INDUCTION OF INDIRECT SOMATIC EMBRYOGENESIS IN CELL SUSPENSION CULTURE OF SIAM PONTIANAK ORANGE (*Citrus nobilis* var *microcarpa*)

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Abstract: *Citrus Vein Phloem Degeneration* (CVPD) disease outbreaks in citrus are one of the limiting factors in conventional vegetative cultivation. This affects the availability of plant material for cultivation, which in turn impacts the productivity and sustainability of Siam Pontianak orange cultivation. Micropropagation through indirect somatic embryogenesis in liquid medium is expected to produce a larger number of disease-free seedlings. Cells from embryogenic callus have the potential to develop into new individuals and are widely used for plant propagation and genetic manipulation. This study aims to determine the best treatment combination for embryogenic callus propagation in solid medium and to observe embryo development in cell suspension culture. The study used a completely randomized design (CRD) consisting of two stages: (1) micropropagation of embryogenic callus on Murashige Skoog (MS) solid medium suplemented with of 0.5 ppm 2,4-D + 0.5 ppm kinetin; 0.5 ppm 2,4-D + 1.5 ppm kinetin; 1.0 ppm 2,4-D + 1.0 ppm kinetin; 1.5 ppm 2,4-D + 1.5 ppm kinetin; and 2.0 ppm 2,4-D + 2.0 ppm kinetin; and (2) proliferation and development of somatic embryos in cell suspension culture on liquid MS medium with the following treatments: without Plant Growth Regulators (D0K0); 1.0 ppm kinetin (D0K1); 0.5 ppm 2,4-D + 1.0 ppm kinetin (D0.5K1); 1.0 ppm 2,4-D (D1K0); and 1.0 ppm 2,4-D + 1.0 ppm kinetin (D1K1). The results showed that the medium with 1.0 ppm 2,4-D + 1.0 ppm kinetin produced the highest average fresh weight (0.568 g), which was significantly different from all other treatments. Somatic embryo formation was observed in all suspension culture treatments 14 days after culture (DAC). Observations at 42 DAC showed that embryo development in the 1.0 ppm 2,4-D + 1.0 ppm kinetin treatment had reached the heart stage (60%), while the other treatments showed embryos only at the globular stage.
Keywords: *Citrus nobilis* var. *microcarpa*; growth; indirect somatic embryogenesis; micropropagation

Abstrak: Serangan penyakit *Citrus Vein Phloem Degeneration* (CVPD) pada tanaman jeruk merupakan salah satu faktor penghambat dalam budidaya vegetatif konvensional. Ketersediaan bahan tanaman khususnya dalam budidaya jeruk Siam Pontianak, sangat penting bagi produktivitas dan keberlanjutan budidayanya. Perbanyakan melalui *indirect somatic embryogenesis* dari kalus embriogenik pada media cair dapat menghasilkan bibit yang bebas penyakit dalam jumlah lebih besar. Sel kalus bersifat embriogenik yang diinduksi dari eksplan kotiledon pada media MS

dengan penambahan 2,4-D dan kinetin memiliki potensi untuk berkembang menjadi individu baru. Penelitian ini bertujuan memperoleh kombinasi perlakuan terbaik sebagai media perbanyakan kalus embriogenik serta mengamati perkembangan embrio pada kultur suspensi sel. Penelitian menggunakan Rancangan Acak Lengkap (RAL) terdiri atas 2 tahap, yaitu: (1) optimasi perbanyakan kalus embriogenik pada media padat MS dengan penambahan 0,5 ppm 2,4-D + 0,5 ppm kinetin; 0,5 ppm 2,4-D + 1,5 kinetin; 1,0 ppm 2,4-D + 1,0 ppm kinetin; 1,5 ppm 2,4-D + 1,5 ppm kinetin, dan 2,0 ppm 2,4-D + 2,0 ppm kinetin; serta (2) proliferasi dan perkembangan embrio somatik pada kultur suspensi sel pada media MS cair dengan perlakuan: tanpa penambahan Zat Pengatur Tumbuh (D0K0); 1,0 ppm kinetin (D0K1); 1,0 ppm 2,4-D (D1K0); 0,5 ppm 2,4-D + 1,0 ppm kinetin (D0,5K1), dan 1,0 ppm 2,4-D + 1,0 ppm kinetin (D1K1). Hasil penelitian menunjukkan bahwa media dengan kombinasi 1,0 ppm 2,4-D + 1,0 ppm kinetin menghasilkan rerata berat basah kalus terbesar (0,568 g) dan berbeda nyata dengan semua perlakuan. Persentase pembentukan embrio somatik sebesar 100% terlihat pada semua perlakuan kultur suspensi umur 14 hari setelah tanam (hst). Pengamatan pada 42 hst menunjukkan bahwa perkembangan embrio pada perlakuan 1,0 ppm 2,4-D + 1,0 ppm kinetin telah mencapai tahap jantung (60%), sedangkan perlakuan lainnya hanya menunjukkan embrio pada tahap globular.
Kata kunci: *Citrus nobilis* var. *microcarpa*; pertumbuhan; embriogenesis somatik tidak langsung; mikropropagasi

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Introduction

West Kalimantan, a province that is one of the centers of local citrus production in Indonesia. Siam pontianak orange (*Citrus nobilis* var. *microcarpa*) is a type of citrus that is widely cultivated. Data from the West Kalimantan Agriculture Office shows that there was a 13.38% decline in Siam pontianak orange production from 2019 to 2020. Furthermore, according to the Central Statistics Agency (2020), in Sambas Regency, within a period of 2 years (from 2018 to 2020), the production of Siam pontianak orange in Sambas Regency decreased from 12.69 to 9.75 tons per hectare, or by 30%. Bagaskara *et al.* (2018) explained that the main cause of the decline in Siam pontianak orange production was crop failure due to an attack by the *Liberobacter asiaticus* bacterium, which causes Citrus Vein Phloem Degeneration (CVPD). The bacterial attack also shortened the life of the orange trees, causing them to die.

Conventional cultivation is a limiting factor for the propagation of Siam pontianak orange in West Kalimantan because it can only be done on a limited scale. Continuous conventional propagation can reduce production and quality (Margareta *et al*., 2019). An alternative step that can be taken to overcome this problem is in vitro propagation through somatic embryogenesis.

The process of plant regeneration from somatic cells, known as somatic embryogenesis, is carried out through a series of growth and development processes that resemble zygotic embryogenesis. The process begins with the formation of a proembryo, followed by the globular stage, heart stage, torpedo stage, cotyledon stage, maturation, and germination. Somatic embryos can be obtained through indirect somatic embryogenesis (Rai *et al*., 2022). Indirect somatic embryogenesis involves the formation of an embryogenic callus as a transitional stage before the formation of somatic embryos. The presence of the stage, enhances cell proliferation capacity, ultimately resulting in a greater number of somatic embryos than the direct somatic embryogenesis method (Ramírez-Mosqueda, 2022). An embryogenic callus comprises actively dividing cells with the competence to undergo differentiation and develop into somatic embryos Peng et al. (2022). The research by Kosmiatin et al. (2014) explains that embryogenic callus was successfully induced from *Citrus nobilis* Lour. cv Simadu endosperm explants grown on MS medium with 3 mg/L BA and 500 mg/L casein hydrolysate or malt extract.

Research results D'Onghia et al.(2001) report that Citrus Psorosis Virus (CPsV)-free plantlets can be formed through somatic embryogenesis induced from callus explants of the pistil stems of three types of citrus fruits, namely Common Mandarin, Sweet Orange, and Sweet Tangor. According to Olah et al. (2022), virus-free plant production has also been successfully achieved through somatic embryogenesis in several plants such as avocado (*Persea americana*, Lauraceae), black pepper (*Piper nigrum*, Piperaceae*)*, cassava *(Manihot esculenta*, Euphorbiaceae*),* different citrus species (*Citru*s spp., Rutaceae), cocoa (*Theobroma cacao*, Malvaceae), grapevine (*Vitis* spp., Vitaceae), and sugarcane (*Saccharum* spp., Poaceae). Keresa et al. (2021) research proves that somatic embryogenesis has shown greater effectiveness in eliminating viral infections from garlic (*Allium sativum* L.). Virus elimination success ranged from 13.3% up to 62.5%.

Induction of embryogenic callus from cotyledon of Siam Pontianak orange cultured on solid MS medium supplemented with 2,4-D (0.5 mg/L – 2 mg/L) + kinetin (0.5 mg/L – 2 mg/L) resulted in white and yellowish-white callus with a crumbly texture. Microscopic observation of 56-day-old callus revealed that embryo development was very slow; the stage of embryo development observed was the pro-embryo phase (Zakiah & Turnip, 2024). Malik et al. (2025) explain that the direct or indirect induction of somatic embryogenesis on solid media poses a challenge for propagation, as each callus cell has different propagation and embryogenic potential. The use of liquid media for propagation via somatic embryogenesis offers advantages because it allows for direct contact between callus cells and the medium, as well as adequate aeration through agitation. The uptake of nutrients and growth regulators proceeds efficiently, thereby enhancing callus growth and differentiation. Golami & Kaviani (2018) explained that the rate

of indirect embryogenesis in hybrid oranges [C. *paradisi* Macf. ('Duncan') × *C. reticulata* Blanco. ('Dancy')] was higher than that of direct embryogenesis, at 68.80% and 26.30%, respectively. Based on the above description, further research was conducted on the proliferation and somatic embryo development stages through indirect somatic embryogenesis via cell suspension culture.

Materials and Methods

Materials and tools

The tools and materials used in this study were glassware (Iwaki), Laminar Air Flow Cabinet as transfer room, an autoclave, shaker, and tweezers. The materials used in this study included solid and liquid Murashige-Skoog (MS) medium supplemented with a combination of 2,4-D and kinetin, Siam pontianak orange purchased directly from farmers in Tebas, Sambas Regency

Propagation of Embryogenic Callus

Callus was induced from cotyledon explants in a previous study. Cotyledons were cultured on solid MS medium supplemented with various combinations of 2,4-D and kinetin concentrations. The treatment combination that produced embryogenic callus was used as a callus propagation medium. The treatment consisted of solid MS medium with the addition of a combination of 0.5 ppm 2,4-D + 0.5 ppm kinetin; 0.5 ppm 2,4-D + 1.5 kinetin; 1.0 ppm 2,4-D +1.0 ppm kinetin; 1.5 2,4-D + 1.0 ppm kinetin and 2.0 ppm 2,4-D + 2.0 ppm kinetin (Zakiah & Turnip, 2024).

Callus propagation was carried out for 56 days of culture (with one subculture at 28 DAC). The culture was maintained at a temperature of 24±2°C, with a 16-hour light period and a light intensity of 2000–3000 lux using fluorescent lamps. Each treatment was replicated five times, resulting in 25 experimental units. The treatment combination that produced the highest wet weight of embryogenic callus was used for embryo proliferation in liquid MS medium.

Proliferation and Development of Somatic Embryos

The treatment with the highest wet callus weight was inoculated at a rate of 0.3 g into 30 mL of liquid MS medium with the following treatments: without PGR addition (D0K0); 1.0 ppm kinetin (D0K1); 0.5 ppm 2,4-D + 1.0 ppm kinetin (D0.5K1); 1.0 ppm 2,4-D (D1K0), and 1.0 ppm 2,4-D + 1.0 ppm kinetin (D1K1). Each treatment was replicated five times, resulting in 25 experimental units. The culture was incubated in an orbital shaker at a speed of 120 rpm. Proliferation was carried out for 42 days, with subculturing performed twice at 14-day intervals. The stage of embryo development was observed every week.

Observation Parameters

The growth parameters observed included: measurement of the wet weight of embryogenic callus by weighing the callus using an analytical balance to obtain the treatment with the highest wet weight of callus (g), the time of somatic embryo formation observed every week until the culture age of 42 days after culture, the percentage of treatments that formed somatic embryos (%), and observation of the percentage of embryo development stage in each treatment.

Data Analysis

The data obtained from the wet weight observations of callus were statistically analyzed using ANOVA with the SPSS 19.0 program. If a significant effect of treatment was obtained, a follow-up test was performed using Duncan's New Multiple Range Test (DNMRT) at a 5% level. Analysis of other observation data was performed descriptively, accompanied by figures and tables.

Results and Discussion

Propagation of Embryogenic Callus

The ANOVA test results show that the addition of 2,4-D and kinetin has a significant effect on the wet weight of callus. The best callus growth with the highest wet weight was obtained in the 1.0 ppm 2,4-D + 1.0 ppm kinetin (D1K1) treatment, which was 0.568 g (Table 1). Duncan's multiple range test showed that the wet weight of callus in the D1K1 treatment was significantly different from the other treatments.

Table 1. Average wet weight of callus (g) of Siam pontianak orange cotyledon culture on solid Murashige-Skoog medium with the addition of 2,4-D and kinetin at 56 DAC

Treatment combination		Average wet weight of callus (g)
2,4-D (ppm)	kinetin (ppm)	
0,5	0,5	0,223 ± 0,043[a]
1,0	1,0	0,568 ± 0,075[c]
0,5	1,5	0,183 ± 0,023[a]
1,5	1,5	0,353 ± 0,075[b]
2,0	2,0	0,370 ± 0,080[b]

Callus growth in all treatment combinations showed different growth rates (Figure 1). The figure shows that growth in the D1K1 treatment was better than other treatments based on the wet weight of the callus produced. These results indicate that to support the growth of Siam pontianak orange cotyledon callus, a balanced ratio between the concentration of auxin and cytokinin added to the medium and the endogenous hormones of the callus tissue is required. Maulida et al. (2020) explain that growth is characterized by irreversible weight gain, in the form of wet weight of callus, which can represent the variable of fresh weight growth. Physiologically, wet weight describes the water and carbohydrate content

in the callus. High water content will result in large fresh weight of callus. Furthermore, Maulana et al. (2019) explain that 2,4-D can stimulate cell division and enlargement and is often used for the induction of embryogenic callus. 2,4-D is also stable and not easily damaged by heat during sterilization. Almeida et al. (2018) explain that 2,4-D added to the medium can maximize somatic embryo formation after embryogenic callus induction. According to Elhiti & Stasolla (2022), the application of exogenous auxin causes an increase in endogenous hormone tissue content, which results in cell reprogramming to stimulate the embryogenesis process.

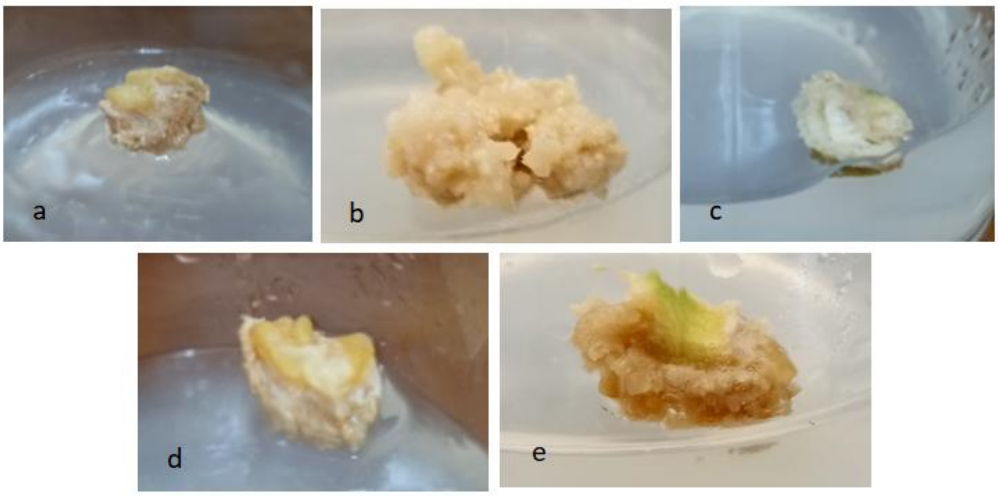


Figure 1. Growth of embryogenic callus from 56-day-old Siam pontianak orange cotyledon culture**.** (a) $D_{0,5}K_{0,5}$; (b) D_1K_1; (c) $D_{0,5}K_{1,5}$; (d) $D_{1,5}K_{1,5}$; (e) D_2K_2.

Proliferation and Development of Somatic Embryos in Suspension Culture

The results of observations of proliferation and somatic embryo development in 14 DAC liquid cultures showed that callus cells in all treatments did not exhibit significant growth (Figure 2). Microscopic observations revealed that two types of callus cells were found in all treatments, namely non-embryogenic cells (Figure 3a) and embryogenic cells (Figures 3b-c).

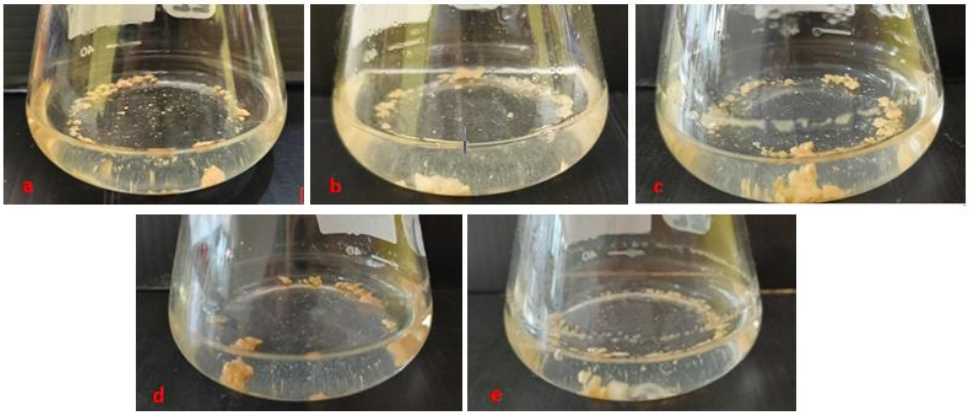


Figure 2. Growth of embryogenic callus on liquid MS medium with the addition of 2,4D and kinetin at 14 days after culture (a) D_0K_0; (b) D_0K_1; (c) $D_{0,5}K_1$; (d) D_1K_0, (e) D_1K_1

The appearance of somatic embryos in Siam pontianak orange suspension culture occurred faster than the appearance of somatic embryos in *Coffea liberica* callus culture. The globular phase somatic embryo in the Siam pontianak orange suspension culture was already visible at 14 DAC. Meanwhile, in the *Coffea liberica* callus culture, the formation of the globular phase embryo was visible in cultures six weeks after culture (Ardiyani et al., 2020).

Forty-two days after culture, the somatic embryo development stages observed in treatments D0K0, D0K1, D0.5K1, and D1K0 were pro-embryo stage (Figure 3d), globular stage with suspensor (Figure 3e), and late globular stage (Figures 3f-g). Based on the results obtained, the addition of 2,4-D was a factor that influenced the somatic embryogenesis process. Delgado-Aceves et al. (2025) explained that one of the synthetic auxins often used is 2,4-D because it increases cell division and maintains totipotency, making it a key component in the induction of somatic embryogenesis.

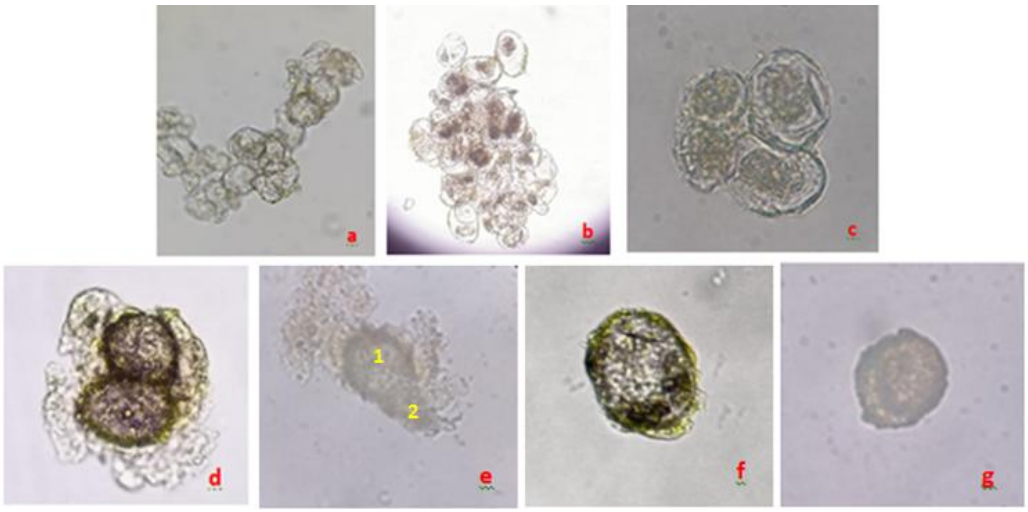


Figure 3. Microscopic observation of cells and somatic embryos formed in liquid culture in MS medium with treatments D0K1, D0.5K1, and D1K0, (a) non-embryogenic cells, (b) embryogenic

cells, (c-d) dividing embryogenic cells, (e) proembryo, with apical (1) and suspensor (2) cells, (f-g) late globular stage somatic embryos. 400x magnification.

Figure 3 e-g shows that proembryo formation is preceded by asymmetric division of embryogenic cells, followed by the formation of a globular stage embryo with a suspensor and finally the final globular stage. This observed process is consistent with the results obtained (Delgado-Aceves et al., 2025), which explain that somatic embryogenesis is indirect, characterized by asymmetric division of embryogenic cells that form apical and basal cells in *Agave maximiliana* cell cultures. Apical cells will develop into somatic embryos, while basal cells will become suspensors. As the proembryo develops, it forms a somatic embryo in the globular stage without a suspensor or with a short suspensor.

Microscopic observations of the D1K1 treatment liquid culture at 42 DAC showed that there were two stages of somatic embryo development, namely globular and heart (Figure 4). These results are in line with the study by Alfian et al. (2019), which explains that the number of embryogenic cells in sugarcane cell cultures can be increased by adding 2,4-D the proliferation stage in liquid media.

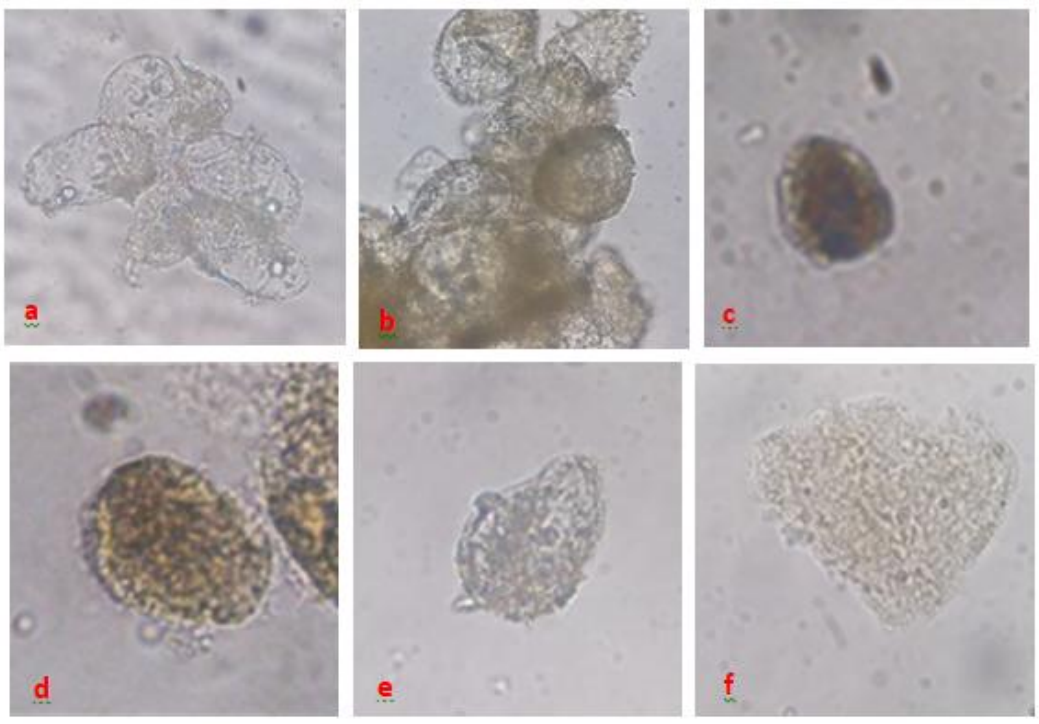


Figure 4. Microscopic observation of cells and somatic embryos formed in liquid culture in MS medium with D1K1 treatment, (a) embryogenic cells, (b-e) globular somatic embryos, (f) somatic embryos at the heart stage, 400x 400x magnification.

Maulidiya et al. (2020) explain that one of the genes that will be expressed and activated in cultures using 2,4-D as auxin for callus induction is Somatic Embryogenesis Receptor Kinase. The active expression of this gene supports somatic embryogenesis in culture. According to Král et al. (2022), the formation of somatic embryos in culture is influenced by 2,4-D, which acts as a plant growth regulator and herbicide. The addition of 2,4-D can increase the synthesis of ABA and ethylene. Increased ABA concentration induces the expression of LEA (Leaf Embryogenesis Abundant) protein, which is responsible for somatic embryo development.

The percentage of somatic embryo development stages is shown in Table 2. All treatments showed the formation of globular-stage embryos. Heart-stage embryo development was observed only in the treatment combination of 1.0 ppm 2,4-D + 1.0 ppm kinetin, at a rate of 60%. This percentage was calculated based on the number of replicates showing heart-stage embryo development in each treatment (Table 2)

Tabel 2. Percentage of embryonic development stages in suspension culture of Siam pontianak orange on MS liquid medium supplemented with 2,4-D and kinetin at 42 days after culture

Treatment combination		Percentage of somatic embryo development (%)	
2,4-D (ppm)	kinetin (ppm)	globular	heart
0,0	0,0	100	0
0,0	1,0	100	0
0,5	1,0	100	0
1,0	0,0	100	0
1,0	1,0	100	60

Based on observations of somatic embryo development, it appears that 2,4-D and kinetin added to MS liquid medium has a greater affects on embryo induction and proliferation. These embryos were unable to develop into mature embryos. The results of this study are inconsistent with those of Riyadi (2010), who found that kinetin was added to the culture medium tends to affect the maturation and germination rates of somatic embryos of *Metroxylon sagu*. These results also contradict the statement to Rai et al. (2022), somatic embryos will form and develop into planlets when cells are cultured in media containing low concentrations of auxin. The development and maturation of embryos through phase changes from globular to cotyledonary phases occur when embryogenic callus is transferred to media without auxin. Based on this, further research is needed to determine the optimal medium combination for somatic embryo maturation, such as by adding of other type plant growth regulator or various types of sugars or nitrogen sources, or a combination of both.

Conclusion

The results showed that MS solid medium with a combination of 1.0 ppm 2,4-D + 1.0 ppm kinetin produced the best callus growth with the highest average wet weight (0.568 g) and was significantly different from all other treatments. Somatic embryo formation was observed in all suspension culture treatments at 14 DAC, with all treatments capable of forming somatic embryo at a rate of 100%. Observations of 42 DAC showed that embryo development in the 1.0 ppm 2,4-D + 1.0 ppm kinetin treatment had reached the heart stage (60%), while the other treatments showed embryos development only at the globular stage. These findings provide a fundamental basis for developing a mass propagation protocol for CVPD-free Pontianak Siamese oranges via suspension culture, although further research is needed to optimize embryo maturation.

Conflict of interest

The authors declare that they have no conflicts of interest. Author roles: Author* (Zulfa Zakiah)/corresponding author: Conceptualization, methodology, and data curation, checking, evaluation, editing, and journal submission.
Author* (Masnur Turnip): Methodology, evaluation, writing, editing

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References

Alfian, F. N., Afdhoria, N. N., Dewanti, P., Restanto, D. P., & Sugiharto, B. (2019). Liquid Culture of Somatic Embryogenesis Cell Proliferation of Sugarcane (*Saccharum officinarum*). *International Journal of Agriculture & Biology*, 21(4), 905–910. https://doi.org/10.17957/IJAB/15.0974

Almeida, R. de O., Silva, S. D. dos A. e, & Dutra, L. F. (2018). Induction of Somatic Embryogenesis and Plant Regeneration of Sugarcane cv. RB925345 From Somatic Embryos. *Scientia Agraria Paranaensis*, 17(2), 165–172.

Ardiyani, F., Setiti, E. D. Y., Utami, W., Purnobasuki, H., & Paramita, S. A. (2020). Development and Regeneration of Somatic Embryos From Leaves-Derived Calli of *Coffea liberica*. *Biodiversitas*, 21(12), 5829–5834. https://doi.org/10.13057/biodiv/d211246

Badan Pusat Statistik. (2020). *Kabupaten Sambas Dalam Angka 2020*. BPS-Statistics of Sambas Regency.

Bagaskara, B. A., Wirawan, I. G. P., Sritamin, M., & Yuniti, I. G. A. D. (2018). Perbanyakan Tanaman Jeruk Siam (*Citrus nobilis* L.) Dengan Teknik

Kultur *In Vitro* Menggunakan Biji Tanaman Terinfeksi Penyakit *Citrus Vein Phloem Degeneration* (CVPD). *AJoAS (Agrotrop Journal of Agricultural Science)*, 8(2), 179–188. https://doi.org/10.24843/AJoAS.2018.v08.i02.p09

D'Onghia, A. M. D., Carimi, F., Pasquale, F. De, Djelouah, K., & Martelli, G. P. (2001). Elimination of *Citrus Psorosis Virus* by Somatic Embryogenesis From Stigma and Style Cultures. *Plant Pathology*, 50(2), 266–269. https://doi.org/10.1046/j.1365-3059.2001.00552.x

Delgado-Aceves, L., Corona, S., Castañeda-Nava, J., Rodríguez, M., & Gutiérrez, A. (2025). Indirect Somatic Embryogenesis of *Agave maximiliana* Baker. *Frontiers in Plant Science*, 16, Pasal 1648362. https://doi.org/10.3389/fpls.2025.1648362

Elhiti, M., & Stasolla, C. (2022). Transduction of Signals During Somatic Embryogenesis. *Plants*, 11(2), Pasal 178. https://doi.org/10.3390/plants11020178

Gholami, A. A., & Kaviani, B. (2018). Somatic Embryogenesis, Encapsulation, Cold Storage, and Growth of Hybrid *Citrus* [*C. paradisi* Macf. ('Duncan') × *C. reticulata* Blanco. ('Dancy')] Shoot Tip Segments. *Indian Journal of Biotechnology*, 17(1), 134–144.

Kereša, S., Kurtović, K., Ban, S. G., Vončina, D., Jerčić, I. H., Bolarić, S., Lazarević, B., Godena, S., Ban, D., & Mihovilović, A. B. (2021). Production of Virus-Free Garlic Plants Through Somatic Embryogenesis. *Agronomy*, 11(5), Pasal 876. https://doi.org/10.3390/agronomy11050876

Kosmiatin, M., Purwito, A., Wattimena, G. A., & Mariska, I. (2014). Induksi Embriogenesis Somatik Dari Jaringan Endosperma Jeruk Siam (*Citrus nobilis* Lour.) cv. Simadu. *Jurnal Agronomi Indonesia*, 42(1), 44–51. https://doi.org/10.24831/jai.v42i1.8213

Král, D., Šenkyřík, J. B., & Ondřej, V. (2022). Expression of Genes Involved in ABA and Auxin Metabolism. *Plants*, 11(21), Pasal 2995. https://doi.org/10.3390/plants11212995

Malik, M., Mazur, J., & Kapczyńska, A. (2025). Optimizing Somatic Embryogenesis and Biomass Proliferation in *Narcissus* L. 'Carlton' Callus Lines Using Solid and Liquid Media. *Agronomy*, 15(11), Pasal 2460. https://doi.org/10.3390/agronomy15112460

Margareta, F., Budianto, B., & Sutoyo, S. (2019). Studi Tentang Metode Perbanyakan Tanaman Jeruk Siam Pontianak (*Citrus nobilis* var. *microcarpa*) Secara Vegetatif Di Kebun Percobaan Punten Desa Sidomulyo Kota Batu. *Jurnal Agrotech*, 2(1), 26–29.

Maulana, M. R., Restanto, D. P., & Slameto, S. (2019). Pengaruh Konsentrasi 2,4-Dichlorophenoxyacetic Acid (2,4-D) Terhadap Induksi Kalus Tanaman Sorgum (*Sorghum bicolor* (L.) Moench). *Jurnal Bioindustri*, 1(2), 138–148. https://doi.org/10.31326/jbio.v1i2.223

Maulida, D., Erfa, L., Sesanti, R. N., & Hidayat, H. (2020). Induction of Kopyor Coconut Embryogenic Callus Using 2,4-D and TDZ. *IOP Conference Series: Earth and Environmental Science*, 411(1), Pasal 012013. https://doi.org/10.1088/1755-1315/411/1/012013

Maulidiya, A. U. K., Sugiharto, B., Dewanti, P., & Handoyo, T. (2020). Expression of Somatic Embryogenesis-Related Genes in Sugarcane (*Saccharum officinarum* L.). *Journal of Crop Science and Biotechnology*, 23(3), 221–229. https://doi.org/10.1007/s12892-020-00024-x

Oláh, R., Turcsán, M., Oláh, K., Farkas, E., Deák, T., Jahnke, G., & Sárdy, D. A. N. (2022). Somatic Embryogenesis: A Tool for Fast and Reliable Virus and Viroid Elimination for Grapevine and Other Plant Species. *Horticulturae*, 8(6), Pasal 508. https://doi.org/10.3390/horticulturae8060508

Peng, C., Gao, F., Wang, H., Tretyakova, I. N., Nosov, A. M., Shen, H., & Yang, L. (2022). Suspension Culture and Somatic Embryogenesis of Korean Pine. *Phyton-International Journal of Experimental Botany*, 91(1), 223–238. https://doi.org/10.32604/phyton.2022.015523

Rai, A. C., Kumar, A., Modi, A., & Singh, M. (2022). *Advances in Plant Tissue Culture: Current Developments and Future Trends*. Elsevier.

Ramírez-Mosqueda, M. A. (2022). Overview of Somatic Embryogenesis. Dalam M. A. Ramírez-Mosqueda (Ed.), *Somatic Embryogenesis: Methods and Protocols* (hlm. 1–8). Springer. https://doi.org/10.1007/978-1-0716-2485-2_1

Riyadi, I. (2010). Pengaruh Kinetin dan BAP Terhadap Pertumbuhan dan Perkembangan Embrio Somatik Tanaman Sagu (*Metroxylon sagu* Rottb.). *Jurnal AgroBiogen*, 6(2), 101–106. https://doi.org/10.21082/jbio.v6n2.2010.p101-106

Zakiah, Z., & Turnip, M. (2024). Induction of Somatic Embryogenesis of Pontianak Siamese Orange Cotyledon Cultures on Murashige-Skoog Media With the Addition of 2,4-D and Kinetin. *Jurnal Biologi Tropis*, 24(2), 241–250. https://doi.org/10.29303/jbt.v24i2.6721