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ACCLIMATIZATION OF SENGON (FALCATARIA MOLUCCANA) MICRO CUTTING PROPAGATION IN VITRO

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Abstract

Sengon (*F. moluccana*) is a legume fast growing, light wood tree species. Legume tree-based farming systems sit at a crucial nexus of agro-ecological sustainability. For the past few decades, the widespread disease of gall rust on sengon trees has affected most areas in Indonesia. The disease has caused serious damage to death and destroys the environment of sengon habitat or community forests. Vegetative propagation is necessary to maintain the disease tolerant genetic material of sengon. Till to date, macro propagation of sengon still encountered obstacles. The use of micro propagation with tissue culture is considered to be alternative technique. The aims of this study were to observe acclimatization, an important stage that requires appropriate environmental treatments, from micro cutting propagation *in vitro*: 1) direct acclimatization (seedlings from axillary bud micro cutting *in vitro*) and 2) indirect acclimatization (seedlings from chamber hood). Explants from each treatment were transferred in three media composition (soil and compost) with no rooting stage *in vitro* as shortcut method. The mean of sprouting ability of sengon plantlet is 7 shoots per plantlets and 3 nodes per shoots. It gives opportunity of high multiply *in vitro*. The highest growth index (0.9) for ability of live (0.97), shooting (0.78) and rooting (0.98) of sengon plantlets was in indirect acclimatization with M2 treatment (50 % sand soil and 50 % compost medium).

Keywords: Acclimatization, Growth Index, Micro Cutting, Falcataria Moluccana.

1. Introduction

Sengon (*Falcataria moluccana*) is a legume fast growing, light wood tree species. Legume tree-based farming systems sit at a crucial nexus of agro-ecological sustainability. For the past few decades, the widespread disease of gall rust on sengon trees has affected most areas in Indonesia. The disease has caused serious damage to death and destroys the environment of sengon habitat or community forests. The census of plants in Indonesia (2003) indicates that there is a very significant increase of the number of sengon trees in community forests across Indonesia. The highest increase of sengon cultivation occurred in Java Island which reached 510.63% (BPPS, 2006). The high number of sengon trees in community forests is a parameter to the high interest of the community to cultivate the species for industrial needs. The widespread cultivation of sengon monoculture has the potential to increase the intensity of gall rust disease in various regions.

Vegetative propagation is necessary to maintain the disease tolerant genetic material of sengon. Till to date, macro propagation of sengon still encountered obstacles. The use of micro propagation with tissue culture is considered to be an alternative technique. *In vitro* propagation techniques provide an opportunity for the rapid and large scale production of such

cultivars which are difficult to produce by traditional methods together with the production of disease free stock material (Sharma et al., 2015). Micro cutting can be defined as micro-propagation process which utilizes tissue culture-based technology to propagate plants by using axillary buds as explants (Haris et al., 2009).

Shoots or plantlets are needs acclimatization stage from *in vitro* culture to the greenhouse; they may desiccate or wilt rapidly and can die as a result of change in environment, unless substantial precautions are taken to accommodate them. In commercial micropropagation, this step is often the limiting factor owing to involvement of more labor and money (Conner & Conner, 1984; Debergh 1986). Acclimatization is defined as the climatic or environmental adaptation of an organism, especially a plant, to a new environment (Aronen, 2016). Although considerable efforts have been directed to optimize the condition for the *in vitro* stages of micropropagation, scant attention has been paid to understand the process of acclimatization of micro propagated plants to the soil environment. Consequently, the transplantation stage continues to be a major bottleneck in the micropropagation of many plants (Hazarika et al., 2006).

Many commercial laboratories do not root micro cuttings *in vitro*, because it is labor intensive and expensive. The process of rooting *in vitro* has been estimated to account for approximately 35-75% of the cost of micro-propagation (Debergh & Maene 1981). Roots formed on micro cuttings *in vitro* were thicker, had thicker root hairs and had an under-developed vascular system compared to that formed in fine sand(McClelland et al., 1990). Rooting shoots after removing from culture produce plants with a higher root: shoot ratio than *in vitro* rooted plants. The aims of this study were to observe acclimatization of sengon, an important stage that requires appropriate environmental treatments, from micro cutting propagation. Transfer explants in acclimatization process without rooting stage *in vitro* to *ex vitro* condition in this research is expected to improve efficiency and effectiveness vegetative propagation clones of sengon.

2. Research Problem

For the past few decades, the widespread disease of gall rust on sengon trees has affected most areas in Indonesia. The disease has caused serious damage to death and destroys the environment of sengon habitat or community forests. Vegetative propagation is necessary to maintain the disease tolerant genetic material of sengon. Till to date, macro propagation of sengon still encountered obstacles. The use of micro propagation with tissue culture is considered to be alternative technique. Attention has been paid to understand of sengon micro propagation; however acclimatization stage of sengon micro cutting to the soil environment has not been reported. There are several constraints to implement micro cutting technology in clonal wood plants. So mastering high proliferation and rooting rates as well as acclimatization survival rate are other challenges in micro cutting process.

3. Review of The Relevant Literature

Most studies report that sengon micro propagation through rooting *in vitro* before acclimatization stage. The highest response for acclimatization and pot establishment of the rooted plantlets of *Albizzia odoratissima* was obtained in soilrite (40%) (Borthakur et al., 2011). After acclimatization, the survival percentage decreased up to 90 % on average due to temperature fluctuation in the field. Thereby, several seedlings wilted and eventually died (Ermayanti et al., 1999; Handini et al., 2018). Hazarika et al., (2006) reported that the natural environment with a reduction in light intensity could be exploited for the acclimatization of micro propagated plants. Douglas *et al.* (1989) reported that the rooting of micro propagated plants improved significantly in compost before they were precultured for2 weeks *in vitro* on sorbarods soaked in liquid medium containing IAA. The percentage survival of micro propagated hybrid roses cvs. Landora, Queen Elizabeth, Virgo, Happiness, and Seapearl varied

from 92 to 98% when transferred to the green house at 82-85% relative humidity and planted in earthen pots containing a mixture of sand, soil and cow dung manure in the ratio of 2:1:1(v/v/v), respectively (Rout *et al.* 1999). Davies (1980) reported that a mixture of coarse pearlite, pith and loam (2:1:1; v/v/v) was good for rooting of rose shoots. He achieved 15-85% variation depending upon the cultivar. Capellades *et al.* (1990) observed that pretreatment of micro shoots with various growth regulators reduce transplant losses and shortened the acclimatization period in the greenhouse. Roberts *et al.*(1992) reported that the use of sorbarod transplantation plugs and growth retardants in the culture medium reduced endogenous gibberellins and prevented the wilting of *in vitro* grown rose after transfer to soil resulting in better survival. Hazarika et al., (2006) reported a simultaneous *ex vitro* rooting and acclimatization using micro shoots from proliferating cultures rooted in Soilrite® after pulsing them with rooting hormone which was an improvement over conventional rooting in agar-based medium.

4. Material and Methods

4.1. Study Site

In vitro and *ex vitro* research has been conducted from January 2013 to December 2015. The bulk seed as material for axillary shoot *in vitro* were collected from Center for Forest Biotechnology and Tree Improvement (CFBTI) arboretum in Yogyakarta, Indonesia (77.66708' SL and 110.42011' EL).

4.2. Plant Materials and Acclimatization Media

The high sprouting ability of sengon plantlet *in vitro* (5-7 sprout/plantlet with 2-3 nodes/sprout in tubes) after 3 sub cultured (1 month/subculture) on MS (Murashige & Skoog 1962) was medium enriched with 1 mg/L BAP (benzylaminopurin) and 0.5 mg/L NAA(naphtalenaceticacid). The media added with 3% sucrose and 0.7% agar, whose pH was adjusted to 5.7 ± 0.1. The all plantlet as explant source were cultured under a 16-hour photoperiod (50~70 μmol.m-2.s-1) at temperatures 20°C for 6 weeks.

Direct acclimatization (seedlings from axillary bud micro cutting *in vitro*) and indirect acclimatization (seedlings from chamber hood) were initiated without rooting stage *in vitro*. Sand soil and peat compost used as acclimatization media in green house (M1: 25 % soil and 75 % compost; M2: 50 % soil and 50 % compost; and M3: 75 % soil and 25 % compost).

5. Data Analysis

All experiments were set up in completely randomized design and repeated thrice. Data pertaining to explants are live ability (l), rooting ability (r), shooting ability (s) and growth index (mean value of m, r and s factor). Analysis data with Excel Program 10.0

6. Result and Discussion

Micro cutting propagation of sengon using the axenic plantlets (no contamination) from nodal segment containing axillary bud with high sprouting ability as source of explants to get more nodes to obtain high plantlet (Fig. 1). Mean of sengon sprouting ability in this research is 7 shoots per plantlets and 3 nodes per shoots. Each plantlet needs 6 sub cultured more for shoot regeneration and can be reused as explant source in micro cutting. This result give opportunity of high multiply *in vitro* due to a low rate of contaminations compared to when the buds of greenhouse mother source, e specially in tropical countries such as Indonesia. The harsh tissue sterilization not only damages the growing regions of the buds but also affects their overall growth potential. Plant propagation from nodal segment containing single axillary bud has been implemented successfully in by using seedling as a source of explants (Lardet et al., 1994; Perrin et al., 1997; Carron et al., 2003). Therefore, the success of handling the contamination during *in vitro* culture was the first challenge for the propagation to get axenic plantlets (Haris et al., 2009).

Figure 1. Node part (arrow) of sengon plantlets with high sprouting ability as explant source of micro cutting (Putri, 2014).

Plantlets are often developed within the culture vessel under low light intensity, aseptic conditions, and on a medium containing sugar and nutrients to allow for heterotrophic growth and in an atmosphere with high levels of humidity. After transfer from *in vitro* to *ex vitro* with indirect treatment (Figure 2) and direct treatment (Figure 3), propagates of sengon have to correct the above mentioned abnormalities.

Figure 2. Chamber hood for indirect acclimatization of sengon micro cutting propagation (Putri, 2014).

Figure 3. Direct acclimatization of micro cutting seedlings of sengon in M2 medium (Putri, 2014).

One of the critical stages in plant tissue culture is the transition phase between the laboratory and field conditions. Humidity is one of the important environmental factors to be gradually weaned and hardened to field conditions. Indirect acclimatization with closed chamber hood incubation for rooting before planting in soil medium treatment has higher daily mean humidity (83 %) and lower daily mean light intensity (61.54 lux)than direct system in polybag media for rooting in green house environment that has lower daily mean humidity(60 %) and higher daily mean light intensity (20 lux).Steps are taken to grow individual plantlets capable of carrying out photosynthesis. Acclimatization of the tissue cultured plantlets is done gradually from high to low humidity and from low light intensity to high light intensity conditions. The live ability, shooting ability, rooting ability and growth index of indirect and direct acclimatization after 6 months in greenhouse were showed in Table 1.

Table 1. Indirect and direct acclimatization plantlets of sengon after 6 months in greenhouse

Treatment	Live ability(l)	Shooting ability (s)	Rooting ability(r)	Growth Index
Indirect acclimatization				
M1	0.64	0.54	0.48	0.55
M2	**0.97**	**0.78**	**0.98**	**0.91**
M3	0.79	0.50	0.59	0.63
Direct acclimatization				
M1	0.36	0.18	0.22	0.25
M2	0.20	0.19	0.22	0.20
M3	0.13	0.18	0.20	0.17

Remark: Growth Index = divided by three of l, s and r addition

The highest growth index for ability of live, shooting and rooting of sengon plantlets was in indirect acclimatization with M2 treatment (50 % sand soil and 50 % compost medium). Indirect acclimatization is an environmental intermediary transition phase between controlled condition in laboratory and uncontrolled condition in greenhouse. Closed chamber hood condition in greenhouse gave optimal environment transition in indirect system for live,

shooting and rooting of plantlets before transfer to polybag media in greenhouse environment. It's the important part and efficient protocol in micro cutting propagation because of shortcut phase without *in vitro* rooting. Many research has reported that micro cutting used axillary bud from seedling mother source *ex vitro* with high contaminated explant, and through rooting phase *in vitro* which need more time incubation and higher cost. The *in vitro* age of sengon plantlets in this research were 6 time subcultures (one month per subculture) with rate varies from 10 to 15 per 6 months. This condition appropriate with Carron et al., 2000, 2007 that plantlets had a well-developed taproot and lateral root system and the multiplication rate varies from 1.3 to 2.3 per month depending on the genotypes and culture age *in vitro* (Carron et al., 2000, 2007). Indirect treatment of micro cutting seedlings of sengon after 6 months transferred to M2 soil medium showed in Figure 4. The seedlings, still grow well until more than 8 months in the green house environment, needs field test for regeneration evaluation of micro cutting of sengon in natural environment.

Figure 4. Indirect treatment of micro cutting seedlings of sengon after 6 months transferred in M2 soil medium.

Even if the water potential of the substrate is higher than the water potential of the media with sucrose, the plantlet may quickly wilt, as water loss of their leaves is not restricted. The leaves that develop *in vitro* generally lack well-developed epicuticular waxes, have raised stomata that may not close normally, have a poorly structured internal anatomy and may not be photosynthetically efficient. These leaves never become "normal". The relatively low light levels and saturated internal atmosphere promotes leaves *in vitro* that anatomically resemble both shade leaves and hydrophytic plant leaves. They often have reduced or absent epicuticular or cuticular wax which can lack the characteristic crystalline structure or differ in chemical composition from that of the control plants. *In vitro* leaves have thinner or somewhat collapsed epidermal layers with a clearly defined but absent or limited palisade layer, sometimes with obonically-shaped palisade cells and a loosely organized spongy mesophyll with an increased percentage of air space. Palisade development is related to light levels and is reduced in vitro as the light levels are relatively low. In addition, water supply can be limiting because of low hydraulic conductivity of roots and root stem connection (Fila et al. 1998). These conditions combine to produce plants uniquely unsuited for survival in the greenhouse and under field conditions. Therefore, most species grown *in vitro* require an acclimatization process in order to insure that sufficient number of plants survive and grow vigorously when transferred to soil. *In vitro* propagation or micropropagation is a challenging plant biotechnological industry that offers new methods of plant production. The most significant advantage offered by the aseptic

method of clonal propagation over the conventional method is that it takes a relatively short period of time and less space, and a large number plants can be produced from a single individual.

Conclusion

The study revealed a part of micro propagation technique findings of micro cutting of sengon (*F.moluccana*) with high sprouting ability for multiply *in vitro*. The research conducted to date have demonstrated that balancing of sand soil and compost medium in indirect acclimatization was important value in high index factor of ability of live, shooting and rooting until greenhouse environment observation, thus field growth test deserves further evaluation to provide alternative propagation technique for disease tolerant clones of sengon.

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