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In vitro propagation of *Dahlia sp.* using different types of explants and plant growth regulators (PGR)

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Abstract

Dahlia sp., a family of Compositeae has potential value as an alternative food and medicinal plant. Its tubers contain carbs, fiber, and many other beneficial substances. *Dahlia* sp. can be propagated via in vitro culture to create uniform seeds, free from pests and pathogens, more stable, and can be scale up in a short time. The addition of cytokinin and auxins in the media can stimulate the shoot formation of *Dahlia* sp planlets. This research aimed to determine the growth response of *Dahlia* sp. cultures on DKW (Driver and Kuniyuki Walnut) medium supplemented with BAP, kinetin, 2-iP, and NAA and to identify the best type of explant used for the micropropagation of *Dahlia* sp. The experimental design was a completely randomized factorial design, with factors tested: type of explant (apical shoots, middle and basal nodes), and plant growth regulator combination between cytokinins (BAP, Kinetin, and 2-iP) and auxin (NAA) at 0 and 1 mg/L. The result demonstrated that the DKW medium supplemented with 1 mg/L 2-iP and 1 mg/L NAA using middle nodes as explants produced the highest values for shoot height, number of shoots, number of roots, fresh weight, and dry weight variables. Apical shoots explant cultured on DKW medium supplemented with 1 mg/L Kinetin and 0 mg/L NAA produced the highest number of leaves and were significantly different from the others.

Keywords: 2-iP, BAP, growth response, kinetin, NAA

Introduction

Dahlia sp. members of the Compositeae family exhibit a wide range of colors, sizes, shapes, forms, and prolific flowers. Dahlia can grow as both annual and perennial plants. All Dahlia cultivars are descended from a single species, *Dahlia variables,* and *Dahlia pinnata* (Rudiyanto et al., 2017; Swedan et al., 2023). Dahlia is known for its colorful inflorescences and the vibrancy of its foliage (Marcinek et al., 2019). *Dahlia* sp. has potential value as an alternative food and medicinal plant. Its tubers contain carbs, fiber, and various beneficial substances, including inulin (41.7–72.6%). Inulin can help sustain the growth of bifidobacterium in the digestive system, stimulate the immune system, and lower the risk of osteoporosis (Rudiyanto et al., 2022; Ismawati et al., 2024).

Dahlia plants can be propagated generatively with seeds and vegetatively with cuttings and tubers. Conventional propagation, whether by seeds, cuttings, or tubers, is very susceptible to pathogens such as bacteria, fungi, and viruses. By using plant tissue culture techniques, *Dahlia* sp. can be propagated under aseptic conditions and can be scale up in uniform seeds in a relatively short time (Ibrahim & Daraj, 2015; Efendi et al., 2021).

The combination of cytokinins and auxins at varying concentrations can influence the growth of plant in vitro culture. Cytokinins can be used to stimulate cell metabolism, encourage cell division, break dormant cell phases, and finally increase shoot formation (Sari et al., 2021). Some cytokinin PGRs, like BAP (Benzyl Amino Purine), Kinetin, and 2-IP (2-Isopentenyl Adenine), can help plants grow faster. On the other hand, auxin is often used in tissue culture to enhance cell growth and adventitious root formation and terminate axillary and adventitious shoot growth. One of the PGRs in the auxin group commonly used in plant tissue culture is NAA (Naphthalene Acetic Acid) (Hapsari et al., 2021).

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Many species exhibit varying responses to in vitro culture using various plant growth regulators. In *Dillenia philippinensis*, MS medium containing 1 mg/L 2-iP gave the best response for plant height. Meanwhile, the highest number of leaves and nodes occurred in the MS medium containing 0.5 mg/L BAP (Wulandari et al., 2021). According to Purwito et al., (2023), the best shoot height, shoot number, and petiole of *Moringa oleifera* were made on a medium that had 1 mg/L Kinetin. The best roots and root lengths were made at 1.0 and 2.0 mg/L IAA.

The aims of this research were to determine the growth response of *Dahlia* sp. cultures on DKW medium supplemented with BAP, kinetin, 2-iP, and NAA, to determine the best combination of growth regulators for Dahlia growth on in vitro culture, and also to identify the best type of explant used for the micropropagation of *Dahlia* sp.

Materials and Methods

Materials and Experimental Design

Experiments were carried out from January to April 2024 at Plant Tissue Culture Laboratory, National Research and Innovation Agency (BRIN), Bogor, Indonesia. *Dahlia* sp. shoot tips with 1.5 cm stem and 2-3 leaves at 8 weeks after culture (WAC) cultivated on DKW media were collected for the explant source. The media used was DKW (Driver & Kuniyuki, 1984; Purwito et al., 2021) supplemented with 30 g/L of sucrose and 4 g/L of gelzan agar (TM Caissonlabs). The media was adjusted to 5.8 pH and then sterilized in an autoclave at 15 psi and 121°C for 20 minutes. The cultures were incubated at 24 ± 2°C with constant light intensity ranging from 4.29 to 7.14 µmol/m^2/s.

The experimental design was a completely randomized factorial design, with factors tested: 1. type of explant (apical shoots, middle nodes, basal nodes), and 2. plant growth regulator (combination between BAP and NAA at 0 and 1 mg/L, combination between Kinetin and NAA at 0 and 1 mg/L, and combination between 2-iP and NAA at 0 and 1 mg/L). The number of replications was 12, and there were 216 experimental units. The variables observed were: plant height, number of shoots, number of leaves, and number of roots which were observed weakly from 0 to 8 WAC. Fresh weight and dry weight were observed at 8 WAC.

Data Analysis

Data were analyzed using an analysis of variance (ANOVA) to find significant differences between treatments. Treatments that differed significantly were further analyzed using Duncan's Multiple Range Test (DMRT) with a 95% confidence level (a = 0.05) using DSAASTAT V.1.1 (open-source software).

Result and Discussion

The optimal concentration of plant growth regulators for promoting plant growth in in vitro cultures varies depending on the species. The type and concentration of plant growth regulators that are effective for a particular plant species and cultivar will provide a different response for others (Poudel et al., 2015; Hapsari et al., 2021). The growth in shoot height of *Dahlia* sp. cultured in DKW medium supplemented with a combination of BAP, Kinetin, 2-iP, and NAA on various types of explants is illustrated in **Figure 1**. All three explant types used in the dahlia culture began to grow after two weeks of culture. The medium supplemented with 1 mg/L of 2-iP + 1 mg/L NAA and 1 mg/L of BAP + 0 mg/L NAA produced the highest shoot height growth of *Dahlia* sp. at 6-8 WAC (Figure 1A). Dahlia shoot height growth was also optimal in medium supplemented with 1 mg/L 2-iP + 1 mg/L NAA at 6–8 WAC (Figure 1B). On the other hand, *Dahlia* cultures using basal nodes had the most shoots grow on media with PGR 1 mg/L Kinetin and 0 mg/L NAA at sixth to eighth WAC (Figure 1C). The lowest shoot height was obtained on DKW media by adding 1 mg/L BAP and 1 mg/L NAA. The growth rate was low during the second to eighth week of culture.

The growth pattern of the number of shoots on *Dahlia* sp. cultured in DKW medium supplemented with PGR (BAP, Kinetin, 2-iP, and NAA) with different types of explants is depicted in **Figure 2**. In the apical shoot explant, the number of shoots increased optimally on media containing PGR 1 mg/L 2-IP + 1 mg/L NAA and 1 mg/L BAP + 0 mg/L NAA. The number of shoots significantly increased in the media containing 1 mg/L 2-IP + 1 mg/L NAA at 5-8 WAC, while the combination of PGR 1 mg/L BAP + 0 mg/L NAA produced steady growth. In the medium containing Kinetin and NAA, the number of shoots significantly increased at 2 WAC, but did not significantly increase in the following week. The number of shoots remained consistent between 2 and 8 WAC during the observation period (Figure 2A). The combination of PGR at 1 mg/L 2-IP + 1 mg/L NAA led to the most optimal growth of dahlia number of shoots from 5-8 WAC. Meanwhile, the number of shoots in the medium containing other various PGR combinations remained stable after two WACs (Figure 2B). The number of shoot variables for growth of *Dahlia* sp varied on the basal node explant used, with the highest number of shoots identified in the media containing PGR at 1 mg/L BAP + 0 mg/L NAA

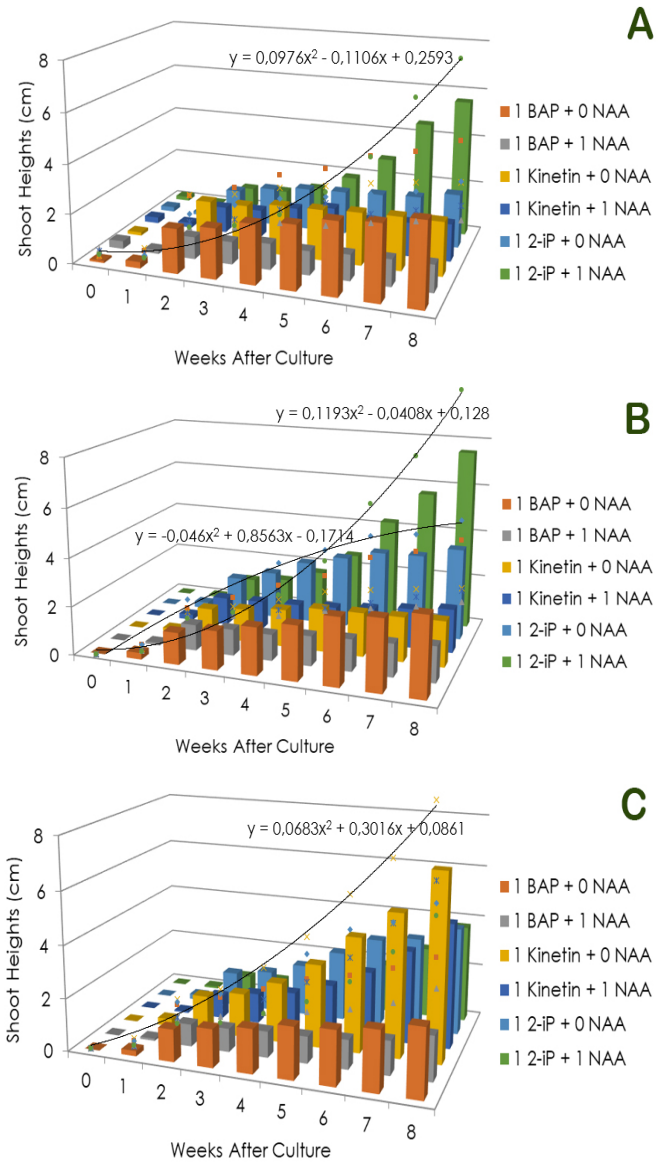

Figure 1. Average shoot height of *Dahlia* sp cultures on DKW media supplemented with 1 mg/L BAP + 0 mg/L NAA, 1 mg/L BAP + 1 mg/L NAA, 1 mg/L Kinetin + 0 mg/L NAA, 1 mg/L Kinetin + 1 mg/L NAA, 1 mg/L 2-iP + 0 mg/L NAA, and 1 mg/L 2-iP + 1 mg/L NAA using different types of explants, apical shoot (A), middle nodes (B), and basal nodes (C) at 0-8 WAC.

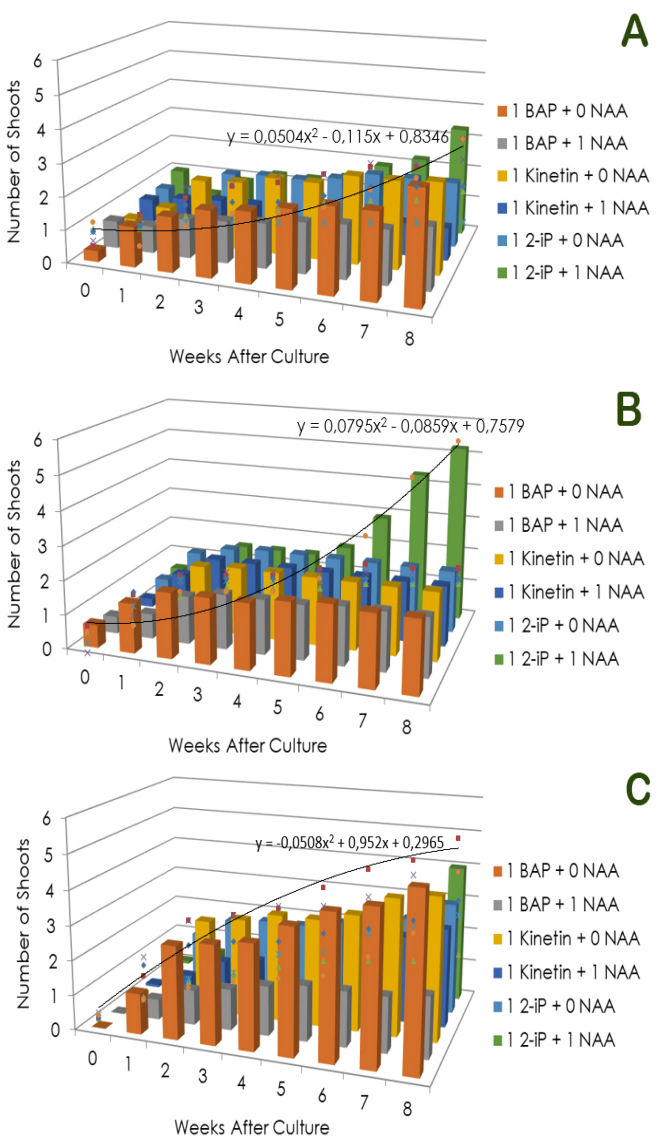

Figure 2. Average number of shoots of *Dahlia* sp cultures on DKW media supplemented with 1 mg/L BAP + 0 mg/L NAA, 1 mg/L BAP + 1 mg/L NAA, 1 mg/L Kinetin + 0 mg/L NAA, 1 mg/L Kinetin + 1 mg/L NAA, 1 mg/L 2-iP + 0 mg/L NAA, and 1 mg/L 2-iP + 1 mg/L NAA using different types of explants, apical shoot (A), middle nodes (B), and basal nodes (C) at 0-8 WAC.

at 8 WAC (Figure 2C). In plant tissue culture, it is common to use Kinetin to stimulate shoot growth. Meanwhile, 2-iP is a very effective cytokinin because its structural formula has an adenine ring like zeatin (Wulandari et al., 2021).

The increasing number of leaf variables of *Dahlia* sp cultured on media added with a PGR combination of BAP, Kinetin, 2-iP, and NAA using different types of explants (apical shoots, middle and basal nodes is illustrated in **Figure 3**. The most prominent number of leaves on *Dahlia* sp. cultured occurred on the media supplemented with 1 mg/L BAP + 0 mg/L NAA, with the number of leaves increasing consistently from 1–8 WAC. The number of *Dahlia* sp. leaves on media containing 1 mg/L 2-iP + 1 mg/L NAA is increasing and reaching its peak in the 6-8 WAC. The media that contained PGR 1 mg/L Kinetin + 1 mg/L NAA exhibited the slowest growth in terms of leaf number. Contamination in the cultured media causes a decrease in the number of leaves at 6 WAC (Figure 3A). In the middle node explant used, the number of leaves significantly increased, particularly in the media, which was supplemented with 1 mg/L 2-iP and 1 mg/L NAA during the 5-8 WAC. When PGR 1 mg/L BAP and 0 mg/L NAA were added to the media, the number of leaves significantly increased at 6–8 WAC (Figure 3B). In the basal node explant, the media containing 1 mg/L BAP + 0 mg/L NAA, as well as 1 mg/L Kinetin + 0 mg/L NAA, exhibited the most optimal increase in leaf number. However, when the media containing 1 mg/L Kinetin + 0

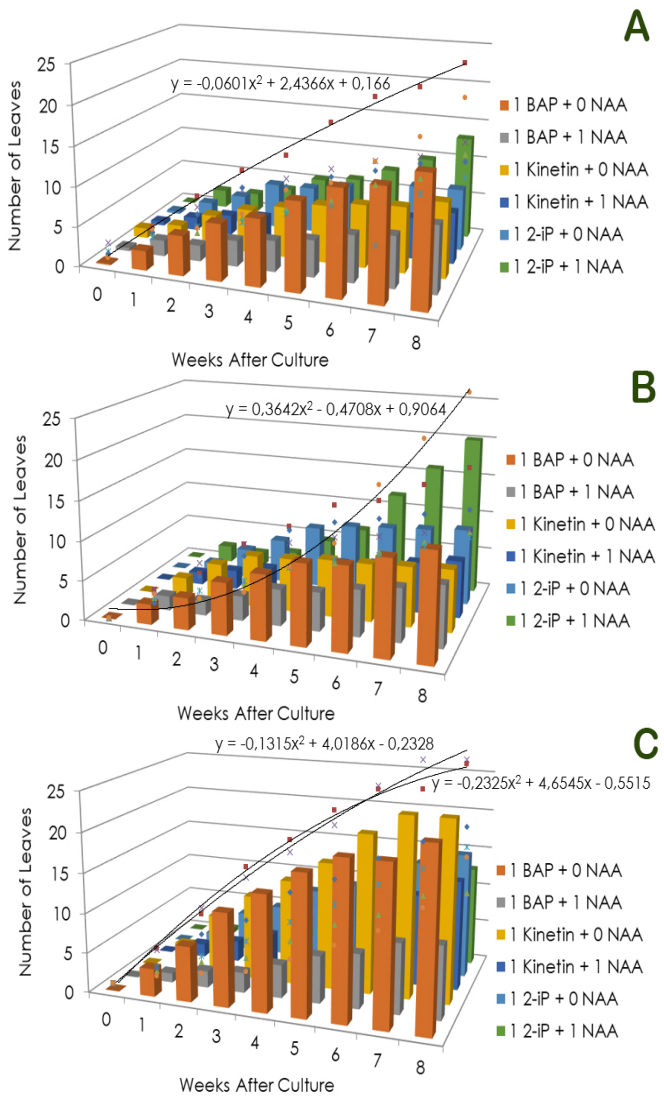

Figure 3. Average number of leaves of *Dahlia* sp cultures on DKW media supplemented with 1 mg/L BAP + 0 mg/L NAA, 1 mg/L BAP + 1 mg/L NAA, 1 mg/L Kinetin + 0 mg/L NAA, 1 mg/L Kinetin + 1 mg/L NAA, 1 mg/L 2-iP + 0 mg/L NAA, and 1 mg/L 2-iP + 1 mg/L NAA using different types of explants, apical shoot (A), middle nodes (B), and basal nodes (C) at 0-8 WAC.

mg/L NAA was used, the number of leaves decreased at 8 WAC and some of the leaves browned and wilted. Meanwhile, the media supplemented with 1 mg/L BAP and 1 mg/L NAA produced the smallest number of leaf variables (Figure 3C). Plant growth regulators are essential medium components for plant growth and differentiation. The absence of PGR in the medium significantly impedes culture growth, potentially preventing it from growing. The proper use of PGR determines the formation of organs. Mitotic plant cell division does not occur without cytokinins. Cytokinins mainly play a role in the formation of microtubules (Rudiyanto et al., 2017; Wulansari et al., 2023).

The increasing number of roots of *Dahlia* sp. cultured on DKW medium supplemented with different combinations of PGR and explant type is described in **Figure 4**. The highest increasing number of roots occurred on medium added with 1 mg/L 2-iP + 1 mg/L NAA using apical shoot as an explant. The number of roots increased continuously at 1–8 WAC, with the highest average number of shoots (3.5 roots) on each plantlet at 8 WAC. On the other hand, the number of roots on the media containing 1 mg/L kinetin and 1 mg/L NAA increased at 3 WAC and then decreased at 6 WAC due to contamination. Conversely, in the other treatment, no roots were formed between 0 and 8 WAC (Figure 4A). In the middle node explant type, it was only media containing 1 mg/L 2-iP and 1 mg/L NAA, which increased the number of roots. After two WAC treatments, the number of roots started

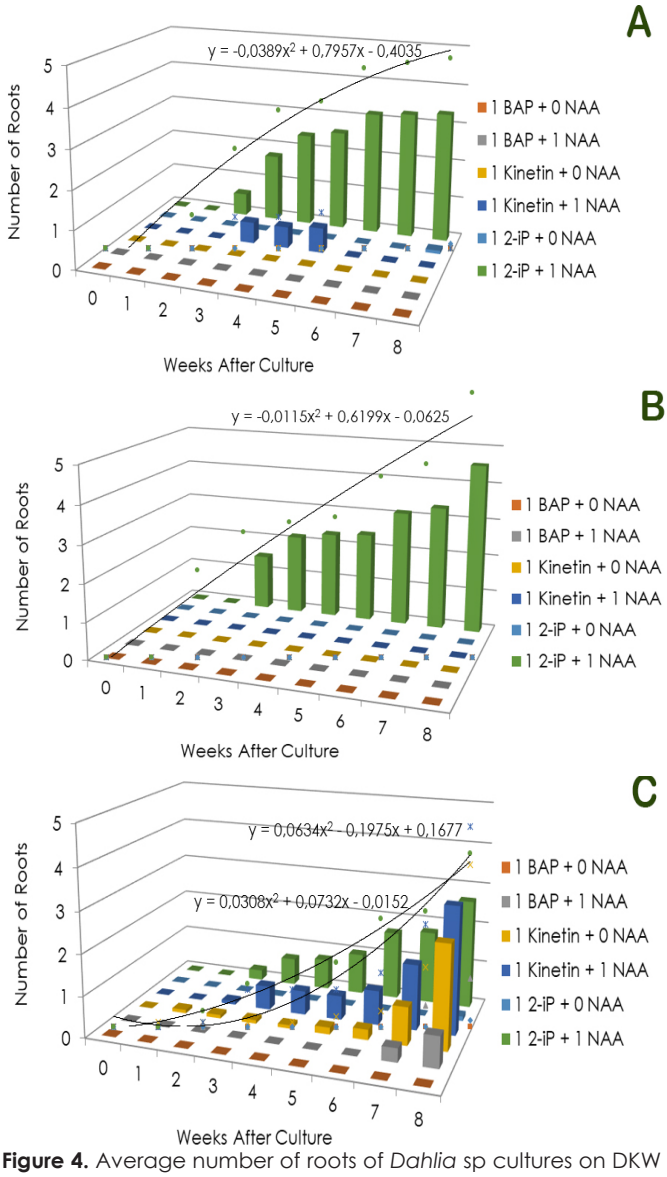

Figure 4. Average number of roots of *Dahlia* sp cultures on DKW media supplemented with 1 mg/L BAP + 0 mg/L NAA, 1 mg/L BAP + 1 mg/L NAA, 1 mg/L Kinetin + 0 mg/L NAA, 1 mg/L Kinetin + 1 mg/L NAA, 1 mg/L 2-iP + 0 mg/L NAA, and 1 mg/L 2-iP + 1 mg/L NAA using different types of explants, apical shoot (A), middle nodes (B), and basal nodes (C) at 0-8 WAC.

to increase. Meanwhile, on the other media, the number of roots did not increase in media containing different combinations of PGR (Figure 4B). When basal node explants were grown in media with 1 mg/L Kinetin and 1 mg/L NAA at 8 WAC, they had the most roots as a result. The number of roots increased significantly from 5–8 WAC. The number of roots increased on media with 1 mg/L 2-iP + 1 mg/L NAA, 1 mg/L Kinetin + 0 mg/L NAA, and 1 mg/L BAP + 1 mg/L NAA. The best growth occurred between the sixth and eighth WAC. The media supplemented with 1 mg/L BAP + 0 mg/L NAA exhibited the lowest growth in the number of roots, with the number of roots not increasing until the eighth WAC (Figure 4C). Bhojwani & Razdan (1996) asserted that excessive cytokinin added in the culture media can inhibit cell division in plant tissue. However, growing the explant in a medium with the right amount of cytokinin can speed up cell division and help plantlets grow properly (Purwito et al., 2021).

The ANOVA analysis of *in vitro* culture of *Dahlia* sp. using different types of explants and combinations of plant growth regulators on plant height, number of shoots, number of leaves, number of roots, fresh weight, and dry weight variables at 8 WAC was presented in **Table 1**.

The types of explants have a significant impact on the parameters of plant height, shoot number, number of leaves, and dry weight. However, the number of roots and fresh weight were not significantly affected by the type of explant. On the other hand, the addition of the PGR combination had a significant impact on all growth parameters. There were interactions between the two factors tested on all variables except fresh weight parameters (Table 1).

Cytokinins are one of the PGRs that can speed up cell division in plant tissue and promote cell extension, cell differentiation, organ formation, and plant growth and development (Ibrahim et al., 2024). Cytokinins also act as cytokinesis inducers, which are processes involved in senescence, apical dominance, root proliferation, and phyllotaxis (Jimenez et al., 2024).

Table 1: Presents the results of an analysis of variance (ANOVA) on *in vitro* culture of *Dahlia* sp. using different types of explants and combinations of plant growth regulators on shoot height, number of shoots, number of leaves, number of roots, fresh weight, and dry weight variables at 8 WAC

No	Variable	F value & Significance		
		Exsplant	PGR	Exsplant x PGR
1	Shoot height	4.80**	12.11**	6.49**
2	Number of shoots	5.36**	6.77**	2.89**
3	Number of leaves	19.42**	10.96**	5.37**
4	Number of roots	1.35ns	41.58**	2.03*
5	Fresh weight	2.03ns	25.15**	1.70ns
6	Dry weight	8.05**	22.03**	4.22**

Noted: * : significance at a: 5%; ** : very significance at a 1%; ns: not significance

The average values for shoot height, number of shoots, leaves, roots, fresh weight, and dry weight of *Dahlia* sp. grown in DKW medium with BAP, kinetin, 2-IP, and NAA using various types of explants at 8 WAC are shown in Table 2. The media supplemented with 1 mg/L Kinetin + 0 mg/L NAA, using basal nodes as explants, and 1 mg/L 2-iP + 1 mg/L NAA, using apical shoots as explants, produced the highest shoots of *Dahlia* sp. cultured, significantly different from than others. Meanwhile, the media containing PGR (1 mg/L BAP + 1 mg/L NAA) using apical shoot explants produced the lowest shoot height (**Table 2**).

Regarding the number of shoot variables, the media containing 1 mg/L 2-iP + 1 mg/L NAA contained a large number of *Dahlia* shoots, with apical shoots and basal nodes being used as explant sources. However, there was no significant difference between the media containing 1 mg/L Kinetin + 0 mg/L NAA and the media containing 1 mg/L BAP + 0 mg/L NAA, both of which used basal nodes as explants. On the other hand, using apical shoots as an explant in media with 1 mg/L Kinetin and mg/L NAA led to the fewest shots, followed by 1 mg/L BAP and 1 mg/L NAA with basal node explants (Table 2).

The number of leaf variables that increased on *Dahlia* sp. cultured at 8 WAC was varied. On the media with the addition of 1 mg/L Kinetin + 0 mg/L NAA and 1 mg/L BAP + 0 mg/L NAA using basal nodes as explants produced the highest number of leaf growth, but it was not statistically different from media supplemented with 1 mg/L 2-iP + 1 mg/L NAA using middle nodes as an explant source. On the media containing PGR, 1 mg/L 2-IP + 0 mg/L NAA and 1 mg/L Kinetin + 0 mg/L NAA, with both explants with apical shoots, produced the lowest number of leaves (Table 2).

Using basal nodes as explants, the media containing 1 mg/L 2-iP + 1 mg/L NAA produced the highest number of roots. Meanwhile, in the media supplemented with 1 mg/L BAP + 0 mg/L NAA and 1 mg/L BAP + 1 mg/L NAA, there are no roots formed. Similarly, the addition of 1 mg/L Kinetin + 0 mg/L NAA, 1 mg/L Kinetin + 1 mg/L NAA, and 1 mg/L 2-iP + 0 mg/L NAA to the media did not cause any root growth, except for the explant of basal nodes (Table 2).

On the media containing 1 mg/L 2-iP + 1 mg/L NAA, using apical shoots as an explant produced the highest fresh weight of *Dahlia* sp. and was significantly different from others. The dahlia with the smallest fresh weight was found on the media supplemented with 1 mg/L BAP + 1 mg/L NAA and 1 mg/L 2-iP + 0 mg/L NAA,

Table 2: Average value of shoot height, number of shoots, number of leaves, number of roots, fresh weight, and dry weight of *Dahlia* sp on *in vitro* culture using different types of explants and plant growth regulators combinations at 8 WAC

PGR	Explants	Shoots Height (cm)	Number of Shoots	Number of Leaves	Number of Roots	Fresh Weight (g)	Dry Weight (mg)
1 BAP + 0 NAA	Apical Shoots	3.32 ± 0.71^{bcde}	3.33 ± 0.76^{bcd}	15.89 ± 3.24^{bc}	0.00 ± 0.00^{c}	0.15 ± 0.03^{c}	20.99 ± 3.95^{de}
	Middle Nodes	3.20 ± 0.29^{bcde}	2.00 ± 0.00^{cde}	14.78 ± 0.92^{bcd}	0.00 ± 0.00^{c}	0.10 ± 0.01^{c}	16.28 ± 1.61^{de}
	Basal Nodes	2.00 ± 0.20^{cde}	3.78 ± 0.46^{abc}	**22.89 ± 2.07^{a}**	0.00 ± 0.00^{c}	0.20 ± 0.03^{c}	27.94 ± 6.06^{de}
1 BAP + 1 NAA	Apical Shoots	1.10 ± 0.03^{e}	1.88 ± 0.26^{de}	8.38 ± 1.04^{def}	0.00 ± 0.00^{c}	0.03 ± 0.00^{c}	4.69 ± 0.27^{e}
	Middle Nodes	1.47 ± 0.09^{de}	1.71 ± 0.14^{de}	7.71 ± 0.91^{ef}	0.00 ± 0.00^{c}	0.04 ± 0.00^{c}	7.11 ± 0.65^{e}
	Basal Nodes	1.14 ± 0.08^{e}	1.33 ± 0.17^{e}	8.56 ± 0.63^{def}	0.00 ± 0.00^{c}	0.05 ± 0.01^{c}	10.16 ± 1.56^{de}
1 Kinetin + 0 NAA	Apical Shoots	1.96 ± 0.09^{cde}	2.00 ± 0.29^{cde}	5.33 ± 0.93^{f}	0.00 ± 0.00^{c}	0.04 ± 0.01^{c}	8.08 ± 1.57^{e}
	Middle Nodes	1.71 ± 0.16^{de}	2.00 ± 0.00^{cde}	7.11 ± 0.96^{ef}	0.00 ± 0.00^{c}	0.04 ± 0.01^{c}	11.83 ± 1.12^{de}
	Basal Nodes	**7.57 ± 1.02^{a}**	**4.00 ± 0.29^{ab}**	**24.56 ± 1.85^{a}**	0.67 ± 0.47^{c}	0.44 ± 0.11^{c}	65.23 ± 14.98^{bc}
1 Kinetin + 1 NAA	Apical Shoots	1.53 ± 0.19^{de}	1.00 ± 0.00^{e}	6.50 ± 0.29^{ef}	0.00 ± 0.00^{c}	0.05 ± 0.01^{c}	7.98 ± 1.49^{e}
	Middle Nodes	1.81 ± 0.21^{de}	1.78 ± 0.15^{de}	7.22 ± 0.43^{ef}	0.00 ± 0.00^{c}	0.04 ± 0.01^{c}	7.16 ± 0.85^{e}
	Basal Nodes	3.76 ± 0.76^{bcd}	2.00 ± 0.33^{cde}	10.67 ± 1.09^{cdef}	0.11 ± 0.11^{c}	0.21 ± 0.11^{c}	23.56 ± 8.36^{de}
1 2-IP + 0 NAA	Apical Shoots	2.19 ± 0.19^{cde}	2.11 ± 0.35^{cde}	5.00 ± 0.71^{f}	0.00 ± 0.00^{c}	0.03 ± 0.00^{c}	8.84 ± 3.08^{de}
	Middle Nodes	3.07 ± 0.19^{bcde}	2.00 ± 0.00^{cde}	9.00 ± 0.78^{def}	0.00 ± 0.00^{c}	0.07 ± 0.00^{c}	14.16 ± 1.12^{e}
	Basal Nodes	4.11 ± 1.21^{bc}	3.22 ± 0.86^{bcd}	15.78 ± 3.48^{bc}	0.11 ± 0.11^{c}	0.25 ± 0.10^{c}	30.97 ± 10.01^{de}
1 2-IP + 1 NAA	Apical Shoots	4.82 ± 1.08^{b}	1.89 ± 0.48^{de}	11.22 ± 2.09^{cdef}	2.56 ± 0.71^{b}	0.94 ± 0.39^{b}	43.90 ± 16.15^{cd}
	Middle Nodes	**7.53 ± 1.71^{a}**	**5.22 ± 1.22^{a}**	20.11 ± 3.46^{ab}	**4.56 ± 0.85^{a}**	**1.91 ± 0.34^{a}**	**130.26 ±19.88^{a}**
	Basal Nodes	3.74 ± 0.87^{bcd}	**4.00 ± 1.22^{ab}**	12.67 ± 4.43^{cde}	2.67 ± 0.87^{b}	1.23 ± 0.50^{b}	79.49 ± 28.82^{b}

Noted: Numbers followed by the same letter in the same column are not significantly different based on Duncan's multiple range test at α = 5%. Presented values are means ± standard error (SE) of 12 replicates.

using apical shoots as an explant. This media did not significantly differ from the others, except for the media added with 1 mg/L 2-iP + 1 mg/L NAA (Table 2). In line with this result, the media containing 1 mg/L 2-iP + 1 mg/L NAA using apical shoots as an explant also produced the highest dry weight of *Dahlia* sp and was significantly different from all other treatments. The *Dahlia* sp. with the lowest dry weight was grown in medium with 1 mg/L BAP and 1 mg/L NAA and an apical shoot explant (Table 2). Based on recapitulation results in Table 2, the DKW medium complemented with 1 mg/L 2-iP + 1 mg/L NAA using middle nodes as explants produced prominent results in five parameters: shoot height, number of shoots, number of roots, fresh weight, and dry weight, and significantly different from others. In the number of leaves variables, the highest result was produced in media containing 1 mg/L BAP + 0 mg/L NAA using basal nodes as explants, even though it is not significantly different from media supplemented with 1 mg/L 2-iP + 1 mg/L NAA using middle nodes as explants. According to Pasternak & Steinmacher (2024), the plant's actual response does not always correlate with the concentration of plant growth regulators. Growth regulators do not work alone to produce a response; instead, they act through the interaction of several compounds. Growth regulators are chemicals that provide "on" or "off" signals, triggering a series of events in cells that ultimately result in physiological changes. Kecis et al., (2023) added that the process of organ formation, such as shoots or roots, is an interaction between exogenous PGRs added to the media and endogenous PGRs produced by the plant itself.

The performance of *Dahlia* sp. cultured in DKW medium containing different combinations of plant growth regulators and different types of explants at 8 WAC is shown in **Figure 5**. On the media supplemented with 1 mg/L 2-iP + 1 mg/L NAA using middle nodes as explants and media containing 1 mg/L Kinetin + 0 mg/L NAA using basal nodes as explants have the highest shoot height

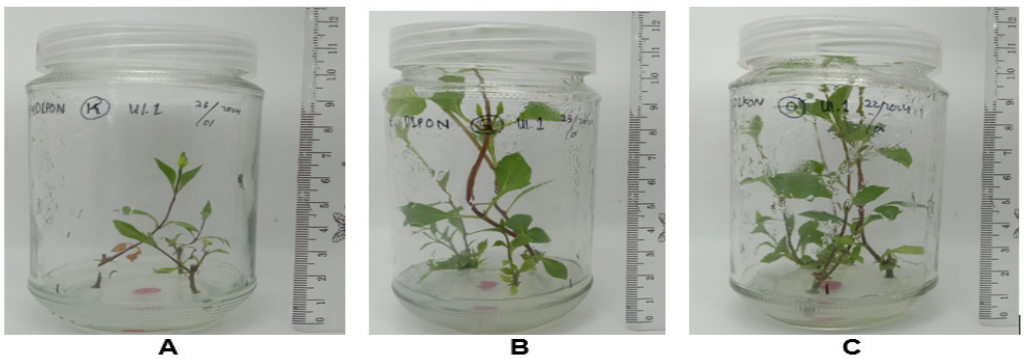

Figure 5. The performance of *Dahlia* sp. cultured in DKW medium containing different combinations of plant growth regulators and different types of explants at 8 WAC. On the media supplemented with 1 mg/L BAP + 1 mg/L NAA using apical shoots as explants (A) On the media supplemented with 1 mg/L 2-iP + 1 mg/L NAA using middle nodes as explants (B) and 1 mg/L Kinetin + 0 mg/L NAA basal nodes as explants (C)

and the largest number of leaves. The performance of leaves looks greener and wider, accompanied by sturdier shoots (Figure 5).

The type and level of cytokinins influence the growth of *Dahlia* sp. shoots cultured in vitro on DKW medium. Likewise, the type and level of auxin also affect root formation. The medium supplemented with 1 mg/L 2-iP and NAA is the most effective for promoting shoot growth, increasing the number of shoots and roots, and determining both the fresh and dry weights of *Dahlia* sp. The combination of these PGRs, using middle nodes as explants, produces the highest number of roots. The selection of growth regulators and explant types is crucial for identifying the best medium composition and the appropriate explant type for the growth and propagation of *Dahlia* sp. Shoots

Conclusions

The explant type significantly affected the parameters of shoot height, number of shoots, number of leaves, and dry weight. Meanwhile, the PGR combination affects all variables' growth metrics. There are interactions between the two factors tested (explant type and PGR) in all parameter growth except in fresh weight. The highest average values of shoot height, number of shoots, number of roots, fresh weight, and dry weight occurred in media supplemented with 1 mg/L 2-iP + 1 mg/L NAA using middle nodes as explants. Meanwhile, the highest number of leaves were produced in the media containing 1 mg/L Kinetin + 0 mg/L NAA using basal nodes as explants.

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