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Response of Morphogenesis and Cell Proliferation to Allelopatic Compounds on Rice Germplasm

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Abstract: Allelopathic compounds are chemical substances produced by weeds, including Imperata cylindrica, that can inhibit the absorption of nutrients and cell growth of neighboring plants. Tissue culture technique can be used to determine the effect of media composition on morphogenesis and cell proliferation, as indicated by related genes. Through the process of somatic embryogenesis, cells regenerate into whole plants via several phases initiated by gene expression. Genes are the signal that connects environmental cues and plant cells. The objective of this study was to determine the effect of allelopathic compounds from Imperata cylindrica root extract on the morphogenesis and cell proliferation of indigenous Indonesian rice plant. The used callus was derived from the seeds of Bondoyudo, Caok, Ciliwung and Situbagendit rice varieties. The results of callus induction were selected and transferred to regeneration media that had been treated with I. cylindrica roots extract of 2.5 g/L and 5 g/L. The development of callus was monitored weekly for six weeks. As for the molecular analysis, four-week-old callus was utilized. The results demonstrated that the root extract of Imperata cylindrica had a specific effect on the morphogenesis and proliferation of rice cells depending on concentration and target plant. At a molecular level, the expression of the OsBBM, OsLEA, OsLEC1, OsSERK, and OsWOX4 genes was affected differently by the administration of allelopathic compounds at a concentration of 5 g/L on a molecular level.

Keywords: Allelopathic compounds, Gene expression, Indonesia local rice, Tissue culture, Morphogenesis

Introduction

Tissue culture is the *in-vitro* or aseptic propagation of cells, tissues, or plant organs on artificial media, resulting in the regeneration of entire organs. Initially, tissue culture techniques were used to obtain large quantities of seeds in a short period of time. However, the development of tissue culture techniques has been applied to numerous purposes, including the assembly of high-yielding varieties. According to (Wahyurini 2009), the role of tissue culture techniques is extremely advantageous because it enables the rapid and large-scale production of plants with improved properties that are unaffected by environmental factors. Several factors, including sterile conditions, the composition of the medium, and the use of plant growth regulator (PGR) and suitable explants, have contributed to the success of tissue culture. The optimal combination of basic media and PGR will stimulate cell division during the morphogenesis process. Consequently, tissue culture can be used to determine the interaction between the

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chemical composition of the media and plant cells. According to (Suparyono & Setyono 1997), tissue culture is an effective and efficient method for determining the response of cells to medium content, primarily because environmental conditions can be controlled in an *in vitro* culture setting. In tissue culture, the composition of the medium influences morphogenesis and proliferation in plants.

Rice (*Oryza sativa* L.) is an important and primary staple crop for a number of nations, including Indonesia. Weeds are a typical problem in rice cultivation (Zarwazi et al., 2016). The presence of weeds on the field will interfere with the growth and development of the rice plants due to competition. Physically, weeds compete with rice plants for nutrients, air, and light as well as growing space, because weeds are capable of growing faster than rice plants. Chemically, the exudate released by weeds inhibits nutrient absorption and cell division. The exudate released by weeds is an allelopathic secondary metabolite. Based on previous research, allelopathic compounds have a profound effect on their targets (Narwal & Sampietro 2009). The presence of allelopathic compounds inhibits the growth and development of plants. One (Menambah pustaka) of the plants that contain allelopathic compounds is reeds, due to the phenolic substances in the plant. Some of the potent allelopathic chemicals were abscisic acid and methyl caffeate which were extracted from cogon grass rhizomes (Suzuki et al., 2015). Another potential allelopathic substance produced by cogon were phenolic and aromatic acid (Hagan et. al., 2013). By preventing nutrient absorption and inhibiting plant cell growth, these substances could stunt the development of other plants (Kurniati et al., 2018). Karmegam et al. (2014) demonstrated that allelopathy could inhibit germination, germination length, total weight, and chlorophyll content in three rice cultivars.

During the phases of morphogenesis and proliferation, the application of allelopathic compounds induces the expression of a number of genes in rice plants. Genes, such as *OsLEA*, *OsLEC1*, *OsSERK*, *OsBBM*, and *OsWOX4* are involved. These genes have an impact on the morphogenesis of rice plant cells. Rice plants that are resistant to allelopathic compounds have a chemical defense mechanism, whereas those that are sensitive to allelopathic compounds lack a chemical defense mechanism, resulting in retardation of growth and development in response to the allelopathic compound (Usman et al., 2016).

Several studies have reported the effect of allelopathic substances on rice plants, but the effect on morphogenesis and cell proliferation levels has not been comprehensively studied. Since the differentiation of explants into plantlets could be precisely followed and analyzed, this phenomenon could be studied using the tissue culture technique of rice callus induction. Therefore, it is possible to use tissue culture method to determine morphogenesis and cell proliferation in response to an allelopathic compound. The objective of this study was to determine the effect of allelopathic substances in the morphogenesis and cell proliferation responses of indigenous rice varieties in Indonesia. This research will provide the foundational knowledge necessary to comprehend the effect of allelopathic substance affects the growth of indigenous rice varieties in Indonesia.

Materials and Methods

Plant Material and Explant Planting

Rice (*Oryza sativa* L). seed varieties of *Bondoyudo*, *Caok*, *Ciliwung*, and *Situbagendit* were used as material in this research. The above-mentioned seeds were surface sterilized using 70% ethanol prior to planting. Our study used 15 rice seeds as explants in each petri dish with two replications. The sterilized rice seeds were then planted on a standard Murashige and Skoog (MS) medium (Macronutrients: NH_4NO_3, KNO_3, $CaCl_2 \cdot H_2O$, $MgSO_4 \cdot 7H_2O$, KH_2PO_4; Iron: Na_2-EDTA, $FeSO_4 \cdot 7H_2O$. Micronutrients: $MnSO_4 \cdot 4H_2O$, $ZnSO_4 \cdot 7H_2O$, H_3BO_3, KI, $Na_2MoO_4 \cdot 2H_2O$, $CuSO_4 \cdot 5H_2O$, $CoCl_2 \cdot 6H_2O$) containing 30 g L^{-1} sucrose , 3 g L^{-1} GELRITE agar supplemented with 2 mg/l (2 ppm) 2,4-D (2,4 Doichlorophenoxyacetic acid) (for the callus induction according to Upadhyaya et al. (2015). The medium pH was adjusted to 5.8 prior to autoclaving at 120 ^{O}C and 1.5 atm for 15 minutes. The medium containing explant were maintained at growth chamber at 26 ^{O}C and 20% relative humidity in the dark condition. The seed explant was kept in for two to three weeks to observe callus formation. The weekly examination assessed the percentage of callus formation (%) and morphology of the callus. Visual observations were made using stereomicroscope and the calculations were performed as follows:

$$\text{Callus Induction Percentage}=\frac{\text{Total Number of Callus}}{\text{Number of Explant}}\times 100\%$$

$$\text{Callus size}=\frac{(\text{width}+\text{length})}{2}$$

Callus Regeneration

After two to three weeks of incubation, calli from individual varieties with the best growth performance in the induction media were then transferred into regeneration media to obtain healthy plantlets and intact plant parts consisting of shoots, roots, stems, and leaves that were unharmed. The regeneration medium used MS media with the addition of 2 mg L^{-1} Kinetin and 1 mg L^{-1} NAA. Subsequently, sterilized reed extract was then added to the MS media according to the treatment. The treatments including control, 2.5 g/l, and 5 g/l of reed extract with three replications.

Afterward, we observe the morphological changes of the callus to plantlets. At 2 weeks and 4 weeks of age, the following parameters were observed: callus diameter, percentage of green spot, embryogenic callus morphology (percentage of callus in globular, scutellar, and coleoptile phases), percentage and number of plantlets formed, and plantlet morphology (shoot length, root length, and the number of leaves).

RNA Extraction and Gene Expression Analysis

Callus 4 days after planting (DAP) on regeneration media were used for RNA extraction. Total RNA was extracted from the callus using the *Ribospin™ Plant* kit (GeneAll, Korea). The extracted RNA was used as template to synthesize cDNA using the *ReverTra Ace® qPCR RT Master Mix* kit (Toyobo, Japan). To measure the expression level of target genes (*OsBBM*, *OsLEA*, *OsLEC1*, *OsSERK*, and *OsWOX4*), semi quantitative polymerase chain reaction (PCR) was performed using *GoTaq® Green Master Mix* (Promega, USA*)*. List of primer used in this study was listed in Table 1. The PCR products were then separated in a 2% agarose gel stained by EtBr and visualized with a UV transilluminator (Bio-Rad, Germany). The gel image of DNA bands were then captured and classified according to their thickness.

Table 1. A list of primers used in semi quantitative RT-PCR

Gene	Primer	NCBI reference sequence
OsSERK	Forward: 5’ TGC ATT GCA TAG CTT GAG GA 3’ Reverse: 5’ GCA GCA TTC CCA AGA TCA AC 3’	XM_015794373.2
OsWOX4	Forward: 5’ CGC TAA CGA AAC CAA AGA GG 3’ Reverse: 5’ GGA AGA GCT CCA GGG TCA CT 3’	XM_015779881.2
OsLEC1	Forward: 5’ CGT CGG TGG GAT GCT CAA GTC 3’ Reverse: 5’ GGT GCT CGA AGT TGA CGG TCT 3’	XM_015769434.2
OsBBM	Forward: 5’ CGA TTT ACC GTG GCG TGA CA 3’ Reverse: 5’ CGT GAA GAG CAT CCT GGA CA 3’	XM_026019980.1
OsActin	Forward: 5’ TCC ATC TTG GCA TCT CTC AG 3’ Reverse: 5’ GTA CCC GCA TCA GGC ATC TG 3’	XM_015774830.2

Experimental Design and Statistical Analysis

This research employed a factorial Completely Randomized Design (CRD) method with two treatment factors and two replications. The first factor was rice varieties (*Bondoyudo*, *Caok*, *Ciliwung*, *Situbagendit*). The second factor was allelopathic treatment (control, 2.5 g/l, and 5 g/l) with three replications. The data obtained from the observations included both qualitative and quantitative data. Qualitative data were analyzed descriptively, whereas quantitative data were analyzed using ANOVA; if the results obtained were significantly different, further analysis was conducted using Duncan's Multiple Range Test (DMRT) with a confidence level of 95%.

Results and Discussions

Callus Induction

Induction of callus plays a crucial step in rice plant propagation. To regenerate into plantlets, callus must be of high quality. A high-quality callus that has a greater potential to regenerate into plantlets callus called embryonic callus. Before being transferred to regeneration media, the callus obtained from induction media must undergo a selection phase. Induction media (MS medium containing 2,4-D 2 mg/L) containing the explants were kept in a growth chamber at 27°C in the dark condition. Then, on day seven, all explants were injured and began to expand within the embryo. 14 days after planting (DAP), induction of callus was observed.

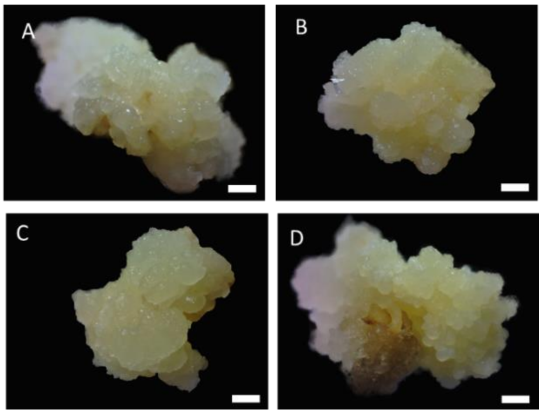

Figure 1**.** Callus morphology of Indonesian local rice on MS medium containing 2,4-D 2 mg/L at 14 days after planting (DAP). (Bar= 1 mm). A: *Bondoyudo*, B: *Caok*, C: *Ciliwung*, D: *Situbagendit*. (Bar scale = 1mm).

Figure 1 depicts the calli morphology at 14 days after planting the seed from *Bondoyudo*, *Caok*, *Ciliwung*, and *Situbagendit* rice as explants. The produced callus appear yellowish-white color at 14 DAP. Callus has a crumbly appearance due to its brittle nature and nodule-covered surface. This observation follows Minarsih et al. (2016), who state that an embryogenic callus has crumbly, nodular, and yellowish-white visual characteristics. Compared to a non-embryogenic callus, an embryogenic callus has a greater ability to regenerate into plantlets, making it an ideal explant for regeneration. In this study, we discovered that the addition of 2,4-D hormone to Murashige and Skoog (MS) medium at a concentration of 2 mg/L is an effective method for inducing embryogenic callus formation in *Bondoyudo*, *Caok*, *Ciliwung*, and *Situbagendit* Rice.

The percentage of explants that were successfully induced to form callus was determined for 14-day-old calluses in the induction medium (Table 2). The percentage was calculated by dividing the number of produced calli by the number of explants planted and multiplying the result by 100%. The callus diameter was then measured using the *Imager Raster* and *Optilab* to determine its size.

Table 2. Percentage and diameter of callus formation on MS media containing 2,4-D 2 mg/L at 14 days after planting

Varieties	Callus induction percentage (%)	Diameter callus (mm)
Bondoyudo	73.5 ± 4.32^{a}	6.61 ± 0.15^{b}
Caok	58.50 ± 0.97^{b}	8.2 ± 0.04^{a}
Ciliwung	59.89 ± 2,76^{b}	6.45 ± 0.6^{b}
Situbagendit	51.20 ± 4.16^{b}	8.22 ± 0.13^{a}

Note: Numbers followed by the same letter showed an insignificant difference on 5% DMRT test

The statistical analysis of the percentage of callus induction variables yielded significantly different results, as shown in Table 2. *Bondoyudo* Rice exhibited the highest percentage of callus formation (73.5%), whereas *Situbagendit* Rice exhibited the lowest percentage (51.2 percent). Similarly, the results for the callus diameter variable varied significantly. *Situbagendit* rice produced the largest callus diameter (8.22 mm), followed by *Caok* Rice (8.2 mm), *Bondoyudo* Rice (6 mm), and *Ciliwung* Rice (6 mm) (6.45 mm). The differences in callus formation response showed by rice varieties might reflect several factors that influence the callus induction including the genotype and composition of the media used (Michel et al., 2008).

Bondoyudo rice responded better in callus formation than the other three rice varieties, although the resulting callus diameter was smaller in comparison to Caok and Situbagendit. Caok, Ciliwung, and Situbagendit shared a similar lower percentage of callus formation in comparison to Bondoyudo rice. The callus diameter also varies among the rice varieties. The callus diameter of Bondoyudo and Ciliwung shared an almost similar size of ± 6 mm, whereas Caok and Situbagendit shared a larger diameter of ± 8.2 mm. Among the 4 rice varieties, Ciliwung rice has a relatively lower percentage of callus formation and callus diameter. This phenomenon is presumably linked to the genotype of each rice variety. Furthermore, each rice seed used as an explant was already accompanied by endogenous growth regulators. The application of the exogenous growth regulator may interact with the plants' endogenous growth regulator. This collaboration of exogenous and endogenous growth regulators result in the formation of specific organs or tissues when exogenous growth regulators are introduced to shape the direction of culture development. Sari et al. (2014) report that the administration of the 2,4-D hormone promoted callus morphogenesis.

Callus Regeneration

The calli obtained at the induction stage were transferred to regeneration media supplemented with the hormones NAA 1 mg/L and Kinetin 2 mg/L, as well as alang-alang root extract as a treatment. There were three types of treatment: P0 as control or without the addition of cogon grass root extract, P1 with the addition of cogon grass root extract 2.5 g/L, and P2 with the addition of alang-alang root extract 5 g/L. To replenish the nutrients in the media, media replacement occurs every two weeks. The transferred calli were incubated for 16 hours of light at 27 ^{o}C (room temperature). During a six-week period, the morphology of a callus was monitored weekly. In the first two weeks, the induced calli were transferred to regeneration media using a Petri dish and at the following week, they were transferred to regeneration media using a tube to facilitate observation of the newly formed plantlets.

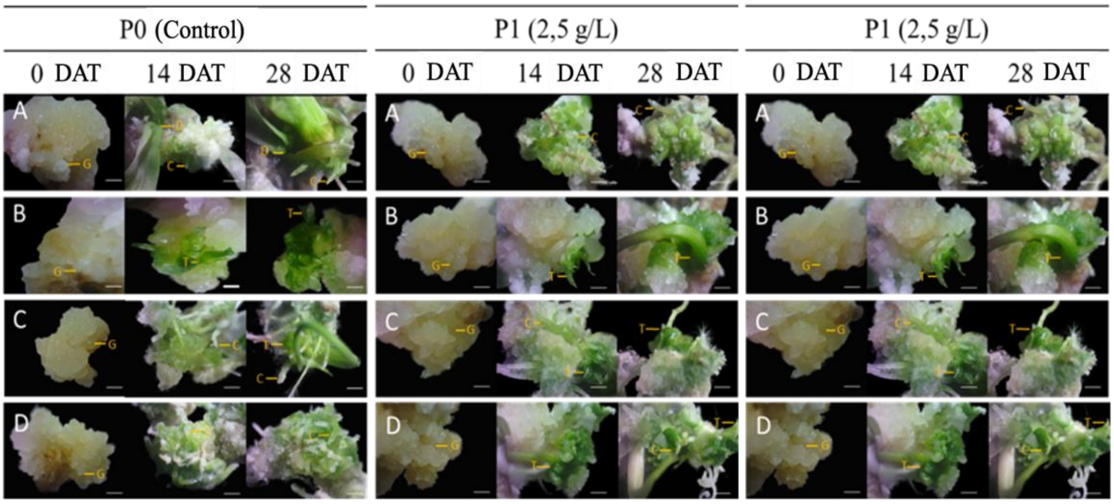

Figure 2. Morphological changes of rice calli after 14 and 28 days grown on the MS medium containing NAA 1 mg/L and Kinetin 2 mg/L. The addition of alang 3a'ytrsIUYTREW-alang extract of P0 = 0 g/L, P1= 2.5 g/L, and P2= 5 g/L. A:*Bondoyudo*, B:*Caok*, C:*Ciliwung*, D: *Situbagendit*.

Plant regeneration can be obtained by somatic embryogenesis, a formation of embryonic cells from somatic cells. Somatic embryogenesis consists of two phases; the induction phase, during which differentiated somatic cells acquire embryogenic competence and proliferate as embryogenic cells; and the expression, during which the embryogenic cells express their embryogenic competence and differentiate into somatic embryos. After the embryogenic induction is completed, the next stages are the globular, scutellar, and coleoptile stage for monocot plants (Mastuti, 2017).

There are several stages of embryogenesis in monocotyledonous plants such as rice, including: pro-embryo, globular, scutellar, and coleoptile. Figure 2 showed the morphological changes 0, 14, and 28 DAP of callus grown on P0, P1, and P2. When the callus was transferred from the induction medium, the surface of each explant was a shiny and dry nodule. This indicated that the callus had reached the pro-embryo mass (PEM) stage. After 4 days of incubation in regeneration media, all local Indonesian rice callus developed green spots, with the exception of *Situbagendit* rice on media treated with 2,5 g/L (P2). Greenspot signified an essential stage in tissue culture because it is an early indicator of callus regeneration (Artadana et al., 2017).

The progression of green spot formation depends on the viability and regeneration of the calli used. At the time of induction, the callus used was two weeks old and was not subcultured. According to Minarsih et al (2016), the longterm use of 2,4-D hormone causes genetic variations and inhibits regenerative capacity of plants. In general, young explants from meristematic tissue that have undergone dedifferentiation are easier to regenerate into plantlets than older explants (Morrish et al., 1987).

On 14 DAP, callus planted on control media (P0) of all rice emerged green and had entered the coleoptilar phase, characterized by the elongation of the callus surface nodules. *Bondoyudo* rice even demonstrated a marginally superior performance, as it has been successful in producing plantlets with the appearance of leaves. All rice varieties produced shoots when treated with alang-alang root extract at 2.5 g/L (P1) concentration. *Bondoyudo* rice has entered the coleoptilar phase in a solution containing 5 g/L cogon grass root extract (P2). 14 days after planting, *Caok* rice grew more slowly and remained in the scutellar stage. This stage is characterized by the formation of a liver-like cell mass in which the cell's center is lower and the right side left protrudes due to faster cell division (Yadav et al., 2020). *Ciliwung* rice has successfully produced shoots at 14 DAP. In the meantime, the *Situbagendit* rice callus had entered the coleoptilar phase despite the absence of green spots and the occurrence of the albino phenomenon.

Without alang-alang root extract (P0), *Bondoyudo* and *Ciliwung* rice produced shoots after 28 DAP. However, neither *Caok* nor *Situbagendit* rice had yet sprouted. Nonetheless, the growth progression of *Caok* and *Situbagendit* rice improved over the previous fourteen days. In contrast, all explants that were exposed to 2.5 g/L of cogon grass root extract (P1) developed shoots. Under the treatment with 5g/L cogon grass root extract, *Bondoyudo*, *Caok*, and *Ciliwung* were successful in producing shoots, whereas *Situbagendit* failed to produce shoots. The calli of *Situbagendit* rice appeared albino and underwent browning in certain areas.

The response of callus from different rice varieties to treatment with cogon grass root extract during plantlet formation was variable (Figure 2). The genetic factor of the rice calli and the concentration of the cogon grass root extract may affect this result. Compared to the control (P0), the administration of 2.5 g/L (P1) of cogon grass root extract showed no significant difference. However, callus treated with 5 g/L of cogon grass root extract demonstrated a slower regeneration process. According to Yulifrianti et al. (2015), allelopathic compounds inhibit the absorption of nutrients in the medium and the division of plant cells. In addition to its inhibitory effect, a concentration of 5 g/L of cogon grass root extract caused the callus of *Situbagendit* rice to become albino. Albinism is caused by DNA damage in the plastid or nucleus, or by the addition of chemical compounds to the media (Sun et al., 1979).

Table 3. Percentage of callus greenspots during the second and fourth weeks of treatment

Varieties	Treatment	Green Spot Percentage(%) 2nd week	 4th week
Bondoyudo	P0	91.67 ± 8.33^a	100 ± 0
	P1	91.67 ± 8.33^a	100 ± 0
	P2	83.33 ± 8.33^a	100 ± 0
Caok	P0	91.67 ± 8.33^a	100 ± 0^a
	P1	91.67 ± 8.33^a	100 ± 0^a
	P2	83.33 ± 16.67^a	91.67 ± 8.33^a
Ciliwung	P0	91.67 ± 8.33^a	100 ± 0^a
	P1	91.67 ± 8.33^a	91.67 ± 8.33^a
	P2	91.67 ± 8.33^a	91.67 ± 8.33^a
Situbagendit	P0	91.67 ± 8.33^a	100 ± 0^a
	P1	91.67 ± 8.33^a	100 ± 0^a
	P2	0^b	8.33 ± 8.33^b

Note: Numbers followed by the same letter showed an insignificant difference at 5% DMRT test

The two-week-old calli were transferred to the regeneration media containing various concentrations of allelopathic compounds and incubated for six weeks at 16/8 hours of irradiation (light/dark). During the second and fourth week, observations were performed regarding green-spotted callus. The percentage of green spots in the second week is displayed in Table 3. In the second week, all rice in the P0 (control) and P1 (2.5 g/L) treatments produced 91.67 percent green spots. However, the P2 (5 g/L) treatment reduced green spot appearance on *Bondoyudo* and *Caok* rice to 83.33 percent, whereas *Situbagendit* rice had not yet entered the green spot phase. In contrast, the administration of allelopathic compounds of cogon grass root extract on P1 (2.5 g/L) and P2 (5 g/L) in *Ciliwung* rice had no effect on the formation of green spots at 2 weeks of age. In the fourth week, the treatment of *Bondoyudo* rice with allelopathic compounds of cogon grass root extract at concentrations of 2.5 g/L (P1) and 5 g/L (P2) did not differ from the control (P0). They have all successfully progressed to the green spot phase. This result suggests that the allelopathic compounds affect the formation of green spot differently among the rice varieties. This effect of cogon grass root extract was likely to be slightly concentration-dependent as seen in *Bondoyudo*, *Caok*, and *Situbagendit*, especially during 2 weeks of culture. *Ciliwung*, on other hand displayed no apparent effect during 2 weeks of culture due to the given treatments. In addition, the effect of P1 and P2 in *Bondoyudo* also appears to be diminished on the 4 weeks of culture. This finding highlights the response and sensitivity or tolerance differences shown among 4 rice varieties.

Treatment of *Caok* Rice with 2.5 g/L cogon grass root extract (P1) had no effect on the formation of green spots; however, treatment with 5 g/L cogon grass root extract (P2) reduced the green spot phase by in to 91.6%. The allelopathic compounds applied at P1 (2.5 g/L) and P2 (5 g/L) in *Ciliwung* Rice could reduce the green spot phase by 91.67 percent. The P1 treatment had no effect on *Situbagendit* Rice, but the P2 treatment drastically reduced the green spot phase by up to 8.33%. The addition of 5 g/L allelopathic compounds to *Situbagendit* callus caused the callus to become albino. The presence of chemical compounds in the medium causes damage to the nucleus or plastids in cells, preventing the formation of green spots in an albino callus (Sun et al., 1979).

Due to photosynthesis induction in the regenerated callus by light exposure, the regenerated calli can produce green spots. It is important to observe the formation of green spots because they are frequently used as an indicator of plant regeneration. The purpose of the plant regeneration parameter is to determine the number of plantlets that will develop from the regenerated callus (Nabors et al., 1982).

Table 4. Percentage of calli that entered globular, scutellar, and coleoptilar phases (%)

Varieties	Treatment	2 weeks			4 weeks		
		Globular	Scutellar	Coleoptilar	Globular	Scutellar	Coleoptilar
Bondo-yudo	P0	0	6.67 ± 6.67	93.33 ± 6.67	0	0	100 ± 0
	P1	0	13.33 ± 6.67	86.67 ± 6.67	0	6.67 ± 6.67	93.33 ± 6.67
	P2	0	20 ± 11.55	80 ± 11.55	0	13.33 ± 6.67	86.67 ± 6.67
Caok	P0	0	6.67 ± 6.67	93.33 ± 6.67	0	0	100 ± 0
	P1	0	6.67 ± 6.67	93.33 ± 6.67	0	0	100 ± 0
	P2	0	26.67 ± 17.64	73.33 ± 17.64	0	20 ± 11.55	80 ± 11.55
Ciliwung	P0	0	13.33 ± 6.67	86.67 ± 6.67	0	6.67 ± 6.67	93.33 ± 6.67
	P1	0	13.33 ± 6.67	86.67 ± 6.67	0	6.67 ± 6.67	93.33 ± 6.67
	P2	0	20 ± 0	80 ± 0	0	13.33 ± 6.67	86.67 ± 6.67
Situ-bagendit	P0	0	33.33 ± 33.33	93.33 ± 6.67	0	0	100 ± 0
	P1	0	13.33 ± 13.33	86.67 ± 13.33	0	6.67 ± 6.67	93.33 ± 6.67
	P2	0	20 ± 11.55	73.33 ± 6.67	0	13.33 ± 13.33	80 ± 11.55

Note: Numbers followed by the same letter showed an insignificant difference at 5% DMRT test

In Table 4, the percentage of callus that entered the globular, scutellar, and coleoptile phases within two and four weeks of subcultivation on regeneration media is displayed. The globular phase is characterized by a spherical shape, which is followed by the formation of a scutellar phase embryo, which is the transitional phase to coleoptile, or the first young shoots to emerge (Zhao et al., 2017).

The induced callus is embryogenic and has reached the pro-embryonic mass phase. One day after being transferred to callus regeneration media, the callus developed and entered the globular phase. All calluses at 2 weeks of age had entered the scutellar and coleoptile phases. Even the percentage of callus that had entered the coleoptile phase was greater than the percentage of callus that had entered the scutellar phase. At four weeks of age, the callus has developed and a greater proportion of callus has entered the coleoptilar phase. According to Table 3, *Bondoyudo* and *Situbagendit* treated with allelopathic compounds of cogon grass root extract grew more slowly. The phase of callus development appeared to be more impeded as the concentration of allelopathic compounds increased. In contrast, the growth of *Ciliwung* and *Caok* rice treated with 2.5 mg/L cogon grass root extract (P1) did not differ from that of rice untreated with allelopathic compounds (P0). However, a concentration of 5 g/L cogon grass root extract (P2) prevented callus formation.

The application of allelopathic compounds had an effect on the explants proportional to the concentration level and the target plant. Table 4 reveals that 2.5 g/L of cogon grass root extract (P1) exhibited allelopathic properties in *Bondoyudo* and *Situbagendit* rice, but showing no effect on *Caok* and *Ciliwung* rice compared to the control (P0) treatment. At a concentration of 5 g/L, allelopathic compounds inhibited the callus development phase in *Bondoyudo*, *Caok*, *Ciliwung*, and *Situbagendit* rice. The allelopathic compounds found in plant cells can inhibit cell division and differentiation, thereby impeding the regeneration process (Cheng & Cheng 2015).

Table 5. Rice callus proliferation at 2 and 4 weeks

Varieties	Treatment	Callus Diameter (mm) 2-week	4-week
Bondoyudo	P0	7.10 ± 0.05^{b}	8.14 ± 0.06
	P1	6.66 ± 0.19^{a}	8.01 ± 0.10
	P2	6.43 ± 0.25^{ab}	7.77 ± 0.11
Caok	P0	7.52 ± 0.06	8.60 ± 0.05
	P1	7.89 ± 0.02	8.65 ± 0.12
	P2	7.19 ± 0.06	7.67 ± 0.08
Ciliwung	P0	7.13 ± 0.09	8.28 ± 0.08
	P1	6.82 ± 0.28	7.83 ± 0.05
	P2	6.42 ± 0.05	6.78 ± 0.04
Situbagendit	P0	7.11± 0.05	8.03 ± 0.17
	P1	7.27± 0.07	8.19 ± 0.01
	P2	6.58 ± 0.14	7.71 ± 0.08

Note: Numbers followed by the same letter showed an insignificant difference at 5% DMRT test

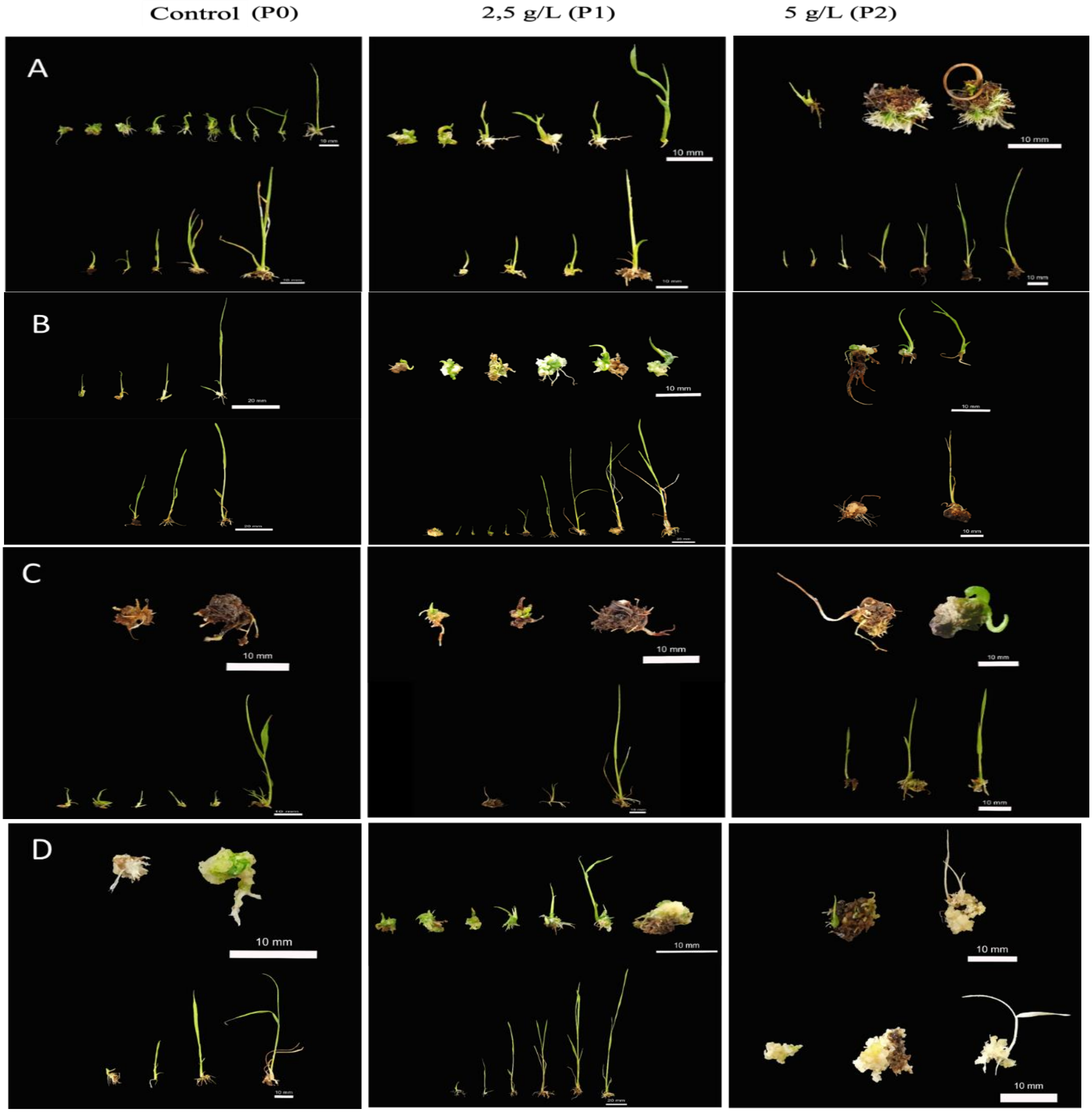

Figure 3. Morphological changes of plantlet emerged from calli at individual explant 42 days of age grown on the MS medium containing NAA 1 mg/L and Kinetin 2 mg/L. The addition of cogon grass root extract into the media were applied as P0 = 0 g/L, P1= 2.5 g/L, and P2= 5 g/L. A: *Bondoyudo*, B: *Caok*, C: *Ciliwung*, D: *Situbagendit*.

Proliferation is the cumulative result of an individual's cell cycle. Proliferation is the capacity of cells to divide, resulting in an increase in cell number. Typically, cells proliferate to increase their number or to replace dead cells. In addition to cell differentiation and death, cell proliferation is a growth and development determinant in plants (Novikova et al., 2013). In the *In vitro* culturing condition, proliferation is tightly regulated by the composition of the culture medium. There are chemical compounds in the medium that can either inhibit or stimulate plant cell proliferation.

It is demonstrated in Table 5, the administration of allelopathic compounds in *Bondoyudo* rice produced a highly significant effect in the second week, with the concentration of allelopathic compounds decreasing callus proliferation. The administration of allelopathic compounds to *Caok*, *Ciliwung,* and *Situbagendit* rice was not statistically significant, but it was observed that the higher the concentration of allelopathy compounds, the more they inhibited the process of cell proliferation. In the fourth week, the progression of callus development in terms of cell proliferation is indicated by an increase in callus diameter over the previous two weeks. Similar to the second week, the proliferation decreased in the fourth week due to the presence of allelopathic compounds.

Table 6. Percentage of plantlet formation during 42 days of age grown on regeneration media with the addition of cogon grass root extract

Varieties	Treatment	Percentage of Plantlet Formation (%) *	Total Plantlet **
Bondoyudo	P0	100 ± 0^a	15
	P1	100 ± 0^a	9
	P2	75 ± 11.18^b	8
Caok	P0	100 ± 0^a	6
	P1	100 ± 0^a	12
	P2	75 ± 7.91^b	3
Ciliwung	P0	50 ± 7.91^a	6
	P1	50 ± 13.69^a	4
	P2	50 ± 13.69^a	3
Situbagendit	P0	100 ± 0^a	5
	P1	100 ± 0^a	12
	P2	70 ± 9.35^b	2

*Average percentage of callus that produced plantlet. Number followed by the same letter showed an insignificant difference at DMRT test 5%. ** Number of plantlets produced from callus

According to Table 6, all explants from the varieties utilized in this study are capable of regenerating into plantlets regardless of treatment. This indicates that the selected explants can be cultured because they have a high capacity for regeneration and plantlet formation. However, when treated with cogon grass root extract at a concentration of 5 g/L (P2), plantlet formation appeared to decrease.

A 100 percent of the callus of *Bondoyudo* rice in the control treatment (P0) produced 15 plantlets, indicating the formation of plantlets. In the presence of 2.5 g/L of cogon grass root extract (P1), all calli were able to produce healthy plantlets. In the subsequent treatment with 5 g/L (P2), 75% of callus has been successfully generated ~~the~~ *Caok* rice in the control treatment (P0) produced six plantlets. In the 2.5 g/L (P1) treatment, all calli generated 12 plantlets. In contrast, the 5 g/L (P2) treatment successfully induced plantlet development in 75% of the planted callus In each treatment, 50% of *Ciliwung* Rice explants produced plantlets. The control treatment produced six plantlets, P1 produced four plantlets, and P2 produced three plantlets. On the control (P0) media, *Situbagendit* rice explants have a 100 percent callus formation rate 75 percent fewer plantlets were produced in the 5 g/L (P2) treatment, namely two plantlets.

The effect of cogon grass root extract concentration on callus affects the proportion of plantlet formation. The higher the concentration, the lower the percentage of plantlet formation, although no effect was found on the formation of plantlets in *Ciliwung* rice. Rice explants also reacted differently to cogon grass root extract. *Bondoyudo* and *Ciliwung* rice responded negatively to an increased plantlet. The greater the concentration, the fewer plantlets are produced. In *Caok* and *Situbagendit* rice, the 2.5 g/L of cogon grass root extract produced a greater total number of plantlets than the control treatment. At 5 g/L of cogon grass root extract, however, the total number of plantlets drastically reduced. The differential effect of allelopathic compounds on plantlet formation was influenced by the target plant's concentration and sensitivity (Gniazdowska et al., 2015). According to Rice (2012), allelochemicals can either inhibit or promote the plant growth, depending on certain concentrations. This was supported by a study by Yar et al. (2020) that low concentrations of allelochemicals can stimulate germination and plant growth.

Table 7. Length of shoot, length of root, and number of leaves of plantlets from each variety from different treatments

Varieties	Treatments	Length of shoot (mm)*	Length of root (mm)*	Number of leaves**
Bondoyudo	P0	75.99 ± 10.65a	10.67 ± 0.78a	27
	P1	43.92 ± 4.55b	8.71 ± 0.66ab	15
	P2	38.8 ± 10.03b	7.57 ± 1.24b	10
Caok	P0	81.69 ± 10.87ab	10.77 ± 1.48a	19
	P1	89.77 ± 26.31a	15.25 ± 2.23a	26
	P2	32.39 ± 5.27b	9.49 ± 2.13a	9
Ciliwung	P0	118.34 ± 3.46a	10.74 ± 1.14a	14
	P1	70.58 ± 22.29b	10.37 ± 1.82a	7
	P2	29.20 ± 5,61c	8.36 ± 1.4a	6
Situbagendit	P0	38.96 ± 11.67b	7.60 ± 0.38ab	15
	P1	106 ± 33.06a	12.78 ± 3.20a	28
	P2	18.34 ± 1.36b	4.24 ± 0.35b	2

*Average percentage of callus that produced plantlet. Numbers followed by the same letter showed an insignificant difference at 5% DMRT test.**Number of leaves that produced in plantlet

Table 7 demonstrates that each type of rice grown on regeneration media responded differently to the treatments. *Bondoyudo* rice responded negatively to shoot length, root length, and leaf number. The shoot length, root length, and number of leaves produced decrease as the concentration of treatments increases (P1 and P2). The length of the shoot was 75.99 mm at the control medium (P0), 43.92 mm at the P1 medium, and 38.8 mm at the P2 medium. P0 root length was 10.67 mm, P1 root length was 8.71 mm, and P2 root length was 7.57 mm. Following a similar trend, the leaves of *Bondoyudo* rice grown in the control (P0), P1, and P2 medium produced 27, 15, and 10 leaves, respectively. Figure 3 demonstrates that the plantlets formed on P2 media emerged from a browning callus, resulting in inferior growth of shoots and number of leaves compared to P0 and P1 media. Nevertheless, *Bondoyudo* callus grown on P2 showed a dense root growth. *Bondoyudo* variety appears to be sensitive to the treatment of cogon root extract in a concentration-dependent manner.

Caok rice grown in P1 media shown a positive effect on shoot length (89.77 mm), root length (15.25 mm), and leaf number (26), compared to control media (81.69 mm, 10.77, and 19 leaves). Meanwhile, the effect of P2 in the callus growth seems to be showing an inhibitory effect. The shoot length, root length, and number of leaves were decreased in comparison to P0 and P1. Moreover, observation of *Caok* callus grown for 42 days revealed that on P2 media, callus exhibited browning and denser root growth than on control (P0) and P1 media (Figure 3). *Caok* variety shown a stimulatory response upon the P1 treatment and inhibitory response upon the P2 treatment.

Ciliwung rice grown in the control medium had the highest values for shoot length (118.34 mm), root length (10.74 mm), and number of leaves (14 strands). Although P1 showed a decrease in comparison to P0, P2 media showed the lowest values for shoot length (29.2 mm), root length (8.36 mm), and number of leaves (6). This indicates the sensitivity shown by *Ciliwung* variety upon the treatment of cogon root extract in a concentration-dependent fashion. Based on plantlet morphology, there were several browning calli in each treatment medium after 42 days. This result may be associated by the endogenous factors working within the rice callus

In comparison to control media, *Situbagendit* rice grown in P1 medium produced the longer shoots (106 mm), roots (12.76 mm), and most leaves (28 strands). The P2 medium yielded the shortest shoot length of 18.34 mm, the shortest root length of 4.24 mm, and few of leaves. Based on the morphology of the callus, *Situbagendit* grown in P2 medium produced white (albino) callus and some browning callus. Albino callus resulted in the development of albino plantlets. Albino is an abnormal condition in which plant cells cannot produce chlorophyll pigment and disrupt the chloroplast membrane's differentiation process. Therefore, albinism causes insufficient photosynthesis and accelerates plant mortality (Kumari et al., 2009).

Gene Expression

In this study, the regulatory genes, such as *OsBBM*, *OsLEA*, *OsLEC1*, *OsSERK*, and *OsWOX4* were analyzed during the development of somatic embryogenesis using the thickness of the band obtained from electrophoresis and PCR results, visualized with a UV-transilluminator. The band was the results of 28-day-old callus RNA from *Bondoyudo*, *Caok*, and *Ciliwung* rice on the regeneration media. Because the callus had visually or morphologically matured after 28 days, it was easier to compare gene expression and morphology.

During the development of somatic embryogenesis, several regulatory genes, including *OsBBM*, *OsLEA*, *OsLEC1*, *OsSERK*, and *OsWOX4*, were analyzed in this study using the band thickness obtained from electrophoresis and PCR results visualized using a UV-transilluminator. The bands were obtained from is the identified RNA of *Bondoyudo, Caok,* and *Ciliwung* rice callus grown on the regeneration media for 28 days. The 28-day-old callus was utilized as it was easier to compare the gene expression with callus morphology.

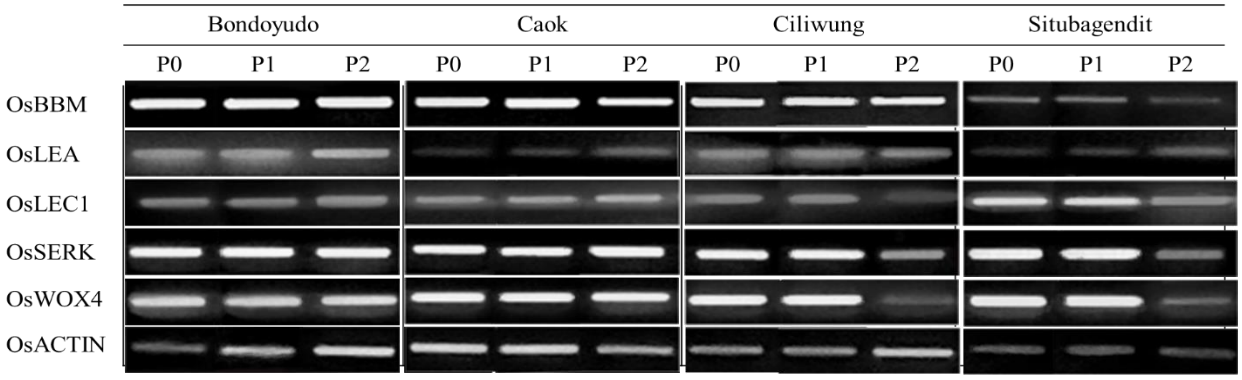

Figure 4. The gene expression of *OsBBM*, *OsLEA*, *OsLEC1*, *OsSERK*, *OsWOX4.* The expression of housekeeping genes, i.e. *OsACTIN*, was used as a reference. RNA used for gene expression analysis were harvested from callus grown for 28 days on the regeneration media.

Our study reveals that the treatment of 2.5 g/L (P1) and 5 g/L (P2) of cogon grass root extract had no effect on the expression level of *OsBBM* gene in *Bondoyudo*, *Caok,* and *Ciliwung* rice. In contrast, the expression of *OsBBM* gene in the *Situbagendit* rice was significantly lower in both control (P0) and treatment (P1 & P2) than in the other three rice varieties. The effect of cogon grass root extract on the expression of *OsBBM* was observed at a concentration of 5 g/L (P2). The band was significantly less intense than P0 and P1. Rice *BABYBOOM* (Os*BBM*) gene regulates somatic embryo induction, cell differentiation, and plant development (Jha & Kumar 2018). *OsBBM* stimulates the expression of Auxin biosynthesis genes which is an essential process in somatic embryogenesis, especially in callus induction (Khanday et al., 2020). In our study, *Situbagendit* showed slower progression of shoot development at 28 DAP in comparison to the other three rice varieties. Given that *OsBBM* also induce the somatic competence (Khanday et al., 2020), the lower expression of *OsBBM* might partially explain the slower progress of shoot development at 28 DAP. The success of callus regeneration to plantlets is essential, since it demonstrates the success of somatic embryogenesis as a cumulative result of the simultaneous collaboration of essential genes in plant development (Figure 2 and 3).

The *OsLEA* gene expression was detected in *Bondoyudo*, *Caok*, *Ciliwung*, and *Situbagendit*, although the intensity of the band differs between rice varieties. The *Bondoyudo*, *Caok,* and *Situbagendit* rice exhibited a slight increase in band intensity between P1 and P2 relative to P0. A slight clear increase of band intensity was observed upon P2 treatment in *Bondoyudo*, *Caok*, and *Situbagendit*. In contrast, the expression of *OsLEA* in *Ciliwung* was comparable between P0, P1, and P3. According to Hong-Bo et al. (2005), the expression of the Late Embryogenesis Abundant (*LEA*) gene indicates the increased allelopathic content of plant media, associated with the regulation of abiotic stresses. According to Cheema et al. (2013), the administration of allelopathic compounds to the growing medium can induce abiotic stress because allelopathic compounds themselves can inhibit plant absorption of nutrients. According to Hong-Bo et al. (2005), *LEA* is a protein that protects the cytoplasm from drought conditions. Based on our results (Figure 4), we hypothesize that cogon grass root extract can increase the *LEA* gene expression, when administered. Therefore, the addition of allelopathic compounds can inhibit the regeneration of plantlets compared to the absence of allelopathic treatment.

Figure 4 demonstrates that the *OsLEC1* gene was expressed differently in each treatment and variety. The *OsLEC1* gene controls the somatic embryo development and embryo maturation (Kumar et al., 2020). The *OsLEC1* gene expression in *Bondoyudo* and *Caok* experience an inconsiderable effect among the control and treatments. Conversely, the *OsLEC1* gene expression was observed at a higher level in P0 and P1 than in P2 in *Ciliwung* and *Situbagendit*. This indicate there is a slight suppression effect of the treatment of cogon grass root extract to the expression of *OsLEC1,* especially at the concentration 5 g/L (P2). According to our findings, the administration of cogon grass root extract has various effects on the expression of *OsLEC1* gene in different rice varieties. Our study pronounces the application of cogon grass root extract has minimum effect on the *OsLEC1* gene expression in *Bondoyudo* and *Caok* rice, but a suppression was found in *Ciliwung* and *Situbagendit* rice under the treatment of 5 g/L (P2). This finding is in the accordance with Gniazdowska et al. (2015), who stated that the effect of allelochemicals is dependent on the concentration and sensitivity of the target plant. The expression of *OsSERK* gene in *Bondoyudo* and *Caok* rice remained constant in control and under the treatment. However, in *Ciliwung* and *Situbagendit*, the *OsSERK* gene expression was relatively high in P0 and P1, and lower in P2. These results indicate that cogon grass root extract administered at up to 5 g/L concentrations had no effect on the expression of *OsSERK* gene in *Bondoyudo* and *Caok* rice. However, in *Ciliwung* and *Situbagendit,* the expression of *OsSERK* gene under the treatment of P2 was slightly downregulated. Although the *OsSERK* gene mainly expressed in callus during morphogenesis (Hu et al., 2005), it also regulates root development, immune responses, and cell death (Kumar & Van Staden 2019).

The expression of *OsWOX4* gene in four rice varieties was depicted in Figure 4. The expression of *OsWOX4* in *Bondoyudo* and *Caok* were distinct in both control (P0) and treatments (P1 & P2). The treatments of 2,5 and 5 g/L of cogon grass root extract apparently did not alter the level of *OxWOX4* expression in both varieties. Meanwhile, although under P1 treatment *Ciliwung* and *Situbagendit* showed no difference in expression level with respect to the control, the expression of *OsWOX4* was significantly reduced under the treatment of 5 g/L of cogon grass root extract. This study showed that the treatment of cogon root extract affects expression of *OsWOX* differently depends on the variety used. The *OsWOX4* gene has multiple functions, including embryogenesis, root elongation, meristem cell maintenance, and early leaf development (Yasui et al., 2018). In addition, the *OsWOX4* gene influences cell proliferation and root tip elongation, which contribute to the formation of primary root length (Chen et al., 2020).

The gene expression analysis using RT-PCR, visualized in a UV-transilluminator revealed that rice varieties grown *in vitro* and treated with cogon grass root extract express genes at different levels. The expression level of *OsBBM* gene in *Situbagendit* rice was affected by the administration of allelopathic compounds at a concentration of 5 g/L (P2) while the P0 and P1 treatment show less differences. Subsequently, the allelopathic compounds can slightly induce *OsLEA* expression in all rice varieties, with *Ciliwung* as an exception which has the similar level of expression in P1 and P2 treatments compared to the control (P0). Rice varieties differ in their expression of *OsLEC1* gene. *Bondoyudo* and *Caok* rice treated with 2.5 g/L (P1) and 5 g/L (P2) exhibited no effect on the *OsLEC1* gene expression level. In contrast, the expression of *OsLEC1* gene was suppressed in *Ciliwung* and *Situbagendit* under the P2 treatment, whereas under P1 and P0, *OsLEC1* expression was maintained at the same level. The *OsSERK* and *OsWOX4* genes exhibited identical patterns of gene expression. *Bondoyudo* and *Caok* varieties at P0, P1, and P2 expressed both genes at a comparable level. Under the influence of *OsSERK* and *OsWOX4* genes, however, a moderate to obvious reduction in band thickness was observed. This means that the expression of *OsLEC1*, *OsSERK*, and *OsWOX4* genes responds similarly to the treatment of allelopathic compounds of cogon grass root extract, particularly at a concentration of 5 g/L (P2), which caused a significant reduction in expression level among the three genes.

Conclusion

The administration of allelopathic compounds has a specific stimulatory/inhibitory effect depending on the concentration administered and the target plant. In tissue culture, the correct quantity of stimulatory substance can promote the growth and development of cultured plant on the media. The administration of 2.5 g/L allelopathic compounds stimulated the morphogenesis and proliferation of *Caok* and *Situbagendit* rice, except in *Bondoyudo* and *Ciliwung* rice. The administration of 5 g/L allelopathic compounds inhibited morphogenesis and cell proliferation significantly in all four rice varieties. Administration of allelopathic compounds can also have diverse effects on the expression of *OsBBM*, *OsLEA*, *OsLEC1*, *OsSERK*, and *OsWOX4* genes at the molecular level. The expression of essential genes in morphogenesis and cell proliferation may influence the growth and development of plants in their respective media. This research investigates the effect of allelopathic compounds in stimulating or inhibiting the growth and development of plant tissue in culture media. Future research should be carried out to dissect the effect of allelopathic compounds on other rice varieties as well as numerous essential genes.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

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References

Artadana, I.B.M., Suhono, G.B.F., Hardjo, P.H., Purwanto, M.G.M., Wang, Y.B., & Supaibulwatana. K. (2017). Plant regeneration induced from mature Embryo-derived callus of Balinese red rice (Oryza sativa Cv. Barak Cenana). *Bali Med J 3*,12–17. https://doi.org/10.15562/bmj.v6i3.710

Cheema, Z.A., Farooq, M., & Wahid, A. (2013). Allelopathy: Current trends and future applications. *Allelopath Curr Trends Futur App*, (pp.l 1–517). https://doi.org/10.1007/978-3-642-30595-5

Chen, R., Xu, N., Yu, B., Wu, Q., Li, X., Wang, G., & Huang, J. (2020). The wuschel-related homeobox transcription factor oswox4 controls the primary root elongation by activating osaux1 in rice. *Plant Sci*, 298. https://doi.org/10.1016/j.plantsci.2020.110575

Cheng, F., & Cheng, Z. (2015). Research progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy. *Front Plant Sci*, *6*,1–16. https://doi.org/10.3389/fpls.2015.01020

Gniazdowska, A., Krasuska, U., Andrzejczak, O., & Soltys-Kalina, D. (2015). Allelopathic compounds as oxidative stress agents: yes or no. *Reactive Oxygen and Nitrogen Species Signaling and Communication in Plants, Signaling and Communication in Plants.* (pp. 155–176). 10.1016/j.cropro.2003.09.004

Hagan, D., Jose, S., & Lin, C.H., (2013). Allelopathic exudates of cogongrass (imperata cylindrica): implications for the performance of native pine savanna plant species in the southeastern us. *Journal of Chemical Ecology 39*(2), 312-322.

Hong-Bo, S., Zong-Suo, L. &, Ming-An, S. (2005). Lea proteins in higher plants: structure, function, gene expression and regulation. *Colloids Surfaces B Biointerfaces*, *45*,131–135 . https://doi.org/10.1016/j.colsurfb.2005.07.017

Jha, P., & Kumar, V. (2018). Baby boom (bbm): a candidate transcription factor gene in plant biotechnology. *Biotechnol Lett* 5. https://doi.org/10.1007/s10529-018-2613-5

Karmegam, N., Kalpana, M., & Prakash, M., (2014). Allelopathic effect of aqueous root bark extract of tamarindus indica L. and rhizosphere soil on germination and seedling growth of oryza sativa L . *Int J Curr Microbiol Appl Sci 3*,505–514

Khanday, I., Medellin, C.S., & Sundaresan, V. (2020). Rice embryogenic trigger baby boom1 promotes somatic embryogenesis by upregulation of auxin biosynthesis genes. https://doi.org/10.1101/2020.08.24.265025

Kumar, V., Jha, P., & Van Staden, J. (2020). Leafy cotyledons (lecs): master regulators in plant embryo development. *Plant Cell Tissue Organ Cult, 140*, 475–487 . https://doi.org/10.1007/s11240-019-01752-x

Kumar, V., & Van Staden, J. (2019). Multi-tasking of SERK-like kinases in plant embryogenesis, growth, and development: current advances and biotechnological applications. *Acta Physiol Plant, 41*,1–16 . https://doi.org/10.1007/s11738-019-2819-8

Kumari, M., Clarke, H.J., Small, I., & Siddique, K.H.M., (2009). Albinism in plants: A major bottleneck in wide hybridization, androgenesis and doubled haploid culture. *CRC Crit Rev Plant Sci, 28*,393–409 . https://doi.org/10.1080/07352680903133252

Kurniati, T., & Daniel, S. (2018). Uji toksisitas dan sifat alelopati ekstrak alang-alang (Imperata cylindrica) terhadap perkecambahan biji padi (Oryza sativa). *J At 3*,54–6

Mastuti, R. (2017). *Dasar-dasar kultur jaringan tumbuhan*. Universitas Brawijaya Press

Méndez-Hernández, H.A., & Ledezma-Rodríguez, M., Avilez-Montalvo, R.N., Juárez-Gómez, Y.L., Skeete, A., Avilez-Montalvo, J., De-la-Peña, C., & Loyola-Vargas, V.M., (2019). Signaling overview of plant somatic embryogenesis. *Front Plant Sci, 10*. https://doi.org/10.3389/fpls.2019.00077

Michel, Z., Hilaire, K.T., Mongomaké, K., Nguessan, A., & Justin, K.Y. (2008). Effect of genotype, explants, growth regulators and sugars on callus induction in cotton (Gossypium hirsutum L.). *Aust J Crop Sci, 2*,115–125.

Minarsih, H., Suharyo., Riyadi, I., & Ratnadewi, D. (2016). Pengaruh jumlah subkultur dan media sub-optimal terhadap pertumbuhan dan kemampuan regenerasi kalus tebu (Saccharum officinarum L.) (Effect of repeated subculture and suboptimum media on the growth of sugarcane calli (Saccharum officinarum L.)). *E-Journal Menara Perkeb, 84,* 28–40 . https://doi.org/10.22302/ppbbi.jur.mp.v84i1.219

Morrish, F., Vasil, V., & Vasil, I.K. (1987). Developmental morphogenesis and genetic manipulation in tissue and cell cultures of the Gramineae. *Adv Genet, 24,* 431–499 . https://doi.org/https://doi.org/10.1016/S0065-2660(08)60014-0

Nabors, M.W., Kroskey, C.S., & McHugh, D.M. (1982). Green spots are predictors of high callus growth rates and shoot formation in normal and in salt stressed tissue cultures of oat (avena sativa l.). *Zeitschrift für Pflanzenphysiologie, 105,* 341–349 . https://doi.org/10.1016/s0044-328x(82)80030-5

Narwal, S. (2009). Allelopathy and allelochemicals. In: Narwal (ed) *Isolation, identification and characterization of allelochemicals/natural products*. Science Publishers, Playmouth, (pp 3–5).

Novikova, G.V., Nosov, A.V., Stepanchenko, N.S., Fomenkov, A.A., Mamaeva, A.S., & Moshkov, I.E. (2013). Plant cell proliferation and its regulators. *Russ J Plant Physiol*, *60*, 500–506 . https://doi.org/10.1134/S102144371304010

Rice, E.L. (2012). *Allelopathy*. Academic Press.

Safitri, F.A., Ubaidillah, M., & Kim, K.M. (2016). Efficiency of transformation mediated by agrobacterium tumefaciens using vacuum infiltration in rice (Oryza sativa L.). *J Plant Biotechnol, 43,* 66–75 . https://doi.org/10.5010/JPB.2016.43.1.66

Sari, N., Suwarsi, R. E., & Sumadi (2014). Biosaintifika. *J Biol Biol Educ, 6,* 51–59 . https://doi.org/10.15294/biosaintifika.v6i1.3785

Sun, C.S., Wu, S.C., Wang, C.C., & Chu, C.C. (1979). The deficiency of soluble proteins and plastid ribosomal RNA in the albino pollen plantlets of rice. *Theor Appl Genet, 55,* 193–197 . https://doi.org/10.1007/BF0026811

Suparyono, & Setyono, A. (1997). Mengatasi Permasalahan Budidaya Padi. Penebar Swadaya, Jakarta

Suzuki, M., Tominaga, T., & Kato-Noguchi, H. (2015). The potent allelopathic substances of cogongrass rhizome extracts. Allelopathic Exudates of Cogongrass (Imperata cylindrica): Implications for the Performance of Native Pine Savanna Plant Species in the Southeastern US

Upadhyaya, G., Sen, M., & Roy, A. (2015). In vitro callus induction and plant regeneration of rice (Oryza sativa L.) var. ‘Sita’, ‘Rupali’ and ‘Swarna Masuri.’ *Asian J Plant Sci Res,* (ISSN 2249-7412) *5,* 24–27

Usman, Purwoko, B.S., Syukur, M., & Guntoro, D.D. (2016). Toleransi galur harapan padi sawah (Oryza sativa L.) pada persaingan dengan gulma echinochloa crus-galli. *J Agron Indones, 44,* 111. https://doi.org/10.24831/jai.v44i2.13476

Wahyurini, E. (2009). Pengembangan teknologi berbasis bahan Baku lokal. In: *Peran kultur jaringan tanaman dalam pengembangan pangan lokal*. Lembaga llmu Pengetahuan Indonesia LlPl, Yogyakarta, (pp 675–682).

Yadav, H., Malik, K., Kumar, S., & Jaiwal, P.K. (2020). Comparative regeneration in six bread wheat (Triticum aestivum L.) varieties from immature and mature scutella for developing efficient and genotype-independent protocol prerequisite for genetic improvement of wheat. *Vitr Cell Dev Biol - Plant, 56,* 610–617 . https://doi.org/10.1007/s11627-020-10070-3

Yar, S., Khan, E.A., Hussain, I., Raza, B., Abbas, M.S., & Munazza, Z. (2020). Allelopathic influence of sorghum aqueous extracts and sorghum powder on germination indices and seedling vigor of hybrid corn and jungle rice. *Planta Daninha, 38,* 1–10 . https://doi.org/10.1590/s0100-83582020380100005

Yulifrianti, E., Linda, R., & Lovadi, I. (2015). Potensi alelopati ekstrak serasah daun mangga (mangifera indica (l .)) terhadap pertumbuhan gulma rumput grinting (cynodon dactylon (l .)) press. *J Protobiont, 4,* 46–51 . https://doi.org/http://dx.doi.org/10.26418/protobiont.v4i1.8719

Zarwazi, L.M., Chozin, M.A., & Guntoro, D.D. (2016). Potensi gangguan gulma pada tiga sistem budidaya padi sawah. *J Agron Indones, 44,* 147. https://doi.org/10.24831/jai.v44i2.13481

Zhao, P., Begcy, K., Dresselhaus, T., & Sun, M.X. (2017). Does early embryogenesis in eudicots and monocots involve the same mechanism and molecular players? *Plant Physiol, 173,* 130–142. https://doi.org/10.1104/pp.16.01406

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