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Fiber carbohydrates enhance embryogenic callus induction and double haploid production in anther cultures of three rice genotypes

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Abstract. *Iriani YF, Harijati N, Munawarti A. 2025. Fiber carbohydrates enhance embryogenic callus induction and double haploid production in anther cultures of three rice genotypes. Biodiversitas 26: 3460-3468.* Anther culture offers a great opportunity to produce spontaneous Double Haploid (DH) plants because the anther contains microspores that have the potential to develop into plantlets. However, the low yield of regenerative callus of Indica types (recalcitrant) compared with Japonica and Intermediate types is a problem in rice (*Oryza sativa*) anther culture. This study's aim was to evaluate and analyze the effect of the addition of dietary fiber carbohydrates, namely Gum Arabic (GA) or Glucomannan (GL), at 0, 5, 10 mg L^{-1} in the induction medium on embryogenic callus formation in the anther culture of rice varieties Japonica (Nipponbare), Indica (Rice Kultur Jaringan [RCKJ] 24), and Intermediate (RCKJ 13). The number of anthers capable of producing callus and callus formed in the three rice varieties induced by the addition of 10 mg L^{-1} GA was higher than that of the treatment of GA with lower concentrations and all concentrations of GL. The addition of GA at a concentration of 10 mg L^{-1} significantly enhanced anther forming callus, number of callus formed, and green plantlets percentage of three rice genotypes. The highest callus induction (17% anther forming callus and 290,67% number of callus formed) was obtained from the Nipponbare genotype with the addition of 10 mg/L GA. In addition, 10 mg L^{-1} GA has the potential to increase the number of DH plants significantly; the number increased from 0 to 35 plants; however, it did not make a difference in the morphological characteristics of the callus formed. The addition of GL at a concentration of 10 mg L^{-1} also has the potential to increase the number of DH plants, although the number of DH plants only increased from 0 to 7. The addition of 10 mg L^{-1} GA or GL to callus induction media can be applied as an alternative to increase the number of rice double haploid plants.

Keywords: Anther culture, double haploid, glucomannan, gum arabic, rice

INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food source that continues to increase in accordance with the demand of the rice market. Increasing rice production can be achieved through conventional breeding efforts or through modern biotechnology procedures to produce superior varieties. Conventional technology requires a long time, more than 5 years, to produce a superior variety, whereas modern technology requires a relatively shorter time. The desired character selection of modern technology may already have been performed in the early generations, and the plants obtained were homozygous (Dewi and Purwoko 2016b; Tripathy 2018).

An alternative approach to the assembly of new varieties involves the induction of Double Haploid (DH) plants. In vitro anther culture techniques are frequently used to produce the generation of homozygous DH pure strains through the embryogenesis process, which typically commences with callus induction (Dewi and Purwoko 2016a; Rout et al. 2016; Karima et al. 2021; Samantaray et al. 2021). However, anther culture is not without disadvantages; chief among them is the significant prevalence of albino plants. Furthermore, not all genotypes respond to anther culture, and the resulting plants frequently exhibit a higher degree of haploid than spontaneous DHs (Jain et al. 2022).

The primary issue in the anther culture of cereals such as rice is the low regeneration of green plants (Tripathy et al. 2019). Shoot regeneration from produced callus in anther culture is a complex process; it is influenced by numerous factors, including explant age, the physiological conditions of the donor plant, pretreatment, growth media composition, and genotype (Rout et al. 2016; Tripathy et al. 2019; Ermawati et al. 2022).

Rice is classified into two primary subspecies: Japonica and Indica. The Intermediate subspecies is a hybrid of Japonica and Indica. Anther cultures often use Japonica because of its high regeneration ability. Indica rice is characterized as recalcitrant because of its inherent recalcitrance when regenerating into whole plants (Yaqoob et al. 2021). Considering the disparate regeneration capabilities exhibited by these rice subspecies, Japonica rice can be used as a point of comparison to evaluate the responses of Indica and Intermediate rice to the utilization of a uniform media composition.

Media composition is also a determining factor in the success of anther culture in producing DH plants through the ability to form embryogenic callus (Ali et al. 2021).

Various organic and inorganic substances affect the ability of pollen grains to produce callus and plantlet formation (Pasternak and Steinmacher 2024). One of the organic substances used is fiber carbohydrates (Navarro et al. 2019; Kshirsagar et al. 2020; Stribling and Ibrahim 2023). Carbohydrates are polysaccharides composed of carbon, hydrogen, and oxygen that function as energy and raw materials for cell proliferation (Lei et al. 2020). Carbon is the primary component of carbohydrates and has been shown to enhance embryogenic callus formation (Lee et al. 2023; Md Saad et al. 2023). Embryogenic callus affects the ability of callus to regenerate into green plantlets (Pan et al. 2024).

Gum Arabic (GA) is classified as a member of the fiber carbohydrate group (Atgié 2018; Mariod 2018; Jarrar et al. 2021; Larson et al. 2021; Özcan et al. 2025). GA, a protein carrier of arabinogalactan, D-galactose, L-arabinose, D-galacturonic acid, and l-ramnose, has a positive effect on the induction and regeneration phase of anther culture in rice (Tajedini et al. 2023). In this study, GA addition was tested on three types of rice, Japonica, Indica, and Intermediate.

Another type of carbohydrate that functions as a solidifying material and contains a carbon source is Glucomannan (GL) (Gómez et al. 2017; Drira et al. 2021). GL is a polysaccharide composed of D-glucose and D-mannose units (Nurlela et al. 2019; Tripetch et al. 2023). However, GL utilization in tissue culture media remains underutilized. Glucomannan is widely used as stabilizer and gelling agent in the food industry (Kumala et al. 2020). The aim of this study was to evaluate and analyze the effect of the addition of fiber carbohydrates, GA or GL at concentrations of 0-5 mg L^{-1}, on the induction of embryogenic callus in anther cultures of rice types. The varieties used in this study were Nipponbare, Rice Kultur Jaringan (RCKJ) 24, and RCKJ 13. The objective was to obtain the optimal fiber carbohydrate concentration to induce embryogenic callus and to enhance the percentage of green regenerant of DH plants derived from rice anther culture. The generation of double haploid plants is a key step in this process, as it allows for the rapid production of homozygous plants, which are critical for accelerating the rice breeding process.

MATERIALS AND METHODS

Study design

The experiment was arranged as a three-factor factorial in a completely randomized design where genotype was considered as the first factor, fiber carbohydrate type as the second factor, and fiber carbohydrate concentration as the third factor with three replications for each treatment combination.

Plant materials

A total of three rice genotypes were obtained from the seed collection of rice breeders at PT. BISI International, Tbk. in Kediri, East Java, Indonesia. The three genotypes were Nipponbare (Japonica rice type), RCKJ 24 (Indica rice type), and RCKJ 13 (Intermediate rice type). The seedlings were planted in groups of 10 clumps for each genotype. Approximately 2-3 months after transplantation, donor plants enter the generative phase.

Procedure

Panicle pretreatment

Prior to harvesting the explants, a selection process was implemented to identify panicles containing uninucleate microspores. The selected panicle explants were harvested, and the distance between the flag leaf and the last leaf was determined based on the outcomes of the microspore development analysis (Figure 1.A). Then, the panicles were wrapped in wet tissue and placed in a plastic bag for pretreatment at 8°C for 8 days. This treatment was designed to induce a shift in microspore development from gametophytic to sporophytic.

Selection and sterilization of the explants

Panicles from each treatment were removed from the rolled wet-tissue wrap. Spikelet selection was executed based on cytological observation or anther position. The anther position in the floret/spikelet should not exceed half of the spikelet. All anthers were tightly packed in a single spikelet (Figure 1.B). After pretreatment and spikelet selection were carried out, sterilization of the spikelet explants was conducted using 70% alcohol for 2 minutes, followed by 20% chlorox, which added 50 µL Tween 20 for 15 minutes and rinsed with sterile distilled water three times for 3 minutes each. Every 100 ml of 20% chlorox need 0.05% Tween 20 (v/v). Sterilized spikelets were drained on a 13.5-cm-diameter Petri dish containing sterile tissue.

Anther culture

Sterilized spikelets were pinched using tweezers and then cut, leaving 1/3 of the base of the spikelet (Figure 1.C). Subsequently, the cut end of the spikelet was clamped using specialized bayonet tweezers and tapped on the edge of a Petri dish containing callus induction media, with the objective of removing the anther within the spikelet. The composition of the callus induction media utilized was as follows: 3.21 g L^{-1} Gamborg's B5, 50 g L^{-1} maltose, 2 g L^{-1} Gelrite, 2.5 mg L^{-1} NAA (Naphthaleneacetic Acid), 1 mg L^{-1} Kinetin, 2 mg L^{-1} 2,4-D (2,4-Dichlorophenoxyacetic Acid), 0, 5, 10 mg L^{-1} GA, or 0, 5, 10 mg L^{-1} GL (Tajedini et al. 2023). The positive effect of the optimum concentration of gum arabic (10 mg L^{-1}) was established in callus induction of anther culture, but higher concentrations (15 mg L^{-1}) reduced callus induction in all rice genotypes tested (Tajedini et al. 2023). Each 90 mm × 15 mm Petri dish contained 100 anthers. The cultured anther was then subjected to incubation in an incubator maintained in the dark at a temperature of 25°C for a period of 4-6 weeks, until callus formation was observed. The evaluation of the androgenic response was determined by the percentage of anther-forming callus, number of callus formed, and calculation of the callus formed.

Plant regeneration

Callus with a size of 1-2 mm in diameter were regenerated in MS (Murashige and Skoog) regeneration

medium plus 30 g L^{-1} sucrose, 2 g L^{-1} Gelrite, 1 mg L^{-1} BAP, 1 mg L^{-1} Kinetin, and 1 mg L^{-1} NAA with pH 5.8. The callus was incubated in the culture room at 28°C and photoperiodicity for 16 hours. The morphology of the callus formed was observed at this stage. Observations began by marking the texture (crumbly and compact) and color of the callus (milk-white and yellowish-white) at the beginning of regeneration. The development of the callus was observed up to 14 days after regeneration to determine if the callus had regenerated to form shoots or not. Two weeks after regeneration, the callus formed green plantlets, while some formed albino plantlets. The green plantlets were transferred to bottles containing rooting media with the composition of MS media plus 30 g L^{-1} sucrose, 2 g L^{-1} Gelrite, and 0.5 mg L^{-1} IBA (Ali et al. 2021).

Preacclimatization and acclimatization

Plantlets with good root development were removed from the regeneration medium and transferred to test tubes containing Yoshida liquid medium, incubated in a culture room at 28°C and 16 hours photoperiod for 7-14 days until ready for acclimatization in the greenhouse. Yoshida liquid medium is formulated to provide essential nutrients for rice growth. The use of liquid media aims to improve the adaptability of green plantlets transferred from solid agar to soil with high water content. Acclimatization was performed using plastic cups filled with clay and manure under the condition of sufficient water.

Ploidy analysis

Ploidy analysis was conducted 7 days after the plants were transferred from the agar medium to the Yoshida liquid medium. At this stage, the leaves of the plantlets had already increased in size and number, allowing for the sampling and subsequent ploidy analysis of a subset of the plantlets. Ploidy analysis was performed using a flow cytometer, which generated a graphical representation of the ploidy level of the plant.

Observation parameters

The measured parameters are percentage of anther-forming callus, number of callus, percentage callus formed, percentage of green plantlets, percentage of haploid plant, percentage of double haploid plant, percentage of tetraploid plant, and percentage of mixoploid plant.

Number of callus was counted from all anthers derived from one petri plate. The calculation of the callus formed was performed using the following formula:

$$\%\ \text{Anther} - \text{forming callus} = \frac{\Sigma\ \text{anther} - \text{forming callus}}{\Sigma\ \text{anther cultured}} \times 100\%$$ (Ali et al. 2021)

Number of callus = Σ callus regenerated at 28-35 days after induction (Dewi et al. 2006)

$$\%\ \text{Callus formed} = \frac{\text{Number of callus formed}}{\text{Total number of anther cultured}} \times 100\%$$ (Ali et al. 2021)

The percentage of green plantlets was calculated using the following formula:

$$\text{Green plantlets} = \frac{\text{Total green plantlets}}{\text{Number of callus regenerated}} \times 100\%$$

The ploidy of the plant is categorized as haploid, DH, tetraploid, or mixoploid, and the ploidy percentage can be calculated using the following formula:

$$\%\ \text{Haploid} = \frac{\text{Total haploid plants}}{\text{Total plants with different ploidy}} \times 100\%$$

$$\%\ \text{DH} = \frac{\text{Total DH plants}}{\text{Total plants with different ploidy}} \times 100\%$$

$$\%\ \text{Tetraploid} = \frac{\text{Total tetraploid plants}}{\text{Total plants with different ploidy}} \times 100\%$$

$$\%\ \text{Mixoploid} = \frac{\text{Total mixoploid plants}}{\text{Total plants with different ploidy}} \times 100\%$$

Data analysis

The data obtained were analyzed using three-way Analysis of Variance (ANOVA) with Duncan further test. The applications used were SPSS Ver. 25 for Windows and Microsoft Excel 2019.

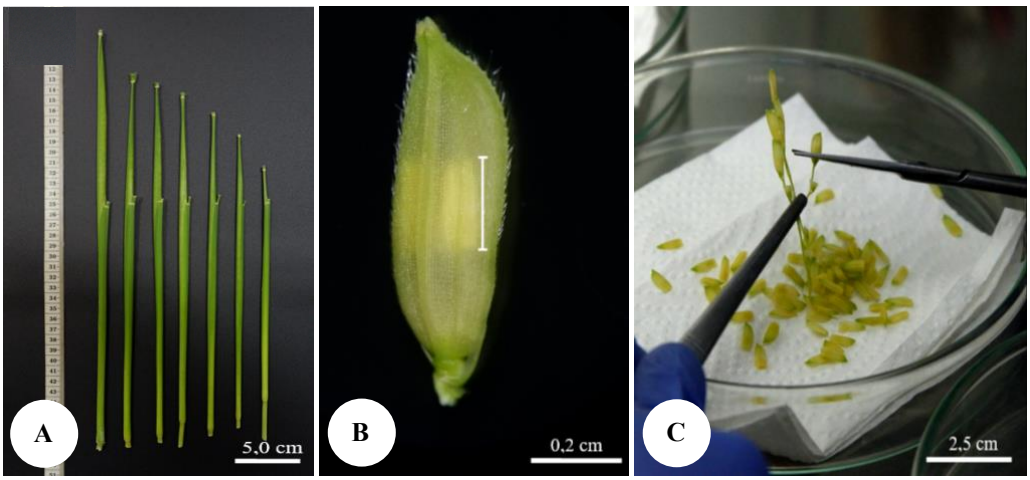


Figure 1. Stage of DH induction of rice plants by anther culture. A. Selection of panicle explants used for culture, B. Position of anthers in grains suitable for explant use, C. Cutting of grain to remove anther

RESULTS AND DISCUSSION

Induction and morphology of the callus from the rice anther culture

Callus formation occurred 21 days after culture in Japonica-type rice (Nipponbare), whereas in Indica-type rice (RCKJ 24) and Intermediate-type rice (RCKJ 13), it appeared 28 days after culture. These results were consistent with the findings of Dewi et al. (2017), which reported that callus formation typically occurs within a timeframe of 21-56 days following the inoculation process. This callus formation occurred directly from the anther explants, causing the anther to appear as if it had opened (Figure 2.A). Subsequently, callus that had reached a size of 1-2 mm was subcultured on a regeneration media (Figure 2.B).

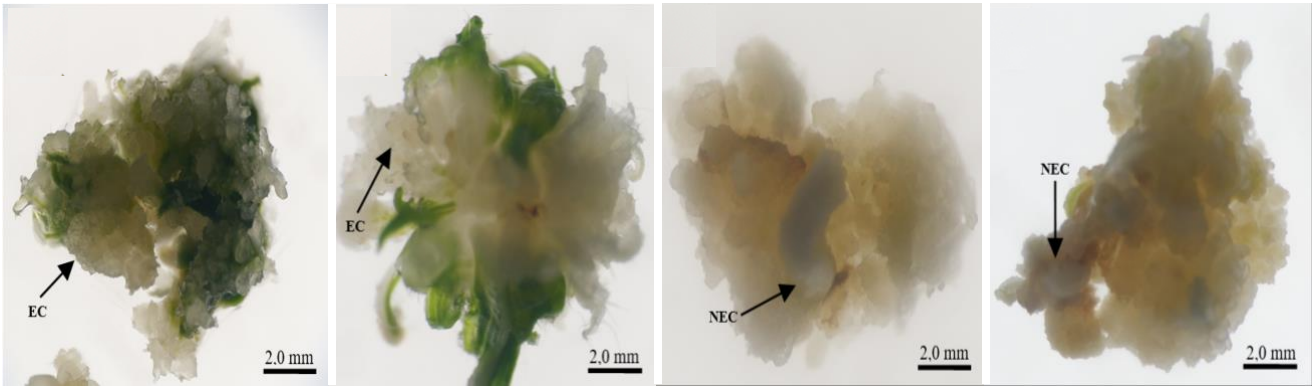
Based on qualitative observations, the callus produced from the rice anther culture in this study showed no differences between genotypes, as well as the treatment of fiber carbohydrate addition. Two types of fiber carbohydrates with three different concentrations also had no effect on the morphology of the callus. However, in one genotype with fiber carbohydrate treatment at the same concentration, it can produce callus with different morphology; this difference includes callus texture and color.

In this study, more shoots were obtained from the callus, which had a crumbly texture and a white-yellowish color (Figures 3.A and 3.B). Meanwhile, the callus, which has a compact texture and milky-white color, tends to change color to brownish starting 7 days after transfer to the regeneration medium (Figures 3.C and 3.D). Crumbly callus formation was caused by the increased cell division process. The growth regulator 2,4-D, an auxin group, which plays a role in stimulating and accelerating cell division and enlargement, such that the cells experience increased growth without the development of cell walls toward thickening; therefore, the callus texture remains crumbly (Mayerni et al. 2020). One component of the callus induction media in the study contained 2,4-D at the same concentration; however, the addition of fiber carbohydrates at concentrations up to 10 mg L^{-1} did not result in significant differences. Further research is required to determine the effect of adding high-concentration fiber carbohydrates on the morphology of the callus formed in response to all treatments.

Analysis of variance revealed that genotype, fiber carbohydrate type, and fiber carbohydrate concentration had a significant effect on anther forms callus percentage ($p<0.05$) (Table 1). In addition to carbohydrate type, the concentration of each dietary fiber showed different effects. The addition of GA or GL fiber carbohydrates increased callus formation compared with the control. The incorporation of GA at a concentration of 10 mg L^{-1} enhanced the capacity of the anthers to form a callus, with a notable increase from 4.00% to 17.00% observed in Nipponbare. As shown in Table 1, the percentage increased from 0.67% to 7.33% for RCKJ 24 and from 2.67% to 16.33% for RCKJ 13. Callus formed per 100 anther has the same result, the addition of GA at a concentration of 10 mg L^{-1} increase 73.33% to 290.67% observed in Nipponbare, from 1.00% to 37.67% for RCKJ 24 and from 4.00% to 141.67% for RCKJ 13 (Table 2).

A B

Figure 2. Stage of callus induction of rice plants by anther culture: A. Callus induction 21-28 days after culture, B. The callus that had reached a size of 1-2 mm



A B C D

Figure 3. Rice callus from anther culture. A and B: Embryogenic callus (EC), C and D: Non-embryogenic callus (NEC). The NEC mostly forms a milky-white solid structure

Research using GL as a chemical additive for tissue culture media, especially rice callus induction media, has not yet been conducted. In this study, GL use showed the same pattern for the three genotypes, where the number of callus formed was highest at a concentration of 10 mg L^{-1}. Genotype Nipponbare showed an increase in the number of callus formed from 73.67 to 244.33 with the addition of GL 10 mg L^{-1}, while RCKJ 24 showed an increase from 0.33 to 12.33 with the addition of GL 10 mg L^{-1}; RCKJ 13 showed an increase from 4.67 to 66.67. This is supported by previous research indicating that in addition to basal media and hormones, the type and concentration of carbon sources, nitrogen sources, and organic plant material can increase haploid induction in cereals (Lu et al. 2016; Tajedini et al. 2023).

In this study, the anther planted contained microspores that developed into the callus. The addition of fiber carbohydrates to the callus induction medium exhibited a positive effect on the androgenic response of rice anther compared to the callus induction medium without the addition of fiber carbohydrates. This result is in agreement with the results of (Tajedini et al. 2023). She mentioned that the B5 induction medium supplemented with 10 mg L^{-1} GA within 10 days of cold pretreatment of panicles produced the highest callus induction among the anther cultures of four rice varieties (Wakara, Sahel 305, Remar, and Hashemi).

The addition of various chemicals, such as the Arabinogalactan Protein (AGP) found in GA, is required to regulate plant growth and yield (Khan et al. 2020). AGP use in liquid and solid media to enhance gamete embryogenesis and subsequently increase the number of green haploid/DH plantlets in anther culture (Niazian et al. 2019). Arabinogalactan has positive effects on the induction and regeneration phases of rice anther culture and genetically enhanced callus induction and embryo regeneration (Tajedini et al. 2023).

Gum arabic has been shown to be more effective than glucomannan in promoting callus induction and callus regeneration. Studies demonstrate that gum arabic enhances both the efficiency of callus formation and the subsequent regeneration process, making it a superior choice compared to glucomannan as a gelling agent or supplement in plant tissue culture media (Li et al. 2021; Sen and Beser 2022). This superiority is attributed to the favorable physical and mechanical properties of gum arabic, which support better callus phenotype and growth (Li et al. 2021; Muzika et al. 2024). These findings suggest that gum arabic is a preferable alternative to glucomannan for applications requiring efficient callus induction and regeneration in plant tissue culture.

Table 1. Analysis of variance for anther forms callus percentage in anther culture of three rice genotypes

Source of variation	Degree of freedom	Mean squares
Genotype	2	162.463**
Fiber carbohydrate type	1	127.574**
Fiber carbohydrate concentration	2	298.352**
Genotype × fiber carbohydrate type	2	6.241**
Genotype × fiber carbohydrate concentration	4	16.519**
Fiber carbohydrate type × fiber carbohydrate concentration	2	44.463**
Genotype × fiber carbohydrate type × fiber carbohydrate concentration	4	6.296
Error	36	2.130

**: Significant at the 0.05 probability level

Table 2. Effect of fiber carbohydrate addition on the callus formation from anther cultures of Japonica-type rice (Nipponbare), Indica-type rice (RCKJ 24), and Intermediate-type rice (RCKJ 13)

Carbohydrate fiber	Concentration (mg L^{-1})	Total anther cultured	Anther forms callus (%)	Callus formed per 100 anthers (%)
Japonica (Nipponbare)				
Gum arabic (GA)	0	100	4.00±1.00^{bcd}	73.33±11.06^{c}
	5	100	10.33±1.53^{fg}	171.67±5.77^{e}
	10	100	17.00±2.00^{h}	290.67±55.19^{g}
Glucomannan	0	100	3.67±1.15^{bcd}	73.67±8.14^{c}
(GL)	5	100	8.00±1.00^{ef}	158.33±20.82^{d}
	10	100	11.00±2.00^{g}	244.33±6.03^{f}
Indica (RCKJ 24)				
Gum arabic (GA)	0	100	0.67±1.15^{a}	1.00±1.73^{a}
	5	100	4.00±1.00^{bcd}	9.33±1.15^{a}
	10	100	7.33±1.15^{e}	37.67±4.16^{b}
Glucomannan	0	100	0.33±0.58^{a}	0.33±0.58^{a}
(GL)	5	100	1.67±1.53^{ab}	3.33±3.06^{a}
	10	100	4.00±1.00^{bcd}	12.33±3.51^{ab}
Intermediate (RCKJ 13)				
Gum arabic (GA)	0	100	2.67±1.53^{abc}	4.00±34.69^{a}
	5	100	4.67±0.58^{cd}	69.33±254.82^{c}
	10	100	16.33±141.67^{h}	141.67±100.24^{d}
Glukomannan	0	100	2.00±1.00^{abc}	4.67±115.47^{a}
(GL)	5	100	2.67±0.58^{abc}	6.00±127.29^{a}
	10	100	6.00±2.00^{de}	66.67±532.39^{c}

Note: The same letter in each parameter indicates not significantly different in the Duncan test with a real level of 5% ($p<0.05$)

Regeneration of the callus from the rice anther culture

The formation of yellowish-white callus accompanied by the emergence of greenspots commenced 4-6 days after the subculture process (Figure 4.A). The greenspot that formed continued to grow, eventually developing into shoots and ultimately into green plantlets within a period of 6-14 days (Figure 4.B). The callus partially regenerates into green (Figure 4.C), albino (Figure 4.D), and browning plantlets. Subsequently, the green plantlets underwent a preacclimatization process, and ploidy analysis was conducted (Figure 4.E).

Effect of the addition of carbohydrate fiber GA or GL on the potential increase of callus regenerants in rice anther culture

Callus induction media with the addition of fiber carbohydrate GA 10 mg L^{-1} produced the most green regenerants in the Nipponbare rice anther culture. Callus induction media without the addition of any fiber carbohydrates produced the least green regenerants compared with callus induction media with the addition of fiber carbohydrates (Figures 5.A and 5.D). The higher the concentration of fiber carbohydrate added to the callus induction medium, the more green regenerants were produced (Figures 5.B and 5.E). The type of fiber carbohydrate added has a different effect on green regenerant formation. GA fiber carbohydrate (Figures 5.A-5.C) was able to produce more green regenerants compared with GL (Figures 5.D-5.F).

The greenspot callus formed differentiate to form shoots and become whole plants called green plantlets. Greenspot callus has been demonstrated to produce multiple green plantlets, thereby enabling the percentage of green plantlets to exceed 100%. In the parameter of green plantlet percentage, the percentage of Nipponbare was higher, followed by RCKJ 13, and RCKJ 24, at 114.48%, 96.47%, and 92.43% (Table 3).

The percentage of green planlet formation showed a significant effect of each factor, genotype, type of fiber carbohydrate, and fiber carbohydrate concentration. In addition, there was an interaction between genotype and fiber carbohydrate concentration added to the induction medium.

The factors affect the success of callus regeneration to produce green regenerants, such as media composition, explant source, genotype, and environment (Jumsu Trisno and Jamsari 2017; Ali et al. 2021; Carsono et al. 2022b). Among these factors, genotype and media composition are considered the main factors that cause differences in regeneration ability (Niroula et al. 2005).

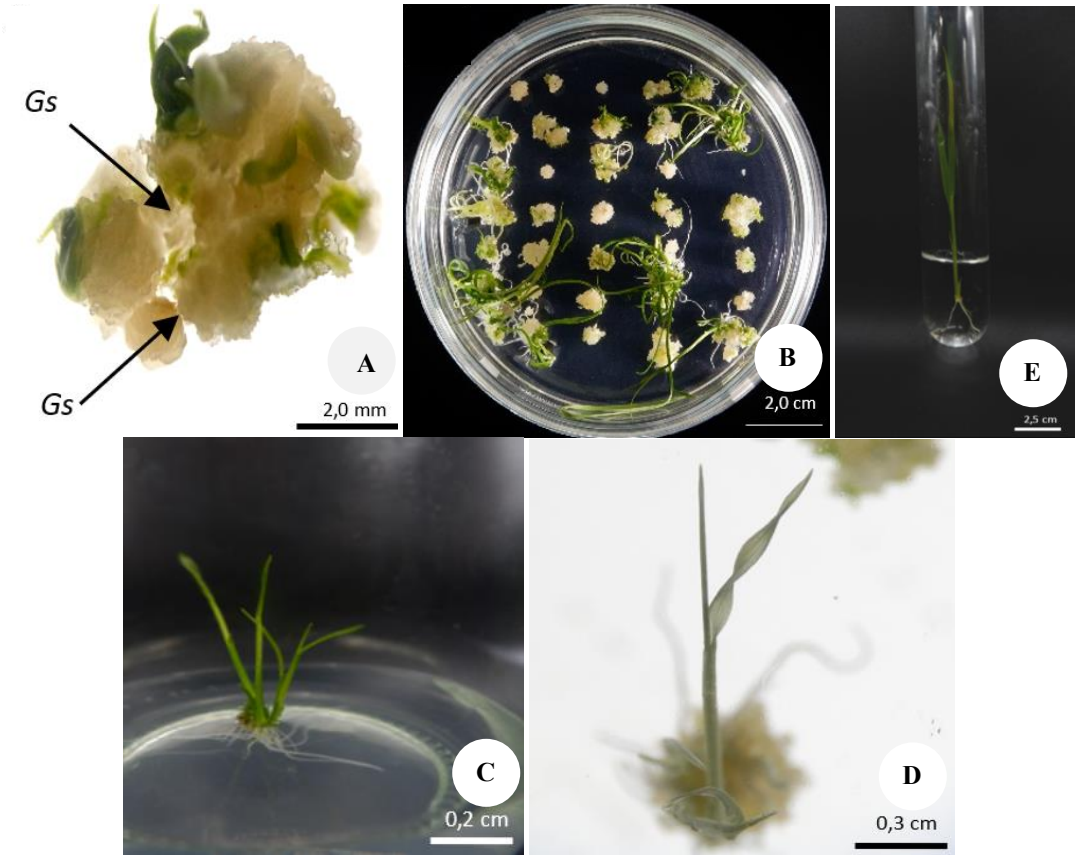


Figure 4. Stage of callus regeneration of rice plants by anther culture: A. Greenspot callus (*Gs*), B. Plantlet regeneration, C. Green plantlets, D. Albino plantlets, E. Preacclimatization of green plants

Table 3. Effect of fiber carbohydrate addition on the potency of callus regeneration from anther cultures of Japonica-type rice (Nipponbare), Indica-type rice (RCKJ 24), and Intermediate-type rice (RCKJ 13)

Carbohydrate fiber	Concentration (mg L^{-1})	Total anther cultured	Callus greenspot (%)	Callus albino (%)	Callus browning (%)	Green plantlets (%)
Japonica type (Nipponbare)						
Gum arabic (GA)	0	100	42.17^{b}	3.76^{ab}	54.07^{abc}	87.43^{defg}
	5	100	59.58^{def}	8.02^{ab}	32.40^{a}	88.74^{defg}
	10	100	76.67^{gh}	9.23^{ab}	14.11^{a}	114.48^{g}
Glucomannan	0	100	43.43^{bc}	5.17^{ab}	51.40^{abc}	82.01^{def}
(GL)	5	100	66.08^{fg}	7.96^{ab}	25.96^{a}	85.26^{defg}
	10	100	57.23^{cdef}	7.51^{ab}	35.26^{a}	111.67^{fg}
Indica type (RCKJ 24)						
Gum arabic (GA)	0	100	0.00^{a}	0.00^{a}	33.33^{a}	0.00^{a}
	5	100	50.83^{bcde}	3.33^{ab}	45.83^{ab}	65.83^{cde}
	10	100	86.37^{h}	2.56^{ab}	11.06^{a}	96.47^{efg}
Glucomannan	0	100	0.00^{a}	0.00^{a}	33.33^{a}	0.00^{a}
(GL)	5	100	13.89^{a}	8.33^{ab}	22.22^{a}	38.89^{a}
	10	100	49.77^{bcd}	9.72^{b}	40.05^{a}	61.11^{cd}
Intermediate type (RCKJ 13)						
Gum arabic (GA)	0	100	6.67^{a}	0.00^{a}	86.67^{cd}	20.00^{ab}
	5	100	64.87^{efg}	1.46^{ab}	33.67^{a}	82.16^{def}
	10	100	88.34^{h}	0.95^{ab}	11.17^{a}	92.43^{defg}
Glucomannan	0	100	5.56^{a}	0.00^{a}	94.44^{d}	0.00^{a}
(GL)	5	100	13.10^{a}	4.17^{ab}	82.74^{bcd}	25.00^{ab}
	10	100	60.23^{def}	3.07^{ab}	36.70^{a}	85.06^{defg}

Note: The same letter in each parameter indicates not significantly different in the Duncan test with a real level of 5% ($p<0.05$)

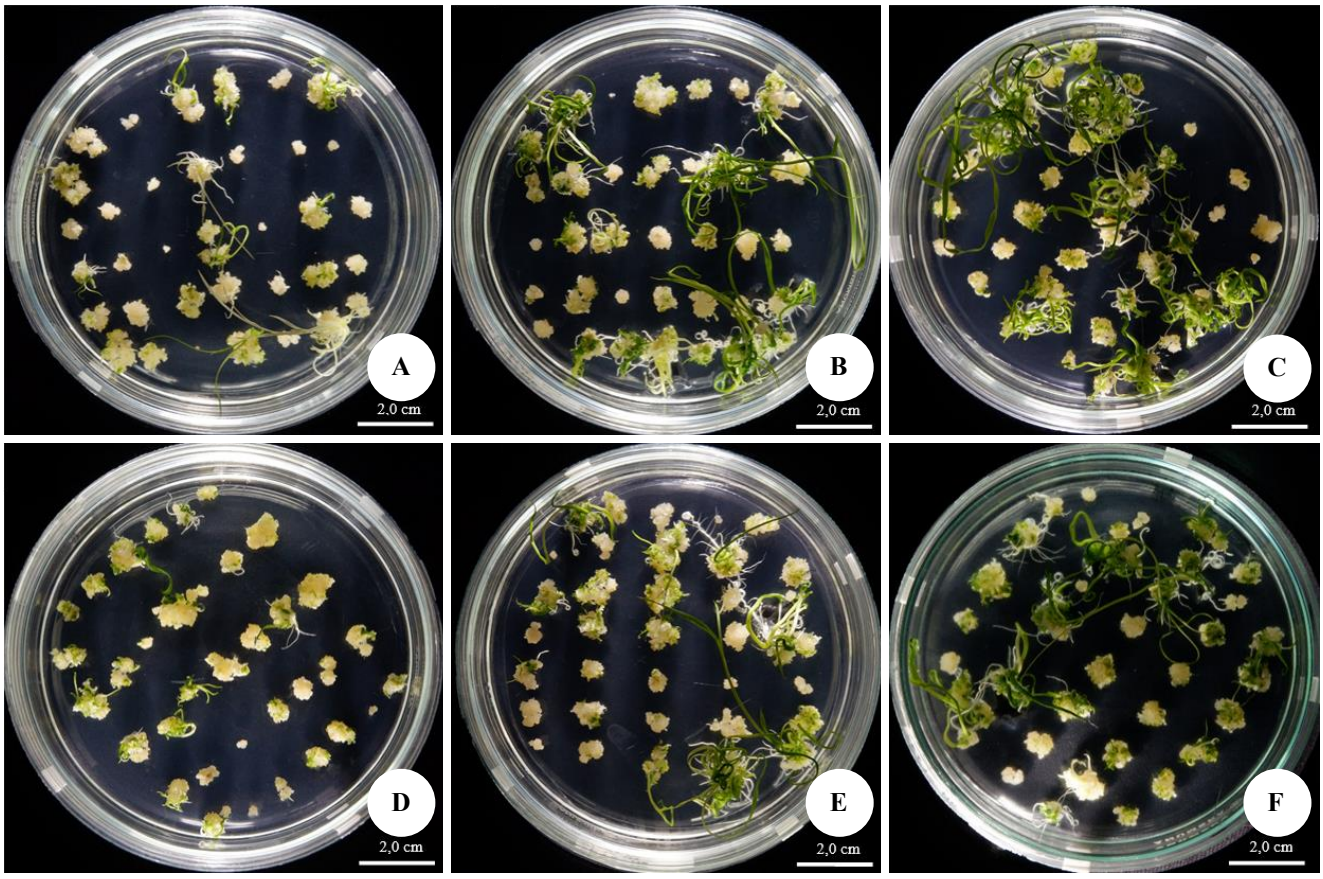


Figure 5. The regeneration of callus from an anther culture of Japonica (Nipponbare) type rice plants with fiber carbohydrate addition was studied. The experiment included five treatments: A. 0 mg L^{-1} of GA (control), B. 5 mg L^{-1} of GA, C. 10 mg L^{-1} of GA, D. 0 mg L^{-1} of GL (control), E. 5 mg L^{-1} of GL, and F. 10 mg L^{-1} of GL

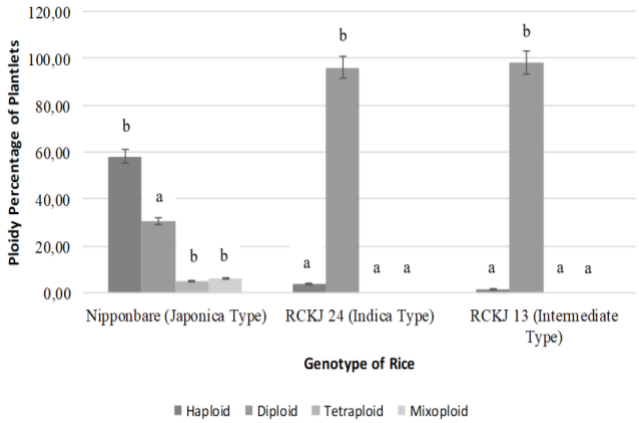


Figure 6. Ploidy percentage of anther-cultured rice plantlets from three rice genotypes. Note: The same letter in each parameter indicates not significantly different in the Duncan test at the 5% real level ($p<0.05$)

Effect of the addition of carbohydrate fiber GA or GL on ploidy level of plants from the rice anther culture

There was a significant difference among the rice genotypes studied in terms of ploidy percentage of plantlets resulting from anther culture. The Nipponbare genotype had the highest haploid percentage of 58.30% compared with RCKJ 24 and RCKJ 13, which had haploid percentages of 3.87% and 1.75%, respectively. Regarding the percentage of double haploid/diploid, RCKJ 24 and RCKJ 13 showed high values, namely 96.13% and 98.25%, respectively (Figure 6). The results of the ploidy analysis revealed the influence of genotype on the ploidy percentage for each genotype used in this study. Plants resulting from anther culture can be haploid plants, spontaneously obtained double haploid/diploid plants, and higher ploidy plants (Gunarsih et al. 2022).

The three rice genotypes utilized in this study possess varying backgrounds, thereby influencing the response to anther culture outcomes. Among the three rice genotypes, Nipponbare, which possesses a Japonica genetic background, exhibited the highest values for all parameters compared with the other two genotypes. Nipponbare was a frequently used model plant owing to its high responsiveness to diverse treatments. Japonica rice exhibits a heightened responsiveness to anther culture compared with its Indica and Intermediate counterparts, thus leading to its categorization as having high anther culturability (Sun et al. 2023; Guo et al. 2024). In contrast, Indica-type rice is classified as recalcitrant; this classification was attributed to the fact that the plant exhibits limited anther responses owing to several problems, including early necrosis, low callus formation, and difficulty in regeneration (Ali et al. 2021).

Genotype is one of the factors that influences the response to anther culture. Kassa et al. (2024) mentioned that the androgenic response (anther culture) is highly dependent on the genotype, which will affect the pollen development pathway and callus induction frequency, where the Turkan genotype used in the study showed a much better callus induction response than the local genotype for all parameters.

In conclusion, the addition of the dietary fiber carbohydrate GA or GL to the callus induction medium of rice anther culture showed an increase in the percentage of anthers producing callus, percentage of callus formed, and formation of green plantlets as the concentration of GA or GL increased in the three genotypes used. Among these two dietary fiber carbohydrates, 10 mg L^{-1} GA had a better effect on all the observed parameters than the control, 5 mg L^{-1} GA, 5 mg L^{-1} GL, and 10 mg L^{-1} GL. Ploidy analysis showed the effect of genotype on ploidy percentage according to genotype. Among the three genotypes used, the Japonica type (Nipponbare) produced the highest percentage of haploid plants at 50.30%, whereas the highest percentage of DH plants was obtained from the Intermediate type (RCKJ 13) at 98.25%. To study the effect of adding GA or GL to callus induction media, which has the potential to increase the acquisition of DH plants, to maximize the results in anther culture activities in rice. Using double haploid technology can speed up the process of creating new rice varieties. It is important to pay attention to how well the greenhouse is working when planting DH lines. This helps to make sure that selfing is correct and that there is no pollen contamination. This can cause DH lines to become non-uniform.

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