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Induction of Organogenesis and Regeneration Species *Tagetes erecta* In Vitro

Induksi Organogenesis dan Regenerasi Tunas pada Spesies *Tagetes erecta* Secara *In Vitro*

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ABSTRACT

Marigold (*Tagetes erecta*) from the Asteraceae family is well-known for its various uses as cut flowers, potted plants, ornamental plants, medicines, dyes, and biopesticides. Micropropagation and optimization of in vitro regeneration of *T.erecta* species provide opportunities for plant propagation and utilization of biotechnology in plant breeding. This study evaluated the regeneration efficiency of in vitro plantlet leaf explants with different combinations of IAA and BAP concentrations in organogenesis induction media. IAA concentration affects the growth of adventitious shoots on explants. Decreasing IAA concentration affects the percentage of shoot growth, roots, and average number of roots in organogenesis induction media. Meanwhile, adding cytokinins to the induction media will modulate auxin movement during organogenesis and plant cell regeneration. Leaf explants on Murashige and Skoog (MS) media supplemented with 2.8 µM IAA + 31.0 µM BAP showed shoot growth reaching 58%, with root growth reaching 32%. Meanwhile, using lower BAP 2.8 µM IAA + 13.3 µM BAP resulted in 63% of explants forming shoots with a lower percentage of root growth. Increasing the concentration of IAA (5.7 µM + 13.3 µM BAP) showed a root formation response reaching 61% with a smaller number of shoots (48%). The explant response began with the explant part differentiating to form a callus with a growing point that would form yellowish-green shoots (RHS / Fan3-N144-C) at 28 days of induction.

Keywords: IAA, BAP, Leaf explants, Organogenesis.

ABSTRAK

Marigold (*Tagetes erecta*) dari keluarga Asteraceae terkenal dengan berbagai kegunaannya sebagai bunga potong, tanaman pot, tanaman hias, obat-obatan, pewarna, dan biopestisida. Mikropropagasi dan optimalisasi regenerasi spesies *T.erecta* secara in vitro memberikan peluang dalam perbanyakan tanaman dan pemanfaatan bioteknologi dalam pemuliaan tanaman. Dalam penelitian ini, kami mengevaluasi efisiensi regenerasi dari eksplan daun planlet in vitro dengan kombinasi konsentrasi IAA dan BAP yang berbeda dalam media induksi organogenesis. Terdapat pengaruh konsentrasi IAA dalam memicu pertumbuhan tunas adventif pada ekspalan. Penurunan konsentrasi IAA mempengaruhi persentasi pertumbuhan tunas, akar, dan rata-rata jumlah akar pada media induksi organogenesis. Sedangkan sitokinin yang ditambahkan pada media induksi berperan dalam memodulasi pergerakan auksin selama proses organogenesis dan regenerasi sel tanaman. Eksplan daun pada media Murashige dan Skoog (MS) yang ditambah 2,8 µM IAA + 31,0 µM BAP menunjukkan pertumbuhan tunas mencapai 58% dengan pertumbuhan akar mencapai 32%. Sedangkan pada penggunaan BAP yang lebih rendah 2,8 µM IAA + 13,3 µM BAP menghasilkan 63% eksplan membentuk tunas dengan persentase pertumbuhan akar lebih rendah. Peningkatan konsentrasi IAA (5,7 µM + 13,3 µM BAP) menunjukkan respon pembentukan akar mencapai 61% dengan jumlah tunas yang lebih sedikit (48%). Respon eksplan diawali dengan bagian eksplan berdiferensiasi membentuk kalus dengan titik tumbuh yang akan membentuk tunas berwarna hijau kekuningan (RHS/Fan3-N144-C) pada induksi 28 hari.

Kata Kunci: IAA, BAP, Eksplan daun, Organogenesis

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INTRODUCTION

Marigold (*Tagetes erecta* L.) belongs to the Asteraceae family, which is widely cultivated in Indonesia. Generally, marigold is used as an ornamental plant and a source of cut flowers. Other uses of marigolds include their application as an insecticide, natural dye, refugia plant, source of vitamin A, essential oil, and as a symbol in religious ceremonies (Priyanka et al., 2013). The Tagetes species commonly cultivated in Indonesia are characterized by their bright and striking flowers, ranging in color from pale yellow to deep yellow and orange.

In vitro plant propagation through the induction of cellular differentiation to form either shoots or roots is called organogenesis (Phillips & Garda, 2019; Kong et al., 2021). Organogenesis is influenced by plant species, type of explant, and the presence of plant growth regulators (PGRs) in the culture medium. The regenerative capacity of plant somatic tissues depends on species-specific factors, explant type, and the presence of exogenous PGRs in the induction medium. The induction of organogenesis largely depends on using auxins and cytokinins in the direction of plant development. The interaction between auxins and cytokinins is crucial in differentiating cells into shoots capable of regeneration. Several previous studies have reported the successful use of auxin-cytokinin combinations with various explant types in the optimal shoot induction of *Tagetes* species (Pal et al., 2012; Motte et al., 2014; Munshi et al., 2021). In vitro propagation of *T. erecta* presents a valuable opportunity for biotechnological improvement, including enhancing and producing secondary metabolites, mutation breeding, and genetic transformation.

The interaction between auxin and cytokinin in organogenesis is influenced by the ratio of their concentrations in the induction medium. A study by Apriliani et al. (2022) demonstrated optimal leaf explant regeneration using 17 µM IAA and 22 µM BAP in the organogenesis medium for *T. erecta*. Majumder et al. (2014) also reported that shoot regeneration using a 1:13 ratio of NAA to BAP resulted in the best regeneration outcomes for marigold species under in vitro conditions. Therefore, evaluating the effectiveness of various hormone concentration combinations in the organogenesis of *T. erecta* is essential. This study aims to determine an effective in vitro organogenesis induction method by identifying a responsive culture medium composition.

MATERIALS AND METHOD

The research was carried out from August to November 2021 in the Plant Tissue Culture Laboratory, Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University.

Tools and Materials

The equipment used included a laminar airflow (LAF) cabinet, culture bottles, sterile planting tools, and an incubation room. Materials included *T.erecta* Maharani F1 plantlets (East-West Seed, Indonesia), culture media, plant growth regulators (PGRs), and documentation tools.

Explant preparation

Seed sterilization was performed using 10% and 5% sodium hypochlorite solutions followed by sterile distilled water. Sterile seeds were germinated and propagated on induction and growth media to serve as explant sources. The explants used in this study were stem segments and leaves, cut to a size of approximately 1.0 cm from 30- to 90-day-old plantlets.

Experimental design

The experiment was arranged in a two-factor, Completely Randomized Block Design (CRBD). The first factor was IAA concentration at two levels: 2.8 µM and 5.7 µM. The second factor was BAP concentration at three levels: 13.3 µM, 22.1 µM, and 31.0 µM. Each treatment consisted of five culture bottles with five explants per bottle, replicated three times. Organogenesis induction was carried out for four weeks under full light conditions. The observed variables included days to callus, shoot, and root formation after planting (DAP); percentage of explants forming callus, shoots, and roots; number of shoots and roots formed; and the color of the callus and shoots.

Data analysis

Data obtained from the experiment were analyzed using analysis of variance (ANOVA) with the F-test in STAR (Statistical Tool for Agricultural Research) version 2.0.1. Significant results were further analyzed using

the Least Significant Difference (LSD) test at a 5% significance level (α = 0.05).

RESULT AND DISCUSSION

Regeneration of *T.erecta*

The Shoot explants were successfully achieved using leaf explants with modified auxin-to-cytokinin ratios in the organogenesis induction medium. The interaction between IAA and BAP, as well as the effect of reduced IAA concentration, had a significant impact on the percentage of shoots, roots, and root number in *T. erecta* organogenesis. Optimization of IAA and BAP concentrations using leaf explants indicated that a lower auxin concentration reduced root formation per explant while promoting greater shoot development (Table 1).

Table 1. Single factor effects of IAA and BAP on leaf organogenesis of *T. erecta*

Concentration	% of shoot formation	% of root formation	Number of callus	Number of roots
IAA (µM)				
2,8	55	43	3	1
5,7	42	52	2	2
Uji F	**	*	ns	**
BAP (µM)				
13,3	55 a	55 a	3	3
22,1	45 a	44 a	2	3
31,0	45 a	43 b	2	3
F test	*	*	ns	ns

Note: ns = not significant; * = significant at p ≤ 0.05; ** = significant at p ≤ 0.01

Higher IAA concentrations relative to BAP in the induction medium favored adventitious root formation, whereas lower IAA concentrations promoted adventitious shoot formation. In the conducted experiment, the use of 5.7 µM IAA successfully induced shoot formation but was accompanied by excessive root development. Conversely, a lower auxin concentration (2.8 µM IAA) led to a higher average number of shoots and fewer adventitious roots per explant (Table 1). Successful shoot induction requires a balanced ratio of IAA and BAP. BAP supplementation stimulates cell division and shoot initiation, particularly when its concentration is higher than that of IAA, but only under appropriate ratios.

Table 2. Interaction effects of IAA and BAP on shoot and root formation in *T. erecta* leaf explants

IAA	BAP	% of callus	% of roots	Number of shoots	Number of roots
2,8	13,3	63 a	49 a	4,4	2,2 a
	22,1	44 b	47 a	2,4	2,3 a
	31,0	58 a	32 b	3,1	0,7 b
5,7	13,3	48 a	61 a	2,2	3,4 a
	22,1	47 a	41 b	2,9	1,9 b
	31,0	31 b	53 ab	2,0	3,1 ab
F test		*	*	ns	*
CV (%)		14,05	14,74	35,15	29,12

Note: Values followed by the same letter within the same column are not significantly different according to the Least Significant Difference (LSD) test at α = 0.05.ns = not significant; * = significant at p ≤ 0.05.

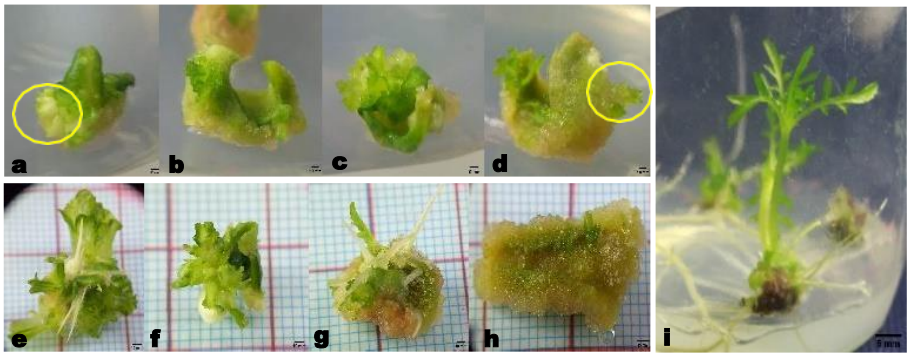

Figure 1. Response of leaf explants on organogenesis induction media. At 14 weeks after culture (WAC): (a) 2.8 µM IAA + 13.3 µM BAP, (b) 2.8 µM IAA + 22.1 µM BAP, (c) 2.8 µM IAA + 31.0 µM BAP, (d) 5.7 µM IAA + 22.1 µM BAP. At 30 days after culture (DAC): (e) 2.8 µM IAA + 13.3 µM BAP, (f) 2.8 µM IAA + 31.0 µM BAP, (g) 5.7 µM IAA + 13.3 µM BAP, (h) 5.7 µM IAA + 13.3 µM BAP, (i) shoot regeneration.

The interaction between IAA and BAP was particularly evident when a higher BAP concentration was used. The combination of 2.8 µM IAA and 31.0 µM BAP resulted in 58% shoot formation with a lower root formation rate of 32%. However, a lower BAP concentration (2.8 µM IAA + 13.3 µM BAP) produced even better shoot formation, with a percentage of 63% and a correspondingly lower average root number (Table 2). The use of BAP with a lower IAA concentration effectively induced shoot organogenesis in leaf explants, leading to a higher shoot percentage and average shoot number. The explant tissue differentiated into a light green-yellowish callus (RHS/Fan3-N144-D) with visible shoot primordia forming on the callus surface (Figure 1). The regenerated shoots exhibited a bright green-yellow coloration (RHS/Fan3-N144-C). These shoots were then transferred to an elongation medium for further growth into complete plantlets.

The type of explant commonly used in in vitro regeneration is young, meristematic tissue. Leaf explants, in particular, undergo dedifferentiation to form meristematic tissue, which can then be induced to generate shoots. Explants that lack pre-existing meristematic tissue require a balanced ratio of auxins and cytokinins to induce cell proliferation and organ formation, whether shoots or roots (Maurya et al., 2013).

The interaction between auxins and cytokinins in shoot induction has been widely studied. Auxins are known to promote cell division, while cytokinins modulate auxin distribution and movement during the process of organogenesis. A study by Vanegas et al. (2002) reported successful organogenesis in *T. erecta* leaf explants using a medium containing 13.0 µM BA and 17.1 µM IAA, achieving a shoot induction rate of 69.4%. Similarly, Apriliani et al. (2022) observed a 36% shoot regeneration rate using a combination of 17.1 µM IAA and 22.1 µM BAP in leaf explants. In the present study, shoot regeneration was successfully achieved at a lower auxin concentration (5.7 µM IAA), resulting in shoot formation and development into plantlets. Auxin concentrations below the physiological threshold required by a given species may not support organogenesis. However, the simultaneous application of exogenous cytokinin and auxin at an appropriate ratio can enhance shoot induction (Zhao et al., 2021).

The desired outcome of plant regeneration is the development of shoots capable of forming complete plants. The polar transport of auxin within plant tissues (both basipetal and acropetal) plays a vital role in cell elongation and modulation of shoot development in meristematic tissues (Davies, 2004; Arteca, 1995). The tendency of explants to form roots rather than shoots may be attributed to the accumulation of endogenous auxin in plantlets derived from in vitro culture. When combined with a high concentration of exogenous auxin in the induction medium, this may lead to increased cell differentiation into root structures. Baidowi & Wiendi (2017) reported that a combination of 1.3 mg L^{-1} IAA and 0.5 mg L^{-1} BAP induced a high average number of roots per plant. Similarly, Belarmino (1992) demonstrated that MS medium supplemented with 5.0 mg L^{-1} NAA and 0.5 mg L^{-1} BA resulted in 100% root formation in both leaf and hypocotyl explants of *T. erecta*.

CONCLUSION

Efficient regeneration of *T. erecta* using leaf explants can be achieved through the use of an induction medium containing a lower auxin concentration relative to cytokinin. The combination of 2.8 µM IAA and 13.3 µM BAP resulted in the highest shoot formation percentage (63%) with minimal root proliferation after a 28-day induction period.

REFERENCES

Apriliani, E., Syukur, M., Sudarsono, S., Aisyah, S.I., Suprapta, D.N., Chan, M.T., Sukma, D., 2022. Regeneration of marigold (*Tagetes* sp.) plants from different types of explants and concentration of plant regulators in vitro. *Acta Horticulturae*, 1334: 199–204.

Arteca, R.N., 1995. *Plant growth substances: Principle and application.* Chapman and Hall. New York.

Baidowi, B., Muhammad, M., Wiendi, N.M.A., 2017. Proliferasi tunas adventif tagetes (*Tagetes erecta* L.) kultivar african crackerjack dengan BAP, GA3, dan IAA secara in vitro. *Buletin Agrohorti*, 5 (1): 55.

Belarmino, M., Takeo, T., 1992. Callus induction and plant regeneration in African marigold (*Tagetes erecta* L.). *Japan J.Breed*, 42: 835–41.

Davies, P.J., 2004. *Plant hormones: Biosynthesis, transduction, action*. Dordrecht: Kluwer Academic Publisher.

Kong, E.Y.Y., Biddle, J., Foale, M., Panis, B., Adkins, S.W., 2021. The potential to propagate coconut clones through direct shoot organogenesis: A review. *Scientia Horticulturae* 289 (January): 110400.

Majumder, M., Jayoti, J., Singh, K.P., Singh, S.K., Prasad, K.V., Verma, M., 2014. In vitro morphogenesis

in marigold using shoot tip as explant. *Indian Journal of Horticulture*, 71 (1): 82–86.

Maurya, R.P., Yadav, R.C., Godara, N.R., Beniwal, V.S., 2013. In vitro plant regeneration of rose (*Rosa hybrida* L.) CV. Benjamin Paul through various explants. *Journal of Experimental Biology and Agricultural Sciences,* 1(28): 111–19.

Motte, M., Hans, H., Vereecke, D., Geelen, D., Werbrouck, S., 2014. The molecular path to in vitro shoot regeneration. *Biotechnology Advances*, 32 (1): 107–21.

Munshi, J.L., Baksha, R., Rahaman, M.Z., Huque, N.N., Zinat, E.A., Momtaz, N., 2021. In vitro plant regeneration from leaf explants of *Tagetes erecta* L. *Bangladesh Journal of Scientific and Industrial Research* 56 (2): 69–74.

Pal, A.K., Acharya, K., Ahuja, P.S., 2012. Endogenous auxin level is a critical determinant for in vitro adventitious shoot regeneration in potato (*Solanum tuberosum* L.). *Journal of Plant Biochemistry and Biotechnology* 21 (2): 205–12.

Phillips, G.C., Garda, M., 2019. Plant tissue culture media and practices: An overview. *In Vitro Cellular and Developmental Biology - Plant* 55 (3): 242–57.

Priyanka, D., Shalini, T., Navneet, V.K., 2013. A brief study on marigold (*Tagetes species*): A review. *International Research Journal of Pharmacy*, 4(1): 43–48.

Vanegas, P.E., Valverde, M.E., Paredes L.O., 2002. Plant regeneration via organogenesis in marigold. *Plant Cell Tissue and Organ Culture*, 69(3): 279–83.

Zhao, X., Song, J., Zeng, Q., Ma, Y., Fang, H., Yang, L., Deng, B., et al., 2021. Auxin and cytokinin mediated regulation involved in vitro organogenesis of papaya. *Journal of Plant Physiology*, 260 (March): 153405.