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Response of Auxins and Cytokinins on *Citrus suhuiensis* Adventitious Shoot Culture Initiation and Growth

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ABSTRACT

Bacterial and viral diseases are the common problems hampering the vast majority of citrus plants which resulted in a decrease of citrus trees development and production yield. While the usage of chemicals to eliminate internal pathogens is harmful to the ecosystem, plant tissue culture is another alternative to develop disease-free plants based on the defined physical and chemical conditions under an aseptic environment. This study aimed to initiate *Citrus suhuiensis (C. suhuiensis)* adventitious shoot culture specifically in response towards different types of plant growth regulators (PGRs). The effects of explants and PGRs were evaluated on the shoot growth within 35 days of culture. *C. suhuiensis* shoot cultures were induced from three different explants which are leaf, callus and seeds on the solid Murashige and Skoog (MS) medium containing different combinations of PGRs which are auxins *i.e.* 1-naphthylacetic acid (NAA) or indole butyric acid (IBA) at 0.5 mg/L, respectively with cytokinins *i.e.* 6-benzylaminopurine (BAP) or kinetin (KN) at various concentrations (0.5 - 4.0 mg/L). Based on the results, the earliest shoot emergence from the cotyledon can be observed after the 8th day of inoculation for PGRs combination of 0.5 mg/L IBA with 2.0 mg/L, 3.0 mg/L KN and 3.0 mg/L BAP, respectively. Meanwhile, based on the ANOVA analysis (*p-value* < *0.05*), the most significant PGRs combination for the establishment of *C. suhuiensis* shoot culture is IBA and KN followed by the treatment of NAA and KN. The findings of this study can serve as a basis for future investigation of micropropagation of shoot culture and cultivation of *C. suhuiensis* plant.

Keywords: Adventitious shoot, *Citrus suhuiensis*, auxins, cytokinins, plant micropropagation

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INTRODUCTION

Citrus *(Citrus spp.)* or *Limau* in Bahasa Malaysia is among popular fruits grown in Malaysia to suit the demand of domestic market. The citrus plant is a variety of blooming trees and bushes in the mourn family, Rutaceae. It is one of the major fruit crops globally, which incorporates mandarins, sweet oranges, limes, lemons, tangerines and grapefruits (Devi et al., 2018). Over 40 percent of world citrus exports is dominated by oranges (FAO,

2021). *Citrus suhuiensis* (*C. suhuiensis*) is a prominent type of orange in Malaysia which is also known as *limau madu* or *limau manis* (Al Zoubi & Mohd. Noor, 2012; Ghazali et al., 2018). It is a good import substitute for oranges and mandarins. It contains high vitamin C amount and fiber as well many other bioactive compounds mainly phenolic compounds, terpenoids and pectin (Puad et al., 2018).

The vast majority of citrus trees problems are high susceptibility to bacterial and viral infections that diminishing the survival rate of these trees such *Diaporthe citri*, *Elsinoe spp.* and *Mycosphaerella citri* (Gabriel et al., 2007). Some diseases that affecting this plant are alternaria stem-end rot, cachexia, citrus tatter leaf, citrus canker and citrus greening. For an instance, citrus canker is a bacterial disease that causes yellow radiance like sores or scabs on the natural product, leaves and twigs of citrus trees which eventually bringing about leaf loss, blemished fruit, and fruit drop (Behlau, 2021; Gottwald et al., 2002). In addition, scale insects are a major pest of citrus with a high population that causes loss to citrus fruits production (Triwiratno et al., 2017).

The application of micropropagation and *in vitro* morphogenesis in citrus evokes interest because potential important cultivars are either monoembryonic or produce a smaller number of seeds and thus hindering cultivar improvement. Besides, the potential of tissue culture in eliminating diseases and providing opportunity for cultivar improvement through somaclonal variation and somatic hybridization become the contributing factor (Krishna et al., 2016; Singh & Rajam, 2010). Therefore, it is a major significance to accelerate the evaluation of commercial traits from the development of a reliable shoot regeneration system for mature tissue of citrus (Curtis & Mirkov, 2012). The upsides of tissue culture method in proliferating disease-free plants can be observed by increasing the susceptibility of these plants towards infections and growths. This expanded vulnerability is influenced by the modified nutritional and physiological conditions of the tissue culture-derived plants. Thus, establishment of *C. suhuiensis* adventitious shoot culture is one of the potential research subjects since this type of culture has been established in several citrus species from various types of explants but still lack for *C. suhuiensis*.

Generally, a high cytokinin to auxin ratio is mandatory for shoot regeneration with the types and concentrations of cytokinins and auxins differing between species (Marutani-Hert et al., 2011; Nowakowska et al., 2019). Mostly for the case of citrus species, 6-benzylaminopurine (BAP) is the cytokinin used to initiate shoot organogenesis, used either singularly or in combination with 1-naphthylacetic acid (NAA) (Chamandoosti, 2017; Kasprzyk-Pawelec et al., 2015; Liang et al., 2020; Marques et al., 2011; Savita et al., 2011; Singh & Rajam, 2010). Besides, the utilization of highly responsive explants and composition of media are vital to the shoot regeneration process to achieve a high number of adventitious shoots (Marques et al., 2011). This study involved the selection of different types of explants which are the leaf, seeds and callus and different combinations of PGRs for the initiation of *C. suhuiensis* adventitious shoot culture. Based on the literature, the most common types of PGR supplemented to the citrus shoot culture media are BAP, NAA and indole butyric acid (IBA). Thus, the functionality of four PGRs which are BAP, kinetin (KN), IBA and NAA towards *C. suhuiensis* shoot initiation was investigated. *C. suhuiensis* shoot growth was then evaluated for their responses to different combinations of PGRs by the observation of shoot initiation period and elongation.

MATERIALS AND METHODS

C. suhuiensis explants

The explants were collected from various sources. *C. suhuiensis* seeds were obtained from the fruits bought from a market (Pasaraya Ong Tai Kim) in Danau Kota, Setapak (GPS Coordinates: 3.21282,101.71143). Meanwhile the leaves were obtained from *C. suhuiensis* potted plant available at the Department of Chemical Engineering and Sustainability, Kulliyyah of Engineering, IIUM. Lastly, callus explant were established previously from the seed (Fathil et al., 2017) and available in Plant Biotechnology Laboratory, Kulliyyah of Engineering IIUM.

Surface sterilization of explants

Freshly gathered leaves and seeds with their seed coats removed were washed under slow running tap water to clean any contaminations on the surface. Surface sterilization method was performed based on Aarifa et al., (2013) with some modifications. Leaves were submerged for 30 seconds in 70% ethanol while seeds for 1 minute before rinsing them twice in sterile distilled water (SDW).

The plant materials were immersed in 5.2% of sodium hypochlorite for about 10 minutes before rinsing them again three times in SDW. Anti-browning treatment was given to control phenol exudation from the cut surfaces by soaking the leaf explants in filtered 0.5 mg/L ascorbic acid (R&M Chemicals) for 30 minutes prior to the inoculation.

Preparation of medium

An amount of 4.4 g of Murashige and Skoog (MS) (Duchefa, USA) powdered medium was weighed for 1 L medium. MS powder was gradually added into 800 mL of distilled water followed by 30 g/L of sucrose (Bendosen Laboratory Chemicals) and 2.5 g/L of Gelrite (Duchefa, Netherlands). The PGR was then added according to the experimental design (Table 1). The remaining distilled water was later added to made up to 1 L medium. The pH of the medium was adjusted to 5.7 by adding 1 M of NaOH or HCl. Lastly, the media was autoclaved at 121 °C for 15 minutes (Puad & Tang, 2016).

Inoculation of explants

The sterile leaves were cut into 10 mm by 10 mm in square and seeds were set on sterile tissue paper to dry. The leaf, callus and seed explants were cultured in 240 mL glass jars containing 30 mL of MS medium in the laminar flow cabinet. Only one seed and one cut leaf was inoculated for each glass jar, respectively while for the callus explant, 0.5 g of 21-days old friable yellow callus was used. Light was provided at an intensity of a day photoperiod light (16 hours) using cool-white fluorescent tube lights at 25 ± 2 °C.

Shoot growth analysis

The observation for the first shoot initiation from the three types of explants was made for every 3 days within the 35 days of incubation period while the shoot formation parameters were recorded based on the days for shoot induction and length of shoots (mm). The days of shoot induction is defined as the first day of shoot started to grow in the media. Meanwhile the length of the shoots was measured by a digital Vernier caliper in mm measurement. The experimental design table for different combinations of auxins and cytokinins which are 1-naphthylacetic acid (NAA) (R&M Chemicals) or indole butyric acid (IBA) (Phyto Tehnology Laboratories) at 0.5 mg/L with 6-benzylaminopurine (BAP) (R&M Chemicals) or kinetin (KN) (Phyto Technology Laboratories) at various concentrations (0.5-4.0 mg/L) were set as in the Table 1.

Table 1. Experimental design for different combinations of IBA or NAA with BAP or KN at various concentrations.

No.	Concentration of auxin		Concentration of cytokinin	
	IBA (mg/L)	**NAA (mg/L)**	**BAP (mg/L)**	**KN (mg/L)**
1	0.5	-	0.5	-
2	0.5	-	1.0	-
3	0.5	-	2.0	-
4	0.5	-	3.0	-
5	0.5	-	4.0	-

6	-	0.5	0.5	-
7	-	0.5	1.0	-
8	-	0.5	2.0	-
9	-	0.5	3.0	-
10	-	0.5	4.0	-
11	0.5	-	-	0.5
12	0.5	-	-	1.0
13	0.5	-	-	2.0
14	0.5	-	-	3.0
15	0.5	-	-	4.0
16	-	0.5	-	0.5
17	-	0.5	-	1.0
18	-	0.5	-	2.0
19	-	0.5	-	3.0
20	-	0.5	-	4.0

Statistical analysis

Each treatment was carried out in triplicate. The data was analyzed using the one-way analysis of variance (ANOVA) using 95% confidence interval. The statistical significance between different treatments was assessed using Boxplot graph. All analyses were done using Minitab version 18 software.

RESULTS AND DISCUSSION

Initiation of *C. suhuiensis* shoot culture from the cotyledon explant

It was observed that the earliest shoot emerged in a number of glass jars after 8 day of inoculation and continued to grow until the end of observation period (35 days) (Fig. 1), and expanded shoots became more visible after day 11. Shoot emergence and development was achieved under all PGR treatments but there were significant differences observed in shoot growth among various PGRs tested (Table 3). In addition, root growth was also present and observed earlier than shoot proliferation for some combinations of PGRs (data is not shown).

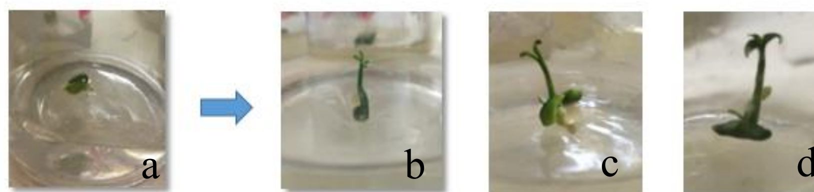

Fig. 1. Shoot induced from *C. suhuiensis* cotyledon in media supplemented by 0.5 mg/L IBA and 2.0 mg/L KN for (a) day 0; (b) day 8; (c) day 29 until (d) day 35 of incubation.

A study of the amenability of cotyledonary explants of *C. reticulata* on MS medium supplemented with different concentrations and combinations of BAP (0.25-6.0 mg/L), NAA (0.25-2.5 mg/L), IBA (0.25-0.5 mg/L) and

KN (0.25 mg/L) was reported by Sarma et al. (2011). A direct shoot regeneration without an intervening callus phase was achieved in which the earliest shoot emergence was after 12th day and 2 mg/L IBA was found to be superior to other auxins for *in vitro* root induction. In comparison with this study, *C. suhuiensis* shoot emergence was observed to be earlier by 4 days (Table 2). It can be deduced that the earliest shoot emergence can be observed after 8th day of inoculation using 0.5 mg/L IBA with 2.0 mg/L, 3.0 mg/L KN and 0.3 mg/L BAP, respectively.

Table 2. Comparison of optimum data between different combinations of PGRs for initiation of *C. suhuiensis* shoot from cotyledon.

Combinations of PGRs (mg/L)		Period of induction
IBA 0.5	KN 2.0	Day 8
IBA 0.5	KN 3.0	Day 8
IBA 0.5	BAP 3.0	Day 8
NAA 0.5	KN 1.0	Day 11
NAA 0.5	KN 2.0	Day 11
NAA 0.5	BAP 2.0	Day 11
NAA 0.5	BAP 3.0	Day 20

Meanwhile, in contrast with BAP treatment study on *C. clementina* Hort. Ex Tan for direct organogenesis from cotyledon, shoots appeared directly from the explants after three weeks of incubation and no callus around the emerging shoots was detected under the stereo microscope (Lombardo et al., 2011). This clearly shows a huge gap in difference from this study in terms of the shoot initiation period. Possible cause that leads to the difference is due to the dissimilarity of PGRs treatment. This study highlights the treatments of combinations of auxin and cytokinin meanwhile, the previous study was emphasizing on the cytokinin treatment only. In addition, different explants and cultivars react differently to different concentrations of PGRs (Fletcher et al., 2006).

Initiation of *C. suhuiensis* shoot culture from the leaf explant

The observation for shoot emergence was performed for 35 days (1st batch). However, there was no growth observed and the experiment was repeated for the second time (2nd batch) resulting the same response as the previous attempt. No indication of shoot growth was observed in this study might be due to tropical plant species that contain phenolic substances that are oxidized when cells are wounded or senescent (George et al., 2015). It was observed that the isolated and cut tissue turned brown or black starting from 7 days after inoculation which might cause them failed to grow (Fig. 2). The prevention of blackening during the experiments was overcome by the usage of ascorbic acid (Aarifa et al., 2013). However, the responses were still negative after 35 days of inoculation.

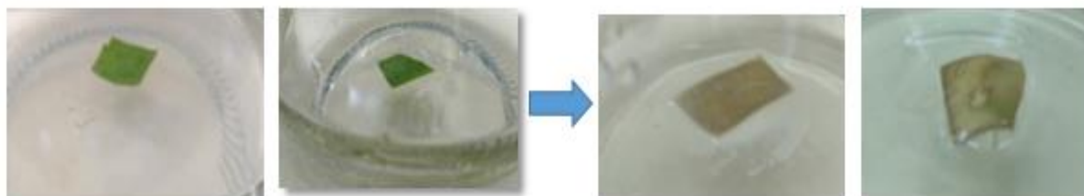

Fig. 2. *C. suhuiensis* growth response for leaf explant after 35 days.

As a comparison to other published study, seed explant was used for germination and leaves were then collected after one month of the germination (Kasprzyk-Pawelec et al., 2015). The shoot culture was successfully grown after the aforementioned steps. In contrast, this study used mature leaves directly for shoot culture initiation. Sharma et al. (2020) concluded that the age of leaf explant of *Populus deltoides* affected its shoot organogenesis. Therefore, the use of leaf as explant for direct *C. suhuiensis* shoot organogenesis needs to be evaluated in terms of types of PGRs, age of leaf and type of medium used for future research.

Initiation of *C. suhuiensis* shoot culture from callus explant

Similar with the leaf explant, no response for shoot culture initiation for all PGR treatments was achieved using *C. suhuiensis* callus (Fig. 3). Callus consists of unorganized cells which theoretically under the stimulus of PGRs added to the medium will actively begin to divide and grow (Fehér, 2019). Some differentiation of cells may occur in plant cultures during the period of slowed and stationary growth. However, normally it is less significant and less complete compared to what occurs in the callus cultures (Espinosa-Leal et al., 2018; George et al., 2007). Therefore, the shoot growth from callus explant may takes longer than the period used in this study that is 35 days.

Fig. 3. *C. suhuiensis* growth response for callus explant after 35 days of incubation period.

Besides, minimum size of explant or quantity of separated cells or protoplasts per unit culture volume plays significant role in a successful culture initiation. Inoculation density of the culture affects the initial rate of growth *in vitro* (Espinosa-Leal et al., 2018). Thus, the small size of callus may cause the late growth of *C. suhuiensis* culture. Large explants usually survive more regularly and grow more rapidly at the beginning than very small ones (George et al., 2007). In this study, the inoculation size of the callus was smaller than the one used in plant regeneration study of *C. jambhiri Lush* from callus culture (Savita et al., 2011).

C. suhuiensis shoot growth from seed explant

The boxplot illustration is a standardized way of displaying the distribution data between PGRs concentration and shoot length. The length of shoot for IBA combinations with BAP and KN are shown in Fig. 4 and 5, respectively. Meanwhile the length of shoot for NAA combinations with BAP and KN are shown in Fig. 6 and 7, respectively. As previously mentioned, root growth was observed earlier than the shoot proliferation for some combinations of PGRs. Therefore, the respective combinations were not included in the box-plot illustration.

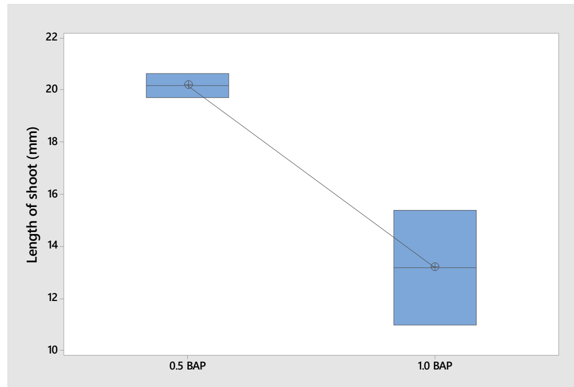

Fig. 4. Length of shoot for the combinations of 0.5 mg/L IBA and 0.5 mg/L or 1.0 mg/L BAP, respectively after 35 days of incubation.

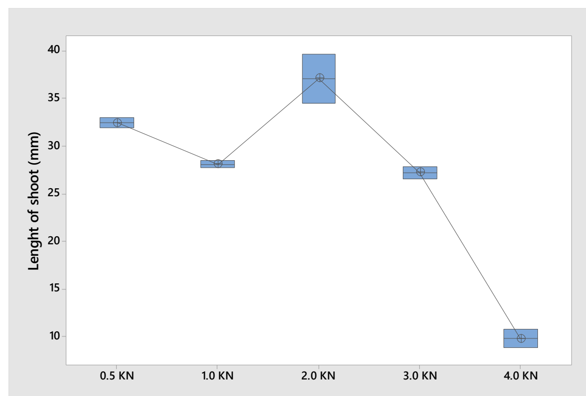

Fig. 5. Length of shoot for the combinations of 0.5 mg/L IBA and 0.5 mg/L, 1.0 mg/L, 2.0 mg/L, 3.0 mg/L or 4.0 mg/L KN, respectively after 35 days of incubation.

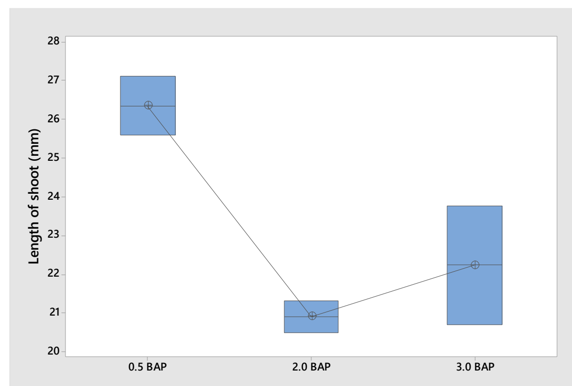

Fig. 6. Length of shoot for the combinations of 0.5 mg/L NAA and 0.5 mg/L, 2.0 mg/L or 3.0 mg/L BAP, respectively after 35 days of incubation.

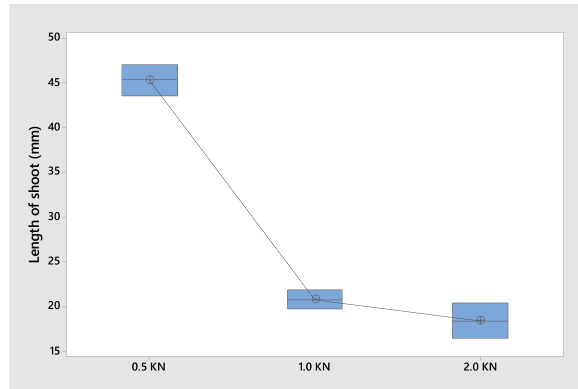

Fig. 7. Length of shoot for the combinations of 0.5 mg/L NAA and 0.5 mg/L, 1.0 mg/L or 2.0 mg/L KN, respectively after 35 days of incubation.

Based on the treatments of 0.5 mg/L IBA with (0.5 mg/L-4.0 mg/L) BAP or KN, the highest shoot length after four weeks of inoculation was observed at 39.7 mm particularly for the combination of 0.5 mg/L IBA with 2.0 mg/L KN. In contrast, by using *C. latifolia Tan.* nodal as the explant, the highest length of shoot 100 mm was favored on the medium with 2.0 mg/L BAP and 0.1 mg/L IBA after 8 weeks of observation (Chamandoosti, 2017). The results can be compared in terms of the different combinations of auxins and cytokinins. However, different protocols such as different types of explants used and the sampling period would cause the difference.

Additionally, for *in vitro* plant regeneration of *C. grandis* (L.) Osbeck using cotyledonary segments, the use of MS medium supplemented with 0.1 mg/L NAA and 2 mg/L BAP produced adventitious shoots directly on the surface of the segments with 12 mm length (Ibrahim, 2012). In this study, the same combination of PGR treatment; 0.5 mg/L NAA and 2 mg/L BAP produced a higher shoot growth which is 20.5 mm. However, the highest shoot proliferation observed in this study for NAA and BAP is the combination of 0.5 mg/L NAA and 0.5 mg/L BAP with 26.26 mm length. In contrast, for the establishment of shoot cultures for *C. sinensis* L. Osbeck, the combination of 2 mg/L NAA and 3 mg/L BAP on MS medium gave the optimum shoot formation after 30 days (Khalil et al., 2011). The differences between these studies are due to different types of plant species.

Nevertheless, the treatment for IBA and NAA with KN is challenging to be compared to any published reports as it is rarely reported for Citrus species. In this study, for IBA and KN treatment, the results were positive on all combinations and the highest shoot length was found to be 39.7 mm for 2.0 mg/L KN. For NAA and KN treatments, shoot growth can be observed for three combinations and the highest shoot length was 46.4 mm. Based on these results in comparison to BAP treatment, it can be deduced that KN combinations are more compatible for *C. suhuiensis* shoot growth than BAP.

Overall, shoot growth was favored for 0.5 mg/L NAA and 0.5 mg/L KN among other combinations. The length of shoot in the medium containing NAA and 0.5 mg/L KN after 35 days were significantly higher and demonstrated the longest shoot proliferation which is 46.4 mm. The induction of *C. suhuiensis* shoot formation towards different types of PGRs seems to be less sensitive or less responsive to the combinations with BAP than KN.

Significant PGRs combination for *C. suhuiensis* shoot growth

After 35 days, PGR combination presented in Table 3 led to a significant difference (f-value = 181.89 and p-value = 0.00) in shoot induction and growth for its all treatments as compared to IBA and BAP (f-value = 9.57 and p-value = 0.091). These results showed that the effect of IBA and KN is significantly greater than the effect

of IBA and BAP on shoot proliferation. Besides, PGR combination of NAA and KN which was found significantly difference with (f-value = 23.9 and p-value = 0.014) than the shoot length of NAA and BAP. ANOVA indicated an insignificant difference (f-value = 7.75 and p-value = 0.065) in the number of shoots induced by NAA and BAP.

The values of sum of square, mean of square degree of freedom, R^2, f-value, p-value are presented in Table 3 for all combinations of treatment. Thus, based on the analysis of data, it can be concluded that the most significant PGRs combination for the establishment of *C. subuiensis* shoot culture was IBA and KN for reliable amount of p-value which is 0.00 followed by treatments of NAA and KN with p-value equals to 0.014. As a comparison to NAA, synthetically prepared IBA tend to be denatured in media and rapidly metabolized within plant tissues (Gaspar et al., 1996). These attributes can be useful when developmental phases in progress require less auxin, or in automatically changing the auxin to cytokinin ratio. Thus, IBA with KN combinations achieved more progressive results. The R^2 which indicated the highest correlation between the experimental and predicted value, was also represented by IBA and KN which is 99.32% followed by NAA+ KN (94.09%), NAA + BAP (83.79%) and IBA + BAP (82.72%). However, based on the p-value, the combinations of IBA and NAA with BAP were not significant to the study.

Table 3. Analysis of ANOVA for all combinations of PGR treatment.

Auxin concentration (mg/L)	Cytokinin concentration (mg/L)		Sum of square	Mean square	F-value	p-value	R^2 (%)
IBA (0.5)	BAP	0.5 1	48.65	48.65	9.57	0.091	82.72
IBA (0.5)	KN	0.5 1 2 3 4	4	217.002	181.89	0	99.32
NAA (0.5)	BAP	0.5 2 3	2	16.094	7.75	0.065	83.79
NAA (0.5)	KN	0.5	2	423.71	23.9	0.014	94.09

CONCLUSION

In this study, a protocol of citrus shoot culture for *in vitro* grown propagation was established for three different explants that were designed by manipulating different combinations of auxin and cytokinin. Based on the findings, cotyledon explant achieved the best shoot growth while the best PGR combination is IBA and KN. Thus, further research on *in vitro* micropropagation of *C. subuiensis* adventitious shoot culture can be developed. For future work, different types of explants can be explored for direct organogenesis to initiate the shoot culture such as nodal segments because it has been proven to be a success for number of citrus shoot culture studies such *C. reticulate Osbeck* and *C. Limonia*. Moreover, to promote better cell growth for this species, different

parameters in the growth medium can be manipulated such as the type of media with different concentrations and type of PGRs.

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REFERENCES

Aarifa, J., Bhat, K., Bhat, S., Mir, M., Bhat, M., Imtiyaz, A., & Rather, J. (2013). Surface sterilization method for reducing microbial contamination of field grown strawberry explants intended for *in vitro* culture. *African Journal of Biotechnology, 12*(39), 5749-5753.

Al Zoubi, M., & Mohd. Noor, N. (2012). Desiccation sensitivity and cryopreservation of excised embryonic axes of citrus suhuiensis cv. limau madu, citrumelo [Citrus paradisi Macf. x Poncirus trifoliata (L.) Raf.] and Fortunella polyandra tn Omar. *Cryo-Letters, 33*(3), 241-251.

Behlau, F. (2021). An overview of citrus canker in Brazil. *Tropical Plant Pathology, 46*(1), 1-12. doi:10.1007/s40858-020-00377-2

Chamandoosti, F. (2017). Effect of interaction between different plant growth regulators on *in vitro* shoot multiplication of Citrus latifolia Tan. (persian lime). *International Journal of Environmental & Agriculture Research, 3*(7), 51-54.

Curtis, I. S., & Mirkov, T. E. (2012). Influence of surfactants on growth and regeneration from mature internodal stem segments of sweet orange (Citrussinensis) cv. Hamlin. *Plant Cell, Tissue and Organ Culture (PCTOC), 108*(2), 345-352. doi:10.1007/s11240-011-0037-1

Devi, M., Jaglan, R. S., Yadav, G. S., & Singh, N. (2018). Population Dynamics of Citrus Butterfly, Papilio demoleus L. (Lepidoptera: Papilionidae) in Kinnow (Citrus nobilis × Citrus deliciosa) as Influenced by Abiotic Factors. *International Journal of Current Microbiology and Applied Sciences, 7*(2), 475-478.

Espinosa-Leal, C. A., Puente-Garza, C. A., & García-Lara, S. (2018). *In vitro* plant tissue culture: means for production of biological active compounds. *Planta, 248*(1), 1-18. doi:10.1007/s00425-018-2910-1

FAO. (2021). *Citrus Fruit Statistical Compendium 2020*. Rome.

Fathil, N. A. M., Puad, N. I. M., Amid, A., Azmi, A. S., & Ibrahim, R. (2017). Optimization of plant growth regulators for Citrus suhuiensis callus induction. *Asia Pacific Journal of Molecular Biology and Biotechnology, 25*(1), 75-81.

Fehér, A. (2019). Callus, Dedifferentiation, Totipotency, Somatic Embryogenesis: What These Terms Mean in the Era of Molecular Plant Biology? *Frontiers in Plant Science, 10*. doi:10.3389/fpls.2019.00536

Fletcher, E., Amoako, T., & Twumasi, P. (2011). Effect of 2,4-D, explants type and cultivar on the callogenesis expression of cassava (Manihot esculenta Crantz) in Ghana. *African Journal of Biotechnology, 10*(46), 9396-9401. doi:10.5897/AJB10.2115

Gabriel, D., Gottwald, T. R., Lopes, S. A., & Wulff, N. A. (2020). Chapter 18 - Bacterial pathogens of citrus: Citrus canker, citrus variegated chlorosis and Huanglongbing. In M. Talon, M. Caruso, & F. G. Gmitter (Eds.), *The Genus Citrus* (pp. 371-389): Woodhead Publishing.

Gaspar, T., Kevers, C., Penel, C., Greppin, H., Reid, D. M., & Thorpe, T. A. (1996). Plant hormones and plant growth regulators in plant tissue culture. *In Vitro Cellular and Developmental Biology - Plant, 32*(4), 272-289. doi:10.1007/bf02822700

George, E. F., Hall, M. A., & De Klerk, G. J. (2007). *Plant Propagation by Tissue Culture: Volume 1. The Background*: Springer Netherlands.

Ghazali, H., Rosman, R., & Sharif, Z. (2018). Soluble Solid Content Determination of Limau Madu Using Microwave Sensing Technique at 2.0 -2.6 GHz. *IOP Conference Series: Materials Science and Engineering, 341*, 012006. doi:10.1088/1757-899x/341/1/012006

Gottwald, T. R., Graham, J. H., & Schubert, T. S. (Producer). (2002, 2 January 2019). Citrus canker: The pathogen and its impact. *Plant Health Progress*

Ibrahim, M. A. (2012). *In vitro* plant regeneration of local pummelo (Citrus Grandis L. Osbek) via direct and indirect of organogenesis. *Genetics and Plant Physiology, 2*(3-4), 187-191.

Kasprzyk-Pawelec, A., Pietrusiewicz, J., & Szczuka, E. (2015). *In vitro* regeneration induced in leaf explants of Citrus limon L. Burm cv. 'Primofiore'. *Acta Scientiarum Polonorum - Hortorum Cultus, 14*(4), 143-153.

Khalil, S., Zamir, R., Ahmad, N., Sajid, M., Fazal, H., Khan, M., . . . Alam, R. (2011). *In vitro* regeneration of plantlets from unpollinated ovary culture in sweet orange (*Citrus sinensis* L. Osbeck). *African Journal of Biotechnology, 10*(67), 15130–15134. doi: 10.5897/AJB11.833

Krishna, H., Alizadeh, M., Singh, D., Singh, U., Chauhan, N., Eftekhari, M., & Sadh, R. K. (2016). Somaclonal variations and their applications in horticultural crops improvement. *3 Biotech, 6*(1), 54. doi:10.1007/s13205-016-0389-7

Liang, H., Xiong, Y., Guo, B., Yan, H., Jian, S., Ren, H., . . . Ma, G. (2020). Shoot organogenesis and somatic embryogenesis from leaf and root explants of Scaevola sericea. *Scientific Reports, 10*(1), 11343. doi:10.1038/s41598-020-68084-1

Lombardo, G., Alessandro, R., Scialabba, A., Sciandra, M., & Pasquale, F. (2011). Direct Organogenesis from Cotyledons in Cultivars of Citrus clementina Hort. Ex Tan. *American Journal of Plant Sciences,, 2*(2), 237-244. doi:10.4236/ajps.2011.22025

Marques, N. T., Nolasco, G. B., & Leitão, J. P. (2011). Factors affecting *in vitro* adventitious shoot formation on internode explants of Citrus aurantium L. cv. Brazilian. *Scientia Horticulturae, 129*(2), 176-182. doi:https://doi.org/10.1016/j.scienta.2011.03.018

Marutani-Hert, M., Evens, T. J., McCollum, G. T., & Niedz, R. P. (2011). Bud emergence and shoot growth from mature citrus nodal stem segments. *Plant Cell, Tissue and Organ Culture (PCTOC), 106*(1), 81-91. doi:10.1007/s11240-010-9896-0

Nowakowska, K., Pacholczak, A., & Tepper, W. (2019). The effect of selected growth regulators and culture media on regeneration of Daphne mezereum L. 'Alba'. *Rendiconti Lincei. Scienze Fisiche e Naturali, 30*(1), 197-205. doi:10.1007/s12210-019-00777-w

Puad, N. I. M., Sarji, M. A., Fathil, N. A. M., & Abduh, M. Y. (2018). Growth Kinetics of Citrus suhuiensis Cell Suspension Cultures. *Biological and Natural Resources Engineering Journal, 1*(1), 1-13.

Puad, N. I. M., & Tang, C. W. (2016). A simple and easy method for preparing solid and liquid media for plant culture. In P. Jamal, I. A. Noorbatcha, & A. S. Azmi (Eds.), *Experimental Methods in Modern Biotechnology* (Vol. 2, pp. 9-15). Malaysia: IIUM Press.

Sarma, C., Borthakur, A., Singh, S., Modi, M. K., & Sen, P. (2011). Efficient *in vitro* plant regeneration from cotyledonary explants of Citrus reticulata L. Blanco. *Annals of Biological Research, 2*(6), 341-348.

Savita, Singh, B., Virk, G. S., & Nagpal, A. K. (2011). An efficient plant regeneration protocol from callus cultures of Citrus jambhiri Lush. *Physiology and Molecular Biology of Plants : An International Journal of Functional Plant Biology, 17*(2), 161-169. doi:10.1007/s12298-011-0055-9

Sharma, S., Reddy, M. S., & Kumar, A. (2020). Direct shoot organogenesis from leaf explants of Populus deltoides and changes in selected enzymatic activities. *Physiology and Molecular Biology of Plants, 26*(2), 399-407. doi:10.1007/s12298-019-00755-4

Singh, S., & Rajam, M. V. (2010). Highly Efficient and Rapid Plant Regeneration in Citrus sinensis. *Journal of Plant Biochemistry and Biotechnology, 19*(2), 195-202. doi:10.1007/bf03263340

Triwiratno, A., Wuryantini, S., & Agustina, D. (2017). Potential entomopathogenic fungi to control scale insect pest on Citrus tangerine (Citrus suhuiensis Tan.) *Russian Journal of Agricultural and Socio-Economic Sciences, 72*, 327-335. doi:10.18551/rjoas.2017-12.46

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