Microscopic callus selection of sengon tree (Falcataria moluccana) putative tolerant to Uromycladium falcatarium

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Abstract. The increase of the cultivation area of the sengon tree (Falcataria moluccana) through monoculture cropping system especially in Java has caused an increase in gall rust disease. Infestation of the disease may lead to 100% death of sengon seedlings. Uromycladium falcatarium has been identified as the fungus causing the gall rust epidemic. This research aimed to select calli putative tolerant to gall rust disease. Seeds of ten individual colonies of 3 clones of sengon trees from Purwobinangun district that have been attacked severely by gall rust were used as explant sources. Gall rust filtrate was used as bioagent selection in vitro and the viable calli were selected under a microscope. The callus viability was determined based on the average living cell percentage stained with fluorescence diacetic acids. These viable cells of callus were found to have a good correlation with the tolerance index value. The results showed that callus colony from clone 3 individual 4, clone 5 individual 5, and clone 6 individual 5 exhibited the highest tolerance index value. The cell with different index values to the biotic pathogen filtrate indicated the presence of an enzymatic defence process of the cells against gall rust attack.

1. Introduction

The increasing cultivation of sengon tree (F. moluccana) in the Java island using monoculture cropping system leads to the increase of gall rust disease. This disease may cause 100% death of the sengon seedlings. Uromycladium falcatarium sp. Nov. has been identified as the fungus that causes the gall rust epidemic in South East Asia [1]. Due to its obligate parasite, this fungal pathogen is the major reason for the tree product loss. The disease not only reduces the yield but also greatly impair the quality and stability of production, undermining efforts to promote sustainable forestry. Environmental and health hazards resulting from the application of numerous chemical fungicides have raised concerns.

One of the forest-breeding programmes is to develop tolerant and/or resistant clones of valuable woody trees. To enhance the efficiency and success rate of this breeding programme, more rapid and cost-effective screening methods are needed. Mass screening methods can be performed with well-specified filtrates or purified toxins applied to the callus organs. Purified toxins are more easily quantified and replicated than inoculum and crude extracts (filtrates). There are many pathogens from which toxins have not yet been isolated (mostly obligate biotrophic parasites). However, before using a toxin as a selection agent for disease resistance, it is important to determine whether the toxin is an essential component of the pathogenicity and disease development [2]. The use of crude extracts or
filtrates on callus as explants are more laborious than the use of toxins. Nevertheless, it may be the better choice for plant-pathogen interactions using unknown toxins such as the gall rust disease.

In vitro selections may also be difficult and time-consuming. In many papers published since 1980, many of the problems related to the theoretical and practical approaches of in vitro selections and their importance for plant breeding have been addressed [3, 4, 5, 6, 7, 8].

This research provides an overview of the basic principles and methodology of in vitro callus selections for rust gall disease resistance in sengon tree. The gall rust filtrate of sengon tree contains toxic secondary metabolites such as alkaloids, steroids, saponins and terpenoids. These metabolites belong to physianicipin group, low molecular weight compounds produced constitutively, in contrast to the phytochemical groups synthesized in the course of pathogen infection. The active compounds were suspected derived from sengon tree as host plant [9]. The objectives of this research were microscopic callus selection of F. moluccana putative tolerant to U. falcatarium sp. Nov.

2. Materials and Methods

This research has been conducted at the Tissue Culture Laboratory, Centre for Forest Biotechnology and Tree Improvement, Yogyakarta. In vitro method by Wagh et al. [10] was used with a modification in this research. Calli were induced from seeds of sengon trees from Purwobinangun district, Yogyakarta. Liquid MS [11] media with callus initiation hormone added with gall rust tumour filtrate were used for cell selection. Gall rust filtrate (25% dimethyl sulfoxide solution) was used as a chemical agent for callus in vitro selection. The observation of cell viability was using the FDA (fluorescence diacetic acid) live cell painting method [12]. Callus cell selection of sengon in vitro was measured based on the highest index values obtained from several factors where 30 day observation period divided by mean callus induction time on explants, an incubation time of LD50 callus infiltrate gall rust treatment, the percentage of explants that form a callus, and living cell percentage on FDA cell paint. Flowchart of microscopic callus selection of F. moluccana putative tolerant to U. falcatarium is shown in Figure 1.

Individual clone SPW-3, clone SPW-5, and clone SPW-6

\[\text{Explants from seeds} \quad \downarrow\]

\[\text{Callus induction} \quad \downarrow\]

\[\text{Multiplication of callus} \quad \downarrow\]

\[\text{Immersion of callus colony sample individually in gall rust filtrate with LD}_{50}\text{ incubation time treatments} \quad \downarrow\]

\[\text{Callus viability observation using FDA (fluorescence diacetic acid) based on living cells at 3 microscopic views in incubation time of LD}_{50} \quad \downarrow\]

\[\text{Regeneration of colonies of selected sengon clones based on the highest index value} \quad \downarrow\]

Figure 1. Flow chart of microscopic callus selection of F. moluccana putative tolerant to U. falcatarium.
3. Results and Discussion

The results showed that callus cells of sengon tree of clone 3 individual 4 (Figure 2), clone 5 individual 5 (Figure 3), and clone 6 individual 5 (Figure 4) had the highest tolerance index values.

Figure 2. The index value of individual sengon clone 3.

Figure 3. Index value of individual sengon clone 5.
Callus colonies from the 3 clones grew well in vitro. Callus clone selections of sengon tree candidate tolerant to gall rust disease are shown in Figure 5 and its regeneration after callus selection is shown in Figure 5.

There are different approaches to acquire genotype selections through the breeding of tolerant trees, among others are indirect selections in vitro. However, until now there has not be known yet exactly what parameters should be used. Index values of callus cells of sengon in vitro based on the day observation period, mean callus induction time on explants, an incubation time of LD₅₀ callus in filtrate gall rust treatment, the percentage of explants form callus and viable cell percentage should be a good alternative for selection in vitro. The individual family of SPW 6-5 colony callus with the highest index value gave the best growth compared to an individual family of SPW 6-7 colony callus with the lowest index value (Figure 6).

Figure 4. Index value of individual sengon clone 6.

Figure 5. Regeneration of sengon callus clone SPW-6.5 after 42 days in culture.
Figure 6. Callus selection of sengon candidate gall rust tolerant: gall rust attacked at sengon trees as seeds source (A), suffering from gall rust attacks (A.1); plantlets of sengon tree (B); callus initiation of sengon in vitro (C); callus immersion in gall rust filtrate (D); individual clone SPW-6.5 had the highest index value (E); individual clone SPW 6.7 had the lowest index value (F).

In vitro selection method has been highly more effective and efficient [5, 13, 14, 15, 16] compared with the tolerance selection directly at the nursery or the selection process in the wild. In this research, good callus colonies that regenerated fast, friable with shiny white and green were obtained only in 42 days of in vitro culture. The maintenance of callus was important for the subsequent research until the seedlings were regenerated from the plantlets. This research was conducted to avoid a lack in developing a woody plant tolerant to a certain disease using conventional ways i.e. the low accuracy selection, the lengthy time needed to develop and to test the progenies of the previous mother plants.

On the indirect selection in vitro, the cell viability indicated with fluorescence diacetic acids has a good correlation with the tolerance index value. The difference in the cell reaction on the index value parameter of the biotic pathogen filtrate indicates the presence of an enzymatic defence process of the sengon callus cell. Kumar et al. [17] reported that the pathogen filtrate induced oxidative pressure during the in vitro selection. During the agent selection treatment, the cells reproduced a radical superoxide which was immensely toxic to protect the cells from the pathogen filtrate pressure.

The presence of steroids, saponins, and terpenoids in the gall rust filtrates was considered as the agent selection compounds in vitro that contribute to the sengon tree defence mechanism [9]. Therefore, sengon cells putative tolerant to gall rust filtrate in vitro that survived from oxidation damage because the cells had high anti-oxidative enzyme activity. Programmed cell destruction (PCD) is a cellular homeostatic reaction as a defensive response against biotic pathogen stress or biochemical gall rust filtrate selection agent. As it reaches closer to a unicellular level, the cell selection process has been narrowing down even further as the homeostatic reaction became more potent, this was able to stabilize smaller internal cells [18].
4. Conclusions
The difference in the cell reaction based on the index value parameter to the biotic pathogen filtrate from microscopic callus selection indicates the presence of an enzymatic defence physiology process in callus cells of *Falcataria moluccana* putative tolerant to *Uromycladium falcatorium*.

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6. References
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