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CALLUS REGENERATION POST GAMMA RAY IRRADIATION FOR PRODUCING SEEDS THAT WERE EXPECTED RESISTANT TO FUSARIUM WILT DISEASE TO SUPPORT ARGO-TOURISM

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ABSTRACT

Banana wilt disease is one of the impediment diseases in banana production. The disease is caused by *Fusarium oxysporum* fs. *cubesense*. It had destroyed most banana production areas in Sumatra and Kalimantan. Currently it has destroyed bananas plantation in Sleman up to 100%, as reported by Agriculture and Forestry Services District of Sleman. Tens hectare of bananas plantations of ambon kuning should be eradicated and then substituted by kepok which is more resistant against wilt disease. High availability of genetic material with high diversity is needed for supporting genetic selection in discovering high resistant varieties against wilt disease. The aim of this preliminary research was to obtain the mutagenic callus of banana that was resistant to selected media and was expected to be resistant to the wilt disease caused by Fusarium. Research has been conducted in three phases: 1) induction of callus, 2) gamma rays irradiation of callus, and 3) callus regeneration in culture media. The growth and development of callus were greatly influenced by various things. The most appropriate growth medium formula was MS + 2,4 D (1mg/L) + NAA (0.1mg/L). This formula was also economically cheaper. The cell of callus was more resistant to low dose (5 gray) of gamma ray irradiation. The plantlets were visually good and grew faster. Plantlets regeneration was highly dependent on the nutrients and additional elements composition of media formula. Low level of auxin with high level of kinetin in MS medium was able to increase the growth rate and the number of shoots, while MS medium with high level of auxin and kinetin was able to enhance the growth of roots.

KEYWORDS: Banana, Wilt disease, Gamma iradiation, Callus regeneration

INTRODUCTION

"Ambon kuning" is the best banana of Ambon cultivar that people preferred most. However it is susceptible to various diseases. One of them is wilt disease caused by *Fusarium oxysporum* fs. *cubesense*. The disease had destroyed the areas of banana plantations, such as in Sumatra, Kalimantan. Currently, Ministry of Agriculture and Forestry Sleman District reported that "Ambon" banana plantation in Sleman had been totally destroyed by the disease (KR, 2009). Tens hectare of "Ambon" banana plantation should be eradicated and replaced by "Kepok" banana that is more resistant to the disease.

Improvement of genetic material variation as selected materials to obtain resistance to *Fusarium* wilt disease is needed since the availability of superior seed of "Ambon" banana that is resistant to the disease is low (Sisworo et al, 2006; Harsanti and Mugiono, 2007). The improvement could be conducted by irradiation in order to obtain potential mutant to be developed (Javed et al., 2002). Sisworo *et al.* (2005) mentioned that in vitro mutagenesis was able to be applied on large amounts of plant material and was more rapidly than ex vitro mutagenesis. Sungkono *et al.* (2008) reported that there were two types of mutagens that could be used for breeding mutations, namely radiation and chemical compounds. Radiation included irradiation with X rays, gamma rays and ultraviolet. The chemical compounds that could be used as mutagens were EMS (Ethyl Methyl Sulphonate), NMH (N-methyl-N-nitroso urea), NTG (Nitroso guanidine) and colchicine.

Gamma ray radiation causes ionization that can penetrate materials, including living tissues, penetrate the cells and ionize molecule substances in the cells inducing abnormal functioning or even damaging the substances. According to Broetjes and Van Harten *cit* Medina (2004), cells exposed-radiation would produce normal cells, cells undergoing mutation, chromosomal damage or cell death. Research conducted by Molla *et al.* (2005) produced a specific dose of gamma rays that were successfully extended the banana roots. Similar result had been gained by Mugiono (2008) and Javed *et al* (2002) on obtaining mutants of rice with a very good growth.

The aim of this preliminary research was to obtain the mutagenic callus of banana that was resistant to selected media and was expected to be resistant to the wilt disease caused by Fusarium.

METHODS

The study comprises several phases: 1) Induction of callus, 2) Irradiation induction of callus by gamma rays, and 3) regeneration of shoots. Research activities were listed in the following flowchart:

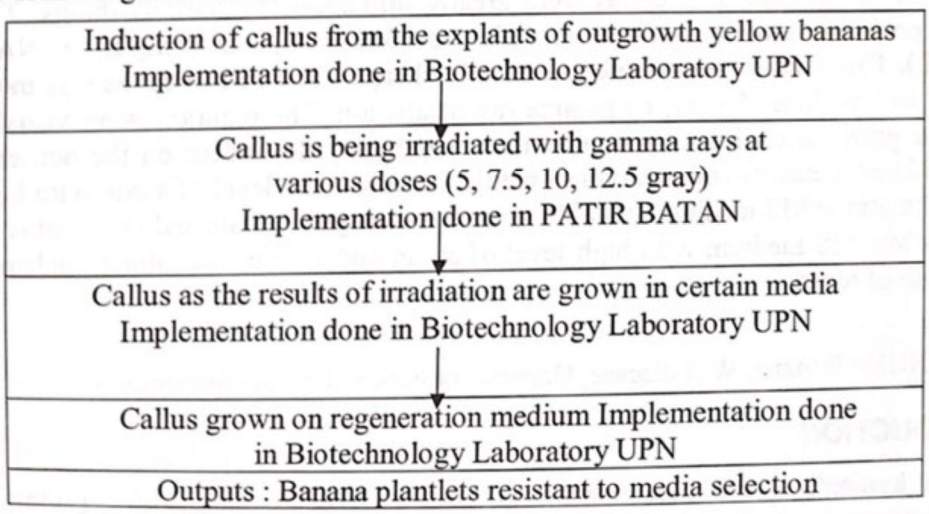

Figure 1. Flow chart of research activities

Final results were expected to obtain callus that were considered resistant to the selection media (remain green), then moved into the shoot regeneration medium.

Research was conducted in laboratory experimental methods and arranged in single factor of Completely Randomized Design (CRD).

Media formulations tested were:

T1 = MS + kinetin 5mg /L + IAA0.1mg / L
T2 = MS + kinetin 5mg /L +IAA 0.2mg /L
T3 = MS + kinetin 5mg /L +IAA 0.3mg / L
T4 = MS + kinetin 5mg /L +IAA 0.4mg / L
T5 = MS + kinetin 5 mg /L + IAA 0.5 mg / L

RESULTS AND DISCUSSION

The explants which had a callus of 0.5-1 cm were selected to be irradiated. Explants bottles directly inserted into the ice gel container to maintain temperature and subsequently brought to PATIR BATAN Jakarta to be irradiated in Gamma Chamber of 4000A machine with the appropriate doses of treatment. The results of the analysis on the percentage of living explants and time needed for forming plantlets after irradiated using gamma rays was described in table 1.

Table 1. The mean percentage of banana living-plantlets on various levels of gamma-ray irradiation

Treatment	Percentage of living (%)	time of growing plantlets (day)
5 gray	100 a	5 a
7,5 gray	100 a	10 b
10 gray	90 a	12 b
12,5 gray	75 a	15 b

Note: numbers followed by the same letter are not significantly different at 5% level of DMRT test

From the results of callus induction, the callus size from 0.5 to 1 cm was chosen to be irradiated using gamma rays. The plantlets with a dose of 5 gray had grown faster than the other doses. It was only need 5 days for emerging the plantlet (Table 1), and was chosen to be developed because it was visually better (bright green, plantlets already looks set to grow) than others. Up to the age of 30 days all treated plantlets had shown the high percentage of living, but after 90 days almost all plantlets with the high dose did not survive. It was suggested that the cells of the callus yellow "Ambon" bananas were too young to be irradiated. Two weeks old callus were not resistant to high doses of gamma rays irradiation. It was only able to survive at doses up to 5 gray. Similar phenomena occurred when plantlets grown. It was needed more time to emerge with the increase of gamma ray doses.

The most important step of this research was to develop plantlets on regeneration medium. The growth of "Ambon kuning" banana callus in vitro was significantly affected by growth media. The effect was occurred on parameters of shoots height,

number of tillers, root length and number of roots (Table 2). The results of further tests using the 5% level DMRT showed that the growth of plantlets height at the age of 8 weeks reached 2.97 cm when it was grown in medium MS + kinetin 5 mg / L + IAA 0.1 mg / L. It was the best growing medium. It was significantly different to medium MS + kinetin 5 mg / L + IAA 0.5 mg / L but not to others. This was presumably caused by the composition of mineral in the media was optimal for the growth "Ambon kuning" plantlets. Nitrogen element contained in the MS medium reached 840.6763 mg / L. That element needed by plant in large quantities to stimulate the growth of plant height. The presence of organic carbon was expected to increase the activity of cell fission below shoot meristem, followed by cell enlargement and cell elongation. This steps addition had increased the plant height. The existence of kinetin growth regulators at 5 mg / L had very high role in triggering the growth of shoots.

Table 2. The mean height of shoots, number of tillers, number of roots and root length of yellow bananas on various regeneration media (cm)

Treatment	Height of shoots (cm)		Number of tillers		Total number of root		length of roots (cm)	
MS + kinetin 5 mg / L + IAA 0.1g / L	2,97	a	4,33	a	2,00	b	1,47	b
MS + kinetin 5 mg / L + IAA 0.2 mg /L	2,33	ab	2,33	b	3,33	ab	1,00	b
MS + kinetin 5 mg / L + IAA 0.3mg / L	2,40	ab	3,33	ab	2,67	b	2,97	ab
MS + kinetin 5 mg / L + IAA 0.4 g / L	2,40	ab	1,33	c	4,67	a	0,75	b
MS + kinetin 5 mg / L + IAA 0.5 g / L	1,80	b	2,33	b	4,79	a	3,83	a

Note: numbers followed by the same letter are not significantly different at 5% level of DMRT test

On the observation and analysis of the number of tillers, the best results occurred on plantlets grown on medium MS + kinetin 5 mg / L + IAA 0.1 mg / L) but did not significantly different to growing medium MS + kinetin 5 mg / L + IAA 0.3 mg / L. Elements for enhancing the growth of the shoots were definitely fulfilled in the MS growth media. Adequate nitrogen supplied would enhance the formation of proteins and other organic compounds, so it would stimulate cell fission, cell elongation and encourage the process of differentiation, stimulate the growth of plants ultimately (Suryowinoto, 2000). Besides the adequate supply of nitrogen element, it was also caused by the optimal supplied of green leaves forming elements such as iron (Fe) and magnesium (Mg). Rai (2002) reported that plant which lack of magnesium nutrients, its chlorophyll was not formed because it was an essential element of chlorophyll molecules, as well as an iron. The presence of chlorophyll will support the process of photosynthesis, which in turn produces carbohydrate which being translocated immediately to the parts of body for elongating of shoots, and increasing the number of shoots.

In observations on the roots of plants, the plantlets grown in growth media MS + kinetin 5 mg / L + IAA 0.4 mg / L and MS + kinetin 5 mg / L + IAA 0.5 mg / L showed the number of roots were relatively similar, but better than other media. It could not be separated from media composition on both medias, which contained high IAA (0.4 and 0.5 mg / l) would stimulate apical meristematic cell fission actively, so

that the formation of epidermal tissue, cortex and the other tissue were run rapidly. According to Salisbury and Ross (1995), the IAA (auxin) caused the cell's walls became loose, then the epidermis cells elongated rapidly, and sub epidermis cells that attached to it elongated too, so the roots would grow long. The increase of the length and the number of the orchid seeds roots would support the absorption of nutrients, thus affecting the growth of the plant, the crown part will develop optimally.

CONCLUSIONS

The growth and development of "Ambon kuning" banana callus in vitro were influenced by various factors. At the step of the gamma-ray irradiation, the cells would be more resistant to low dose (5 gray), looked good visually and plantlets growing faster. At the step of regeneration of plantlets, it was very dependent on the composition of the nutrients contained in the growing media and the additional elements. MS medium which was given low auxin, and high kinetin levels would be able to increase the height and number of shoots, while the MS which was given a high auxin with high kinetin would enhance the growth of root.

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