Rapid Propagation from Hypocotyl and Shoot Tip Explants of *Sterculia foetida* Linn

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***Abstract*—Sterculia foetida Linn. is a large deciduous tree belonging to Sterculiaceae. Seed of S. foetida is used for treating skin diseases and rheumatism also can be used as a source of alternative bioenergy and medicine. The main problem of S. foetida cultivation is the limited supply of seed plants. Seeds development were very easy, but to produce fruit have to wait for approximately 5 years. The objective of this research was to obtain kepuh plants through direct in vitro regeneration. Hypocotyls and shoot tips explants were excised from sterile germinated seedlings and placed on shoot induction medium containing basal salts of Woody Plant Medium (WPM) and various concentrations of plant growth regulators. The results showed that the best medium of shoots elongation were WPM + 1 mgL-1 kinetin + 5 mgL-1 GA3. Highest percentage of roots (65%) occurred on WPM with 5 mgL-1 IBA which average number of roots was 3.1. Rooted plantlets were acclimatized to field conditions by placing them in pots containing sterilized and compost medium (1:1) and subsequently transferred to filed with 80% survival.**

***Index Terms*—Sterculia foetida Linn., hypocotyl, shoot tip, propagation, bioenergy, medicine**

I. Introduction

Sterculia foetida Linn. is a large, straight umbrella shaped spreading decidious tree belonging to the family Sterculiaceae. It is also called as Java olive, Poon tree, Wild almond, Hazel Sterculia and Sterculia nut. It was first described in the year 1753 by Carolus Linnaeus. The origin of the name *Sterculia* genus comes from the Roman God, Sterquilinus, who was the God of fertilizer or manure. *Sterculia foetida* seeds can be eaten raw or roasted, so their usage is not harmful to human and other animals.

S. foetida trees are now only found in a few places that are considered sacred as the grave, staircase or other sacred places. Because the plant is often regarded as a plant billowing *genderuwo*. Especially with odd shapes and large fruit to fruit also called *genderuwo*, *kepuh*, *pranajiwa* and *kelumpah.* In English this plant called Hazel Sterculia. It is also often referred to as Indian Almond, Indian-Almond, Olive Java, Java Olives, Java-Olive, Peon, Skunk Tree, and Sterculia Nut [1].

Seed of *S. foetida* is used for treating skin diseases and rheumatism also contains several compounds that can be used as raw material for alternative bioenergy and medicine [2]. Oil content in the seed of *S. foetida* was reported that the yield of kepuh oil high enough that reaches 60% - 70% [3]. In addition, the level of purity is also good enough to be used as biofuel, so the cost of production for the extraction can be as low as possible.

The existence of *S. foetida* plants in Central Java has been relatively rare [4] even in some districts this plant has not be found [2]. Research on breeding activities both vegetative and generative, it is as one of the forms of plant germplasm conservation efforts *S. foetida* [5]. Natural regeneration has major constraints such as loss of viability with passage of time, long life cycles and seasonal response; therefore *in vitro* regeneration was preferred. This fascinating technique has the advantage to maintain superior, genetically table, disease free stocks with minimum space and plant material independent of season [6].

In vitro regeneration also expected to provide seed mass, and uniform flowering and fruiting can be faster than the seedlings derived from generative propagation [5]. In vitro regeneration also accounts for the *ex situ* conservation of plant diversity [7], [8]. In Sterculiaceae *in vitro* propagation was reported in some members such as *Sterculia foetida* [9] *Sterculia urens* [10], [11] and *Hildegardia populifolia* [12]. In view of its medicinal, industrial applications and lack of tissue culture reports the present work was undertaken as a means of rapid propagation.

The Murashige and Skoog (MS) (1962) culture medium is basically and widely used for plant tissue culture. Its components of salts are responsible for significant gains in tissue and cell development and growth. Additional media were also available for woody plants. The Woody Plant Medium (WPM) developed by Lloyd and McCown (1981) is the second most used medium for *in vitro* cultivation of woody species.

The objective of this research was to obtain *S. foetida* plants through direct *in* vitro regeneration for propagation of plants quickly, uniformly and faster production.

II. Materials and Methods

A. Plant Material and Sterilization Protocol

The apical and axillary buds explants from seedling *S. foetida* were surface sterilized for 2 min in 70% ethanol solution followed by continuous agitation for 15 min in

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20% Clorox solution (0.53% sodium hypochlorite) added with two drops of Tween 20.The apical and axillary buds explants were rinsed three to four times in sterile distilled water and cultured on germination medium containing half-strength Woody Plant Medium (WPM) salts supplemented with 2.5 gL^{-1} agar and 30 gL^{-1} sucrose.

B. Stage of Experiment

The study is divided into four stages of experiment, namely (1) the induction of shoots from apical and axillary bud explants, (2) the apical and axillary shoot multiplication, (3) elongation of shoots, (4) rooting induction, and (5) acclimatization.

C. Treatment and Parameter Recorded

The sterile apical and axillary buds explants from seedling were cultured on WPM incorporated with different concentrations of plant growth regulators for shoot proliferation and root formation [9]. Shoot induction and multiplication from shoot apical and axillary buds explants on various concentrations of (1, 1.5 and 2.0 mgL^{-1}) BAP; (1, 2 and 3 mgL^{-1}) Kinetin; (1, 2, 3, 4 and 5 mgL^{-1}) Kinetin + 5 mgL^{-1} GA were test. Parameters recorded were the percentage of explants producing shoots (%). And the mean number of shoots produced per explant. Data were collected after eight weeks of culture, while growth characteristics were observed every week.

Rooting experiments performed using WPM basic medium at two levels formula (0.5 and 1 WPM formula) and two levels IBA (1.5 and 3 mgL^{-1}) and WPM + (2 and 6 mgL^{-1}) IBA + 0% activated charcoal. Variables that are observed are the percentage of rooted culture and the average length of the roots. Rooted culture percentage is calculated by dividing the amount of culture that is rooted in the culture of the total number tested. Plantlets formed from acclimatized rooting experiment using a growing medium is soil + compost in the greenhouse. Plantlets / shoots were acclimatized using plastic lid for two months. Greenhouses are used shaded by using paranet 75%. Variables measured is increasing the number of leaves and the height of the plant. In addition, induction of rooting was also performed *ex vitro*, i.e. using the buds that have not been induced *in vitro*, but its roots soaked in a solution of IBA (100-200 ppm) for one or two hours and acclimatized on compost + soil (1: 1). Variables observed is the percentage of life and the percentage is growing. Life or death of the plant is determined by the color of the plant tissue. If the network is still dark green then expressed are still alive. The growth of plants is determined by the emergence of new leaves.

III. Results and Discussion

A. Induction of Shoots from Apical and Axillary Bud Explants

The initial apical and axillary buds explants showed increase in the rate of growth. With more nutrient content and formulation of appropriate media, WPM +(1.5 and 2 mgL^{-1}) BAP and (0.5 and 1 mgL^{-1}) IAA, indicated reach 3.5 cm in length of shoots. It seems that the basic WPM medium with growth regulators BAP and IAA was positive synergism activity in the growth of shoots.

B. The Apical and Axillary Shoot Multiplication

In the first through third number of shoots from the media WPM + (2 mgL^{-1}) BAP + (0.5 mgL^{-1}) IAA showed more than the WPM + 2 mgL^{-1}BAP + 1mgL^{-1}IAA. Increased concentrations of BAP to 2 times not in line with the increase in the formation of buds. Subcultures can increase the rate of germination, even in the third month high enough primordia shoots on WPM medium supplemented with 2 mgL^{-1}BAP and 0.5 mgL^{-1}IAA.

C. Elongation Shoots

Shoots were cultured on WPM medium containing BAP and IAA showed no growth in the direction of elongation after the 3rd month period of culture. Subcultures on the media WPM + (1,2 and mgL^{-1}) kinetin + (0 and 5 mgL^{-1}) GA3 showed elongation growth after 2 months period of culture. Longest shoots mainly derived on WPM medium + 1 mgL^{-1} kinetin + 5 mgL^{-1}GA3l, which is 1 cm. Without GA3 shoots ranged from 0.5 to 0.9 cm long. With the addition of GA3 on medium containing kinetin then shoots can reach 3 times longer than medium without GA3 (Fig. 1). In accordance with the role, physiologically GA3 can stimulate cell elongation and books that are in line with the results of this study [13]. Increasing concentrations of kinetin from 1 to 3 mgL^{-1}can not spur the growth of shoots in the direction of elongation. Thus, the use of low concentrations of kinetin in combination with GA3 more effective in promoting cell elongation and plant tissue books. Furthermore shoots elongated and best treatment subcultured on rooting medium.

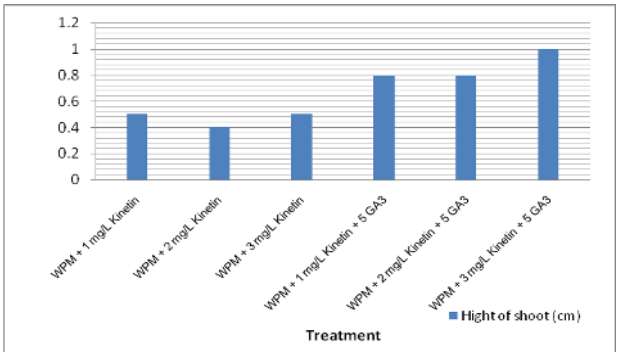

Figure 1. The average height of shoots on WPM containing Kinetin and GA on 2 months culture.

D. Rooting Induction

Shoots were cultured on WPM medium containing IBA and activated charcoal to stimulate roots induction (Fig. 2). Some culture begin to swell on the base of shoots after wo months of culturing,. This condition indicates the occurrence of organized callus formation mechanism followed by root initiation and media especially WPM + (2 and 6 mgL^{-1}) IBA + 0% activated charcoal. The average number of roots and the average length of roots showed more than 1.5 cm when cultured

in medium with 6 mgL^{-1} IBA and in the absence of activated charcoal. Treatment of other media formulation already seen the formation of root nodules expected will then be differentiated to form roots. In general, cultures can not form roots except for (2 and 6) mgL^{-1} IBA without activated charcoal. Similarly cultures grown on IBA treatment plus 0.5% activated charcoal none of which can form the root. It seems that in addition to the activated charcoal to absorb phenols can also absorb auxin, so that root growth is inhibited. Subsequent experiments using different concentrations of IBA, NAA and macro salt dilution to 0.5 formula. Using this new media formulations treatment seems to give better results than subcultured on (1, 0,5) WPM medium + (0, 1, and 3 mgL^{-1}) IBA or (1, 2, and 3 mgL^{-1}) NAA. The successful formation of roots ranged between 25-65% of various treatments of rooting medium. The highest rooting percentage, namely 65% originated and 0.5 WPM + 1 mgL^{-1} IBA, WPM + 3 mgL^{-1} IBA, and 0.5 WPM + 1 mgL^{-1} NAA (Fig. 3).

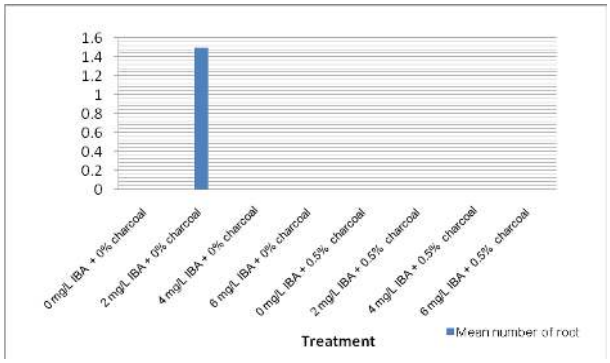

Figure 2. Root induction on WPM containing IBA and activated charcoal on 3 months culture.

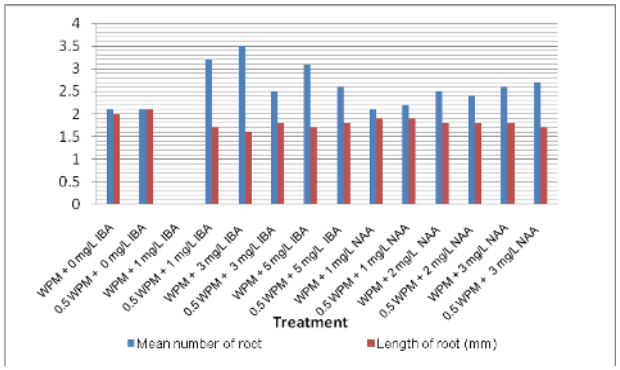

Figure 3. Rooting on WPM containing IBA or NAA at 2 months culture.

Root formation showed NAA treatment stimulated to form callus at the base of shoots culture. The stem are able to form callus to produce root because the relationship between vascular tissue, so the acclimatization stage of success are low. Mostly, total roots on WPM + 3 mgL^{-1}IBA was 3.5, and the treatment of MS + 5 mgL^{-1} IBA was 3.1. The number of roots that many very necessary because it can expand the field of nutrient uptake during acclimatization [13]. The number root per plantlets produced showing more than one root, while the plants derived from seeds grown only one root is a taproot. Rooting induction can also be done at this stage of *ex vitro* acclimatization. The number of roots more than one would be better for growth resulting in faster production [14]. In addition to the reforestation program more real because *S. foetida* rooted more than one is able to withstand the ground that are not easily landslides [15]. This study has been attempted in the mangosteenusing IBA solution without going through a phase of rooting induction in vitro [16].

E. Acclimatization.

Furthermore, the appearance of the plantlets have reached optimal growth, among others, has its roots be perfect, so do acclimatization in the greenhouse by using a mixture of sterile soil and compost medium (1:1). Growing success rate achieved was 80% (Fig. 4).

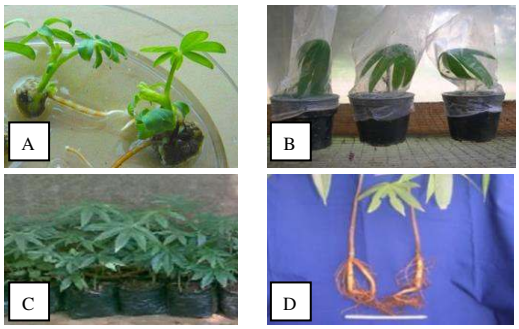

Figure 4. A: Response of shoot tip and hypocotyls explants on WPM + 1 mgL^{-1} kinetin + 5 mgL^{-1} GA3. Highest percentage of roots (65%) occurred on WPM with 5 mgL^{-1} IBA which average number of roots was 3. B: Aclimatization of *in vitro* regenerated plantlet. C: Potted *in vitro* regenerated plantlet ready to transfer to the field. D: Compare root derived from *in vitro* culture (left) and seeds (right).

The new leaves growth looked at the age of 1 week after acclimatization. The success rate of plantlets which grow into seedlings ready for planting at the age of 2 months after acclimatization to 90%. The long and the short root does not affect the level of success. Unsuccessful because too much water so the roots. Plants produced from acclimatization then prepared for planting seeds in the field. Plants showed the difference between the results of tissue culture and seedlings that is the number of root are more on the plants derived from tissue culture than plants derived from seeds after 3-month of acclimatization.

Roots of *S. foetida* derived from plant tissue culture have potential as a good greening plants to avoid land slides. Micropropagation of woody plants have managed since 1960 by Caton with Ericaceaous plants [17]. In further studies reported that the roots can be induced *in vitro* and *ex vitro*. *Ex vitro* treatment intended to obtain plants that will be used as seed [18]. Direct Rooting of shoots more economical results micropropagation techniques *in vivo* than *in vitro*. Rooting can be induced *in vivo* using the medium of peat, perlite, vermikuliute [19].

Research for the recalcitrant seed plants successfully growing woody plants outside their habitat. This is done in the context of the conservation program at the University of Helsinki botanical garden [20]. The selection criteria for the plant micropropagation result is that local climate suitability and appearance anthocyanin

phenological time to improve the adaptability of growth in high and low light intensity [21]. Micropropagation of woody plants would be better to increase the induction of shoots and roots routinely using 10 mgL^{-1} triacontanol combined with 0.5 mg of IBA [22]. WPM content 4 ppm BAP and 0.5 ppm IAA is able to produce complete plantlet, whereas 4 ppm BAP and 1 ppm IAA has the best growth of shoot and leaves [23].

IV. Conclusion

Several conclusions can be drawn from this study are as follows. Shoot induction of apical and axillary bud explants of germination on WPM +(1.5 and 2 mgL^{-1}) BAP and (0.5 and 1 mgL^{-1}) IAA on average very slow growth. Increasing concentrations of BAP to 2 time is not in line with the increase in the formation of buds. Subcultures can increase the rate of germination, on WPM supplemented with 2 mgL^{-1}BAP and 0.5 mgL^{-1}IAA. The second subculture of measuring 1-2 cm shoots on WPM + 1.5-2 mgL^{-1}BAP + 0.5 mgL^{-1}IAA can increase the number of shoots into 3.9 to 4.8. Then shoot elongation medium WPM + 1 mgL^{-1}kinetin + 5 mgL^{-1}GA3is the best formulation. Highest rooting percentage, namely 65% with the most number of roots, ie 3.1 obtained and media WPM + 5 mgL^{-1}IBA, while the acclimatization success achieved by 80%. The number of roots of plants derived from tissue culture more than the number of roots of plants from seed.

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