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EFFECT OF THE COMBINATION OF THE GROWTH REGULATORS AND PUTRESCINE ON THE SOMATIC EMBRYOGENESIS OF WHEAT (*Triticum aestivum* L.) ON SOME TYPES OF EXPLANTS

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Abstract*. Plant breeding programs need to be carried out in order to improve the genetics of wheat that is able to adapt to tropical environments through hybridization, mutation induction, tissue culture, and genetic transformation. In vitro culture through somatic embryogenesis pathways plays an important role in genetic improvement and its integration with other breeding programs can positively affect the improvement of wheat quality, quantity, and development in Indonesia. The purpose of this study was to obtain an embryogenic callus induction method from the Dewata variety using five different types of explants, namely mature Seeds, immature embryos, immature seeds, leaf, stem, and* to obtain combination of plant growth regulators and putrescine on somatic embryogenesis of wheat. *The experimental design was prepared based on a complete randomized design with a combination treatment of embryogenic callus induction media consisting of 9 levels, namely: 1 ppm 2.4- D, 1 ppm 2.4-D + 1 ppm Picloram, ppm 2.4-D + 1 mg / L Picloram, 1 ppm 2.4-D + 10^{-4} M Putrescine, 1 ppm 2.4-D + 1 ppm Picloram +10^{-4} M Putrescine,2 ppm 2.4-D + 1 ppm Picloram + 10^{-4} M Putrescine, 1 ppm 2.4 D + 10^{-3} M Putrescine, 1 ppm 2.4 D + 1 ppm Picloram + 10^{-3} M Putrescine, 2 ppm 2.4 D + 1 ppm Picloram + 10^{-3} M Putrescine. The results showed that the media used was able to induce embryogenic callus using mature seed and immature embryo, but immature seed and leaf were not able to produce embryogenic callus. The best media that produced the highest percentage of embryogenic callus was 2 ppm 2.4-D + 1 ppm Picloram + 10^{-4} M Putrescine with as much as 85.9% in young embryo explants.*

Keywords: Dewata; embryogenic callus; immature embryos; in vitro; variety

1. Introduction

Wheat (*Triticum aestivum* L.) is a cereal crop of a grain tribe native to the subtropics. Wheat contains carbohydrates, a fairly high content of gluten and protein at 13.8 grams compared to 7.5 grams of rice and 9.2 grams of corn per 100 grams of ingredients. The consumption of foods derived from wheat and its derivatives is increasing beyond the consumption of tubers (Ariani, 2010). Indonesia's wheat imports from year to year are increasing, causing it to become the world's largest wheat importer country beating Egypt. Wheat imports in 2020 reached 11.17 million tons (Central Statistics Agency, 2020). Food dependence from abroad is feared to be a threat to food security, especially wheat crops.

Wheat development is a challenge for Indonesia to produce wheat on a large scale in order to reduce wheat imports. Wheat cultivation in the highlands is limited, due to land competition with horticultural crops that have more selling value. Plant breeding programs need to be carried

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out in order to improve genetics and superior character (Lee & Huang, 2014; Hesami *et al.*, 2018). Somatic embryogenesis if integrated with plant breeding programs such as mutation induction, somaclonal variation, protoplast fusion and genetic engineering can have a positive effect on improving the quality, quantity, and development of wheat in Indonesia (Komamine *et al.*, 2005).

Many factors affect the success of somatic embryogenesis including growth regulators, types of explants, medium, and storage condition. Research on embryogenesis has been conducted by Setiawan (2015) which states that the use of more immature embryo (22.85%) produces embryogenic callus when compared to mature embryo (0.56%). Smit and Weijers (2015) states that auxin inducing promoters have reported that auxin spatial and temporal distribution mediate key steps in early embryo settings. Auxin is a regulator of the early add stages of somatic embryo establishment and postembryonic plant development, such as 2.4-D and picloram (Asghar *et al.*, 2022). The use of polyamine compounds can increase the percentage of embryogenic callus. Polyamine compounds commonly used are putrescine, spermine and spermidine (Aydin *et al.*, 2016). The purpose of this study was to obtain an embryogenic callus induction method from the Dewata variety using five different types of explants, namely mature Seeds, immature embryos, immature seeds, leaf, stem, and to obtain combination of plant growth regulators and putrescine on somatic embryogenesis of wheat.

2. Methods

This research was conducted from June to October 2020 at the Network Culture Laboratory of the Faculty of Agriculture, Andalas University, Padang. The explants used were mature seeds, immature embryos, immature Seeds, leaves and stems. The explants were sterilized using fungicide solutions (*mancozeb* active ingredients) and bactericides (*streptomycin sulfate* active ingredients) with aconcentration of 2 g/L each for 30 minutes, continued in *laminar air flowcabinet* (LAFC). Then, the explants were soaked in 1.25% of *Na-hypochlorite* solution for 30 minutes and rinsed with sterile aqueous. After that, the explants were soaked in 70% of alcohol for 3 minutes and rinsed with aqueous sterile 3 times while being stirred with a spatula and then transferred into a petri dish to proceed to the planting process. The medium used was MS (*Murashige* and *Skoog*) media, while other materials used were PGR 2,4-D, Picloram and Putrescine, sucrose 30 g/L, *bacto agar* 8 g/L and pH 5.8.

The design used was a Complete Randomized Design. The treatment used was a combination of plant growth regulator (PGR) and putrescine, which consists of 9 levels, namely: 1 mg / L 2.4-D, 1 mg / L 2.4-D + 1 mg / L Picloram, 2 mg / L 2.4-D + 1 mg / L Picloram, 1 mg / L 2.4-D + 10^{-4} M Putrescine, 1 mg / L 2.4-D + 1 mg /L Picloram +10^{-4} M Putrescine, 2 ppm 2.4-D

+ 1 ppm Picloram + 10^{-4} M Putrescine,1 ppm 2.4 D + 10^{-3} M Putrescine, 1 ppm 2.4 D + 1 ppm Picloram + 10^{-3} M Putrescine, 2 ppm 2.4 D + 1 ppm Picloram + 10^{-3} M Putrescine. The experiment used type f explants that were not on treatments. Each level of treatment was repeated 10 times, so that 90 experimental units obtained per explant were used. Observations include the percentage of callus formation, the time of callus induction, the percentage of embryonic callus formation, the fresh weight of the callus, the percentage of callus color, and the percentage of callus texture.

The data obtained were statistically analyzed using the STAR (*Statistical Tool For Agriculture Research*) program. Data were analyzed using Test F at a real level of 5%. Real different data was continued using the Duncan multiple hose test (DMRT) at a real level of 5%.

3. Results and Discussion

3.1 The percentage of forming callus

The combination of PGR and putrescine in Table 1 shows no influence on the percentage of explants forming the callus of wheat crops. The treatment given is able to induce 100% of callus in the explants of mature seed and immature embryos. This indicates a combination of PGR and putrescine used precisely to induce callus. The use of a combination of PGR and putrescine on immature seed and leaf explants has not been able to induce callus, while in stem explants, a combination of 2 ppm 2.4-D + 1 ppm Picloram + 10^{-3} M Putrescine is able to induce the highest percentage of callus formation at about 20.00%.

Table 1. Percentage of callus formation on some media and types of explant (%)

Media	Mature Seeds	Immature Embryos	Immature Seeds	Leaf	Stem
1 ppm 2.4-D	100.00	100.00	0.00	0.00	0.00 b
1 ppm 2.4-D + 1 ppm Picloram	100.00	100.00	0.00	0.00	0.00 b
2 ppm 2.4-D + 1 ppm Picloram	100.00	100.00	0.00	0.00	0.00 b
1 ppm 2.4-D + 10^{-4} M Putrescine	100.00	100.00	0.00	0.00	0.00 b
1 ppm 2.4-D + 1 ppm Picloram + 10^{-4} M Putrescine	100.00	100.00	0.00	0.00	0.00 b
2 ppm 2.4-D + 1 ppm Picloram + 10^{-4} M Putrescine	100.00	100.00	0.00	0.00	0.00 b
1 ppm 2.4-D + 10^{-3}M Putrescine	100.00	100.00	0.00	0.00	0.00 b
1 ppm 2.4-D + 1 ppm Picloram + 10^{-3}M Putrescine	100.00	100.00	0.00	0.00	0.00 b
2 ppm 2.4-D + 1 ppm Picloram +10^{-3}M Putrescine	100.00	100.00	0.00	0.00	20.00 a

Description: Data followed by different lowercase letters in the column showed anoticeable effect based on the DMRT test level of α = 5 %.

The success of explants in forming callus is influenced by several factors including the types of media and explant used. The use of explants and the right type will result in a high percentage of callus. Sharma *et al.* (2005) explained that different types of explants will give different responses. The immature embryo produced 94.00% of callus, the mature embryos produced 69% of callus and the scutellar did not induce callus. The use of putrescine was able to increase the percentage of callus formation. This is supported by Rakesh *et al.* (2021) that reported spermine and putrescine have effect on embryogenic calli development and meristemoid formation.

3.2. **The timing of the callus induction**

The results showed that the combination of PGR and putrescine had different influences. Callus in mature seed appears in the range of 9.0-10.0 day after culture (DAC), while in Immature Embryos, it ranges from 4.0 to5.3 DAC. On stem callus, it only appears with combination of 2 ppm 2.4 D+ 1 ppm Picloram + 10^{-3} M Putrescine at about 16.6 DAC (Table 2)

Table 2. Induction time of wheat callus on some media and explant type (DAC)

Media	Mature Seeds	Immature Embryos	Immature Seeds	Leaf	Stem
1 ppm 2.4-D	9.3	4.6	0.0	0.0	0.0 b
1 ppm 2.4-D + 1 ppm Picloram	9.6	4.0	0.0	0.0	0.0 b
2 ppm 2.4-D + 1 ppm Picloram	9.0	4.0	0.0	0.0	0.0 b
1 ppm 2.4-D + 10^{-4} M Putrescine	9.0	5.3	0.0	0.0	0.0 b
1 ppm 2.4-D + 1 ppm Picloram + 10^{-4} M Putrescine	9.0	4.3	0.0	0.0	0.0 b
2 ppm 2.4-D + 1 ppm Picloram + 10^{-4} M Putrescine	9.6	4.0	0.0	0.0	0.0 b
1 ppm 2.4-D + 10^{-3} M Putrescine	10.0	4.6	0.0	0.0	0.0 b
1 ppm 2.4-D + 1 ppm Picloram + 10^{-3}M Putrescine	9.0	4.0	0.0	0.0	0.0 b
2 ppm 2.4-D + 1 ppm Picloram + 10^{-3} M Putrescine	9.0	4.3	0.0	0.0	16.6 a

Description: Data followed by different lowercase letters in the column shows anoticeable effect based on the DMRT test level of α = 5 %

The appearance of callus was characterized by the swelling of the explants, then a clear gel-like layer appears on the surface of the explants. Young embryo explants give rise to callus faster when compared to other explants. This shows the use of young tissue is more effective because it has meristematic properties (still actively dividing). The PGR most commonly used for callus induction of wheat crops are 2,4-D, Picloram and NAA (Asghar *et al.*, 2022). Plant growth regulators and type of explant play an important role in the initiation of callus because each explant requires a particular concentration of exogenous and endogenous hormones for callus production

(Adhikari *et al.*, 2013).

3.3. Percentage of embryogenic callus

The results showed that the influence of the combination of PGR andputrescine on the type of explants used was able to form embryogenic callus. Aged seed explants produced the highest embryogenic callus in a combination of 1 ppm2.4-D +10^{-4} M Putrescine by 30.0%, which was no different from a combination of 1 ppm 2.4-D + 10^{-3} M Putrescine (20.0%). Meanwhile, in other combinations, embryogenic callus was not formed. In Immature Embryos, embryonic callus was formed in the range of 20.0-85.0%, while in young seed explants, the leaves and stems of embryogenic callus were not formed (Table 3).

Table 3. Percentage of wheat embryogenic callus in some media and type of explans (%)

Media	Mature Seeds	Immature Embryos	Immature Seeds	Leaf	Stem
1 ppm 2.4-D	0.0 b	20.0 c	0.0	0.0	0.0
1 ppm 2.4-D + 1 ppm Picloram	0.0 b	20.0 c	0.0	0.0	0.0
2 ppm 2.4-D + 1 ppm Picloram	0.0 b	55.0 b	0.0	0.0	0.0
1 ppm 2.4-D + 10^{-4} M Putrescine	30.0 a	50.0 b	0.0	0.0	0.0
1 ppm 2.4-D + 1 ppm Picloram + 10^{-4} M Putrescine	0.0 b	70.0 a	0.0	0.0	0.0
2 ppm 2.4-D + 1 ppm Picloram + 10^{-4} M Putrescine	0.0 b	85.0 a	0.0	0.0	0.0
1 ppm 2.4-D + 10^{-3}M Putrescine	20.0 a	30.0 bc	0.0	0.0	0.0
1 ppm 2.4-D + 1 ppm Picloram + 10^{-3}M Putrescine	0.0 b	20.0 c	0.0	0.0	0.0
2 ppm 2.4-D + 1 ppm Picloram + 10^{-3}M Putrescine	0.0 b	40.0 b	0.0	0.0	0.0

Description: Data followed by different lowercase letters in the column shows anoticeable effect based on the DMRT test level of α = 5 %

Embryogenic callus has a crumb texture, has a yellowish-white or yellow- white color with a green spot (Figure 1). The characteristics of embryogenic callus were also expressed by Tamimi and Othman (2021) report that a yellowish-yellow callus with a crumb texture.

The combination of PGR and putrescine given to young embryo explants was able to produce embryogenic callus in the entire combination. Meanwhile, the combination of 2 ppm 2.4-D + 1 ppm Picloram + 10^{-4} M Putrescine produced the highest embryogenic callus with 85.0%. The addition of putrescine can increase embryogenic callus and this is in line with what Aydin *et al*. (2016) report which stated that the addition of 10^{-4} M putrescine results in 100% embryogenic callus in Kirik variety wheat. Several research reported that maximum callus induction for indica rice species was obtained on MS medium with 2–3 mg/L (Toppo *et al.*, 2014; Abiri *et al.*, 2017;

Kalhori *et al.*, 2017). In grass tissue culture, the auxin 2,4-D has been utilised in different proportion such as, in Paspalum scrobiculatum 20 mg/L, in switch grass 5 mg/L and in Panicum maximum 10 mg/L (Grant *et al.*, 2017). Auxin was effective in inducing 'Fengdan' somatic cembryos (Ren *et al.*, 2020).

Figure 1. Calluses that have the potential to become embryogenic calluses.

3.4. Callus weights

The results showed that the combination of plant growth regulator have no effect on the weight of the callus. Mature seeds have callus weights ranged from 107.55 to360.30 g. Meanwhile, in immature embryo explants, it ranged from 274.90 to503.10 g. A combination of 2 ppm 2.4-D + 1 ppm Picloram +10^{-3} M Putrescine on stem explants resulted in a callus weight of 132.40 g (Table 4).

Table 4. Weight of wheat callus in some medium and type of explant age 8 MST(g)

Media	Mature Seeds	Immature Embryos	Immature Seeds	Leaf	Stem
1 ppm 2.4-D	258.65	394.55	0.00	0.00	0.00
1 ppm 2.4-D + 1 ppm Picloram	275.80	362.55	0.00	0.00	0.00
2 ppm 2.4-D + 1 ppm Picloram	192.65	288.95	0.00	0.00	0.00
1 ppm 2.4-D + 10^{-4} M Putrescine	279.10	503.10	0.00	0.00	0.00
1 ppm 2.4-D + 1 ppm Picloram +10^{-4} M Putrescine	197.90	458.40	0.00	0.00	0.00
2 ppm 2.4-D + 1 ppm Picloram + 10^{-4} M Putrescine	259.45	368.75	0.00	0.00	0.00
1 ppm 2.4-D + 10^{-3}M Putrescine	360.30	341.80	0.00	0.00	0.00
1 ppm 2.4-D + 1 ppm Picloram + 10^{-3}M Putrescine	107.55	317.25	0.00	0.00	0.00
2 ppm 2.4-D + 1 ppm Picloram + 10^{-3}M Putrescine	156.20	274.90	0.00	0.00	132.40

Description: The data has no real effect based on the F test level α = 5%

It is expected that the heaviest callus weight obtained with explants of Immature Embryos, which is 503.10 g, would lead to higher callus weights produced and also a higher likelihood of callus becoming embryogenic. Numerous reports are emerging that suggest auxin and its gene

regulatory network could play a crucial role in cell division, callus induction and governing storage root development (Kolachevskaya *et al.*, 2019; Hoang *et al.*, 2020)

3.5. Callus texture

The results showed that the old seed explants produced a compact textured callus in the overall combination of PGR and putrescine, while in Immature Embryos, it had a diversity of callus textures, namely crumb and compact. In the resulting callusstem escrow, it had a compact texture (Table 5).

Table 5. Dominant Texture of Wheat Callus on some media and explant types

Media	Mature Seeds	Immature Embryos	Immature Seeds	Leaf	Stem
1 ppm 2.4-D	Compact	Compact	-	-	-
1 ppm 2.4-D + 1 ppm Picloram	Compact	Compact	-	-	-
2 ppm 2.4-D + 1 ppm Picloram	Compact	Crumbs	-	-	-
1 ppm 2.4-D + 10^{-4}M Putrescine	Compact	Crumbs	-	-	-
1 ppm 2.4-D + 1 ppm Picloram + 10^{-4}M Putrescine	Compact	Crumbs	-	-	-
2 ppm 2.4-D + 1 ppm Picloram + 10^{-4}M Putrescine	Compact	Crumbs	-	-	-
1 ppm 2.4-D + + 10^{-3}M Putrescine	Compact	Compact	-	-	-
1 ppm 2.4-D + 1 ppm Picloram + 10^{-3}M Putrescine	Compact	Compact	-	-	-
2 ppm 2.4-D + 1 ppm Picloram + 10^{-3}M Putrescine	Compact	Compact	-	-	Compact

The texture of the callus describes the direction of callus formation. The texture of callus in Immature Embryos is a callus with a crumb texture and the callus of crumbs has the potential to produce embryogenic callus (Mostafiz *et al.*, 2018). Tamimi and Othman (2021) reported that crumb texture on callus indicates that the callus is potentially embryogenic. Compact callus textures have the potential to form organogenic calluses.

3.6. Callus color

The resulting color of the combination of PGR and putrescine given was diverse.The callus produced from the explants of Mature Seeds produced a uniform color, namely white. Meanwhile, in Immature Embryos, it produced white and white-greenish-yellowcolors. The explants of the stem produced a callus of white color (Table 6).

The color of the callus indicates the identity of the callus to be formed. Calluses in young embryo explants got a greenish-yellow white outgrowth. This color indicates that callus has the potential to produce embryogenic callus. Setiawan (2015) reported that embryogenic callus has a yellowish-white and greenish-yellow color. Tamimi and Othman (2021) also states the characteristics of embryogenic callus are greenish-yellow.

Table 6. Dominant Color of Calus Wheat on some media and explant types

Media	Mature Seeds	Immature Embryos	Immature Seeds	Leaf	Stem
1 ppm 2.4-D	White	White	-	-	-
1 ppm 2.4-D + 1 ppm Picloram	White	White	-	-	-
2 ppm 2.4-D + 1 ppm Picloram	White	White Greenish-Yellow	-	-	-
1 ppm 2.4-D + 10^{-4} M Putrescine	White	White Greenish-Yellow	-	-	-
1 ppm 2.4-D + 1 ppm Picloram + 10^{-4} M Putrescine	White	White Greenish-Yellow	-	-	-
2 ppm 2.4-D + 1 ppm Picloram +10^{-4} M Putrescine	White	White Greenish-Yellow	-	-	-
1 ppm 2.4-D + 10^{-3}M Putrescine	White	White	-	-	-
1 ppm 2.4-D + 1 ppm Picloram + 10^{-3}M Putrescine	White	White	-	-	-
2 ppm 2.4-D + 1 ppm Picloram +10-^{3}M Putrescine	White	White	-	-	White

4. Conclusions

The results showed that the medium used was able to induce embryogenic callus using exploits from Mature Seed and Immature Embryos, whereas explants from Immature Seed and leaf failed to produce embryogenic callus. Medium to produce the highest percentage of embryogenic callus was 2 ppm 2,4-D + 1 ppm picloram + 10^{-4} M putrescine, with up to 85.0% in immature embryo explants.

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