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Picloram-Induced Embryogenic Callus Formation and Proliferation from Nucellar Embryo of Mandarin 'Berastagi' (*Citrus reticulata* Blanco)

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Abstract

Mandarin 'Berastagi' (*Citrus reticulata* Blanco) is a key horticultural commodity in North Sumatra, Indonesia, with increasing demand for high-quality, genetically uniform planting material. Biotechnological approaches, particularly somatic embryogenesis, hold significant potential enhancing propagation efficiency and crop quality. This study aimed to determine the optimal culture medium and picloram concentration for the induction and proliferation of embryogenic callus from nucellar explants of Mandarin 'Berastagi' in vitro. Two experiments were conducted: (1) induction of embryogenic callus from nucellar embryos isolated from young fruits (60–70 days after anthesis) cultured on different basal media supplemented with varying picloram concentrations, and (2) proliferation of embryogenic callus on media containing different carbohydrate concentrations. The Murashige and Wetmore (MW) medium supplemented with 1.5 mg/L picloram resulted in the highest callus induction rate (37.8%) and largest mean callus diameter (17.8 mm). For proliferation, MW medium containing 4% sucrose produced the best response, with an average callus diameter of 14 mm and 96% of explants developing globular-stage somatic embryos. These results establish an efficient somatic embryogenesis protocol that can support large-scale propagation and future crop improvement programs in Mandarin 'Berastagi'.

Keywords: carbohydrate, lactose, picloram, somatic embryogenesis, sucrose

Introduction

Mandarin 'Berastagi' (*Citrus reticulata* Blanco) is one of the most widely cultivated citrus varieties in Karo Regency, North Sumatra, Indonesia (Simbolon et al., 2025). This cultivar is distinguished by its thick yellow peel with a smooth texture, sweet flavor, and high vitamin C content (Anggreani et al., 2021). It contains approximately 13.5 °Brix of soluble solids (Balai Penelitian Jeruk dan Buah Subtropika, 2020) and 51 mg/100 g of vitamin C (Tarigan, 2017), which is substantially higher than the average vitamin C content reported for most tangerine varieties (31 mg/100 g) (Lathifah et al., 2022). These nutritional attributes position Mandarin 'Berastagi' as a superior local cultivar with strong market potential. However, the increasing year-round demand for mandarins has intensified the need for high-quality planting material. Citrus production in Karo Regency faces several constraints, including high susceptibility to pests and diseases, aging orchards, suboptimal seedling quality, and inadequate post-harvest handling practices (Sembiring, 2024). Among these challenges, the limited availability of high-quality rootstock seedlings represents a critical bottleneck, highlighting the need for advanced propagation strategies to improve seedling quality and production efficiency (Bhardwaj et al., 2025).

In vitro culture is a well-established technique for the aseptic cultivation of plant tissues or organs from donor plants on artificial nutrient media (Baviskar & Patil, 2021). One of the advanced techniques in plant tissue culture

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is somatic embryogenesis, in which somatic cells develop into embryos without fertilization. This process mimics zygotic embryogenesis and progresses through distinct stages: proembryo, globular, heart-shaped, torpedo, cotyledonary, and ultimately germination (Qi et al., 2021). Somatic embryogenesis plays a vital role in plant biotechnology and offers several advantages, including rapid production of uniform seedlings, independence from seasonal constraints, and applicability to genetic transformation. In citrus, somatic embryogenesis has been successfully induced in various explants, such as endosperm tissue (Kosmiatin, 2014), epicotyls (Kazmi et al., 2015), nucellar embryos (Murugan et al., 2023), stigma (Catalano et al., 2022), roots and leaves (Sidhu et al., 2023), and cotyledons (Zakiah, 2024). Citrus seeds are typically polyembryonic, and nucellar embryos originating from maternal tissues are produced without fertilization. Consequently, these embryos are genetically identical to the mother plant and lack the heterozygosity observed in zygotic embryos, making them candidates for clonal propagation (Wahyudhi, 2020).

The success of somatic embryogenesis is strongly influenced by the composition of the culture medium, plant growth regulators, explant type, and environmental conditions (Chandoliya et al., 2025). The use of nucellar embryo explants requires a detailed understanding of their growth characteristics (Murugan et al., 2023). These explants consist of highly mitotically active cells that respond rapidly to nutrient availability; however, they are also highly sensitive to stress. Kazmi et al. (2015) reported that nucellar embryos of 'Kinnow' mandarin can produce soft, friable, and embryogenic callus on Murashige and Tucker (MT) medium supplemented with 2,4-D (2.5 mg/L) and BAP (0.5 mg/L). Nevertheless, prolonged exposure to high concentrations of 2,4-D has been associated with epigenetic and genetic instability, including alterations in DNA methylation patterns and increased mutation frequency, which may result in abnormal embryo development (Garcia et al., 2019). Consequently, the use of alternative auxins is recommended to promote cell division while minimizing somaclonal variation and albino regenerants.

Picloram is a highly effective synthetic auxin known to induce stress-related responses that promote cellular dedifferentiation and embryogenic callus formation. In young leaf explants, picloram has been shown to induce significantly higher frequencies of embryogenic callus (73.3%–93.3%) compared with 2,4-D (16.7%–23.3%) (Wahyuni, 2025). The resulting calli are typically friable and loosely organized, with high proliferation capacity, traits advantageous for plant regeneration and transformation studies (Ahansal et al., 2022). In *Gerbera jamesonii*, picloram at 1 mg/L induced callus formation as early as 8 days after treatment, producing the largest callus size and highest proliferation rate, with a diameter of 43.0 ± 2.1 mm, fresh weight of 651.7 ± 6.9 mg, and dry weight of 66.7 ± 2.3 mg (Gantait et al., 2021). Furthermore, picloram has been reported to promote the regeneration of green, morphologically normal plants (Takamori et al., 2015).

Both culture medium composition and picloram concentration play critical roles in determining explant responses. Moderate concentrations of plant growth regulators (approximately 1–8 mg/L) generally optimize callus induction, whereas insufficient levels fail to induce dedifferentiation and excessive concentrations can inhibit biomass accumulation, increase phenolic browning, and exacerbate oxidative stress in explant tissues (Bahrunnisa et al., 2025; Gantait et al., 2021). This variability presents a challenge in defining optimal conditions for specific genotypes. Therefore, this study aimed to determine the optimal culture medium and picloram concentration for the induction and proliferation of embryogenic callus from nucellar explants of Mandarin 'Berastagi' in vitro.

Materials and Methods

This study was conducted from April 2024 to March 2025 at Tissue Culture Laboratory 1, Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University, Bogor, Indonesia. The materials comprised nucellar

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embryos isolated from young citrus fruits (60–70 days after anthesis, with a diameter of 2–3 cm) obtained from Mandarin 'Berastagi' parent plants cultivated in Karo Regency, North Sumatra, Indonesia. Additional materials included macronutrients, micronutrients, vitamins, plant growth regulators (PGRs), malt extract, agar, distilled water, potassium hydroxide (KOH), sodium hydroxide (NaOH), rubber, and plastic.

The first experiment was arranged in a completely randomized factorial design (CRD) with two factors. The first factor was the type of basal medium, consisting of three levels: Murashige and Skoog (MS), Murashige and Tucker (MT), and Murashige and Skoog medium supplemented with Wetmore vitamins (MW). The second factor was the concentration of picloram, consisting of five levels: 0.0, 0.5, 1.5, 2.5, and 3.5 mg/L (Sharma et al., 2024). The experiment comprised 15 treatment combinations, each replicated four times, resulting in a total of 60 experimental units. Each unit consisted of three culture bottles, each containing three nucellar embryos. In total, 180 culture bottles were used, representing 540 explants. Explant development was observed weekly over a 12-week period. The variables observed included the percentages of explants forming callus and embryogenic callus, callus diameter (mm), callus color, and callus type. Callus type was assessed by visual examination of physical characteristics. Callus color was observed using the Munsell Color Chart to ensure standardized color evaluation.

Embryogenic crumb-type callus from the best treatment in the first experiment was transferred to a proliferation medium consisting of MW at 7 mg/L agar, 500 mg/L malt extract, and 2.5 mg/L picloram. Callus was cultured under varying carbohydrate treatments to evaluate the effect of sugar type and concentration on callus proliferation (Kayim et al., 2006). This experiment was conducted using a completely randomized design with a single factor: carbohydrate treatment. The treatments included 4% and 5% lactose and 4% and 5% sucrose. Each treatment was replicated four times, resulting in 16 experimental units. For each replicate, a 5-mm-diameter clump of embryogenic callus was planted into a culture vessel and incubated in complete darkness. After eight weeks of incubation, the following parameters were recorded: callus diameter (mm), percentage of explants forming globular-stage embryos, callus color, and callus type. Callus diameter was measured using a millimeter scale for precision. The percentage of explants forming globular embryos was calculated by dividing the number of explants that developed into globular embryos by the total number of embryogenic calli and multiplying by 100%. Somatic embryo maturation was performed using MW medium supplemented with 1 mg/L BAP.

The data obtained from the experiment were analyzed to determine the effects of the treatments using analysis of variance (*F* test) at a significance level of α = 5%. Further testing was conducted using the Duncan's multiple range test (DMRT) test at a significance level of α = 5%, and polynomial regression analysis was performed using the Statistical Tool for Agricultural Research (STAR) and Microsoft Excel.

Results and Discussion

Induction of Embryogenic Callus

Percentage of Explants Forming Callus

The results of the callus induction experiment showed that the interaction between medium type and picloram concentration was not statistically significant for the percentage of explants forming callus. However, both factors independently significantly increased callus formation ($p < 0.05$) (Table 1). Picloram a potent synthetic auxin, can enhance callus induction at specific concentrations by promoting rapid cell division and callus formation (Gerdakaneh & Zohori, 2013). To estimate the optimal concentration for callus induction, a quadratic polynomial regression model was fitted to the data (Figure 1).

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Table 1

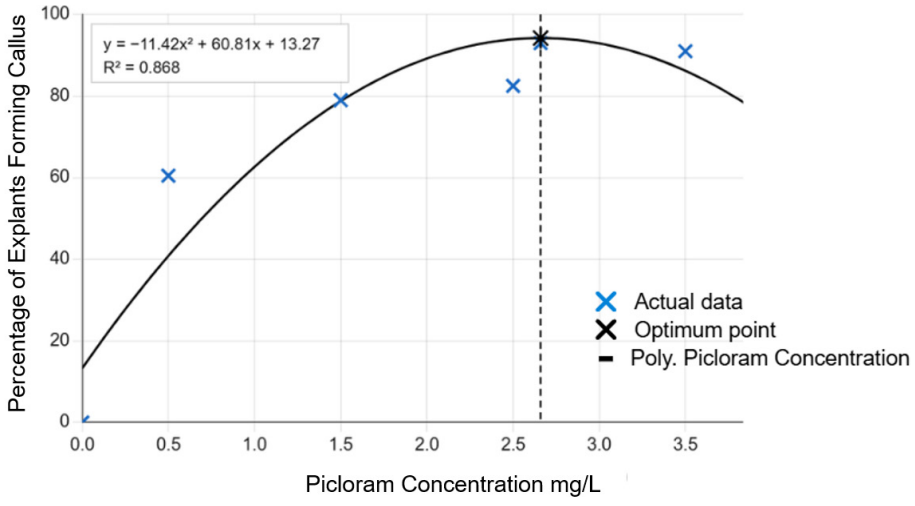
Effects of Medium Type and Picloram Concentration on Callus Color and Callus Type for Eight Weeks

Types of media	Picloram (mg/L)	Callus type	Callus color
MS	0.5	Friable	Brownish white
	1.5	Friable	White
	2.5	Friable	Brownish white
	3.5	Friable	Brownish white
MT	0.5	Friable	White
	1.5	Intermediate	Greenish white
	2.5	Compact	White
	3.5	Friable	Brownish white
MW	0.5	Friable	White
	1.5	Friable	White
	2.5	Friable	Yellowish green
	3.5	Compact	Greenish white

Note. MS = Murashige and Skoog medium, MT = Murashige and Tucker medium, MW = Murashige and Wetmore medium.

Figure 1

Effect of Picloram Concentration on the Percentage of Explants Forming Callus in Mandarin 'Berastagi' at 12 Weeks After Planting (WAP)



Picloram concentration exhibited a quadratic effect on callus formation at 12 weeks after planting, as described by the equation $Y = -11.42x^2 + 60.81x + 13.27$ with a coefficient of determination of $R^2 = 0.868$, indicating a strong model fit. The model predicted an optimal picloram concentration of 2.66 mg/L, corresponding to a maximum callus induction rate of 94%. In contrast, the highest observed callus formation in the experimental data occurred at 3.5 mg/L, with a slightly lower value of 91%. This discrepancy suggests a plateau in the response near the optimum, where further increases in picloram concentration do not yield

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proportional gains in callus induction and may begin to exert inhibitory effects. Overall, both the regression analysis and empirical observations confirm that picloram enhances callus formation within an optimal concentration range, beyond which its effectiveness stabilizes or declines.

In the present study, Murashige and Wetmore (MW) medium was significantly more effective than Murashige and Skoog (MS) and Murashige and Tucker (MT) media, resulting in a 10% higher callus induction rate. This enhanced performance may be attributed to the presence of biotin in MW medium, a key cofactor involved in essential metabolic pathways, including fatty acid biosynthesis and amino acid metabolism, which support active cell division and callus formation (Abdelsalam et al., 2018). Variations in nutrient and vitamin composition among tissue culture media necessitate optimization based on the specific physiological requirements of each plant species (Abrahamian et al., 2011).

Callus initiation on MW medium was initially indicated by explant enlargement, followed by slight tissue browning and the emergence of callus, predominantly at the edges and basal regions of the explant. These responses were not observed in the control treatment lacking picloram. The absence of callus formation in the control highlights the critical role of auxin in inducing cell elongation, division, and subsequent dedifferentiation processes required for callus development (Junairiah et al., 2021).

Percentage of Explants Forming Embryogenic Callus

The results showed that the interaction between the medium type and picloram concentration did not significantly influence the formation of embryogenic calluses in Mandarin 'Berastagi'. Among the treatments, the highest percentage of explant-forming embryogenic callus was observed at 1.5 mg/L picloram (Table 2). This response, however, was not significantly different from that observed at 2.5 mg/L, which resulted in 32.4% embryogenic callus formation. These findings suggest that moderate concentrations of picloram are more effective for inducing embryogenic potential in nucellar embryo explants of Mandarin 'Berastagi'.

The effect of picloram concentration on the percentage of explants forming embryogenic callus exhibited a quadratic response, as illustrated in Figure 2. The graph indicates that not all calli formed were embryogenic. The fitted regression equation was $Y = -8.68x^2 + 35.69x - 0.01$, with a high coefficient of determination $R^2 = 0.983$, indicating an excellent fit between the model and the observed data. Based on this polynomial model, the predicted optimal picloram concentration for embryogenic callus formation was 2.06 mg/L, with a corresponding estimated maximum embryogenic callus formation of 36%. However, the actual data revealed that the highest observed percentage (36.58%) occurred at 1.5 mg/L picloram. This close alignment between the model and experimental results confirms the validity of the quadratic regression model

Table 2

*Effects of Carbohydrate Type and Concentrations on Mandarin 'Berastagi' Callus Characteristics**

Carbohydrate	Callus diameter (mm)	Percentage of explants forming globular-stage embryos (%)	Callus color	Callus type
Lactose 4%	9.17 b	83	Brownish white	Friable
Lactose 5%	9.33 b	75	Brownish green	Friable
Sucrose 4%	14.00 a	96	Greenish white	Friable
Sucrose 5%	11.17 a	88	Greenish white	Compact

Note. *After 8 weeks of culture. Values followed by the same letter within each column are not significantly different ($p > 0.05$) according to Duncan's multiple range test (DMRT).

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and its potential to identify optimal picloram concentrations that promote embryogenic callus development in Mandarin 'Berastagi'.

Embryogenic Callus Diameter (mm)

The results showed that a significant interaction was observed between media types and picloram concentration, indicating that their combined effect influenced embryogenic callus diameter. The largest callus diameter (17.8 mm) was obtained from nucellar embryos cultured on MW medium supplemented with 1.5 mg/L picloram; however, this value was not significantly different from those obtained at 0.5 mg/L (16.6 mm), 2.5 mg/L (17.5 mm), and 3.5 mg/L (17.5 mm). A quadratic polynomial regression model was applied to determine the optimal treatment combination for callus growth (Figure 3).

Figure 2

Effect of Picloram Concentration on the Percentage of Explants Forming Embryogenic Callus in Mandarin 'Berastagi' After 12 Weeks After Planting (WAP)

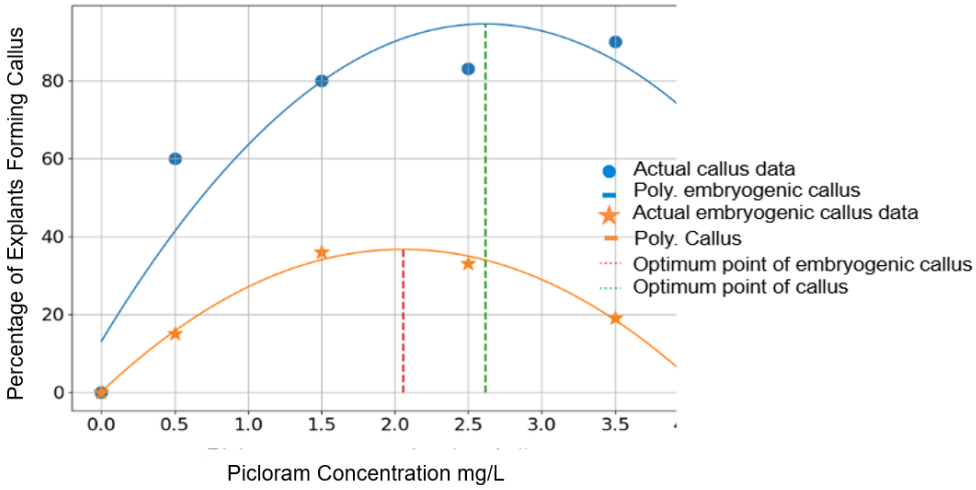
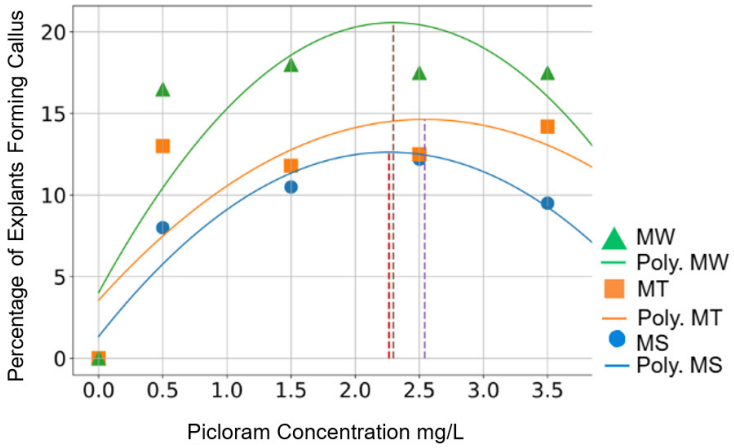


Figure 3

Effect of the Interaction Between Media Type and Picloram Concentration on the Embryogenic Callus Diameter of Mandarin 'Berastagi' at 12 Weeks After Planting (WAP) and Cultured on an MW Medium



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The type of basal medium used and the concentration of picloram exhibited a quadratic effect on the diameter of the embryogenic callus at 12 WAP. As shown in Figure 3, the callus diameter on the MW medium was consistently greater than that observed on the MS and MT media at most picloram concentrations. A quadratic polynomial regression model was fitted to describe the callus diameter response to picloram concentration on an MW medium. The resulting equation was $Y = -3.10x^2 + 14.27x - 4.07$, with a coefficient of determination (R^2) = 0.729, indicating a moderately strong fit. Based on the model, the predicted optimal picloram concentration for maximum callus growth on MW medium was 2.3 mg/L, corresponding to a theoretical maximum diameter of 20.5 mm. However, empirical data showed that near-optimal diameter was already achieved at 1.5 mg/L, with an average embryogenic callus diameter of 17.8 mm. These findings confirm the effectiveness of MW medium, particularly when combined with moderate picloram concentrations, in supporting robust embryogenic callus development in Mandarin 'Berastagi'.

Further analysis of Figure 3 revealed that callus diameter responded quadratically to picloram concentration across all three media types. For MS medium, the regression was $Y = -2.19 x^2 + 9.93x - 1.37$, with a coefficient of determination (R^2) = 0.908, indicating a strong model fit. The model predicted an optimal picloram concentration of 2.26 mg/L, corresponding to a maximum callus diameter of approximately 12.9 mm. However, empirical data showed that the actual peak diameter on MS medium was 12.2 mm at 2.5 mg/L, then decreased to 9.5 mm at 3.5 mg/L. This decline suggests that excessively high picloram concentrations in MS medium may be toxic to developing embryogenic callus, likely inhibiting cell division and differentiation. For the MT medium, the regression equation was $Y= -1.72 x^2 + 8.75x - 3.60$, with a coefficient of determination (R^2) = 0.629, indicating a moderate fit. The predicted optimal concentration was 2.54 mg/L, yielding a theoretical callus diameter of 14.7 mm. However, the highest observed diameter on MT medium was 14.3 mm at 3.5 mg/L picloram. Comparatively, the MW medium consistently produced the largest callus diameters across a range of picloram concentrations, confirming its superior efficacy in supporting embryogenic callus growth in Mandarin 'Berastagi'. The combination of enhanced vitamin content in MW medium, particularly the inclusion of biotin, and optimal picloram levels likely improved cellular metabolism and morphogenetic competence.

Callus Texture and Color

Based on qualitative observations, three types of embryogenic callus were identified: friable, intermediate, and compact (Figure 4). The callus type serves as a useful indicator for assessing cellular activity and helps distinguish actively dividing cells from those that have become quiescent or are undergoing senescence (Ariani et al., 2016). Compact calluses are typically characterized by a dense structure and are often associated with lignification, a process influenced by specific growth regulators that also affect nutrient transport within the tissue (Mahadi et al., 2016). In contrast, the intermediate callus type consisted of heterogeneous masses, in which both friable and compact regions coexisted, reflecting variable cellular activity across the callus mass. The friable callus type was considered the most favorable for in vitro plant propagation because it readily disaggregates into individual cells of small cell clusters capable of forming somatic embryos. Conversely, compact calluses are more suitable for secondary metabolite production due to their stable structure and metabolic characteristics (Indah & Ermavitalini, 2013).

The callus color varied depending on the type of culture medium used. Callus on MS medium was generally off-white to brownish, indicating reduced cell viability and possible oxidative stress. In contrast, callus culture on MW medium exhibited a light greenish-white appearance, which is commonly associated with healthier, metabolically active cells and a greater potential for somatic embryogenesis. The improved color and quality of callus on MW medium can be attributed to the presence of biotin

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and calcium pantothenate, which are absent from the standard MS and MT formulations. This finding aligns with the results reported by Wulansari et al. (2015), who demonstrated that MW medium is more effective than MS and MT media for inducing embryogenic callus.

Effect of Carbohydrate Source on Embryogenic Callus Proliferation

Embryogenic Callus Diameter

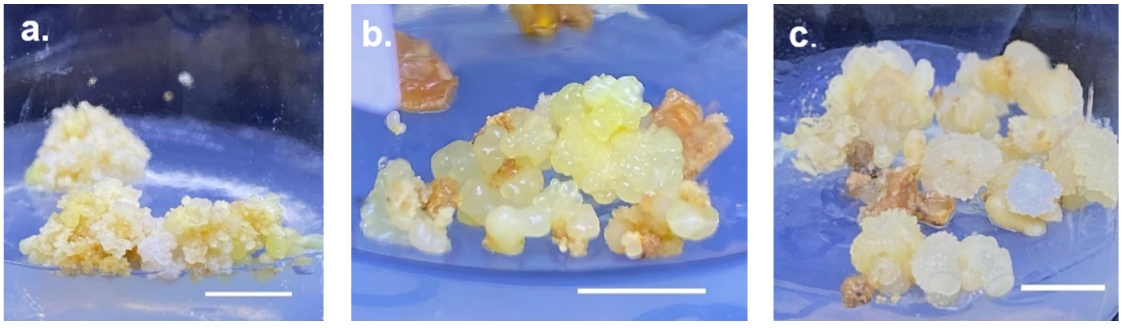
The results showed that carbohydrate type and concentration had a significant effect on embryogenic callus diameter but did not significantly affect the percentage of globular-stage somatic embryos (Table 2). Among the treatments, sucrose resulted in the largest callus diameter, with values significantly higher than those produced by lactose. The application of 4% sucrose resulted in the largest average callus diameter (14.00 mm), although this was not significantly different from the diameter observed with 5% sucrose (11.17 mm). These findings confirm that sucrose is a more effective carbohydrate source than lactose for supporting embryogenic callus proliferation in Mandarin 'Berastagi'.

The effectiveness of carbohydrate sources in supporting callus growth is closely related to their chemical structure and metabolic compatibility with plant cells. Lactose is a disaccharide composed of glucose and galactose; however, in the absence of specific enzymes, such as β-galactosidase, plant cells are unable to hydrolyze lactose efficiently. Consequently, lactose remains in a polymeric form and cannot be readily absorbed or utilized by the callus for energy or biosynthetic processes (Syahrizal & Puspita, 2020). In contrast, sucrose and glucose are more metabolically accessible. These sugars are either directly absorbed or rapidly hydrolyzed into usable monosaccharides, providing a more efficient source of carbon and energy for cell division, morphogenesis, and tissue development. This metabolic advantage explains their superior ability to support embryogenic callus proliferation. The structural limitation of lactose, combined with the lack of enzymatic capacity in plant tissue cultures to degrade it, renders it ineffective as a primary carbohydrate source during callus induction (Zhang et al., 2020).

The objective of callus proliferation is to increase the biomass of embryogenic callus, thereby enhancing the potential for somatic embryo production and ultimately increasing the number of regenerated plantlets compared to the direct regeneration method. Previous studies support this approach; for example, Wulansari et al. (2015) reported that embryogenic callus nearly doubled in size when sub cultured on MW medium. Similarly, Sidhu (2023) demonstrated that increasing the sucrose concentration from 3% to 10% significantly improved callus induction in *Citrus jambhiri* Lush, and was more effective

Figure 4

Morphological Types of Embryogenic Callus in Mandarin 'Berastagi' after 12 Weeks of Culture



Note. a. friable; b. intermediate; c. compact. Scale bar 5 mm.

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than treatment using maltose and glycerol. In the present study, the combination of MW medium and elevated sucrose concentration (4%) resulted in a nearly threefold increase in callus size, from an initial diameter of 5 mm to 14 mm, after 8 weeks of culture. These findings highlight the critical role of both medium composition and carbohydrate source in optimizing callus proliferation and provide new insights into tissue culture of Mandarin 'Berastagi'.

Percentage of Explants Forming Globular Embryos

Carbohydrate treatments did not significantly affect the percentage of explants forming globular-stage embryos (Table 2). However, qualitative differences were observed in embryo development and callus characteristics. In this study, the percentage of explants forming globular embryos in the 4% sucrose treatment reached 96%, which was the highest among all carbohydrate treatments, although the difference was not statistically significant. The callus color in the proliferation medium ranged from greenish-white to brownish-white and brownish-green, indicating variable physiological responses across treatments. Although the type of carbohydrate did not significantly affect the initiation of somatic embryos, it appeared to influence the rate of embryo maturation. Notably, lactose promoted the formation of embryos at more advanced stages, including the heart and torpedo stages, but was also associated with increased callus browning, likely due to oxidative stress. By the end of the observation period, the lowest percentage of globular-stage embryo formation was recorded for the lactose treatment (75%). These findings are partially in agreement with Schuller (1993), who reported that lactose could outperform other carbohydrate treatments, such as glucose, fructose, galactose, and sucrose, in promoting synchronized somatic embryo maturation and the formation of morphologically normal embryos.

The 5% lactose treatment predominantly induced the formation of compact callus, whereas other treatments primarily produced friable callus. Initially white, the callus gradually turned brownish and initiated somatic embryos at the heart and torpedo stages after three weeks of culture (Figure 5c). Compact callus typically contained

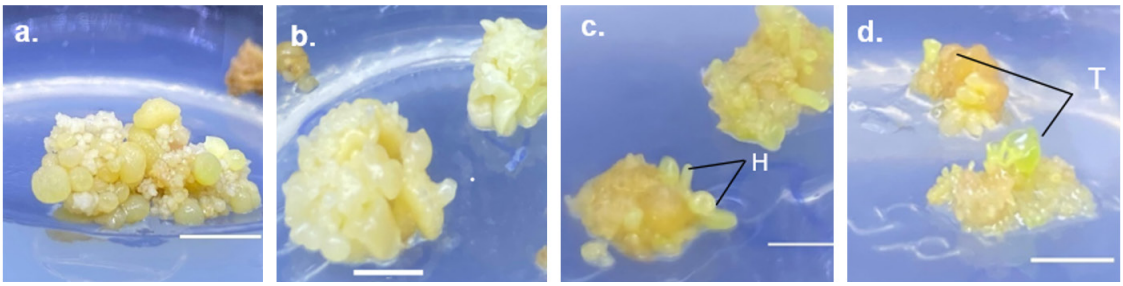
lower water content than friable callus, which coud enhance embryonic potential. The reduction in cellular water content may stimulate mild water stress, which in turn triggers a physiological shift and reprograms the cells toward embryogenic competence (Avila-Victor et al., 2023). Although it promotes embryonic development, lactose appears to be less effective for callus proliferation. Jin et al. (2025) reported that lactose induces the formation of globular structures with developmental abnormalities, whereas 5% sucrose significantly enhanced embryogenic callus mass in Citrus 'Minihyang'. Taken together, these findings suggest that while lactose may accelerate somatic embryo maturation, it is suboptimal for the proliferation phase, especially when the objective is to produce large quantities of high-quality embryogenic tissue.

Globular-shaped embryos have a high potential to develop into young plants, as shown in Figure 6. The addition of BAP at a low concentration (1 mg/L) without auxin has been proven to support the development of somatic embryos and promote the differentiation and growth of pre-embryos. In this study, the size of Mandarin 'Berastagi' embryos increased at each stage of development. However, it is visually difficult to distinguish normal from abnormal young embryos due to their high morphological similarity. Therefore, further analysis, such as histological examination or molecular marker assessment, is needed to ensure the quality and viability of the resulting embryos.

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Figure 5

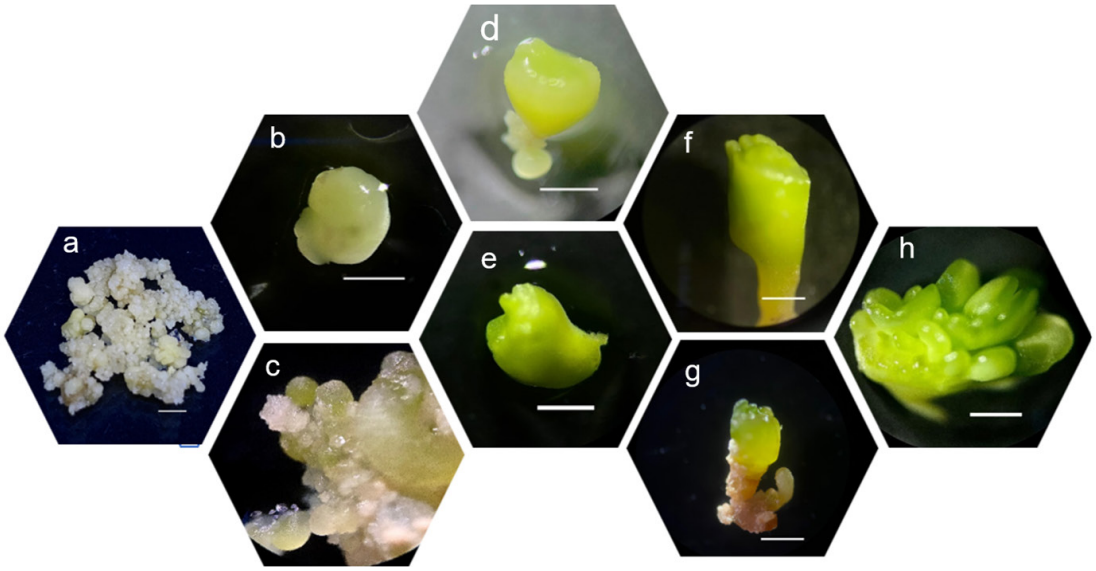
Embryogenic Callus and Somatic Embryos of Mandarin 'Berastagi' after 8 Weeks of Proliferation under Different Carbohydrate Treatments



Note. a. 4% sucrose treatment; b. 5% sucrose treatment; c. 4% lactose treatment; d. 5% lactose treatment; H: Heart; T: Torpedo. Scale bar: 5 mm.

Figure 6

Development of the Mandarin 'Berastagi' Somatic Embryo



Notes. a. Pro-embryo, b. late globular stage, c. early globular stage, d. early heart stage, e. late heart stage, f. late torpedo stage, g. early torpedo stage, h. cotyledon. Scale bar: 1 mm.

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Conclusions

Murashige and Skoog medium supplemented with Wetmore vitamins (MW) was the most effective for inducing embryogenic callus in 'Berastagi' mandarin. The optimal concentration of picloram was 1.5 mg/L, which produced the highest percentage of embryogenic callus formation (37.8%) and the largest average callus diameter (17.8 mm) on the MW medium. For callus proliferation, the MW medium supplemented with 4% sucrose yielded an average embryogenic callus diameter of 14 mm and the numerically highest percentage of explants forming globular-stage embryos (96%), although this value did not differ significantly from those of the other carbohydrate treatments.

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