shoot initiation and the number of micro shoots per explant produced. Each of the plant growth regulators consists of the same concentration range of 0.5 - 5.0 mg/L. Generally, the combination of BAP and NAA showed higher results for percentage of initial shoots and number of micro shoots/explants compared to the combination of BAP with Kin.

Table 2. Effect of different plant growth regulators (BAP, NAA, Kin) on percentage of shoots initiation and number of shoots per explants produced after 60 days of culture.

BAP (mg/l)	NAA (mg/l)	Kin (mg/L)	Initial shoots (%)	Mean number of micro shoots/explants
0	0	0	22 ± 10.36 ^{bcd}	1 ± 0 ^{a}
0.5	0.5	-	72 ± 5.70 ^{ijkl}	1.20 ± 0.44 ^{ab}
	1	-	73 ± 9.74 ^{jklm}	1.20 ± 0.44 ^{ab}
	3	-	88 ± 8.36 ^{lmn}	1.20 ± 0.44 ^{ab}
	5	-	86 ± 8.94 ^{lmn}	1 ± 0 ^{a}
1	0.5	-	97 ± 4.47 ^{n}	1.20 ± 0.44 ^{ab}
	1	-	1000 ± 0 ^{n}	1 ± 0 ^{a}
	3	-	80 ± 7.07 ^{lm}	3 ± 3.93 ^{abc}
	5	-	80 ± 7.07 ^{lm}	1 ± 0 ^{a}
3	0.5	-	98 ± 4.47 ^{n}	3.20 ± 0.83 ^{bc}
	1	-	86 ± 5.47 ^{lmn}	3.20 ± 0.83 ^{bc}
	3	-	53 ± 6.70 ^{gh}	1.60 ± 0.54 ^{ab}
	5	-	41 ± 8.21 ^{efg}	1.20 ± 0.44 ^{ab}
5	0.5	-	90 ± 10.00 ^{mn}	5.80 ± 0.83 ^{d}
	1	-	98 ± 4.47 ^{n}	4.80 ± 0.83 ^{cd}
	3	-	86 ± 5.47 ^{lmn}	1.60 ± 0.54 ^{ab}
	5	-	78 ± 4.47 ^{lm}	1.80 ± 0.83 ^{ab}
0.5	-	0.5	56 ± 5.47 ^{ghi}	1.20 ± 0.44 ^{ab}
	-	1	57 ± 4.47 ^{ghij}	1.20 ± 0.54 ^{ab}
	-	3	11 ± 4.18 ^{abc}	1.40 ± 0.44 ^{ab}
	-	5	11 ± 5.47 ^{abc}	1.20 ± 0.44 ^{ab}
1	-	0.5	74 ± 5.47 ^{klm}	1.20 ± 0.44 ^{ab}
	-	1	60 ± 10.00 ^{hijk}	1.20 ± 0.44 ^{ab}
	-	3	46 ± 5.47 ^{fgh}	1 ± 0 ^{a}
	-	5	15 ± 7.07 ^{abc}	1.20 ± 0.44 ^{ab}
3	-	0.5	26 ± 6.51 ^{cde}	1.20 ± 0.44 ^{ab}
	-	1	33 ± 9.74 ^{def}	1.20 ± 0.44 ^{ab}
	-	3	7 ± 4.47 ^{ab}	1.20 ± 0.44 ^{ab}
	-	5	15 ± 7.07 ^{abc}	1 ± 0 ^{a}
5	-	0.5	25 ± 6.12 ^{cde}	1.20 ± 0.44 ^{ab}
	-	1	33 ± 8.36 ^{def}	1 ± 0 ^{a}
	-	3	0 ^{a}	1 ± 0 ^{a}
	-	5	0 ^{a}	1 ± 0 ^{a}

When BAP (5 mg/L) was combined with NAA (0.5 mg/L), the initial shoots acquired was 100%. From this combination, the highest mean number of micro shoots/explants obtained was 5.8 (**Figure 1(f)**). According to [8], MS media supplemented with 5 mg/L BAP was the most successful for C. aromatic shoot multiplication, obtaining an average of 3.3 shoots per explant. Similarly, results were reported by [9] in their studies on the micropropagation of *Crambe orientalis* L. var. *orientalis* L. They observed that the concentration of BAP (2.00 mg/L) combined with NAA (0.25 mg/L) produced the highest mean shoot number per explant (18.14 ± 1.36) compared to another concentration of BAP (0.25 mg/L) combined with NAA (0.50 mg/L) which resulted in the lowest mean number of shoots per explant (6.12 ± 0.98). In accordance with another study by [10], the highest number of multiple shoots (5) was produced by shoot buds of *Boesenbergia rotunda* (L.) Mansf cultivated on MS medium supplemented with 2 mg/L BAP and 0.5 mg/L NAA. Different concentrations of both BAP and NAA gave different percentages of initial shoots and a mean number of shoots/explant after 60 days of culture. The use of both BAP and NAA in tissue culture media is known to contribute to the development of shoot formation.

Hence, the combination of BAP and Kin showed a decreasing pattern for initial shoots and the number of micro shoots/explants. Most of the number of micro shoots/explants for this combination was only 1, and the percentage of initial shoots was in the range of 0% up to 74% (**Table 2**). The combination of BAP (5 mg/L) and Kin (5 mg/L) resulted in 0% of initial shoots, and there were no shoots. Kinetin had a poor response in terms of shoot development per explant for all the concentrations examined. On the other hand, [11] found that Kinetin alone at 2.22 µM produced a single micro shoot per nodal explant (sixth to ninth from the shoot tip). Due to the interaction impact of the two cytokinins, a combination of 2.22 µM BAP and 2.22 µM kinetin improved multiple micro shoots on nodal explants [11]. [12] discovered that in MS media supplemented with 2.32 M kinetin, 80 percent of rhizomatous eye/buds produced single micro shoots. Research by [13], the steps in plant tissue culture introduce a series of PGRs, which can be divided into two groups: auxins and cytokinins. The suitability of these hormones is dependent on the type of tissue used for culture.

4.3. Effect of Sucrose and Plant Growth Regulators (BAP and NAA) on Mean Number and Length of Shoots and Roots

Kaempferia angustifolia Roscoe was further studied in terms of shoot length and roots by measuring the effectiveness of sucrose and plant growth regulators with the combination of BAP and NAA or only BAP. The concentration of sucrose used was 15, 30 and 45 g/L respectively, for BAP (1, 3 mg/ml) and NAA (0.5, 1 mg/ml). Based on **Table 3**, the highest number of shoots produced was 11.40 at 30 g/L sucrose combined with 3 mg/L BAP + 0.5 mg/L NAA (**Figure 1(g)**, **Figure 1(h)**) while the lowest number of shoots was 4. In terms of shoot length, the highest shoot length recorded ranged from 6 - 8 cm at sucrose (15 g/L) with only BAP used as the plant growth regulator. When single BAP was used as a plant

Table 3. The effect of sucrose and plant growth regulators (BAP and NAA) on number of shoots, length of shoots, number of roots and root lenght.

Sucrose	BAP (mg/l)	NAA (mg/l)	No. of shoots	Shoot length (cm)	No. of roots	Roots length (cm)
15	1	-	2.80 ± 0.83 [a]	6 - 8	3.94 ± 0.58 [ab]	6 - 7
	3	-	4.40 ± 0.89 [ab]	6 - 8	3.46 ± 0.57 [a]	6 - 7
	1	0.5	3.60 ± 1.14 [a]	4 - 5	5.44 ± 0.42 [abcd]	7 - 8
	3	0.5	4.60 ± 1.51 [ab]	3 - 5	5.06 ± 0.40[abc]	7 - 8
	1	1	3.60 ± 1.67 [a]	5 - 6	6.20 ± 0.91 [cde]	7 - 8
	3	1	3.20 ± 0.83 [a]	3 - 5	4.84 ± 0.65 [abc]	7 - 8
30	1	-	7.40 ± 1.14 [bcdef]	5 - 6	5.90 ± 1.27 [bcd]	4 - 5
	3	-	10.20 ± 1.92 [fg]	5 - 6	5.52 ± 1.12 [abcd]	3 - 6
	1	0.5	9.40 ± 2.07 [fg]	4 - 5	5.74 ± 1.11 [bcd]	7 - 8
	3	0.5	11.40 ± 2.07 [g]	3 - 5	5.54 ± 0.76 [abcd]	7 - 8
	1	1	7.60 ± 1.14 [bcdef]	5 - 6	7.34 ± 0.97 [def]	7 - 8
	3	1	6.00 ± 1.58 [abcde]	3 - 5	5.34 ± 0.41 [def]	7 - 8
45	1	-	5.60 ± 1.14 [abcd]	3 - 5	11.82 ± 0.61 [h]	5 - 6
	3	-	5.00 ± 1.58 [abc]	3 - 5	8.16 ± 1.21 [efg]	5.6
	1	0.5	8.21 ± 1.38 [cdefg]	2 - 4	9.58 ± 1.22 [g]	5 - 7
	3	0.5	9.20 ± 1.30 [efg]	2 - 4	8.16 ± 1.21 [efg]	5 - 7
	1	1	8.80 ± 0.83 [defg]	2 - 4	9.58 ± 1.22 [efg]	5 - 6
	3	1	7.20 ± 1.30 [bcdef]	2 - 4	8.24 ± 1.23 [efg]	5 - 6

growth regulator, it showed the highest number of roots (11.8) at sucrose (45 g/L) with a BAP concentration of 1 mg/L compared with the combination of BAP and NAA. According to [14], adding BAP to MS medium was necessary for the regeneration of plantlets from shoot apices. In general, a medium containing both cytokinin (BAP) and auxin (NAA) would promote the proliferation of shoots in the culture. However, in this research, BAP alone outperformed BAP in combination with NAA in terms of the number of shoots. One of the most essential factors in determining the success of shoot multiplication is the number of shoots [15]. In addition, BAP promoted a shoot length range of 3 - 8 cm, as opposed to BAP and NAA, which promoted a shoot length range of 2 - 6 cm.

This finding was contrary to the findings by [16], where the highest shoot height recorded was 12 cm, with a mean of 11.6 cm in the combination treatment of BAP and NAA plant growth regulators. However, because hormones such as BAP help to induce cell division in tissue explants and growth shoots, according to [15] the result for increasing shoot length by only using BAP was acceptable. Also be supported by the study from [17] where BAP's involvement in encouraging shoot growth was more successful when a medium with enough auxin culture was available. According to [18], if NAA hormone was added at a

higher concentration than BAP, root growth would be stimulated more than shoot growth.

In the present study, the obtained plantlets with roots (**Figure 1(i)** were transferred to polybags containing organic soil and topsoil for hardening and kept under controlled conditions with 75% shading in the greenhouse (**Figure 1(j)**, **Figure 1(k)**). The plantlets successfully acclimatized with a survival rate of 97%. After 8 months of planting, the plants was harvested and it can be seen that rhizomes was produced (**Figure 1(l)**, **Figure 1(m)**).

5. Conclusion

This research used direct regeneration with plant growth regulators to develop a micropropagation method for *Kaempferia angustifolia* Roscoe. When BAP was combined with a lower concentration of NAA, it increased the number of shoots while decreasing the length of the shoots. When BAP was combined with a greater concentration of NAA, it increased the number of roots. The development of the micropropagation protocol for genetically uniform *Kaempferia angustifolia* Roscoe plants will meet the demand for this plant in the pharmaceutical and medicinal fields, curb overexploitation of the herb for traditional medicine, and allow for better development in order to enhance traditional medicine usage and production.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Pancharoen, O., Prawat, U. and Tuntiwachwuttikul, P. (2000) Phytochemistry of the Zingiberaceae. *Studies in Natural Products Chemistry*, **23**, 797-865. https://doi.org/10.1016/S1572-5995(00)80142-8

[2] Yeap, Y.S.Y., Kassim, N.K., Ng, R.C., Ee, G.C.L., Saiful Yazan, L. and Musa, K.H. (2017) Antioxidant Properties of Ginger (*Kaempferia angustifolia* Rosc.) and Its Chemical Markers. *International Journal of Food Properties*, **20**, 1158-1172. https://doi.org/10.1080/10942912.2017.1286508

[3] Ashton, H. (2004) The Royal Horticultural Society New Encyclopedia of Herbs & Their Uses (Revised Edition) London: Dorling Kindersley 2002. 448 pp., ISBN: 1 45053 0059 0 £30. *Reference Reviews*, **18**, 42-43. https://doi.org/10.1108/09504120410528289

[4] Tewtrakul, S. and Subhadhirasakul, S. (2007) Anti-Allergic Activity of Some Selected Plants in the Zingiberaceae Family. *Journal of Ethnopharmacology*, **109**, 535-538. https://doi.org/10.1016/j.jep.2006.08.010

[5] Edwards, H. (2003) Plant Resources of Southeast Asia (PROSEA) No. 17: Fibre Plants. *Phytochemistry*, **64**, 1174-1175. https://doi.org/10.1016/j.phytochem.2003.08.006

[6] Tang, S.W., Sukari, M.A., Neoh, B.K., Yeap, Y.S.Y., Abdul, A.B., Kifli, N. and Cheng Lian Ee, G. (2014) Phytochemicals from *Kaempferia angustifolia* Rosc. and Their

Cytotoxic and Antimicrobial Activities. *BioMed Research International*, **2014**, Article ID: 417674. https://doi.org/10.1155/2014/417674

[7] Moreno-Vázquez, S., Larrañaga, N., Uberhuaga, E.C., Braga, E.J.B. and Pérez-Ruíz, C. (2014) Bacterial Contamination of *in Vitro* Plant Cultures: Confounding Effects on Somaclonal Variation and Detection of Contamination in Plant Tissues. *Plant Cell, Tissue and Organ Culture* (*PCTOC*), **119**, 533-541. https://doi.org/10.1007/s11240-014-0553-x

[8] Nayak, S. (2000) *In Vitro* Multiplication and Microrhizome Induction in *Curcuma aromatica* Salisb. *Plant Growth Regulation*, **32**, 41-47. https://doi.org/10.1023/A:1006307316393

[9] Ceylan, Y., Ozdemir, F.A. and Bulbul, A.S. (2017) Effect of Different Concentrations of BAP and NAA on Micropropagation of *Crambe orientalis* L. *var. orientalis* L. *Journal of Animal and Plant Sciences*, **27**, 1671-1677.

[10] Yusuf, N.A., Annuar, M.S. and Khalid, N. (2011) Rapid Micropropagation of *Boesenbergia rotunda* (L.) Mansf. Kulturpfl. (a Valuable Medicinal Plant) from Shoot Bud Explants. *African Journal of Biotechnology*, **10**, 1194-1199.

[11] Walia, N., Kaur, A. and Babbar, S.B. (2006) An Efficient, *in Vitro* Cyclic Production of Shoots from Adult Trees of *Crataeva nurvala* Buch. Ham. *Plant Cell Reports*, **26**, 277-284. https://doi.org/10.1007/s00299-006-0239-x

[12] Chirangini, P. and Sharma, P.J. (2005) *In Vitro* Propagation and Microrhizome Induction in *Zingiber cassumunar* (Roxb.)—An Antioxidant-Rich Medicinal Plant. *Journal of Food, Agriculture & Environment*, **3**, 139-142.

[13] Hill, K. and Schaller, G.E. (2013) Enhancing Plant Regeneration in Tissue Culture. *Plant Signaling & Behavior*, **8**, e25709. https://doi.org/10.4161/psb.25709

[14] Firoozabady, E. and Gutterson, N. (2003) Cost-Effective *in Vitro* Propagation Methods for Pineapple. *Plant Cell Reports*, **21**, 844-850. https://doi.org/10.1007/s00299-003-0577-x

[15] Yuniastuti, E., Widodo, C.E., Samanhudi and Delfianti, M.N.I. (2018) Effect of Benzyl Amino Purine and Indole-3-acetic Acid on Propagation of *Sterculia foetida in Vitro*. *IOP Conference Series: Earth and Environmental Science*, **142**, Article ID: 012011. https://doi.org/10.1088/1755-1315/142/1/012011

[16] Lestari, K.D., Deswiniyanti, N.W., Astarini, I.A. and Arpiwi, L.M. (2019) Callus and Shoot Induction of Leaf Culture *Lilium longiflorum* with NAA and BAP. *Nusantara Bioscience*, **11**, 162-165. https://doi.org/10.13057/nusbiosci/n110209

[17] Sari, H.S., Murni, D. and Iman, B. (2015) Efek NAA dan BAP terhadap pembentukan tunas, daun, dan tinggi tunas stek mikro Nepenthes ampullaria Jack. *Biosfera*, **32**, 194-201. (In Indonesian)

[18] Kristina, N.N. (2009) Induction of Tabato barito (*Ficus deltoidea* Jack) Shoots *in Vitro* Using Benzyl Adenine (BA) and Naphthalene Acetic Acid (NAA). *Industrial Crops Research journal*, **15**, 33-39. https://doi.org/10.21082/jlittri.v15n1.2009.33-39