Vegetative propagation in vitro with content analysis of ginger oil from calluses of “Jahe Gajah” (Zingiber officinalis) on treatments of types of mediums and carbohydrates

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Abstract. Rhizomes of “Jahe Gajah” were made to be explants in this research because of their contents of Ginger Oil were especially liked by consumers as they are not very spicy. The calluses of this research would also be developed for the propagation of the ginger “Jahe Gajah” itself. The purpose of this research was to discover the ideal type of carbohydrate to be added to the mediums MS and VW, in order to increase the biosynthesis process of Ginger Oil in the “Jahe Gajah” calluses. The method of this research was using Complete Random Design with Factorials of two factors: Factor I: Mediums MS and VW, and Factor II: types of Carbohydrates: glucose, fructose and sucrose. So, there were 6 combinations of treatments, repeated 5 times, with 4 samples each. From those, were research the highest contents of Ginger Oil. The publications of this research were of the type of medium and carbohydrate that can increase the content of secondary metabolites of Ginger Oil. The results of this research were: [1] The highest quantity of calluses was found on the treatment of Medium MS and Glucose (C1M1); [2] The quality of calluses on Medium MS and Sucrose (C3M1) & Fructose (C2M1) were friable calluses with embryogenic characteristics, while on other treatments the calluses were compact and organogenic; [3] Embryogenic calluses are friable, and can be developed into ginger planlets; [4] The calluses of “Jahe Gajah” that were compact and organogenic (C2M2) had the highest content of Ginger Oil (Gingerol – Shogaol – Zingerone 0.184 – 0.118 – 0.982 %)

1. Introduction

According to [1] “Jahe Gajah” is an important export commodity, as its primary function as the ingredient of traditional medicines. [2] had stated that the production of secondary metabolites (Ginger Oil) in “Jahe Gajah” (Zingiber officinalis) via tissue culture technique is possible. The species can also be used for herbs, its essential oils, aroma/fragrance giver, as well as for materials for the pharmaceuticals industries [3]. The contents of chemical compounds in ginger are Gingerol, Shogaol and Zingerone. These compounds have pharmacological effects, such as: anti-oxygen, anti-inflammation, analgesic, and anti-carcinogenic [4]. Tissue culture is a very effective technology to be used in this research. In order to produce and increase the secondary metabolites compounds in the calluses of “Jahe Gajah”, the explants of “Jahe Gajah” were planted in the mediums MS and VW with additional treatments of types of carbohydrates, which were glucose, fructose and sucrose [5]. The production of secondary metabolites through plant tissue cultures process is a more effective way to improve these contents than conventional methods [6].
Purpose of research was to discover the type of medium, MS & VW, and type of carbohydrate, glucose, fructose and sucrose, to be added that can better increase the biosynthesis of compounds Gingerol, Shogaol and Zingerone (Ginger Oil), as well as to develop the ginger calluses of “Jahe Gajah”

The urgency of this research was that currently the extraction of the secondary metabolites was only from ginger rhizomes. While it is widely known that the needs of rhizomes are very high as seeds and other requirements. As the high demand of Ginger Oil for ingredients of medicines are still largely unfulfilled, therefore this research were also aimed to discover the best composition of Ginger Oil in ginger calluses through the analysis of the Ginger Oil contents of this variety of ginger [7].

Results of this research were hoped to be beneficial as :
1. To discover the type of Medium (MS or VW) that can produce the best contents of Ginger Oil.
2. To discover the type of Carbohydrate (glucose, fructose, sucrose) to be added to the aforementioned mediums that can increase the biosynthesis process in the calluses of “Jahe Gajah” to produce the best contents of Ginger Oil.
3. To produce ginger Planlets of “Jahe Gajah” variety from embryogenic calluses.

The Plant Systematics

Kingdom : Plantae
Division : Spermatophyta
Sub Division : Angiospermae
Class : Monocotiledonae
Ordo : Zingiberales
Família : Zingiberaceae
Genus : Zingiber
Species : Zingiber officinale
“Jahe Gajah”

Figure 1. “Jahe Gajah” Zingiber officinale R

In world market, the need of ginger are mostly fulfilled from India, for about 50%. According to [8], the low production of ginger in Indonesia, was primarily caused by the lack of superior seeds. The effort to produce this superior seeds and to increase the contents of Ginger Oil were the purpose of this research, using the tissue culture technique and biosynthesis of secondary metabolites extracted from the calluses of three varieties of ginger. According to [9] the tissue culture is a technology to reproduce a part of and tissues of plants in order to propagate them, for genetic engineering and even to produce secondary metabolites in plants. [10] had stated that tissue culture processes are :

a. A part of tissue were taken from the original plant
b. The part were planted/grown in a artificial medium
c. A planlets would grow
The technology of tissue culture was often said as the propagation in Vitro, which means the cultivation was implemented in tubes or artificial mediums in a sterile environment. This system can produce new plants in huge quantity in a relatively short time period. While the characteristics of the original plants were retained in the new produced plants [11].

Some benefits of the usage of tissue culture technique in plants to produce secondary metabolites compounds are:

a. Independent of environmental factors, i.e.: climate, diseases, pests, geographical and seasonal hindrances.
b. The production system can be arranged, as to when and what quantity to be produce.
c. Quality and the results of the production will be consistent.
d. Reducing the need of lands.

Carbohydrates are a carbon source need in the metabolism process as an energy provider. The sources of carbohydrates oftenly used in tissue culture’s mediums are: sucrose, glucose and fructose. The effect of sucrose concentrate in tissue culture’s mediums to the production of secondary metabolites were already widely tested by many researchers groups. Among carbohydrates are monosaccharides, disaccharides and polysaccharides. In the dissolvement of carbohydrates, there are three important reactions, which are glucose, fructose and sucrose. Fructose is a simple sugar of carbohydrate that mostly found in honey and fruits [12]. The degree of sweetness in fructose are higher compared to glucose and sucrose, so there is more energy to be used in the cell’s metabolism [13].

The composition of the medium is also a deciding factor for the success of tissue culture’s implementation. Differences in the medium’s composition can caused the difference in growth and development in explants in Vitro [12]. The production of secondary metabolites compounds through tissue culture technique are highly affected by several factors, i.e.: 

a. Genetic factors, and
b. Environmental factors outside the culture [14]

These factors are varied, depending on the culture used and artificially manipulated to produce desirable compounds in as much as possible quantity. The expression of secondary metabolites compounds depends on the development phases of the producing organism [15]. In order to gain secondary metabolites compounds in huge quantity through tissue culture technique, the planted explant should be grown to form calluses [16].

2. Methods

2.1 Time and Location of Research
This research was conducted in the Laboratory of Tissue Culture at Department of Agriculture, University of Wijaya Kusuma Surabaya, started in January until December 2019.

2.2 Materials and Equipments
Materials used were: Explants (of rhizomes of “Jahe Gajah”), Basic Mediums of Murashige and Skoog (MS) & VW, Growth-control nutrients (5 ppm NAA and 3,5 ppm BAP [17]. Coconut water, Glucose, Fructose, Sucrose, Alcohol 70% and 96%, Clorox, Betadine, Aluminium foils, and Plastik wraps.

Equipments used during research were: Sartorius scales, Autoclave, Oven, LAF, pH meter, Pintets, Scalpel, Erlenmeyer, Measuring cups, Measuring Pipettes, Drop Pipettes, Petridishes, Spatula, Culture tubes, and Magnetic stirrer.

2.3 Method of Research
“Jahe Gajah” are liked by consumers due to their not-so-spicy flavour, and their ability to produce contents of Gingerol, Shogaol and Zingerone in calluses are among the best. Those were the reasons they were used as explants in this research. The research were using precursors of types of carbohydrates added to the mediums MS and VW. This year research method was using Complete
Random Design with Factorials of two factors. Factor 1 : Basic Mediums (MS and VW) and Factor 2 : types Carbohydrates (Glucose, Fructose and Sucrose). So there were 6 combinations of treatments, repeated 5 times with 4 trial samples each. From these, were researched the best contents of Gingerol, Shogaol and Zingerone.

Factor I 
M1 = MS
M2 = VW

Factor II
C1 = glucose
C2 = fructose
C3 = sucrose

2.4 Implementation of Research
2.4.1 Making of Mediums. The making of mediums were adjusted to the composition of MS and VW mediums (Table 3 and 4). These mediums were modified with 5 ppm NAA and 3,5 ppm BAP, as well as the additional precursors of types of carbohydrates concentrates as designated.

2.4.2 Planting. The explants of ginger rhizomes were strelized with HgCl 0.1% for 1 minute, Clorox 20% and Tween were added 1 drop for 5 minutes, then continued with Clorox 10% + Tween 1 drop for 10 minutes and Clorox 5% + Tween 1 drop for 20 minutes, then rinsed 3 times with sterilized water. Then they were cut in sizes of ±0,5 cm3 and planted in the culture tubes containing the mediums as designated treatments [18].

The observations were done visually once a week, of the explants’ growth, consisted of :
1. Quantity of Calluses
Observed once a week, visually with the scoring :
1 = no calluses
2 = few calluses (<1 time size of explant)
3 = medium calluses (1-2 times size of explant)
4 = many calluses (>2 times size of explant)
2. Quality of Calluses
Observed once a week, visually with the scoring:
1 = no calluses
1-2 = compact calluses
2-3 = friable calluses

3. Contents of Secondary Metabolites
Gingerol, Shogaol and Zingerone on the ginger calluses were extracted and observed using a spectrophotometer for analysis.

Table 1. Composition of Medium Murashige and Skoog

| No | Nutrients                  | Quantity (mg/L) of medium |
|----|----------------------------|---------------------------|
| 1  | Macro Nutrients            |                           |
|    | NH₄NO₃                    | 400                       |
|    | KH₂PO₄                    | 170                       |
|    | Ca(NO₃)₂.4H₂O              | 556                       |
|    | MgSO₄.7H₂O                 | 370                       |
|    | K₂SO₄                     | 990                       |
|    | CaCl₂                     | 96                        |
| 2  | Micro Nutrients            |                           |
|    | Na₂-EDTA                   | 32.8                      |
|    | FeSO₄.7H₂O                 | 27.6                      |
|    | MnSO₄.4H₂O                 | 16.9                      |
|    | ZnSO₄.4H₂O                 | 8.6                       |
|    | H₂BO₃                     | 6.2                       |
|    | KI                         | 0.83                      |
|    | NaMoO₄.2H₂O                | 0.25                      |
|    | CuSO₄.5H₂O                 | 0.025                     |
|    | CoCl₂.6H₂O                 | 0.025                     |
| 3  | Vitamins and Amino Acids   |                           |
|    | Thiamine                   | 1                         |
|    | Nicotinic acid             | 0.5                       |
|    | Pyridoxin HCl              | 0.5                       |
|    | Glycine                    | 2                         |
|    | Myo-Inositol               | 100                       |
|    | Sucrose                    | 30.000                    |
|    | Jelly                      | 7.000                     |

Source: [19]

Table 2. Composition of Medium Vacin And Went

| No | Components | Quantity / liter of medium |
|----|------------|----------------------------|
| 1  | NH₄NO₃     | 200 mg/L                   |
| 2  | KNO₃       | 525 mg/L                   |
| 3  | KH₂PO₄     | 250 mg/L                   |
| 4  | MgSO₄.7H₂O | 250 mg/L                   |
| 5  | (NH₄)₂SO₄  | 500 mg/L                   |
| 6  | Fe (EDTA)  | 37 mg/L                    |
| 7  | FeSO₄.7H₂O | 28 mg/L                    |
| 8  | MnSO₄.7H₂O | 7.5 mg/L                   |
| 9  | Sucrose     | 20 gr/L                    |
| 10 | Jelly       | 8 gr/L                     |

Source: [10].
2.5 Data Analysis
Data analysis were done using One-Way Anova, univariate with SPSS-18, and if there were real differences then continued with LSD 5% test.

3. Results and Discussion

3.1 Quantity of Calluses
Results of the observations of the quantity of calluses from “Jahe Gajah” with treatments of types of carbohydrates (glucose, fructose and sucrose) on types of mediums of MS and VW, were reported at Table 3 below.

Table 3. Average Results of the Quantity of Calluses with Treatments of Types of Carbohydrates and Mediums

| Treatment          | WAP (Weeks After Planting) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|--------------------|-----------------------------|---|---|---|---|---|---|---|---|
| MS Medium + Glucose|                             | 1 | 1 | 1 | 1 | 2.2 | 2.2 A | 1.3 B | 2.3 A |
| VW Medium + Glucose|                             | 1 | 1 | 1 | 1 | 1.2 | 1.2 C | 1.3 B | 1.3 C |
| MS Medium + Fructose|                            | 1 | 1 | 1 | 1 | 1.7 | 1.2 B | 1.8 B | 2.9 A |
| VW Medium + Fructose|                            | 1 | 1 | 1 | 1 | 1.2 | 1.2 C | 1.2 B | 1.2 C |
| MS Medium + Sucrose|                              | 1 | 1 | 1 | 1 | 2.2 | 1.2 C | 1.2 B | 1.2 C |
| VW Medium + Sucrose|                              | 1 | 1 | 1 | 1 | 1.7 | 1.2 C | 1.2 B | 1.2 C |
| BNT 5%             |                              | TN | TN | TN | TN | 0.37 | 0.38 | 0.38 | 0.38 |

Notes: The average values followed by the same letter in the same column showed that there were no real differences based on BNT 5% test.

The Table had shown that there were no real differences on Week 1-5 WAP, while from Week 6-7 WAP had shown real difference, with the best average value in Week 8 was of C1M1 (Glucose in Medium MS) compared to other treatments.

The results on the above Table had shown real differences with the treatments of types of carbohydrates and mediums in the week 6 to 8 WAP. From the week 8, the best average value of the quantity of calluses was found in the treatment C1M1, which was of Medium MS and glucose. This was caused as every commodity has a specific response to a treatment [20].

Explant of a plant commodity has to have a compatibility to a medium to be able to grow into callus. The medium composition also affects the growth in relation to the treatments of the research. On the quantity of calluses, the factors that affected the results were the treatments of types of carbohydrates and mediums. The composition of medium was also a deciding factor for the success of tissue culture implementation. Differences in medium composition could cause different growth and developments in explants In Vitro (Marлина, 2004). In this case, the different medium composition of medium MS and VW, as well as the difference in the types of carbohydrates added, were affecting the growth of the quantity of calluses of “Jahe Gajah”. Treatment C1M1 had the highest quantity of calluses. Medium MS had more complete composition than medium VW, while fructose had the highest sweetness [15]. The quality of callus were the characteristics of callus produced by the explants.

3.2 Quality of Calluses
Results of the observations of the quantity of calluses from “Jahe Gajah” with treatments of types of carbohydrates (glucose, fructose and sucrose) on types of mediums of MS and VW, were reported at Table 4, there were differences in growth shown started from Week 6. This had shown that there were interactions with the treatments as seen in week 6 to 8 WAP. On treatment MS Medium and Sucrose (C3M1), the calluses formed were tended to be friable. While on the other treatments, the calluses were compact. The quality of calluses on C3M1 were tended to be friable. According to [21].
the brownish-yellow calluses with nodules are embryonic calluses with the regeneration ability. Embryonic calluses are calluses that can grow and develop to form embryonic structures. While on the other treatments were formed compact calluses with organogenic characteristics [15].

### Table 4. Average Results of the Quality of Calluses with Treatments of Types of Carbohydrates and Mediums

| Treatment                        | WAP (Weeks After Planting) |
|---------------------------------|----------------------------|
|                                 | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  |
| MS Medium + Glucose             | 1  | 1  | 1  | 1  | 1.6| 2.2 B | 1.2 B | 1.2 C |
| VW Medium + Glucose             | 1  | 1  | 1  | 1  | 2.2 | 1.2 C | 2.2 A | 1.2 C |
| MS Medium + Fructose            | 1  | 1  | 1  | 1  | 1.7 | 2.9 A | 1.9 A | 1.9 B |
| VW Medium + Fructose            | 1  | 1  | 1  | 1  | 2.2 | 1.2 C | 1.2 B | 1.2 C |
| MS Medium + Sucrose             | 1  | 1  | 1  | 1  | 1.2 | 1.3 C | 1.3 B | 2.3 A |
| VW Medium + Sucrose             | 1  | 1  | 1  | 1  | 1.2 | 1.2 C | 1.2 B | 1.2 C |
| BNT 5%                          | TN | TN | TN | TN | TN | 0.37 | 0.37 | 0.37 |

**Notes**: The average values followed by the same letter in the same column showed that there were no real differences based on BNT 5% test.

#### 3.3 Contents of Secondary Metabolites

Analysis of contents of secondary metabolites of Ginger Oil was conducted at Laboratory of BPKI Surabaya, Jl. Ketintang Baru XVII No.14, Surabaya, Jawa Timur.

### Table 5. Contents of Ginger Oil

| Treatments                  | GINGER OIL (%) |
|-----------------------------|----------------|
|                            | I     | II    | III   | IV    | V     |
| VW Medium + Glucose         | 0.123 | 0.122 | 0.121 | 0.122 | 0.121 |
| VW Medium + Fructose        | 0.143 | 0.145 | 0.146 | 0.144 | 0.143 |
| VW Medium + Sucrose         | 0.102 | 0.106 | 0.104 | 0.105 | 0.103 |
| MS Medium + Glucose         | 0.110 | 0.111 | 0.113 | 0.111 | 0.114 |
| MS Medium + Fructose        | 0.119 | 0.118 | 0.121 | 0.117 | 0.115 |
| MS Medium + Sucrose         | 0.100 | 0.111 | 0.107 | 0.106 | 0.105 |

The highest amount of Ginger oil in VW and fructose medium treatment can be explained as follows: every commodity has a specific response to a treatment [10]. Explant of a plant commodity has to have a compability to a medium, in this case the medium VW has more compability with the calluses of “Jahe Gajah”, to be able to produce better Ginger Oil than medium MS. As the fructose, it can be explained that fructose has a degree of sweetnes higher than other carbohydrates, so in fructose there is more energy to produce Ginger Oil [13]. On the Table 5 above, the treatment of VW and fructose (C2M2) had produced 0.146% of Ginger Oil as the highest result. In the special analysis of “Jahe Gajah”, the composition of Ginger Oil of Gingerol - Shogaol - Zingerone are 0.184 - 0.118 - 0.982 %.

**Figure 3. Ginger Gajah Plants**
4. Conclusion

1. The highest quantity of calluses of “Jahe Gajah” was found on Medium MS and Glucose.
2. The quality of calluses of “Jahe Gajah” on treatments MS and Sucrose & MS and Fructose were tended to be friable and embryogenic, while on other treatments the quality were tended to be compact with organogenic characteristics.
3. The embryogenic calluses on treatments MS and Fructose and on treatments MS and Sucrose were to be developed into planlets, to be used in vegetative propagation in vitro.
4. The highest contents of Gingerol - Shogaol - Zingerone (Ginger Oil) was produced on treatment of medium VW with fructose.

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