International Journal of Economic, Technology and Social Sciences
e-ISSN 2775-2976
International Journal of Economic, Technology and Social Sciences
url: https://jurnal.ceredindonesia.or.id/index.php/injects
Volume 4 Number 1 page 79 - 85

Callus Induction of Marigold Plants (*Tagetes Erecta L*) with a Combination of 2,4D and Kinetin

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ABSTRACT
Marigold flower (Tagetes erecta L.) is an ornamental flowering plant that belongs to the Astarecae family. Marigold flowers are used as ornamental plants in pots and as flower bouquets. The demand for marigold flowers is very high, so efforts are needed to quickly propagate marigold plants and produce good quality. Efforts to provide marigolds are carried out by increasing the production and quality of marigold plants. Tissue culture is an in vitro technique of plant propagation and is effective in producing large, uniform and disease-free plants. Giving ZPT 2,4-D and Kinetin can stimulate good callus growth and affect the success of callus induction. This study aims to determine the effect of giving 2,4-D and kinetin on the growth of marigold callus induction. The experiment used a randomized block design consisting of two factors, namely 2, 4-D with four concentration levels, namely 0 ppm, 0.5 ppm, 1 ppm and 2 ppm and kinetin with four concentration levels, namely 0 ppm, 0.5 ppm, 1 ppm and 2 ppm with three replicates. The results showed that the combination treatment without 2,4-D and 0.5 ppm kinetin gave the best response when callus appeared 11.67 HST and the treatment of 1 ppm 2,4-D and 2 ppm kinetin gave the best response on the percentage of successful callus induction marigolds.

Keywords: Marigold, Axksin, Cytokinin, In-Vitro

INTRODUCTION

Indonesia has a very high diversity of plants. One of the plants that is often found is the marigold flower (Tagetes erecta L.) or commonly called the kenikir flower. Marigold is an ornamental plant that has compound flowers and belongs to the family/family of Astarecae or belongs to the kenikir-kenikir tribe which belongs to the genus Tagetes. (Bunga et al., 2021). Marigolds are spread all over the world with various types of species. In Indonesia marigolds are often found on the islands of Java, Bali and various other areas in Indonesia. The majority of people call marigolds as kenikir or gumitir. Many other local names are known as the ades flower or Randa Kencana.

Marigold is an annual herbaceous plant or what is called an annual plant with an average height of 0.5 m to 1.5 m from the ground and has tap roots and is a dicot plant.(Sahara, 2022). Marigold has flowers with bright colors, namely white, yellow and orange. The existence of marigold flowers in Indonesia is an introduced species originating from Mexico, South America and grows both in tropical and subtropical regions. Marigolds are adaptable to even drought conditions and have a shorter flowering period from mid-summer to winter(Hormon et al., 2022). Marigold flowers are used as ornamental plants in pots and as flower bouquets. On the island of Bali, especially marigold plants are often found because of their use as Hindu religious ceremonies and other religious activities. This high consumer demand for marigold flowers causes the need for efforts to provide marigold flowers. Efforts to provide marigolds are carried out by increasing the production and quality of marigold plants.

Tissue culture is an in vitro technique of plant propagation and is effective in producing

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a large number of plants that are uniform and free from plant diseases. By using tissue culture techniques, demand fulfillment can be fulfilled in large quantities and in a short time. Another advantage of plant propagation techniques through tissue culture is that they can produce plants that have superior potential according to their parents. Tissue culture can be carried out using organogenesis and somatic embryogenesis methods, the first step that needs to be done is callus induction.(Rasud, 2020).

One way that can be done to increase callus growth is to add growth regulators to the media. There are two types of growth regulators that need to be used in tissue culture, especially during the callus induction phase, namely the use of auxin and cytokinin growth regulators. The use of cytokinins and auxin in the callus induction medium allows us to get callus that is crumbly and allows the desired type of morphogenesis. One of the auxins that is often used is 2,4-D and the cytokinin that is often used is kinetin(Perlakuan & Kinetin, 2015).

Administration of cytokinin growth regulators to callus induction media plays an important role in cell division and elongation, thereby accelerating callus growth and development. According to(Perlakuan & Kinetin, 2015)administration of auxin and kinetin with the right concentration can stimulate callus formation on explants. Research result(Silvina & Ningsih, 2021)Callus growth on binahong plants showed that a concentration of 1 ppm auxin gave rise to callus and the fastest callus formation, while kinetin with a concentration of 0.5 ppm produced the highest percentage of callus. This study aims to determine the effect of growth regulator 2,4-D and auxin on callus growth in marigold callus induction and to obtain the best combination in inducing marigold callus.

METHODS

This research was conducted at the Jember State Polytechnic from October 2022 to May 2023. The materials used in this study included: marigold leaves (Tagets erecta L), Murashige and Skoog (MS) media, sucrose, Agar, 2,4-D , Kinetin, Aquades, Chlorok, 70% alcohol, and dithane. The tools used in this research areThe equipment used in making media is measuring cup, micropipette, magnetic stirrer, a set of equipment for making MS media (analytical balance, pH meter, autoclave). The equipment used for planting is culture bottles, petridishes, Laminar Air Flow Cabinet (LAF), handsprayer, plastic wrap, aluminum foil, scalpel, tweezers, filter paper, orbital shaker. The equipment used for observing explants was a stereo microscope and a camera.

The preparation of the planting medium was first carried out by weighing 2.2 grams of instant MS media, then 12 grams of sucrose was added, the addition of ZPT was carried out according to the concentration of the treatment. Adjust volume to 500 ml. then take a pH measurement with a recommendation of 5.8 after that add agar and heat it using a microwave until the mediam solution becomes clear and then remove it. The prepared media is then filled into disposable petridishes and 20 ml of media bottles. then sterilize using an autoclave at a pressure of 17.5 psi and a temperature of 121°C for approximately 10 minutes.

The study used a randomized block design (RBD) which consisted of 2 factors, namely 2.4-D with four levels of 0 ppm, 0.5 ppm, 1 ppm, 2 ppm and kinetin with four levels: 0 ppm, 0.5 ppm, 1 ppm, 1.5 ppm. The combination of all factors was repeated three times to produce 48 experimental units. Observations were made covering qualitative observations made on callus

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color and callus texture while quantitative observations were made on the time when callus appeared by counting the days the callus appeared since the explants were planted, callus weight by weighing the callus using an analytical balance. The data obtained were analyzed by Sidik Variety and continued with Duncan's Multiple Range Test at 5% level.

RESULTS AND DISCUSSION

Callus color and callus texture

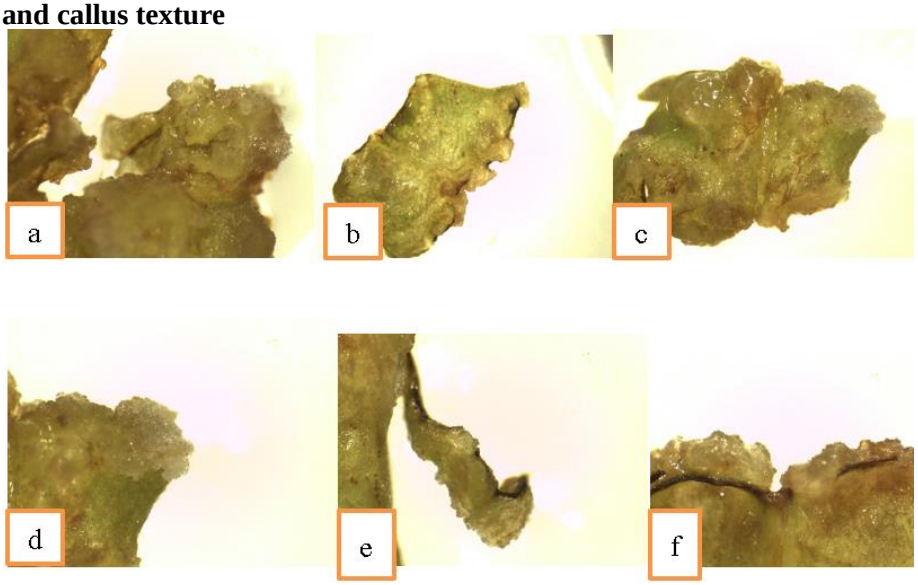

Figure 1.
Appearance of Marigold Callus Color Induced on MS Media with the Addition of ZPT 2,4-D and Kinetin at 12 WAP. As for all treatments, the color of the callus was uniform, namely brownish green and whitish.

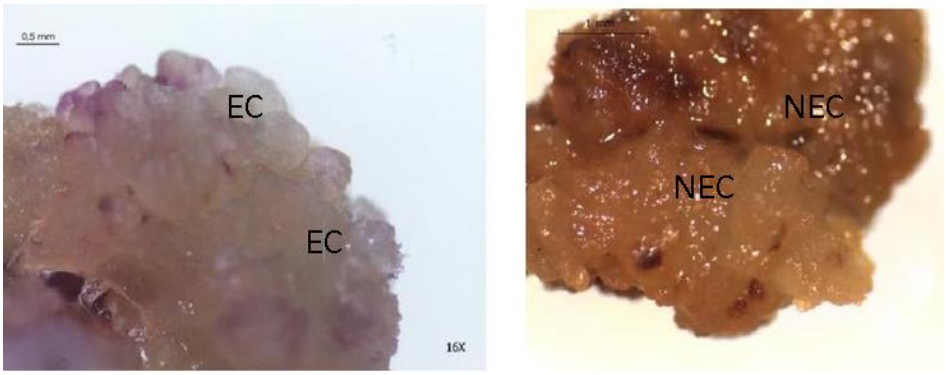

Figure 2.
Appearance of Marigold Callus Texture Induced by Using 2,4-D and Kinetin at 12 WAP. a. Callus with Crumb Structure (1 ppm 2,4-D and 2 ppm Kinetin). b. Compact Structured Callus (0 ppm 2, 4-D and 2 ppm Kinetin).

The morphology of the callus includes the texture and color of the callus, the texture and

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color of the callus are used as indicators of the quality of the resulting callus which is shown in Figures 1 and 2. Based on the observation of callus color in Figure 1, the entire callus has a brownish green and white color. According to(Silvina & Ningsih, 2021)the characteristics of callus that are not well developed are characterized by a brown color, which results from the presence of phenol. The presence of phenol can affect the absorption of nutrients from the media into the callus. As a result, the callus will experience a condition of failure to thrive. While callus that has a green color indicates the presence of chlorophyll, white to yellowish callus is a well-developed callus and is actively experiencing division.(Suhesti et al., 2015)

Whereas in Figure 2 it shows that there are differences in the callus formed from marigold leaf exlans. Morphologically, it has two types of callus structures, namely embryogenic (EC) and non-embryogenic (NEC) callus. The characteristics of EC callus are yellowish white, shiny glossy, visible dry and not compact and crumbly. While EC callus has the characteristics of brownish, wet, and has a compact texture. marigold callus texture, in figure a (1 ppm 2,4-D and 2 ppm Kinetin) shows a crumbly and friable callus texture. Where the indicator of well-developed callus is that it has crumb characteristics and is not compact. This is in accordance with the statement(Hormon et al., 2022)that giving the concentration of 2,4-D at the right dose can produce callus that is crumbly. Whereas in Figure 2.b (0 ppm 2,4-D and 2 ppm Kinetin) shows a compact texture appearance, this indicates that the callus is not developing properly. The compact callus undergoes a process of thickening the cell wall so that it has a hard texture(Rasud, 2020). A compact callus texture indicates slow cell division activity, which affects the absorption of nutrients found in the planting medium.

Callus Time

The results of the analysis of variance showed that there was a significant effect of 2,4-D and kinetin on the time of marigold callus appearance.The addition of growth regulators to the culture media is the main factor that will coordinate cell division thereby stimulating callus formation. The following

Table.1 The Stages of Callus Formation

Callus Formation Phase	Formation Time (hst)
Swelling on the explant surface.	7 (1 week)
Nodular formation on the explant side.	12-14 (2 weeks)
Pre-embryo mass.	28 (4 weeks)
Early globular phase.	42 (6 weeks)

Embryogenic callus formation begins at the age of 7 days after incubation which is characterized by swelling on the surface due to cell division. At the age of 12-14 days after incubation, nodular formation occurred on the surface of the explants. After that, the callus enters the pre-embryonic stage which is characterized by a soft, crystal-like callus structure at the age of 28 days after incubation. (Suhesti et al., 2015). Furthermore, the callus entered the globular phase which was characterized by white color, crumbs, and there were many nodules at 42 days after incubation. Based on microscopic observations, callus induced results from embryogenic and non-embryogenic callus. The characteristics of embryogenic callus are light yellowish white in color, friable and not wet.

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Table 2. Treatment of ZPT 2,4-D and Kinetin

	Kinetin			
Dosage of 2,4-D (ppm)	**0**	**0.5**	**1**	**2**
0	67,15a	12,37ab	25.00a	26,20b
	A	A	A	A
0.5	19,10a	27.00a	25,55a	29.65a
	AB	A	B	AB
1	43,17a	0.00b	33,33a	14,17b
	A	B	AB	B
2	41,28a	0.00b	0.00b	0.00b
	A	B	B	B

Numbers followed by capital letters with the same horizontal direction (rows) and numbers followed by lowercase letters with the same vertical direction (columns) were not significantly different based on Duncan's test results at the 5% level.

Table 2. Shows that in the treatment of ZPT 2,4-D and Kinetin, the fastest callus appearance was in the 0.5 ppm kinetin treatment without 2,4-D compared to other treatments. And not significantly different with the administration of 1 ppm 2,4-D and 2 ppm kinetin. Marlin et al 2012 stated that to stimulate the formation of callus a high concentration of auxin is required. The difference in the time the callus appeared on the explants was influenced by many factors, including an increase in unequal cell division, the influence of hormone administration, and genetic conditions, the age of the tissue and the type of plant as well as these environmental factors.

Environmental factors can be in the form of light, temperature, air humidity and the ability of a tissue to absorb nutrients available in the media environment. Administration of auxin and cytokinins in tissue culture media can have a good effect on the initiation of callus formation. Auxins can stimulate swelling in explants and cytokinins can stimulate the division and expansion of plant cells. Subsequent divisions will stimulate the multiplication of the number of cells which will then form a callus.(Perlakuan & Kinetin, 2015)

Callus Fresh Weight

The results of the analysis of variance on the callus weight parameter showed that there was a significantly different effect on the administration of auxin 2,4-D as well as kinetin which is shown in Table 3. The callus weight produced in each treatment depended on the speed at which the plant cells divide, reproduce cells, so as to produce an increasingly large callus. Enlarged callus makes the weight increase. The combination of giving 2,4-D and kinetin 1 ppm 2,4-D and 2 ppm kinetin was shown to increase callus when compared to the combination of kinetin and 2,4-D in the treatment of 0 ppm 2,4-D and 0.5 ppm kinetin . This allows the administration of 2,4-D and kinetin not to significantly affect the weight of the callus. The role of 2,4-D can increase the acceleration of callus growth, increase osmotic pressure and cell permeability,(Silvina & Ningsih, 2021).

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Table 3. Marigold Callus Weight

	Kinetin			
Dosage of 2,4-D (ppm)	**0**	**0.5**	**1**	**2**
0	1.50b	0.60b	3.00ab	3.45ab
	B	B	A	A
0.5	3,20a	3,10a	4.50a	4.50ab
	A	A	A	A
1	4,15a	0.00b	1.00b	5,70a
	A	B	AB	A
2	3,10ab	0.00b	0.00b	0.00b
	A	B	B	B

Numbers followed by capital letters with the same horizontal direction (rows) and numbers followed by lowercase letters with the same vertical direction (columns) were not significantly different based on Duncan's test results at the 5% level.

Based on the parameters above, it can be seen if there is a successful percentage of marigold callus induction with a combination of ZPT 2,4-D and Kinetin. this is shown by the significant effect on the combination of 2,4-D 1 ppm and 2 ppm kinetin. The role of auxins and cytokinins can significantly influence cell division of marigold explants to initiate the formation, division, cell elongation and cell multiplication. So that in this case callus can be formed in accordance with the theory of totipotency in which every part of a plant cell has the ability to grow into a new individual if it is in an appropriate environment. The existence of an insignificant effect is possible due to explant factors and the dose of auxin and kinetin administration.

CONCLUSION

Based on the results of research that has been done, it can be concluded that the best combination of giving auxin 2,4-D and kinetin which can provide the best response when callus appears is in the treatment of 2,4-D 1 ppm and 2 ppm kinetin.

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e-ISSN 2775-2976
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