Annals of R.S.C.B., ISSN: 1583-6258, Vol. 27, Issue 1, 2023, Pages. 88 - 99
Received 25 January 2023; Accepted 05 February 2023.

Callus Induction from Anther and Seed Explants of Local Rice (Oryza sativa L.) from Siak, Riau

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ABSTRACT

Formation of new superior varieties can be obtained using in vitro tissue culture techniques such as anther culture and somaclonal variation through callus culture. Somaclonal variation in rice can be obtained from callus induction using rice anthers and seeds. This study aims to examine the effect of growth regulator 2,4-D and NAA combined with kinetin on callus induction from anthers and to examine the effect of growth regulator 2,4-D on callus induction from the seeds of tasikmalaya rice . The results of this study indicate that the treatment of growth regulators 2,4-D and NAA combined with kinetin did not respond to callus formation from explants of anther of rice var. Tasikmalaya. Treatment of growth regulator 2,4-D stimulated callus formation from seed explants of rice var. Tasikmalaya with the highest percentage of callus formation 40% in 2 mg/l 2,4-D treatment, with a compact and friable texture and a white color.

Keywords
2,4-D, anther, seed, callus, NAA, rice

Introduction

Riau Province, Indonesia is one of the provinces in Indonesia that has a low level of rice production. This is due to land conversion from agricultural land to plantation land for commodities that are considered to have high economic value such as oil palm and rubber (Erwandari, 2017). It is feared that the conversion of rice fields into oil palm plantations could lead to the loss of potential rice fields in Riau Province. This must be addressed by increasing the productivity of existing rice fields and developing other potential lands (Dewi & Purwoko, 2016). One way to increase the productivity of rice fields is by developing new superior rice strains or new superior varieties. The development of new varieties can be done by means of conventional and biotechnological systems. Conventional breeding systems have the main problem of requiring a long time in the assembly of new superior rice varieties. One of the biotechnology techniques to develop new plant varieties by using in vitro culture. In vitro culture is the richest source of producing genetic variation through callus culture and in vitro selection to obtain superior varieties (Lestari, 2016); (Bednarek et al., 2021).

Callus is able to induce diversity and is capable of undergoing embryogenesis and organogenesis. Callus culture of rice plants can be carried out through anther culture and callus induction from rice seed explants (Hussain et al., 2010); (Carsono et al., 2021). The formation of callus in vitro cultures is determined by the proper use of the substrate and growth regulators. Exogenous growth regulators commonly used in the field of in vitro culture are from the auxin and cytokininin groups.

Rice (Oryza sativa L.) var. Tasikmalaya is one of the local rice originating from Bunga Raya Village, Bunga Raya Sub district, Siak District, Riau, Indonesia.This rice is a type of rice with a planting period of 6 months until the harvest period. The advantage of this rice is that in terms of taste, it is preferred by farmers and when cooked, the rice does not get stale quickly. However, the disadvantage of this rice is that it has a long harvest period and is not resistant to pest attacks. Therefore, it is necessary to make efforts to obtain superior varieties of Tasikmalaya rice, among others, through callus culture from anther explants and seeds. This callus culture is expected

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to produce genetic diversity so that selection can be made to obtain high-yielding varieties. This study aims to determine the effect of giving 2,4-D and NAA growth regulators in spurring callus induction from anthers and rice seeds from Siak, Riau, Indonesia.

Literature Review

Anther culture is an important biotechnological tool for breeding haploid rice plants. The application of anther culture techniques in rice plants has two advantages, namely being able to accelerate the acquisition of pure strains so that they can be utilized in accelerating the assembly of new superior varieties of rice plants and can also produce somaclonal variations through callus culture (Tripathy et al., 2019); (Cha-Um et al., 2009). Some researchers have successfully carried out callus induction from the explants of rice anthers. The explant of rice anther genotype BS6444G produced the most callus (22.67 %) at a treatment of 2 mg / l 2.4-D (*2,4-Dichlorophenoxyacetic acid*) combined with 0.5 mg / l kinetin in N6 media (Naik et al., 2017). The use of modified N6 media can increase callus induction of japonica, Nipponbare, and indica × japonica crosses (CXY6, CXY24) (Nicolas et al., 2021).

Callus culture with rice seed explants is one of the techniques for exploiting somaclonal variations and for improving seed quality (Upadhyaya et al., 2015). Some researchers have successfully carried out callus induction from rice seed explants. Based on the results of research (Upadhyaya et al., 2015) Sita variety rice produced the highest callus frequency, namely 92.3% which was induced from rice seed explants on MS media with the addition of 2.0 mg / l 2.4-D. Treatment of 1.0 2,4-D mg/l on MS medium, on rice seed explants, Biris variety produced callus with a percentage of 92.5% (Libin, 2012).

Methods

This research was conducted at the Integrated Biology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Riau. Plant materials used for callus induction were anthers and seeds of rice var. Tasikmalaya. This rice comes from Bunga Raya Village, Bunga Raya Sub-district, Siak District, Riau.

The treatment for callus induction from anther explants was 5 treatments on MS medium (Murashige and Skoog, 1962). Treatment consisted of: control (without growth regulators); 1 mg/l NAA (*Nephthalene acetic acid*) + 0.5 mg/l kinetin; 2 mg/l NAA + 0.5 mg/l kinetin; 1 mg/l 2,4-D + 0.5 mg/l kinetin; 2 mg/l 2,4-D + 0.5 mg/l kinetin. The treatment for callus induction from rice seed explants consisted of 4 treatments: control (without growth regulators); 1 mg/l 2,4-D; 2 mg/l 2,4-D; and 4 mg/l 2,4-D. Each treatment was repeated 5 times.

Panicle retrieval and pre-culture treatment followed the method of Sharma (Sharma et al., 2017). Panicles were selected based on the distance between the auricles of the flag leaf and the next leaf between 5-9 cm. Panicles are washed under running water, sprayed with 70% alcohol. Panicles were incubated at 8-10 ^{o}C for 8 days. Then panicles were sterilized with 70% alcohol for 30 seconds. Anthers planted as many as 5 anthers on the culture medium. The cultures were incubated in the culture room in the dark for 30 days. Anther explants for callus induction followed the method of Lee et al. (Lee et al., 2003).

Planting of seed explants for callus induction followed the method of Libin et al. (A. Libin, 2012). The hulled rice seeds were sterilized using detergent for 15 minutes then rinsed with clean water and soaked under running water for 1 hour. Furthermore, the explants were sterilized using fungicides and bactericides for 15 minutes each. The explants were then peeled, then sterilized with

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25% sodium hypochlorite for 10 minutes. Furthermore, the explants were soaked with 10 drops of povidone iodine in 50 ml of water for 10 minutes and dried on filter paper. Rice seeds were planted as much as 2 seeds on the culture medium. The cultures were incubated under light conditions in the incubation room for 14 days.

Parameters observed included the percentage of live explants, the percentage of explants forming callus, callus growth and callus morphology. Observation data consisted of quantitative data (percentage of live explants, percentage of explants forming callus, percentage of callus emergence time and percentage of explants forming shoots) and qualitative data (callus growth and callus morphology). Furthermore, to determine the effect of each treatment, Analysis of Variance (ANOVA) was performed using SPSS 17.0, further testing is carried out using Duncan's Multiple Range Test (DMRT) at a level of 5%. For qualitative data (callus growth and callus morphology) descriptive analysis was performed.

Results

Callus Induction from Rice Anther Explants

The results of observing the callus induction response from rice anther explants in 5 treatments given a combination growth regulator of 2,4-D and NAA which were combined with kinetin and cultured for 90 days after planting, did not show any results.

All treatments with the addition of a growth regulator 2,4-D with a combination of kinetin and NAA with a combination of kinetin on the anther explants of the Tasikmalaya accession did not respond to callus formation. In the treatment of 2 mg/l NAA + 0.5 kinetin, browning occurred on the explants which can be seen in Figure 1.e. The condition of rice anther explants after 90 days of culture can be seen in Figure 1. Figure 1 shows that all treatments with growth regulators 2,4-D and NAA combined with kinetin did not respond to callus formation.

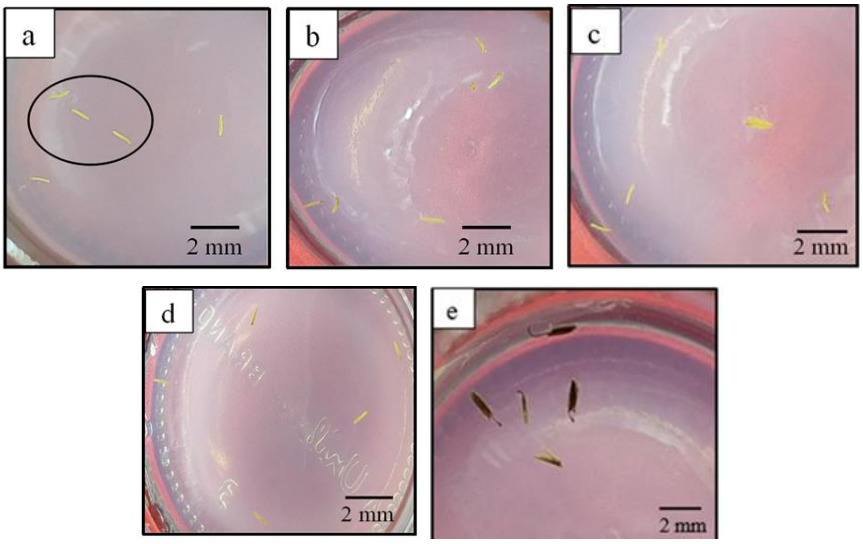

Fig 1. Response of anther explants 90 days after culture: a. MS control, b. treatment 1.0 mg/l NAA + 0.5 mg/l kinetin, c. treatment 2.0 mg/l NAA + 0.5 mg/l kinetin, d. treatment 1.0 mg/l 2,4-D + 0.5 mg/l kinetin, e. treatment 2.0 mg/l 2,4-D + 0.5 mg/l kinetin. The circled image shows the anther.

Callus Induction from Seed Explants

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The results of analysis of variance showed that the treatment with the growth regulator 2,4-D had no significant effect on the percentage of live explants and the percentage of callus induction. However, based on the average number, it was shown that the treatment of 2,4-D was able to stimulate callus induction. The results of observations on the average percentage of live explants and the percentage of callus formation in seed explants of rice var. Tasikmalaya on MS media with the addition of a growth regulator of 2,4- can be seen in Table 1.

Table 1. Percentage of live explants, percentage of callus induction and callus growth from seeds of rice var. Tasikmalaya in 2,4-D treatment after 14 days of culture

Treatment 2,4-D (mg/l)	Percentage of live explants (%)	Percentage of callus induction (%)	Callus Growth
0	50	0	-
1	60	30	++
2	70	40	+++
4	30	30	+

Note: The positive sign (+) indicates that callus was formed on the explant. Different positive (+) signs, i.e. little callus growth (+), moderate (++) and large callus growth (+++).

Table 1 shows that the addition of growth regulator 2,4-D increased the percentage of explant survival only at a concentration of 1 and 2 mg/l 2,4-D. The highest percentage of live explants (70%) occurred in the 2 mg/l 2,4-D treatment.The lowest percentage of live explants was treated with 4 mg/l 2,4-D (30%). Explants that are still alive are indicated by seeds that are still white and not browning.

Rice seed explants grown on MS medium without growth regulators (control treatment) did not form callus. The addition of 2,4-D growth regulator could stimulate callus formation with the highest percentage (40%) in 2 mg/l 2,4-D treatment.

In this study, callus formation begins with swelling of the zygotic embryo to form nodules, followed by changes in callus structure, callus color, and callus texture which can be seen in Figure 2. There is also callus growth that begins with the formation of plumules which then form shoots, then there is a change in the color, structure and texture of the callus.

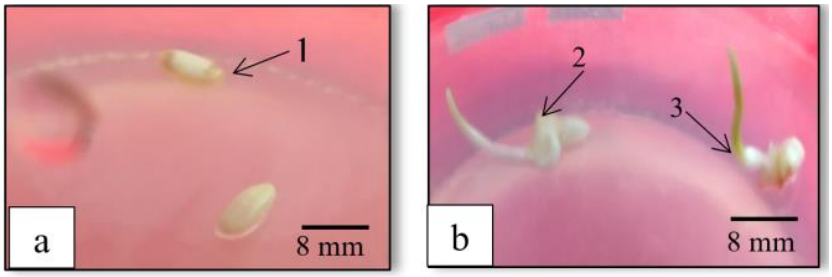

Fig 2. Callus formation in explants treated with 2 mg/l 2,4-D: a) explants formed nodules, b) explants formed shoots and callus. Description: arrows indicate: 1) Nodule, 2) Callus, 3) shoot

Callus growth on seed explants of rice var. Tasikmalaya with 2,4-D treatment after 14 days of culture is presented in Table 1. The results of the study showed that the treatment of various concentrations of 2,4-D on rice seed explants showed different growth of callus. The results showed

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that the highest callus growth was found in treatment of 1 mg/l 2,4-D, while the lowest callus growth was found in treatment 4 mg/l 2.4-D. The results showed that there was a slight callus growth at the tip of the seed embryo in the 4 mg/l 2,4-D treatment. Moderate callus growth, slightly formed callus around the shoots was found in the 1 mg/l 2,4-D treatment and large callus growth was found in the 2 mg/l 2,4-D treatment. Callus growth on seed explants can be seen in Figure 3.

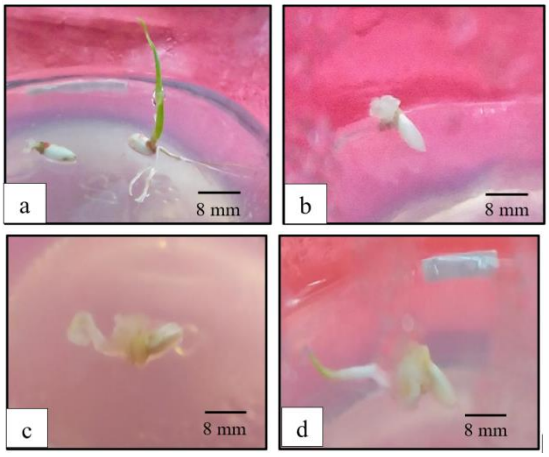

Figure 3. Callus growth on seed explants after 14 days of culture: a. no callus growth in control treatment, b. little callus (+) treatment 4 mg/l 2,4-D, c. medium callus (++) treatment 1 mg/l 2,4-D, d. many callus (+++) treatment 2 mg/l 2,4-D.

One of the hormones that is often used and is very effective in stimulating callus formation is 2,4-D which belongs to the group of auxin growth regulators. In this study, callus was induced from seeds by adding exogenous auxin, namely 2,4-D. Several studies have shown that various growth regulators that play a role in the formation of lateral roots can promote callus formation. Auxin is a growth regulator which is generally used to promote root and callus formation (Ikeuchi et al., 2013).

The callus morphology observed in this study was callus texture and color. Callus is a proliferating mass of undifferentiated and actively dividing cells. Callus texture and color are important indicators in in vitro culture techniques because each explant will produce a different callus morphology. The addition of various concentrations of 2,4-D single in this study resulted in different callus textures and colors. The response of the 2,4-D treatment to the texture and color of callus is presented in Table 2.

Table 2. Callus morphology of seed explants of rice var. Tasikmalaya with 2,4-D treatment after 14 days of culture.

Treatment	Callus texture		Callus Color
2,4-D (mg/l)	Crumb (%)	Compact (%)	
0	0	0	-
1	20	10	Yellowish white
2	10	30	White
4	20	10	White

Table 2 shows that there is a crumbly and compact callus texture. The difference in callus texture is influenced by the composition of growth regulators contained in the culture media. Based on the results of the study, the callus texture obtained from the seed explants of rice var.Tasikmalaya had a crumb (brittle) and compact (not friable) texture. The highest proportion of crumb callus texture

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was found in the 1 mg/l 2,4-D and 4 mg/l 2,4-D treatments, each of 20%. The highest percentage of compact callus (30%) occurred in the 2 mg/l 2,4-D treatment.

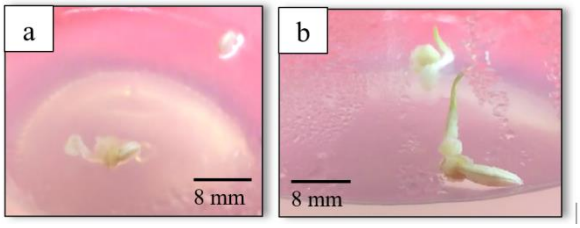

Figure 4. Friable and compact callus texture of rice seed explants after 14 days of culture: a) friable callus in 1 mg/l 2,4-D treatment, b) compact callus in 2 mg/l 2,4-D treatment

The results showed that callus from rice seed explants was white and yellowish white. White callus morphology was found in 2 mg/l 2,4-D and 4 mg/l 2,4-D treatments. Yellowish white callus morphology occurred in 1 mg/l 2,4-D treatment. This difference in callus color indicates that the growth rate of callus in each treatment is different. This is due to differences in explant physiology and concentrations of growth regulators. The morphology of white and yellowish callus can be seen in Figure 5.

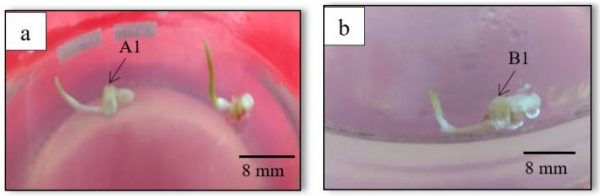

Figure 5. Callus color of rice seed explants after 14 days of culture. a) white callus on 2 mg/l 2,4-D treatment (A1: callus), b) yellowish white callus on 1 mg/l 2,4-D treatment (B1: callus).

Shoot Formation

2,4-D treatment affected shoot growth in rice seed explants. The results of observing the percentage of shoot formation in the 2,4-D treatment can be seen in Table 3.

Table 3. Percentage of shoot formation from seed explants of rice var. Tasikmalaya with 2,4-D treatment after 14 days of culture

Treatment 2,4-D (mg/l)	Shoot formation (%)
0	20
1	20
2	40
4	0

Table 3 shows that not all treatments of growth regulator 2,4-D in rice seed explants formed shoots. Percentage of bud formation 0 to 40%. The 2,4-D growth regulator treatment gave the highest shoot formation response in the 2 mg/l 2,4-D treatment, which was 40%. Rice seed explants treated with 4 mg/l 2,4-D did not form shoots. Treatment of 2 mg/l 2,4-D gave the highest percentage of callus formation, namely 40%. Shoot formation in the treatment without growth regulators occurred directly from the embryo axis, whereas shoot formation in seeds treated with 2,4-D was formed after callus formation, formed from callus (Figures 2 and 3)).

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Discussions

The results of this study indicated that there was no callus formation from anther explants of Tasikmalaya accessioned rice in various growth regulator treatments (1 mg/l NAA + 0.5 mg/l kinetin; 2 mg/l NAA + 0.5 mg/l kinetin; 1 mg/l 2,4-D + 0.5 mg/l kinetin; 2 mg/l 2,4-D + 0.5 mg/l kinetin). Several factors may have caused the growth of callus from anther explants to fail, including improper sterilization techniques, generally difficult callus formation from anther explants, and treatment of growth regulators that were not optimal to stimulate callus formation. Improper sterilization technique maybe because rinsing using distilled water is only done once for each stage of sterilization. Rinsing only once for each stage resulted in the sterilizing material still sticking to the explants, causing toxicity and inhibiting anther growth. In addition, sterilization techniques that are not appropriate are the use of high concentrations of sterilizing materials, namely panicle sterilization using 70% alcohol and anther sterilization using 25% sodium hypochlorite. Sterilization using too much sterilant also results in explant toxicity, inhibits explant growth and results in explant death. To reduce the toxicity of sterilants in explants, it is possible to use sterilants with lower concentrations, for example 0.2% HgCl2 for 10 minutes (Lal et al., 2014); (Sharma et al., 2018); (Cha-Um et al., 2009); (Sikder et al., 1994), 0.1% HgCl2 for 8 minutes (Bishnoi et al., 2000), 4% sodium hypochloride for 3-5 minutes (Joshi & Bimb, 2003), 1% sodium hypochloride for 3 minutes (Arisandi et al., 2020).

The percentage of callus formation from anther explants is generally low. For example, the percentage of callus formation from anthers treated with growth regulator 2,4-D either alone or in combination with NAA and Kinetin, most of the explants did not form callus (Lal et al., 2014). Anther explants of various rice genotypes grown on N6 medium showed a low percentage of callus formation (0.66 - 3.03%) (Joshi & Bimb, 2003). Anther two varieties of rice grown on N6 medium with the addition of 0.5 mg/l Kinetin + 2 mg/l NAA showed a callus induction percentage of 3.08 to 18.33% (Nurhasanah et al., 2016). The addition of 2 mg/l Kinetin + 0.5 mg/l NAA in N6 medium in anther culture of 3 rice varieties (Merah Wangi, Pendok, Dwarf Arum) formed a callus of 0 to 13% (Arisandi et al., 2020). The addition of 1 mg/l 2,4-D + 1 mg/l kinetin in MS medium did not form callus (0%) of the rice anther variety Azucena (Sharma et al., 2018). Treatment of 2,4-D and NAA combined with Kinetin on MS medium from anther explants of two rice genotypes showed a low percentage of callus formation (0.18 to 7.66%) (Hooghvorst et al., 2018). The 2,4-D treatment combined with NAA, Kinetin and BAP on N6 medium from Malaysian indica rice MR219 anther explants, most of them did not form callus (Rahman et al., 2021).

To increase the percentage of callus formation from anthers, it can be done by modifying the culture medium. For example, modification of N6 medium by reducing the concentration of sucrose, by adding several carbon sources (sorbitol, maltose), glycine, and by adding 1 mg/l 2,4-D and 1 mg/l NAA to several rice genotypes can increase the percentage of formation by about 50%. % (Nicolas et al., 2021). Single 2,4-D treatment with higher concentrations (1,2; 2; 2,5; 3 mg/l) on MS medium can increase the percentage of callus formation (100%) of aromatic rice (Sikder et al., 1994). Treatment of 2 mg/l 2,4-D + 1 mg/l kinetin increased the percentage of callus formation (48%) in the rice anther variety Azucena (Sharma et al., 2017).

The results showed that treatment with growth regulator 2,4-D increased the percentage of callus formation from seed explants of the Tasikmalaya variety, with the highest percentage of callus formation (40%) in the 2 mg/l 2,4-D treatment. Callus formation on explants is characterized by the presence of irregular cell masses. The addition of 2,4-D to the media will stimulate cell division and enlargement in explants so as to stimulate callus formation and growth. One of the hormones that is often used and is very effective in stimulating callus formation is 2,4-D which belongs to the group of auxin growth regulators. In this study, callus was induced from seeds by

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adding exogenous auxin, namely 2,4-D. Several studies have shown that various growth regulators that play a role in the formation of lateral roots can promote callus formation. Auxin is a growth regulator that is commonly used to promote root and callus formation (Ikeuchi et al., 2013).

The results of this study indicated that 2,4-D treatment at concentrations of 1, 2 and 4 mg/l could stimulate callus formation. Treatment of auxin can increase osmotic pressure, protein synthesis, increase cell permeability and soften cell walls, so that water can enter and increase cell volume (Ibrahim et al., 2020). The difference in the rate of callus growth and the increase in the rate of cell division, in addition to the influence of growth regulator 2,4-D, is also influenced by tissue conditions, plant species, environmental factors and the ability of tissues to absorb available nutrients.

Based on the results of the study it was found that the highest percentage of callus formation (40%) was found in the 2 mg/l 2,4-D treatment on MS media from rice seed explants var. Tasikmalaya. Several studies of callus induction from rice seed explants also showed a low percentage of callus formation. 25% of callus formation occurred from seed explants of rice cultivar Hom Kra Dang Ngah on MS medium treated with 2 mg/l 2,4-D (Sivakumar et al., 2010). Seeds of rice variety Basmati-370 grown on MS medium with 2.5 mg/l 2,4-D + 0.5 mg/l BAP showed the highest percentage of callus induction (27%) (Ullah et al., 2007).

Several other research results showed a higher percentage of callus formation than seed explants on various cultivars. Callus formation reached 100% of the seed explants of Pajam cultivar rice treated with 2.5 mg/l 2,4-D on MS medium (Ahmad et al., 2010). The percentage of callus formation was 94% for rice variety BAS-370 grown on MS medium with 3 mg treatment /l 2,4-D (Hussain et al., 2010). Himalayan rice seeds of genotype SR-4 grown on MS media with 3.0 mg/L 2,4-D treatment formed callus with the highest percentage (96%) (Noor et al., 2022). The highest percentage of callus formation (90%) was obtained from rice seed explants var. 'Sita', 'Rupali' and 'Swarna Masuri' which were grown on MS medium treated with 2 mg/l 2,4-D (Upadhyaya et al., 2015). The highest callus induction (97%) was found in MS medium with 2 mg/l 2,4-D treatment of the seeds of the Biris variety (A. Libin, 2012). The best callus induction (86.84%) was obtained from seed explants of ASD 16 variety from RRS Ambasamudram, India which were grown on MS media with 2 mg/l 2,4-D + 30 g/l maltose treatment (N, 2020). The highest callus induction (90%) was found on MS medium treated with 2 mg/l 2,4-D from rice seed explants of the Kalijira variety, Bangladesh (Khan, 2019). The highest callus induction (74.2%) was obtained from seed explants of indica rice cv. ADT 43 grown on LS medium treated with 2.5 mg/l 2,4-D (Karthikeyan et al., 2009). The best callus induction (64%) was from Sangyod seed explants grown on media treated with 2 mg/l 2,4-D (Ho et al., 2018). The best callus induction (100%) was obtained from seed explants of Sadamota cultivar rice on MS media with 2 mg/l 2,4-D + 0.5 g/l proline (Rahman et al., 2021).

The results of this study showed a higher percentage of callus formation even with the same treatment as induction of shoots from rice seeds var. Tasikmalaya (2 mg/l 2,4-D) or a slightly higher concentration (2.5 to 3 mg/l 2,4-D). The difference in shoot induction was due to differences in rice genotypes. Another possibility is due to differences in the age of the rice seeds and sterilization techniques. The seeds we use are ripe rice seeds and are cultured after a few weeks of harvest. This can reduce seed viability due to a decrease in seed quality. Our sterilization technique uses 25% sodium hypochlorite (NaOCl) for 10 minutes to reduce contaminants, but may reduce the ability of the seeds to grow, namely callus formation. Several studies that produced a higher percentage of shoot induction used a lower concentration of serilicization material, for example sterilization using 3.5% NaOCl and 1-2 drops Tween-20 for 15 min (M. T. Rahman et al., 2021), 5% NaOCl for 30 min (29), 5.25% NaOCl (Ullah et al., 2007), 5% NaOCl and Tween-20 for 20 minutes (Sidek

et al., 2022), 15% NaOCl and 3 drops of Tween 20 for 10 minutes (Libin, 2012), 0.2 % HgCl for 10 min (Ahmad et al., 2010), 0.1% HgCl for 5 minutes (Upadhyaya et al., 2015), 0.1% HgCl for 4 min (Karthikeyan et al., 2009), 0.1% HgCl for 5 minutes (Sivakumar et al., 2010), and 0.2% HgCl2 for 15 minutes (Mamun et al., 2022). Therefore, some efforts to optimize callus induction from rice seeds var. Tasikmalaya can be done through selecting rice seed explants at various ages, optimizing culture media and using low concentrations of sterilants.

All 2,4-D treatments showed the presence of friable and compact calluses. Crumb callus texture at the end of the observation experienced faster proliferation than the compact callus texture. Friable callus has the characteristics that cells easily decompose, cells grow easily and do not easily oxidize phenolic substances. The formation of friable callus is triggered by the presence of the endogenous hormone auxin which is produced internally by explants that have formed callus. The role of 2,4-D as an exogenous growth regulator for the formation of friable callus is to stimulate cell elongation by adding plasticity to the cell wall to become loose, so that water can enter the cell and cause the cell to elongate, this causes the friable textured callus to contain lots of water. (Ibrahim et al., 2020).

The callus produced from the results of this study is still small (callus growth is still low). This is because the incubation time is still short (only 14 days of culture) and sub-culture has not been done. The callus that is formed has the potential to proliferate and become numerous because the callus has not shown any signs of damage, which is indicated by its color which is still white and nothing is brown yet. Some research results show higher callus growth due to longer incubation time, namely 3 weeks (Ullah et al., 2007); (Khan, 2019), 4 weeks ((Noor et al., 2022); (Poeaim et al., 2016); (Ho et al., 2018); (Yinxia & Te-chato, 2012); (Rahman et al., 2021); (Ho et al., 2018)), 35 days (Sidek et al., 2022), and 6 weeks (A. Libin, 2012). Callus growth can be increased through sub-culture, for example after 15 days of incubation, subculture for 15 days (Sivakumar et al., 2010), after 14 days of incubation, subculture for 24 days (Karthikeyan et al., 2009), after 4 weeks, subculture for 4 weeks (Bano et al., 2005).

The results of this study showed that most calluses were white and formed shoots. The callus has the potential to undergo proliferation and differentiation to form somatic embryos and shoots. If the callus is subcultured on media for callus induction (MS medium + 2 mg/l 2,4-D) callus proliferation will occur. Furthermore, when subcultured on culture media for regeneration can form somatic embryos and shoots (Yinxia & Te-chato, 2012), (Poeaim et al., 2016), (Ho et al., 2018), (Sidek et al., 2022), (Husna et al., 2022).

Conclusion

Treatment of growth regulators 2,4-D and NAA combined with kinetin did not stimulate callus formation from explants of anther of rice var. Tasikmalaya. Treatment of growth regulator 2,4-D stimulated callus formation from seed explants of rice var. Tasikmalaya with the highest percentage of callus formation 40% in 2 mg/l 2,4-D treatment.

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