



Discussion of Growth And Content of *Papain* In *Papaya* Callus (*Carica Papaya L.*) By Tissue Culture Technique With Elicitor *Saccharomyces Cerevisiae* Treatment In Ms And Vw

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ABSTRACT

Plants of papaya were have the main sources of chemical compounds that were generally used in pharmaceuticals and food-additive industries. One of the effort to increase the content of Papain in papaya is through tissue-culture and elicitation technique. The purposes of this research are *To investigate the content of Papain in calluses of papaya leaves with treatment of elicitor *Saccharomyces cerevisiae* in MS and VW mediums. This research was conducted in the month of November 2017 to January 2018, using Complete Random Design (CRD) with factorial using two factors, repeated four times and each repetition contained 4 samples. The main results of this research had shown that The treatment of VW medium and elicitor *Saccharomyces cerevisiae* 25 mg Dry Matter/L had produced the highest content of Papain.

Keywords : Elicitor *Saccharomyces cerevisiae*; Medium; Papain; Papaya leaves callus.

1. INTRODUCTION

Papaya (*Carica papaya L.*) is a type of tropical plant that can grow well in all region of the world with various characteristics depended on the condition and climate of the region. (Ludwig-Muller, J. 2000) Papaya plant was originated from Central America and was spread throughout the world by the Spanish traders. Countries that are best known as producers of Papaya are Republic of Dominica, Puerto Rico, Costa Rica, also Brazil, India, and Indonesia (Warisno, 2003).

Papaya fruit is a fruit with high nutritious content and high fiber, so it is good for health and digestion, with affordable price to be enjoyed by all people in society (Nisak, Pratiwi, & Ali, 2017). The content of anti-oxidant in Papaya fruit is carotenoids in Vitamin A and Vitamin C (Maisarah et al, 2013) . Papaya fruit helps to lower the risk of heart diseases and stroke caused by the blockage in blood vessels. Almost all parts of Papaya plant can be used, aside from the fruit itself. The enzyme of *Papain* is known as an anti-inflammation agent that can reduce inflammation for asthma, arthritis, and other diseases that caused by inflammation. (Dalimartha, S. 2003.)

The enzyme is a protein bio-molecule that functions as a catalyst to hasten anorgano-chemical reaction. Papaya produced the enzyme *Papain* that is a *proteolytic* enzyme secreted through the isolation of Papaya's sap. The sap from Papaya plant can be secreted through the fruits, stems, and leaves (Akujobi CN, Ofodame CN, Enweani CA. ,2010).



One way to increase the production of *Papain* contents in Papaya plant is through the use of tissue culture technique (Yusnita, 2004). Through this tissue culture process, the contents of *Papain* that is a secondary metabolite can be increased with the addition of elisitor *Saccharomyces cereviceae* (Flora, 2010). One of the determinant factor in the success of reproduction is the use of culture mediums. Some culture mediums used had went through various developments in the formula of the medium composition for better optimization of growth and development of cultured plants (Nugroho, 2006).

Polysaccharides, proteins, lipids, and nucleic acids are the main components of plants, which are the primary metabolites. In the plant cells, beside the obvious photosynthesis process, there are also other biochemical reactions to breakup the primary metabolites (Nurhidayati, 2003). Compounds that are the results of the breakup of these primary metabolites are known as the secondary metabolites. Some of these secondary metabolites have economical values and have been intensively used by the food and pharmaceutical industries as the sources of natural anti-oxidants (Vickery, 1981).

Secondary metabolites produced by the culture of plants cells can be increased in content by elicitation technique. The elicitation technique to increase the contents of these secondary metabolites through tissue culture is an induction process that aimed to increase the formation process of secondary metabolites in tissues or calluses by using an elisitor. According to Rahmawati (2006), elisitors are divided to two types Abiotic elisitors and Biotic elisitors. Abiotic elisitors were formed from the anorganic chemical compounds, i.e : ultraviolet, heavy metal, etc. While abiotic elisitors came from organic compounds such as : carbohydrates, proteins, volatile compounds, etc.

Mediums that were widely used are the Murashige and Skoog (MS) & Vacin and Went (VW). These mediums were oftenly used in the tissue culture process because these mediums were considered qualified because of the contents of macro and micro nutrients, as well as vitamins, for the plants' growth that needed nutrients of organic salts and substances to support their growth (Inglett, G.E. and Charalambous, G. 1979).

The plant's medium should contain all the nutrients needed to ensure the growth of explants. The nutrients were mixed to contain mineral salts, macro and micro nutrients, sugar, proteins, vitamins and hormones (Arijanti, 2008). These are the types of medium widely used in the tissue culture process, according to Widiyana (2013) :In this research, there were used explants of the meristems of Papaya leaves. The apical meristem cultures were explants cultured from the tip of meristem to tissues of 0,3 to 1 cm below.



Based on those explanations above, this research was hoping to give informations on the growth and development as well as the contents of calluses of *Carica papaya L.* with treatment of elisitor *Saccharomyces cerevisiae* in MS and VW mediums.(Wohing, P., et al. 2012) The purpose was to gain a better growth and development of calluses so that can be used as based for vegetative reproduction, as well as to investigate the best contents of *Papain* to be used as materials for pharmaceutical industries (Yuniarti, T. 2008).

2. MATERIALS AND METHOD

2.1. Place and Time

This research was conducted in the Laboratory of Tissue-Culture in the Department of Agriculture of Wijaya Kusuma University of Surabaya. This research was conducted in the month of November 2017 to January 2018. While the analysis of the contents of *Papain* were conducted in the Laboratory of Industrial Research and Consultation of Surabaya – East Java.

2.2. Materials :

Materials needed during this research were explants of Papaya's young leaves taken from the researcher's home garden, Basic mediums (MS and VW), growth control nutrients of 20 ml/L NAA and 30 ml/L BAA, Glucose 30 gr, *Saccharomyces cerevisiae* (as treatments), coconut water 150 ml/L. Alcohol 70% and 96%, tween betadine, aluminium foils, and plastic wraps.

2.3. Equipments :

Equipments used during this research were analytic scales, autoclave, oven, Laminar Air Flow (LAF), pH-meter, pinset, scalpel, Erlenmeyer, measuring cups, drop pippette, measuring pippette, petridish, spatula, cultures tubes, hotplate magnetic stirrer.

2.4. Method of Research :

This research were conducted directly at the laboratory. The method used was Complete Random Design (CRD) with six treatments repeated four times and each repetition contained four samples.

Factor 1: *Saccharomyces cerevisiae* :

S1 : 0 mg Dry Matter/L

S2 : 25 mg Dry Matter/L

S3 : 50 mg Dry Matter/L

Factor 2: Type of mediums :

M1 : MS medium

M2 : VW medium

2.5. Implementation of Research

Glasses and metal equipments could be sterilised in the oven on temperature of 160°C for 2-4 hours after wrapped in brown-paper or aluminium-foil. After sterilised, those equipments were



taken out and brought inside the LAF, then sterilised further using ultraviolet ray for 30 minutes. Before actually used, these equipments were unwrapped from the brown-paper and then soaked in the alcohol 96% then burned. Every time they would be used, they were soaked in alcohol and then burned again before used. These were implemented during the research.

Making of medium

Composition of mediums for tissue cultures varied depended on the commodities that would be used. Basically almost every commodity were using MS medium modified with vitamins and hormones as per needed by the commodities. For efficiency, there were made a stock of solutions, so every making of mediums would need only taken some volume from the stock.

Planting

Explants that were used in this research were of young leaves of Papaya, that would be forming the callus. Medium used in this research were MS and VW medium.

Parameters

Quantities of Callus

Observed visually in 1-week interval with scoring as follows :

- 1 = no callus
- 2 = few callus (<1 time size of the explant)
- 3 = medium callus (1-2 times size of the explant)
- 4 = many callus (>2 times size of the explant)

Qualities of Callus

Observed visually in 1-week interval with scoring as follows :

- 1 = no callus
- 2 = compact callus
- 3 = friable callus

Data Analysis

Datas gained from observations and research would be compared using random test analysis to determined whether there was a real differences, with multiple tests (BNT 5%), processed using application of SPSS 18.0.



3. RESULTS AND DISCUSSION

3.1. Quantities of Callus

Results of random analysis had shown interactions between treatments on the parameters of quantities of Papaya callus (*Carica papaya L.*) through visually observed within 1-week interval could be found in Table 1.

Table 1. Average Results of the Observations of Quantities of Callus Formed in Various Treatments from Time-Periods 1-10 MST (Weeks After Planting)

Treatments	Time Periods (weeks after planting)									
	1	2	3	4	5	6	7	8	9	10
<i>Saccharomyces 0 mg/L MS medium (S1M1)</i>	1.00	1.00	1.00	1.00	1.78	1.88	2.57	2.72	2.63	2.63
<i>Saccharomyces 0 mg/L VW medium (S1M2)</i>	1.00	1.00	1.00	1.00	1.66	1.75	1.91	2.35	2.41	2.41
<i>Saccharomyces 25 mg/L MS medium (S2M1)</i>	1.00	1.00	1.00	1.00	1.72	1.88	2.13	2.69	2.75	2.75
<i>Saccharomyces 25 mg/L MS medium (S2M2)</i>	1.00	1.00	1.00	1.00	1.63	1.75	1.85	2.28	2.03	2.03
<i>Saccharomyces 50 mg/L MS medium (S3M1)</i>	1.00	1.00	1.00	1.00	1.66	1.75	1.88	2.28	2.13	2.13
<i>Saccharomyces 50 mg/L MS medium (S3M2)</i>	1.00	1.00	1.00	1.00	1.61	1.85	1.91	2.32	2.19	2.19
LSD 5%	NS	NS	NS	NS	NS	0.13	0.23	0.28	0.44	0.44

Note: S = Significant, NS = Not Significant, (1) no callus, (2) few callus (<1 times of explant size), (3) medium callus (1-2 times explant size), (4) many callus (>2 times explant size).

Table 2. Single Factor of Quantities of Callus Within 1-Week Interval

Treatment	Periods (weeks after planting)				
	1	2	3	4	5
S1	1.00	1.00	1.00	1.00	1.72
S2	1.00	1.00	1.00	1.00	1.69
S3	1.00	1.00	1.00	1.00	1.63
LSD 5%	NS	NS	NS	NS	0.004
M1	1.00	1.00	1.00	1.00	1.72
M2	1.00	1.00	1.00	1.00	1.64
LSD 5%	NS	NS	NS	NS	0.004

From the random analysis shown in Table 1, in week 1 to week 5, there were no interactions yet. In week 6, there were interactions. These were because in week 1 to 5 MST, the Papaya leaves explants might still adapted to the treatments of mediums and *Saccharomyces cerevisiae*.

While in time periods of 5 to 10 MST, the results of random analysis had shown real differences, because the callus of the Papaya leaves had grown and started disassembled their food reserves as responses to the treatments of mediums and elicitor of *Saccharomyces cerevisiae*



in the mediums. The observations' results above had shown that the explants of *Carica papaya L.*'s leaves in MS medium with treatments of elisitor *Saccharomyces cerevisiae* 25 mg BK/L had produced more calluses than other treatments. The response of growth were varied because of the effect of mediums and treatments to the commodities of calluses were very specific (Arijanti, 2008).

On the Table 1, the treatment of 25 mg Dry Matter/L of *Saccharomyces cerevisiae* in MS medium had better effect on the growth and development of the explants than other treatments. This was postulated due that the effect of *Saccharomyces cerevisiae* were more optimal in quantity of 25 mg Dry Matter /L and the more complex composition of MS medium had caused better growth and development for the calluss. Generally the growth and development of explants' cells in-vitro would be better to reach the optimum when the nutrients in the medium's composition were more complex. In this instances, the MS mediums had the optimum composition for growth. Thus, the MS mediums were more suitable for the commodities of Papaya leaves' calluses (Widya, 2014).

On Table 2 were shown the results of single factor of quantities of calluses within 1-week interval. In week 5, it was appeared that on week 5 had shown real differences. As according of Arijanti (2008), the factor of medium's composition is very affecting to the growth of calluss.

3.2. Qualities of Calluss

Results of random analysis had shown interactions between treatments on the parameters of qualities of calluss through visually observations within 1-week interval could be found in Table 3.

Table 3. Average Results of the Observations of Qualities of Calluss Formed in Various Treatments from Time-Periods 1-10 MST (Weeks After Planting)

Treatments	Time Periods (weeks after planting)									
	1	2	3	4	5	6	7	8	9	10
<i>Saccharomyces</i> 0 mg/L MS medium (S1M1)	1.00	1.00	1.00	1.00	1.50	1.57	1.57	1.97	1.85	1.85
<i>Saccharomyces</i> 0 mg/L VW medium (S1M2)	1.00	1.00	1.00	1.00	1.57	1.69	1.69	1.69	1.69	1.69
<i>Saccharomyces</i> 25 mg/L MS medium (S2M1)	1.00	1.00	1.00	1.00	1.53	1.72	1.72	1.97	2.00	2.00
<i>Saccharomyces</i> 25 mg/L MS medium (S2M2)	1.00	1.00	1.00	1.00	1.32	1.41	1.41	1.78	1.82	1.82
<i>Saccharomyces</i> 50 mg/L MS medium (S3M1)	1.00	1.00	1.00	1.00	1.35	1.47	1.47	1.79	1.72	1.72
<i>Saccharomyces</i> 50 mg/L MS medium (S3M2)	1.00	1.00	1.00	1.00	1.19	1.26	1.26	1.91	2.00	2.00
LSD 5%	NS	NS	NS	NS	0.15	0.20	0.20	0.23	0.27	0.27

Note: S= Significant, NS = Not Significant, (1) no calluss, (2) compact calluss, (3) friable calluss.



Table 4. Single Factor of Qualities of Callus Within 1-Week Interval

Treatment	Periods (weeks after planting)			
	1	2	3	4
S1	1.00	1.00	1.00	1.00
S2	1.00	1.00	1.00	1.00
S3	1.00	1.00	1.00	1.00
LSD5%	TNS	NS	NS	NS
M1	1.00	1.00	1.00	1.00
M2	1.00	1.00	1.00	1.00
LSD 5%	NS	NS	NS	NS

On Table 3 were shown that on week 1 to 4 MST, the Papaya callusS were still adapting, thus there were no real differences and the callusS still in the form of Papaya leaves' explants, as can be seen in Figure 1.

While in the week 5 to 10 MST, by random analysis there were shown real differences as the callusS tended to have qualities of compact callus with organogenesis characteristics. The organogenesis callus were callus that their morphogenesis growth had needed mediums with different concentrates of auxin and cytokinen for growing shoots and roots. The forming of compact callus in this research were due to the specific characteristics of Papaya commodity itself. When planted, the organogenesis callus would form their own plants' organs (Ribkhawati, 2012).

There were shown analysis of single factor of qualities of calluses within 1-week interval, from week 1 to 4, in Table 4. The treatments of mediums and *Saccharomyces cerevisiae* had not shown any interactions as the Papaya leaves' callus were still adapting (Ribkhawati, 2012).

3.3. Analysis of Secondary Metabolites

Results of analysis by spectrophotometer using wavelength of 225 μ m to investigate the contents of *Papain* in the callus of Papaya leaves of time period of 10 weeks.

Table 5. Contents of *Papain* in callus of Papaya leaves on Various Treatments in the Week 10 MST

Treatment	Contents of <i>Papain</i> enzyme in %
S1M1 (<i>Saccharomyces c.</i> 0 mg Dry Matter /L, MS medium)	0.126
S1M2 (<i>Saccharomyces c.</i> 0 mg Dry Matter/L, VW medium)	0.129
S2M1 (<i>Saccharomyces c.</i> 25 mg Dry Matter /L, MS medium)	0.146
S2M2 (<i>Saccharomyces c.</i> 25 mg Dry Matter /L, VW medium)	0.148
S3M1 (<i>Saccharomyces c.</i> 50 mg Dry Matter /L, MS medium)	0.144
S3M2 (<i>Saccharomyces c.</i> 50 mg Dry Matter /L, VW medium)	0.139



In Table 5, the results of observations on the contents of *Papain* with all treatments of *Saccharomyces cerevisiae* had shown that, based on the laboratory analysis, the treatments of addition of *Saccharomyces cerevisiae* 25 mg Dry Matter/L tended to caused higher contents of *Papain*. This was due to the *Papain* response in Papaya plant to the medium and elisitor of *Saccharomyces cerevisiae*. According to Ribkhawati (2012), the addition of *Saccharomyces cerevisiae* concentrates must be in optimum quantity, because it would affect the production of the secondary metabolites. Calluss that had grown in lower quantities tended to produce better contents of secondary metabolites. In this research the treatments of VW medium tended to produce lower quantities. And every plant commodity responses were specific, thus the varied production of secondary metabolites. The optimum addition of elisitor *Saccharomyces cerevisiae* concentrates in best medium for Papaya plant was 25 mg Dry Matter /L and had produced the highest contents of secondary metabolites than any other treatments.

Figure 2 showed the comparison of production of secondary metabolites, the best was of S2M2 (*Saccharomyces cerevisiae* 25 mg Dry Matter /L in VW medium).

4. CONCLUSIONS

From the observations on the quantities, qualities, and analysis of contents of *Papain*, with different treatments on mediums and addition of elisitor *Saccharomyces cerevisiae*, on young leaves explants of Papaya (*Carica papaya L.*) could be concluded as follow. Treatment of MS medium and elisitor *Saccharomyces cerevisiae* 25 mg Dry Matter /L had produced the highest quantity of calluss than other treatments. All treatments of medium and elisitor *Saccharomyces cerevisiae* had produced compact calluss and Treatment of VW medium and elisitor *Saccharomyces cerevisiae* 25 mg Dry Matter /L had produced the highest contents of *Papain*.

Pictures of Calluss from the Research



Figure 1.

Kuantitas Kalus *Carica papaya L.* yang terbaik pada media MS dengan elisitor *Saccharomyces cerevisiae* 25 mg Dry Matter/L



Figure 2.

Kalus *Carica papaya L.* yang menghasilkan metabolite sekunder terbanyak pada media VW dengan elisitor *Saccharomyces cerevisiae* 25 mg Dry Matter/L

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