Induction of Bacterial wilt-free Microrhizome of Ginger in a Modified MS Basal Medium and Macronutrient Concentration

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Abstract

Healthy seedlings is a prerequisite to produce healthy plants. An experiment was conducted to determine an optimum concentration of macronutrient in MS basal medium, and a modified medium for stimulating microrhizome formation of big-white ginger B*acterial wilt-free* ginger plantlets derived from 8 weeks cultured meristem in MS basal medium were cut into ± 5 mm length and used as the experimental material. Two-factor treatments were arranged in complete randomized design with three replications. The first factor was macronutrient concentration *e.g*. 25%, 50%, 75% of MS macronutrient, and full of MS macronutrient. The second factor was physic of medium *e.g*. soild medium, *double layer*, and *double layer* + CaP 1 ppm. Orthogonal polinomial tests showed that macronutrient concentration of 83.5% was the optimum concentration to produce microrhizome formation of ginger. In that microrhizome formation was the fastest (12.5 dap). On the otherhands, *double layer* medium with 25% of MS macronutrient promoted the highest number of shoot (5.5 shoots/explant) and root (33.4 roots/explant), and micro-rhizome dry weight (0,023 g). There was only 2% of sample showing *R. solanacearum* infection under microscopic observation. These implied that disease-free rhizome of ginger can be produced by *in vitro* propagation.

Key Words : *Zingiber officinale* Rosc., *bacterial-wilt-free, in vitro, macronutrient, microrhizome, physic of medium*

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I. Introduction

Ginger (*Zingiber officinalle* Rosc.) is a multi purpose crop belong to *Zingiberaceae.* Various compounds were contained in the rhizome fresh of ginger, *i.e* proteins (2.3%), carbohydrates (12%), fats (1%), minerals (1.2%), fibre (2.5%) and water (81%) (Vernin and Parkanyi, 2005) and small amount of vitamin A and B.

Improvement of ginger by conventional breeding technique is not possible due to incapability to form seed. Ginger is usually propagated by using a piece of rhizome with shoot, with low multiplication rate (Kavyashree, 2009). Besides, this technique has an advantages in term of the high risk of in spreading the systemic infection diseases (Sultana, *et al.,* 2009). A Bacterial wilts diseases caused by *Ralstonia solanacearum (*Dohroo, 2005), produced poor quality of rhizome and decrease the crop productivity. This causal agent is present systemically in rhizome seeds as both and latent infection that could be contaminated the seed-pieces for field planting (Hepperly, *et al*., 2004)

A tissue culture techniques have an increasingly important role to play in the deseases free plantlet propagation of certain economic important plants, such as ginger. However, pursuing the regeneration of plant from culture is not an easy one, the trick in achieving regenerations seem to be how to trigger the gene segment that initiated and control the differentiation and development. The problem of inducing plant differentiation and development is so fundamental that, despite a certain concentration and modification of culture medium. A culture medium is the important factor due to contains an important nutrient that it may influence plant growth and development (Marlin, 2001). Some modification in culture medium improved plant growth *in vitro,* in solidified or in liquid medium, or in a *double layer* medium. Some essential elements should be added in culture medium to support plant growth and differentiation, such as macronutrient. An optimal concentration of macronutrient may determine the type of organ formation occurring *in vitro.* The experiment was aimed to determine an optimum concentration of macronutrient in MS basal medium, and a modified medium for stimulating microrhizome formation of big-white ginger.

II. Material and Method

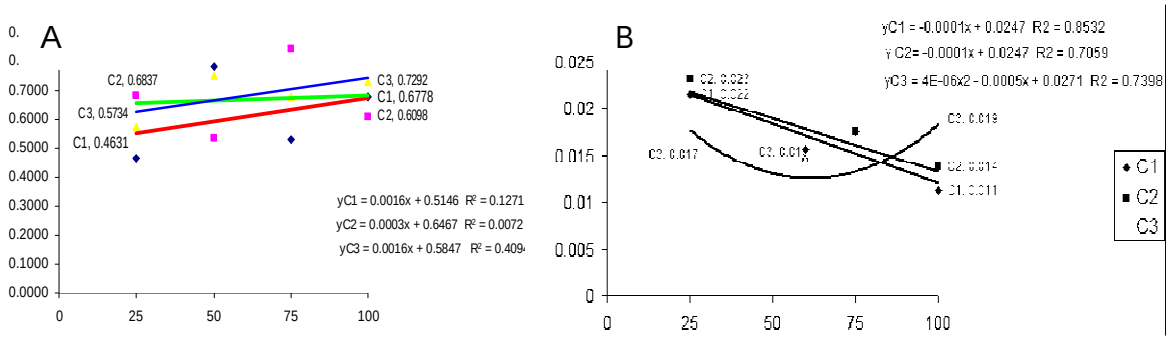
The experiment was conducted in Laboratory of Plant Biotechnology, Faculty of Agriculture, Bengkulu University. The experiment was arranged in completely randomized design with two factors. The first factor was concentration of macronutrient, consist of 25% of MS macronutrient (M_1), 50% of MS macronutrient (M_2), 75% of MS macronutrient (M_3) and 100 % of MS macronutrient (M_4). The second factor was physic medium, consist of MS solidified medium with 8 $g.L^{-1}$ agar powder (C_1), a *double layer* medium (C_2), and a

double layer medium + *Calsium Panthothenat* 1 ppm (C_3). Plantlets derived from meristem culture of big-white ginger in 8 weeks of cultured were used as plant material. Basal part of plantlets were cut into ± 5 mm and cultured in 20 ml culture medium, in each treatments. The explants were then placed and maintained in a culture room with 18 - 20 ^oC and 16 hours of light illumination of about 1000 Lux. D*ouble layer* media were made by adding a 10 ml of MS liquid medium with or without *calsium panthothenat* after 4 weeks of culture.

Microrhizome formation were observed in diameter, fresh weight, and dry weight of microrhizome during 10 weeks of culture. Meanwhile, plants growth were observed at the days of shoot and root formation, root length, number of shoot and root per explants. The data were collected and analyzed by analysis of varians (p 0.05). Analysis were continued with *orthogonal polynomial* to determine the optimum concentration of macronutrients, and then continued to Duncant's Multiple Range Test (p 0.05) to compared physic medium application.

III. Results and Discussion

There were interaction effects of macronutrient concentration and physic medium to root formation and dry weight of microrhizome of ginger within 10 weeks of culture. Increasing macronutrient concentration affected to the day of root formation, in all levels of physic medium culture. This interaction was also showed in a linier negative response to dry weight of microrhizome (Figure 1).



Concentration of macronutrient (%)

Figure. 1. Interaction between a macronutrient concentration and physic medium to days of root formation (A), and the dry weight of microrhizome (B) of ginger, 10 weeks after culture.

The fastest day to root formation was occurred in solid medium with 25 % of macronutrient concentration (4.6 dap). The highest dry weight of microrhizome was occurred in medium culture with 25 % of macronutrient concentration in ~~a~~ form of double layer without CaP. The higher concentration of macronutrient the more time needed to form root in these culture medium. The medium was also caused the decrease of dry weight of ginger microrhizome. It was proved that in a medium with higher concentration of macronutrient, the transport of element in medium culture was obstructed. In this condition, nutrient in medium could not be transferred optimally to the explants.

In this research, it was shown that macronutrient concentration had a significant effects to days of microrhizome formation, fresh weight of microrhizome, and number of root of ginger, 10 weeks ~~of~~ after culture (Figure 2).

Orthogonal polinomial tests ($Y = 0.0026X^2 – 0.4342X + 30.722$), showed optimum concentration of 83.5% macronutrient caused the fastest microrhizome formation (12.5 dap). Macronutrient as essentials nutrient were needed in plant growth *in vitro*, especially in microrhizome formanation. However, 25% of MS macronutrient concentration was able to promot~~e~~ fresh weight of ginger microrhizome (21,5 g) with highest root number (33.4 roots/explant). The results proved that low concentration of macronutrient enhanced nutrition uptake by explant, *in vitro*. In low concentration, water diffused easily into plant cell and increased plant fresh weight.

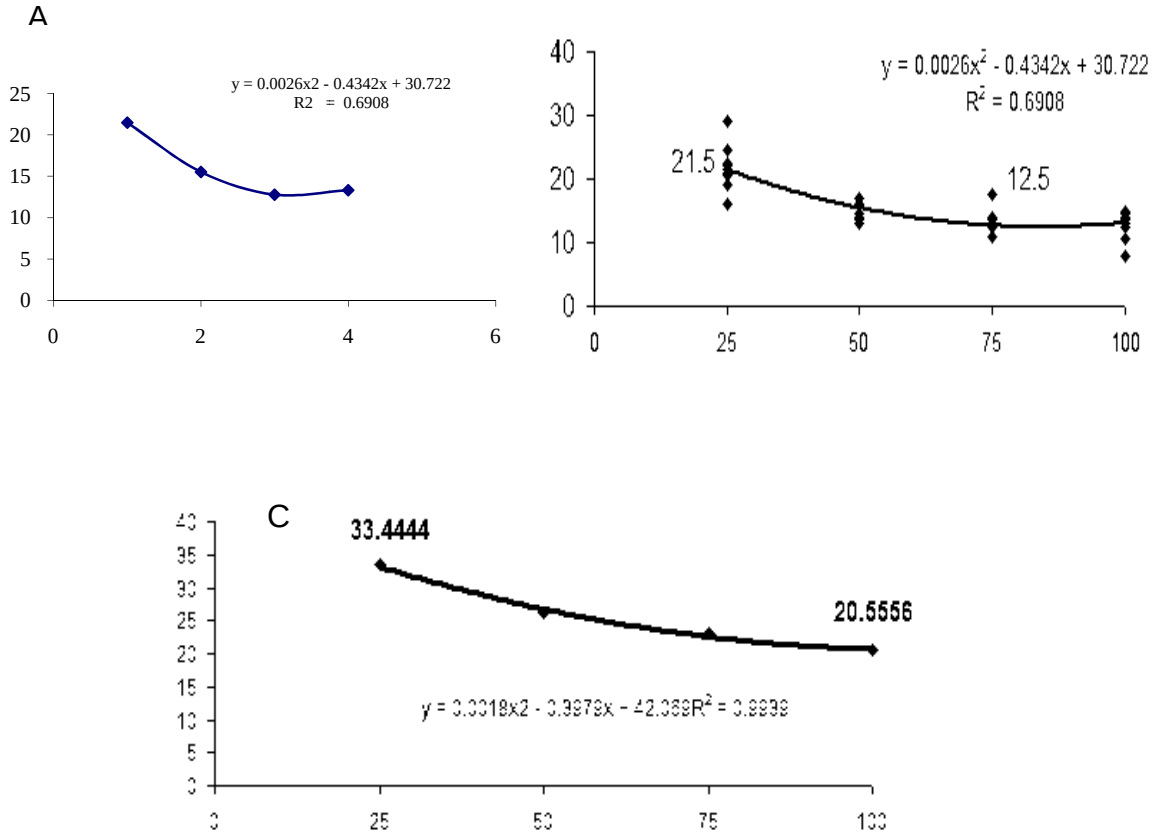


Figure 2. Effects of macronutrients concentration to fresh weight of microrhizome (A), days of microrhizome formation (B), and number of root of ginger (C), 10 weeks after culture

Results showed that physic of medium has a significant effect to length of microrhizome, fresh weight of microrhizome, number of shootlet, and number of rootlet (Table 1).

Table 1. Effect of physic of medium to length of microrhizome (LM), fresh weight of microrhizome (FWM), number of shootlet (NS), and number of rootlet (NR), 10 weeks of culture.

Treatments	LM	FWM	NS	NR
Solid medium	0.57 a	0.14 a	4.00 a	23.79 a
Double layer medium	0.56 a	0.18 ab	5.46 b	33.44 b
Double layer + CAP	0.64 a	0.22 b	4.54 ab	20.96 a

Note : Number followed by same letter has no significant effect according to 5% DMRT

The results showed that physic of medium had no significant effect to length of microrhizome. Testing to examine microrhizome free of *Ralstonia solanacearum* was

done by diluting liquid cell in PGA (*pepton glucose agar*) medium. A microscopic investigation showed that *in vitro* tehnique decresed infection of *R. solanacearum.* There are only 6 sample of 225 sample (2%) were infected. It is proved that *in vitro* tehnique has significant effect to diminish *R. Solanacearum* infection untill 98%. A microrhizome derived from a meristematic tissue is a beneficial material as a problem solving in ginger seedlings equipping.

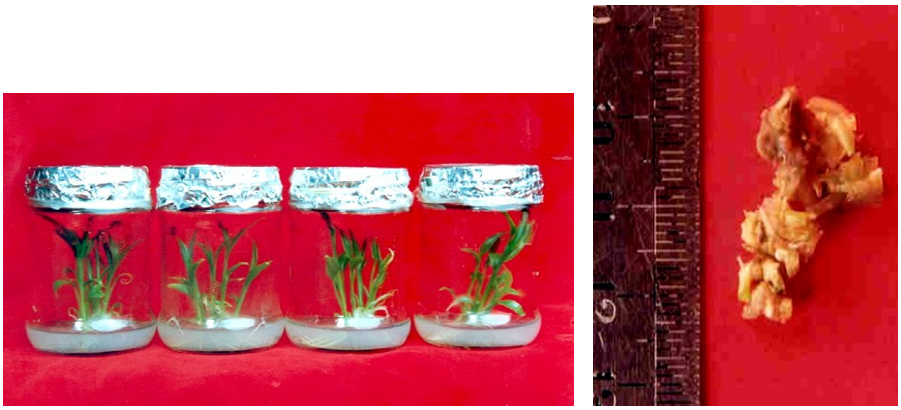


Figure 3. Plantlets growth in double layer + CaP and supplemented by macronutrient of 25, 50, 75, and 100% (A), microrhizome formation in 12 weeks of culture (B)

IV. **Conclusion**

1. Macronutrient concentration of 83.5% was an optimum concentration to produce microrhizome formation of ginger. In this medium, microrhizome formation was growth fastest (12.5 dap). While, in a *double layer* medium with 25% of MS macronutrient promoted highest number of shoot (5.5 shoots/explant) and root (33.4 roots/explants), and micro-rhizome dry weight (0,023 g).
2. A microscopic test ~~of~~ on microrhizome cell showed that there was only 2% of sample ~~is~~ infected by~~.~~ *R. solanacearum*. These experiment~~,~~ was proved that microrhizome regenerated from an *in vitro* technique could be an alternative path to produce a disease-free rhizome of ginger.

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