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Kinds of Co-Cultivation In Soybean Plantlets Support Regeneration And Acclimatization (New Kind Tissue Culture Release)

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Abstract - *Plant breeding activities with the insertion of genes can also be done, this technique is easier done on tissue culture technique was compared with similar techniques in normal plants. This technique is made possible on the degree of success of the insertion of the gene are more successful than if insertion is done in mature plants, as well as the insertion of resistance genes against fungal pathogens on soybean plants. Genes glucanase and chitinase from Trichoderma harzianum, a fungal parasite that produces enzymes chitinolytic and glucanolytic. Therefore, exploration and trial are needed to be able to infect Agrobacterium easily into soybean plants. So that a healthy and strong tissue culture plantlet is obtained to be continued into the next stage. Successful insertion explain will be able to regenerate their cells to grow into planlet. Furthermore, planet with media nutrient intake will develop into plants in tissue culture bottles, where good plants will produce fresh primordial and radicular growth. After about twenty-eight days, the micro plant is ready to be moved in the grower medium. The Methods of this research are Callus as experimental material taken from three sources and grouped according to these sources: (1) The callus on the results of previous regeneration; (2) callus from hypocotyl; (3) Callus of Cotyledon. Each of the callus sources used as a component of the first factor (Kl) Regeneration of Calli Calli material; (Kh) Material callus from hypocotyl and (Kt) of Cotyledon Calli material. The co-cultivation method used is a modification in 3 ways after co-cultivation modification. The third way is used as the basis for modification of the design of the second-factor components: (Ks) Filter Paper Method After cultivation; (Md) Solid Media Method After cultivation; (Pc) Washing Method After Cultivation. From the research, observations and discussions can be concluded as follows: (1) There was an interaction between the modified method with source material Soybean Callus; (2) Modification of a very good method of this study was Laundering and Paper Filter; (3) Callus excellent source of this research is of regeneration callus and Cotyledon*

Keywords; *Plasmid, Regenerate, Tissue Culture, Callus, Acclimatization*

I. INTRODUCTION

One obstacle to soybean cultivation in Indonesia is the attack of various diseases caused by pathogenic fungi, among others; Sclerotinia rolfsii, Phakospora pachirizi, and Rhizoctonia solani.

Attacks pathogen - the pathogen make soybean farmers pay extra to overcome both chemically as well as organic, which in turn makes the cost of production increases. Increased production costs have increased the selling price of soybeans, which compete with purchasing power.

Development of Soybean Plants that are resistant to pathogens is necessary given the problems of food insecurity, especially soybean commodity has been to the extent of the crisis a national source of vegetable protein. Because if we only rely on imported soybeans, there will be a backlash if the International market soybean prices jumped, or dependency on foreign soybean seed will cause soybean crop will be destroyed and become rare plants in Indonesia.

Plant breeding activities with the insertion of genes can also be done, this technique is easier done on tissue culture technique was compared with similar techniques in normal plants. This technique is made possible on the degree of success of the insertion of the gene are more successful than if insertion is done in mature plants, as well as the insertion of resistance genes against fungal pathogens on soybean plants.

Several endophytic fungi that have been isolated from the stem known to be antagonistic to fungal pathogens, and reportedly produce chitinase (Chn) and β-1,3-glucanase (βGlu). Therefore chitin and β-1,3-glucan is the main constituent of fungal cell walls of pathogens (except mushrooms Oomycete like Phytophthora, containing β-glucan and cellulose) then Chn and β Glu is the main enzyme for lysis of the cell wall of fungal pathogens [1] [2]

Genes glucanase and chitinase from Trichoderma harzianum, a fungal parasite that produces enzymes chitinolytic and glucanolytic [3][4], has been used for engineering various types of plants, such as potatoes, tobacco, apples, petunia, Wine, broccoli, and others [5][6][7][8].

Many researchers tried to insert Agrobacterium into

citrus [9] but transform soybean crop needs to be done for each variety and plant species have characteristic traits including Agrobacterium to infection are different from each other [10][11].

Therefore, exploration and trial are needed to be able to infect Agrobacterium easily into soybean plants. So that a healthy and strong tissue culture plantlet is obtained to be continued into the next stage. Successful insertion explan will be able to regenerate their cells to grow into planlet. Furthermore, planlet with media nutrient intake will develop into plants in tissue culture bottles, where good plants will produce fresh primordial and radicular growth. After about twenty-eight days, the micro plant is ready to be moved in the grower medium [12][13][14].

The transfer of plants from the cuticle tissue cannot be done directly but must go through the stages of acclimatization. Where the selection of acclimatization media is also very important to note besides environmental control which is gradually adjusted from the controlled environment in the bottle to the natural environment. But before we can produce plants that are ready to be acclimatized, to later grow into perfect plants, the stages of Agrobacterium insertion become very important. Planlets that are successful in a living will be able to survive into Micropropagules which then grow into normal plants in tissue culture bottles to be ready to be grown as acclimatization material [15][16]. So that the selection of materials for induction of Agrobacterium is very important, besides how to do agrobacterium infection into explants is also the most important concern. Because after all the good and healthy explant material that will be used in the activity of agrobacterium insertion, of course, it will not work well if using an improper method.

Selection of materials or materials explan to determine the success of transgenic transformation itself, where each will own a plant or species of the different response at each growth in the cellular phase.

- Formulation of the problem From the above background, some problems can be formulated as follows:
  - Tissue Culture which is the most efficient material used as the basis of gene insertion to improve resistance to pathogens on soybean plants
  - How that Modification right to co-cultivation
- Research purposes This study has the objective to answer all the above issues, where the efficiency of the selection method of modifications and material culture main target made towards achieving the long-term form of the pathogen-resistant plants.
- Significance Research In terms of the expected scientific results of this study can add knowledge more insight about genetic and pathogen resistance in general and the insertion of genes in tissue culture techniques in particular. In practical terms expected, by doing some optimization methods, it can also be used as a reference for subsequent genetic engineering.
- Scope of the Problem In this study was used as the main object is a soybean plant tissue culture material in the form of callus. To avoid bias focus of research and discussion, the limitations in this research problem as follows: Soybean plants • Induction material used is of regeneration Calli Calli, Calli From Cotyledon and callus from hypocotyl

II. RESEARCH METHODS

(Size 10 & Normal)Do not change the font sizes or line spacing to squeeze more text into a limited number of pages. Use italics for emphasis; do not underline.

Callus as experimental material taken from three sources and grouped according to these sources: (1) The callus on the results of previous regeneration; (2) callus from hypocotyl; (3) Callus of Cotyledon. Each of the callus sources used as a component of the first factor (Kl) Regeneration of Calli Calli material; (Kh) Material callus from hypocotyl and (Kt) of Cotyledon Calli material.

The co-cultivation method used is a modification in 3 ways after co-cultivation modification. The third way is used as the basis for modification of the design of the second-factor components: (Ks) Filter Paper Method After cultivation; (Md) Solid Media Method After cultivation; (Pc) Washing Method After Cultivation

The first stage

1. Soybean seed var. Wilis selected and in sprouts in media that are sterile. Cotyledons that have been included in the media to grow a callus induction, as well as hypocotyls have been selected. After growing callus in hypocotyls and cotyledons, callus well separated and grouped according to treatment.
2. Stock Murashige and Skoog media are made of two kinds of media are liquid media and solid media with the addition of agar. Timentin antibiotic added in liquid media, the media in the majority there Timentin antibiotic and partly without Timentin antibiotic (amount adjusted for treatment)
3. Isolates Agrobacterium inoculum containing genes Chin and Glu respectively cultured in liquid LB in Shaker 28ºC water bath for 4 days. Harvested by centrifugation at 5000 rpm for 15 minutes, that pellet was taken. Then washes that pellet with ddH2O.

Second Stage

1. Each - each callus material soaked in construct Agrobacterium no 440cDNA used Plasmid pB2GW7 binding Chitinase gene and Agrobacterium no 440cDNA used Plasmid pB2GW7 binding Glucanase gene (following the treatment of Glu or Chin), Callus material is removed and drained

2. After 7 days Move all Media Into Solid containing BASTA Herbicide. Was observed for growth and stagnant.

III. RESULT AND DISCUSSION

To know the exact effect of the interaction of both the treatment of the induction of the gene, then the statistical testing performed by Analysis of Variance – Randomized Complete Design. These tests show the results of the interaction between source material and modification methods. The interaction occurs in both activities transgene well with glucanase and chitinase.

Table 1. The result of live callus after cocultivation treatment Agrobacterium no 440cDNA used Plasmid pB2GW7 binding Chitinase gene

Method Treatment	*Average Calli Lives*	*Level of Live*
KsKl	6,666667	***
MdKl	5,666667	***
PcKl	7,666667	****
KsKh	5	***
MdKh	3,666667	**
PcKh	5,666667	***
KsKt	7,666667	****
MdKt	6,333333	***
PcKt	9	*****

From Table 1. AG4404 seen that transformation Agrobacterium no 440cDNA used Plasmid pB2GW7 binding Chitinase gene with various treatment combinations, which gain value level very good is the combination treatment (PcKt) Washing with source material from cotyledon callus. While that scored the worst Harkat is a combination treatment (MdKh) with source material Solid Media callus from hypocotyl.

Table 2 The result of live callus after cocultivation treatment Agrobacterium no 440cDNA used Plasmid pB2GW7 binding Glucanase gene

Method Treatment	*Average Calli Lives*	*Level of Live*
KsKl	8	****
MdKl	7	****
PcKl	9	*****
KsKh	6	***
MdKh	5	***
PcKh	7	****
KsKt	9	*****
MdKt	8	****
PcKt	10,33333	√

From Table 2. AG4404 seen that transformation Agrobacterium no 440cDNA used Plasmid pB2GW7 binding Glucanase gene with various treatment combinations, which gain value Harkat very good is the combination treatment (PcKl) Washing with source material of Regenerating Calli and (KsKt) Paper Filter with source material from Cotyledon Calli, But there is one treatment (PcKt) Washing with source material from cotyledon callus on the transformation with AG4404 Agrobacterium pB2GW7:: :: cDNA-GFR (Table 1) Gets value Harkat Very Well, the treatment time is getting value Harkat Perfect. While that scored the worst Harkat is a combination treatment (MdKh) with source material Solid Media callus from hypocotyl and (KsKh) Paper Filter with source material from hypocotyl callus.

When we look overall at the results Table 1. and Table 2. it will be seen that the gain value Harkat Very Good Is Washing Method Source Material premises Callus Regeneration (PcKl) and Paper Filter with source material Cotyledon Calli (KsKl). As well as obtain value Perfect Harkat is Washing Method with callus source of Cotyledon. So that the outline can be drawn a picture of that method is good to be done is the Filter Paper Method and Washing Method, while the good material is derived from Cotyledon Calli and Regeneration.

IV. CONCLUSION

From the research, observations and discussions can be concluded as follows:

1. There was an interaction between the modified method with source material Soybean Callus
2. Modification of a very good method of this study was Laundering and Paper Filter.
3. Callus excellent source of this research is of regeneration callus and Cotyledon

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