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In Vitro Study: The Potential for Papain Production from Papaya Leaf Callus

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Abstract

The advantage of papain are so assorted thatb it has been broadly need for improvement perposes in industry, medication and creature feed. Papain creation can be developmentt with Biotechnology tissue culture with improving the creation of callus plantled outgrowth leaves can expanded the cantaint of papain. The exploration goals were to decide the sorts of papaya assortments whose leaf callus could create the best papain. Realizing the papain content that can be created from three assortments of papaya leaves planted on MS and VW media. The exploration strategy utilized a totally randomized plan. Factor I was papaya assortments with 3 levels (California, Bangkok, and Gantung). The subsequent factor is the sort of culture media (MS and VW). There are 6 mixes. Every treatment mix is rehashed multiple times with 10 test tests. The outcomes appeared; (1) There was a cooperation between the treatment of the concentrate source from the leaves of 3 sorts of Papaya assortments and the utilization of MS and VW media on callus amount and callus quality boundaries. (2 The amount and nature of callus on MS media was better compared to in VW media. (3) Callus of leaves California assortment produce Papain best than Bangkok and Gantung Variety. (3) Research result show that: all callus produces papain. The callus papaya all assortment containing papain range 11,86 % - 19,50%. The best found in treatment papain M1V1 (MS Media - California (19,50 %); M2V1 (VW Media – California) 18,06 % papain content

Keywords: callus, MS media, papaya varieties, papain, VW media

A. Introduction

Papaya organic product is known to have a sweet taste. This natural product has a high nourishing substance and fiber so it is useful for the wellbeing of the body. The cell reinforcement content in papaya as carotenoids and nutrient an and nutrient C will help diminish the danger of coronary illness and stroke because of stopped-up veins. The substance of nutrient E in papaya natural product capacities to shield cells from harm brought about by malignant growth causing free revolutionaries and its fiber content is valuable in restricting disease-causing poisons in the colon and helps smooth the stomach related framework. Papaya additionally creates sap with Papain catalyst content which is helpful as an answer. protein atoms, and are regularly utilized by numerous individuals in different sorts of mechanical fields. Compounds are biomolecules as proteins that capacity as impetuses that speed up responses in natural substance measures. Papaya produces Papain Enzymes, which are proteolytic proteins created through the tapping seclusion of Papaya sap. The sap in papaya can be delivered through the organic product, stems, and leaves

One approach to build the creation of Papain contained in Papaya plants is by utilizing the tissue culture strategy. Indarwati, Sri Arijanti, Jajuk H, Ristani, Primawan Putra N. (2020), stated that tissue culture biotechnology can be utilized to create secundairy metabolites separated from the callus As a deciding component for the accomplishment of expanding papain creation, the elements that should be considered are the utilization of different assortments of papaya; different culture media, just as the expansion of different sorts and centralizations of carbs. A portion of the way of life media utilized has gone through different recipes created in the piece of the media expected to improve the development and advancement of refined plants.

The media that are regularly utilized are Murashige and Skoog (MS); Vacin and Went (VW). These three sorts of media are regularly utilized in the tissue culture measure on the grounds that these media are considered adequate to meet the full-scale and micronutrients and nutrients required for plant development. each plant requires supplements as natural salts or natural substances to help its development. Supplements in tissue culture are provided through media to incorporate macronutrient components (which are given in enormous amounts) as carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, calcium, sulfur, and magnesium. Then, microelements (which are given in limited quantities) and should be accessible incorporate chlorine, manganese, iron, copper, zinc, boron, and molybdenum. The expansion of sugars to the media can likewise expand optional metabolic items like papain (Arijanti and Dwie Retno, 2018).

This examination has a direness that so far papain has been gotten from the extraction of the organs of the papaya plant. This extraordinarily upsets the utilization of papaya plants for human requirements. Culture media likewise impact the development of the amount and nature of callus. Creation papain can be expanded by improving the amount and nature of callus that produce with biotechnology tissue culture. Consequently, in this investigation, endeavors were made to acquire papain content through the callus of papaya leaves, as it is realized that not all that a large part of the papaya leaves was utilized as explants.

The after effects of this examination are relied upon to give benefits, among others; (1). Can deliver papain from papaya leaf callus. (2). Get papaya assortments that contain the best papain. (3.) Obtain in vitro media that can deliver the best papain So that it tends to be utilized to create papain which can be utilized as crude material for the drug business and food added substances.

B. Literature Review

1. Overview of Soybean

Carica papaya L. is a plant beginning from Mexico on the South American mainland, and has now spread and is broadly filled in the jungle. *C. papaya* is the solitary species in the sort *Carica* (Wadekar, A. B., Nimbalwar, M. G., Panchale, W. A., Gudalwar, B. R., Manwar, J. V., & Bakal, R. L., 2021). Papaya is an organic product item that has numerous capacities and advantages, both as a new natural product since it contains a ton of good nourishment (The Golden of Fruit) at a generally reasonable value contrasted with different organic products (Hadiyanto, 2020).

Nations that are known to be very huge makers of Papaya are India and Indonesia. Papaya is a natural product ware that has a sweet taste, is exceptionally nutritious, and furthermore has high fiber which is useful for wellbeing. These nutritious organic products feed the body and safe framework (Wadekar, Ashish.B. *et al*., 2021)

Papaya in Indonesia is very well known in light of the fact that it tends to be utilized for different purposes; as a new natural product, the leaves are for vegetables and for restorative fixings. The presence of papain content which has multi-capacities makes papain very well known for modern purposes and to improve the nature of creature feed.

2. Tissue Culture Biotechnology for Papaya

Tissue culture is a method for isolating parts of plants such as protoplasm, cells, tissues, or organs and growing them in aseptic conditions so that these parts can regenerate into whole plants again. Through tissue culture, it is hoped that the seeds can produce the same plant seeds as their parents, as well as the seeds obtained, are free from pests and disease, and produce uniform seeds (Arijanti, Ribkahwati, and Retna, 2009). Given the importance of the role of the papaya plant which is quite a lot and one way of propagation techniques to increase the production of these plants by vegetative propagation methods through tissue culture becomes a business opportunity (Arijanti and Dwie Retno, 2018). Tissue culture technology has also been widely used to produce secondary metabolic compounds (anthocyanin content) in dragon fruit (Indarwati, *et al*., 2020).

In tissue culture techniques, several media are often used in implementation but Murashige and Skoog (MS) and Vacin and Went media are relatively good media because nutrients, both macro and micro, and vitamins for plant growth and development can be fulfilled. In the method of propagation through in vitro culture, the growth and development of the explants are strongly influenced by the type of basic media and growth regulators. MS medium is the basic medium that is generally used for the propagation of a large number of plant species. The basic media is rich in minerals that stimulate organogenesis.

In this study, papain was obtained through in vitro propagation, namely tissue culture techniques by planting explants of young leaves from 3 (three) varieties of papaya plants through secondary metabolite biosynthesis in this case "papain". Biosynthesis is a process of forming metabolic products from simple molecules to more complex molecules that occur in living things. in this biosynthetic process, includes the process of growth and development of living things. whereas metabolism in living things consists of 2 stages, namely primary and secondary metabolism. Secondary metabolism is a process that is not essential for the life of an organism. Secondary metabolism is produced in the respiration process and occurs in cells in the differentiation process stage into more specific cells in the stationary phase (Arijanti, *et al.,* 2009).

3. Enzim Papain

Normally papaya plants can create sap white when tapped or in hur. Sap white it very well may be delivered on the entirety of its parts like the organic product, blossoms, and leaves petiole (Yuniarti, T., 2008). Nonetheless, the part that contains the most papain is the organic product. Moussaoui A, Nijs M, Paul C, Wintjens R, Vincentelli J, Azarkan M, Looze Y. (2001) said the sap contains water (85%) and the rest is disintegrated materials comprising of salt, fat, starches, and a few other biomolecules. Papaya sap contains a few components, for example, the catalyst papain, lysosin, and other synthetic segments. Aravind, G (2013) added a few phytochemical parts contained in papaya sap,among others, a combination of terpenoids, natural acids,and alkaloids which can restrain the development of a few sorts of microorganisms like flavonoids, glycisides, saponins, and steroids wich have antibacterial properties. Fajriyah, S. N., Lestari, Y. E., Suaka, N. I., & Darmawan, E. (2021), further exspressed that the entire plant papaya contains, lycopene,isothiocyanide,flavonoids and papain compound.

Papain is a proteolytic protein that is created by separating papaya sap (Suryaningsih, D. R., Prakoeswa, S. A., & Eryanto, A. (2021). Papain chemical is known as a calming which can diminish aggravation, including joint inflammation and different illnesses. Osman, A., Merwad, A. R. M., Mohamed, A. H., & Sitohy, M. (2021) said, papain likewise can deliver peptides with natural exercises. The advantage of papain is so assorted that it has been generally needing for improvement purposes in industry, medication, and creature feed. Papain creation can be developed with tissue culture technology. Improving the creation of callus plant-led outgrowth leaves can improve the content of papain. Papain is helpful as a breakdown of protein particles and is frequently utilized by numerous individuals in different kinds of modern fields.

Amri and Mamboya, (2012) said compound papain is a proteolytic chemical fit for separating proteins to amino corrosive to be all the more effectively absorbable. Such as the statement of Khati, A., M. Danish, K. S. Mehta dan N. Pandey. 2015, that the papain enzyme is a protease catalyst that hydrolyzes protein, which is a vital factor for expanding protein edibility and absorption, which at last influences development. Chemical papain goes about as an impetus to improve the

natural summary feed of bad quality, with the goal that the feed cost can be minimized. Further (Amri & Mamboya, 2012), clarifying that this compound is fit for separating natural atoms made of amino acids, known as polypeptides and assumes a significant part in different organic cycles in physiological states, drug plan, mechanical uses like meat tenderizers and drugs. Papain's extraordinary construction gives usefulness that clarifies how proteolytic catalysts work and makes them significant for different purposes.

Chemical papain can lessen the negative factor of phytic corrosive determined of elementary substance vegetable feed. In the tobacco business compound papain used to assemble quality tobacco. Wulan M. (2017), further added that chemical papain is a compound of proteases that can catalysis just as proteolytic microbes actives on tobacco in a cycle of debasement nicotine. The cycle produces a compound that is less difficult yet didn't rever to nicotine content. The compound papain in response is required to help the movement of catabolism microscopic organisms proteolytic religation in the process with the goal that the nicotine isn't just the metabolism but additionally a decrease tobacco nicotine levels. A few investigations of papain treatment showed the treatment of papain compound focus level had had a genuine effect (alfa 5 %) on nicotine and the all-out sugar of tobacco leaves treatment; yet not actually affected the water content, levels protein content, splendor, ruddy, and yellowish. Best treatment dependent on substance examination; physical and organoleptic found in the treatment splashing with the compound papain level of grouping of 1,200 ppm. (Jeki, D., & Isna R., 2017). Papain likewise can be utilized to caffeine espresso beans (Arif, M. M., 2020).

The natural impacts of papain are different to the point that is broadly utilized for different motivations behind the food business as well as improving the nature of creature feed and fish feed. Alongside the advancement of biotechnology.

Normally papain can be created by tapping the sap; likewise can be delivered with biotechnology tissue culture. Papain can be conveyed from ekstraktion callus by planting organ papayas in tissue culture. Cultur media additionally impact the development of the amount and quality of callus. Creation papain can be extanded with improving the amount and nature of callus that produce with biotechnology tissue culture.

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C. Methodology

1. Place and Time

The study was conducted at the Tissue Culture Laboratory; Faculty of Agriculture, University of Wijaya Kusuma Surabaya., Starting in September 2020 and finishing off in Februari 2021.

2. Material and Method

The materials utilized during this exploration are: explants starting from the youthful leaf of 3 assortments of papaya (California, Bangkok dan Gantung) just as the fundamental media of Murashige and Skoog (MS). what's more, VW Media. NAA and BAP Growth Regulators, Coconut Water, Glucose, Fructose, Sucrose, 70%, and 90% Alcohol. Plantlet from leaves 3 Papaya assortment.

Equipment required during this research are: Sartorius Scales, Autoclave, Oven, LAF, pH meter, Tweezers, Scalpel, Erlenmeyer, Measuring cup, Measuring pipette Petri dish, Dropper Pipette, tweezers, spatula, Culture tube, Magnetic stirrer, and other.

3. Research Design

The study was conducted using a completely randomized design with two factors. The Factor I: sorts of culture media there are 2 levels; M1 = MS media; M2 =VW media (Table 1). Factor II. Kinds of papaya varieties there are 3 levels; V1 = California; V2 =Bangkok ; V3 : Gantung. Each treatment was repeated 4 times, with 10 replications each.

4. Instrumens

The gear utilized was enveloped by earthy colored paper and cleaned at 121 ° C for 30 minutes. Sterile culture tubes with Autoclave 17 psi 30 minutes. essential media utilized by MS media and the other treatment utilized VW Media.

Sterile youthful leave explants from 3 sorts assortment of Papaya at that point cut into ± 1 cm pieces and absorbed betadine, planted in culture tubes that as of now contain media as indicated by treatment. In the wake of planting it is set on a brooding rack which comprises hatching stages.

5. Variable

a. Callus quality. Seen at timespans weeks outwardly utilizing scoring: 1 = no callus; 2 = reduced callus; 3 = friable.callus

b. Callus Quantity: Observed at timespans weeks outwardly by scoring: Scor 1 = no callus; 2 = growing of explants; 3 = little callus (<1 times the explant size); 4 = medium callus (1-2 times the explant size); 5 = many callus (> multiple times the explant size)

c. The content of secondary metabolites in the callus (Enzim Papain): Observed ruinously through papain content examination at about two months subsequent to planting (56 days) Secondary Metabolite Analysis of the material extricated utilizing total liquor at that point broke down by gas chromatography

Table 1 . Comparison of composition between MS media and VW media (Arijanti and Dwi Retno S. 2018)

Media Murashige & Skoog (MS)			Media Vacin Went (VW)		
	Unsur Hara	Jumlah (mg/L) Media		Unsur Hara	Jumlah (mg/L) Media
1	*Unsur Hara Makro*				
	KNO_3	1.900	1	*Unsur Hara Makro :*	
	NH_4NO_3	1.650		NH_4NO_3	200 mg/L
	$CaCl_2.2H_20$	440		KNO_3	525 mg/L
	$MgSO4.7H_20$	370		KH_2PO_4	250 mg/L
	KH_2PO_4	170		$MgSO_4.7H_2O$	250 mg/L
2	*Unsur hara mikro*			$(NH_4)_2SO_4$	500 mg/L
	$FeSO_4.7H_2O$	27.8	2	*Unsur Hara Mikro :*	
	Na_2EDTA	37.3		Fe(EDTA)	37 mg/L
	$CoCl_2.6H_2O$	0.025		$FeSO_4.7H_2O$	28 mg/L
	$CuSO_4.5H_2O$	0.025		$MnSO_4.7H_2O$	7,5 mg/L
	H_3BO_3	6.2	3	*Sumber Energi :*	
	Kl	0.83		Sukrosa	20 g/L
	$MnSO_4.H_2O$	16.9	4	*Pemadat*	
	$Na_2MoO_4.2H_2O$	0.25		Agar - agar	8 g/L
	$ZnSO_4.7H_2O$	8.6			
3	*Senyawa organic*				
	Sukrosa	30			
	Myo-inositol	100			
	Asam Nikotinat	0.5			
	Pyridoxine	0.5			
	Thiamin-HCl	0.1			
4	*Pemadat*				
	Agar-agar	8			

6. Technique of Data Analysis

The data obtained were processed using Variance Analysis (Test F) using a completely randomized design patterns at the level of 5%. If there were any real differences between treatments. If there is an influence that is a significant difference between treatments then the test is continued with a comparison test between treatments using the Least Significant Difference Test (LSD) at the 5% level.

D. Result

1. Observation of Callus Quantuty

Analysis of callus quantity showed that there were no interactions between the single factor of kinds media and the Varieties of papaya on callus quantity parameter at 1,.3, 5, and 7 WAP. The interaction between the treatment of the use of kinds of Media Culture and kinds varieties of papaya (California, Bangkok, and Gantung) were seen in the callus quantity parameters at 8, 9, and 10 WAP observation. The results of observations of the growth of the quantities callus of papaya starting from the age of 1 to 7 weeks after planting can be seen in Table 2

Table 2. Average scoring Quality callus due to kinds of Media Cultur at Various Age Obsevation

Treatment	Weeks after planting (wap)			
	1	3	5	7
MS Media	1.0	1.0	1.0	1.1
VW Media	1.0	1.0	1.0	1.0
LSD 5%	ns	ns	ns	ns

Note: ns = Not Significant

Table 2 it can be seen that there until the age of 7 weeks the treatment of different kinds of culture media had the same affect on quantities of callus with score of 1,00 , both MS and VW

media callus had not been formed. While from the age of 7 WAP on MS media calus growth began to occur with a score of 1,10 and on the VW media had a score still 1,00. In this study pertumbuhan kalus dari plantlet daun pepaya pada media MS baru terlihat pada minggu ke 7 dengan nilai scor kuantitas calllus 1,1.

Table 3. Average skoring Quantity of calus due to Kinds of Varieties Papaya

Treatment	Weeks after planting (wap)			
	1	3	5	7
California	1.0	1.0	1.0	1.0
Bangkok	1.0	1.0	1.0	1.0
Gantung	1.0	1.0	1.0	1.0
LSD 5%	ns	ns	ns	ns

Note: ns = Not Significant

Table 3 it can be seen that there until the age of 7 weeks the treatment of different kinds varieties of papaya had the same affect on quantities of callus with score of 1,00 , both Varieties California, Bangkok and Gantung callus had not been formed. Until the age of 7 WAP the score quantities calis still of 1,00. The interaction between culture media typies and papaya varieties on callus quantity parameters occurred starting at the 8 -10 week observation has seen at figure1.

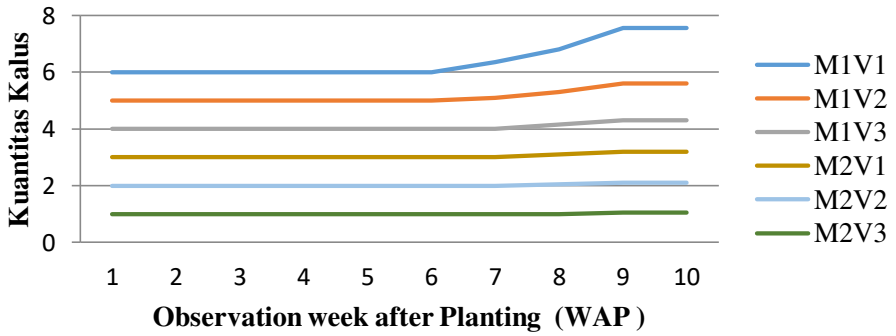

Figure 1. A curve tren quantity of callus growth observation 1 – 10 WAP

On Figure 1; It look that from time to time observation all treatment that is trying show trent better growth. Combination M1V1 Treatment exhibit picture growth quantity callus is better than the other treatment.

2. Observation of Callus Quality

Analysis of callus quality showed that there were no interactions between the single factor of kinds media and the Varieties of papaya on calus qualiy parameter at 1, 3, 5, and 7 WAP. The interaction between the treatment of the use kinds of Media Cultur and kind of papaya varieties (California, Bangkok and Gantung) were seen in the callus quality parameters at 9 and 10 WAP observation. The results of observations the growth of the callus qulity of papaya starting from the age of 1 to 8 Weeks after planting can be seen in Table 4.

Table 4. Average scoring quality callus due to kinds of Media Cultur at Various Age Obsevation

Treatment	Weeks after planting (wap)			
	1	3	5	7
MS Media	1.0	1.0	1.0	1.1
VW Media	1.0	1.0	1.0	1.0
LSD 5%	ns	ns	ns	ns

Note: ns = Not Significant

From table 4. It can be seen that until the age of 7 weeks the treatment of different kinds of culture media had the same affect on quality of callus with score of 1,00 , both MS and VW media callus had not been formed. While from the age of 8 WAP on MS media callus growth began to occur with a score of 1,0 and the VW media had a score of 1,0. The results of

observations the growth of the callus qulity of papaya starting from the age of 1 to 7 Weeks after planting can be seen in Table 5.

Table 5. Average skoring quantity of calus due to Kinds of Varieties Papaya at various age observation

Treatment	Weeks after planting (wap)			
	1	3	5	7
California	1.0	1.0	1.0	1.0
Bangkok	1.0	1.0	1.0	1.0
Gantung	1.0	1.0	1.0	1.0
LSD 5%	ns	ns	ns	ns

Note: ns = Not Significant

From table 5 It can be seen that until the age of 7 weeks the treatment of different kinds varieties of papaya had the same affect on quality of callus with score of 1,00 . Both of Varieties California, Bangkok and Gantung had not been formed callus until the age of 7 WAP the quanlity of callus still ascore of 1,00 . The interaction between culture media typies and papaya varieties on callus quality parameters occurred starting at the 9 -10 weeks observation has seen at figure 2. Figure 2. It can be seen that interaction M1V1 ; On calus quality parameter treatment between culture media MS and papaya California resulting the best score of quality callus (score 1,70) and significanly different than other treatments. It is also shows that the use of MS media treatment resulted better callus quality growth than VW media.

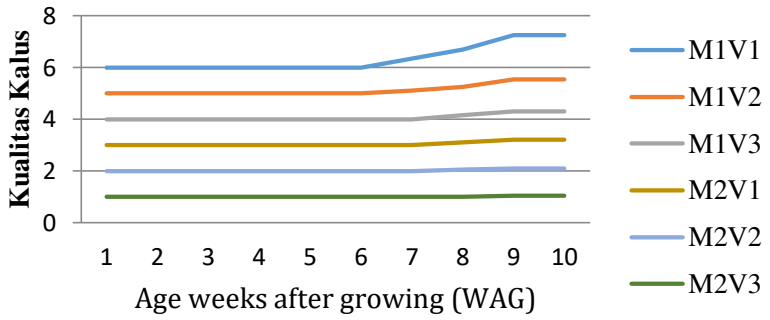

Figure 2. Trend Curve of Callus Quality Development at 1-10 WAG

On Figure 2; It look that from time to time observation all treatment that is trying show trent better growth . M1V1 Treatment show trent growth the quality of a callus most better than another treatment. From a analysis data it seen that scor Quality of calus (1,70) was the best callus seen in treatment M1V1; combination MS media treatment and varietas California papaya .

3. Observation of Callus Quality

The analysis of the papain content of three leaf callus varieties can be seen in table 6. Table 6. shows that the lab analisys, the callus formed in M1V1 Treatmant (MS – California) can produced the best papain content (19,5 %) higher than other treatment. The network culture technology with various varieties of papaya leaf plantlets that were tried was able to form callus with papain content ranging from 11,86 % - 19,5 %. While in M2V1 treatment (VW- California) callus which is formed from papaya leaves contains 18,06 % papain.

Table 6. Analisis of the *papain* content of three varieties of papaya leaf callus on MS and VW media

Treatmant	Papain %
M1V1 (MS Media + California)	19,50
M1V2 (MS Media + Bangkok)	17,20
M1V3 (MS Media + Gantung)	14,80
M2V1 (VW Media + California)	18,06
M2V2 (VW Media + Bangkoak)	14,05
M2V3 (VW Media + Gantung)	11,86

E. Discussion

Osman *et al.,* (2021), from the results of observations on the quantity of callus can be seen at the treatment. From the result of analysis of the observation score for the quantity and quality of callus table 1; 2; 4 and table 5; it can be seen that the leaf plantlets of some papaya varieties show slow growth. Until the 7th week after planting; the use of MS media plant-let still looks fresh and It is just starting to show callus growth with a score of 1,1. Meanwhile, the use of VW media in response to callus growth was greater; the quantity and quality of the callus still showed a score of 1,1 (Figure 1). Until week 7, the use of MS and VW media for the papaya leaf plant-tet culture activities showed a low response. It is assumed that up to7 weeks the planting material/plant-let that was tried was still in the stage of adapting to the media and the environment.

The interaction between the use of various culture media (MS and VW)and the varieties of papaya leaf plantlets (California, Bangkok, and Gantung only visible from 8 – 10 week after planting (WAP). This showed that the explant is starting to adapt and begins to respond to callus growth.) The success of engineering the tissue culture of the plant is highly dependent on the medium used. Further Indarwati *et al.* (2020), added that MS media has a real effect and is very good to use as a culture medium to produce metabolic sekundair (Anthocyanin) from the shoots of dragon fruit stems.

Once of factors that determine the succes of plant regeneration is the availability of carbohydrate sources. For plants bred through tissue culture, carbohidrates serve as a sources of carbon needed to produce energi. From thebresult of the analysis of calus quality observation on table 6. It can also be seen that were tried was to produce various callus qualities. This is inaccordance with Ariani R, Anggraito YU, Rahayu ES. (2016), that the ability of explant to form callus in influenced by the genotype of the explants, the age of the explants, the culture infironment and the responsiveness of each explant

The aftereffects of the experiment of papain content (table 7) show that the callus delivered from different papaya leaf assortments all contain papain. Auxiliary metabolites can be shaped through the biosynthetic pathway in plant cells in papaya, which results from an optional metabolite called papain. Further Arijanti, Ribkahwati, and Retna (2009) clarified that optional digestion is an interaction that isn't fundamental for the existence of an organic entity. Optional digestion is created in the breath interaction and happens in cells in the separation cycle stage into more explicit cells in the fixed stage .

The aftereffects of the investigation showed that the mix of M1V1 treatment delivered the most quieting and was altogether unique in relation to the others. California variety was likewise seen to contain higher papain both on MS medium and on VW medium than in Bangkok and Gantung assortments. This is on the grounds that California papaya leaves have generally many leaf bones so they have high papain content contrasted with Bangkok papaya assortments which have slender leaf bones and Hanging papaya has uncommon leaf bones (Hadiyanto, 2020). The papain content in varietas papaya plants is hereditary.

F. Conclusion

There was an interaction between the treatment of the extract source from the leaves of 3 kinds of Papaya varieties and the use of MS and VW media on callus quantity and callus quality parameters. The quantity and quality of callus on MS media were better than in VW media. Callus of leaves California variety produce Papain best than bangkok and Gantung Variety. Research result show that : all callus produce papain. The callus papaya all variety containing papain range 11,86 %-19,50%. The best faund in treatment papain M1V1 (MS Media- California (19,50 %); M2V1 (VW Media – California) 18,06% papain content.

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