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Clonal Propagation of Two Clones *Eucalyptus Pellita* F. Muell By Mini-Cutting

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Abstract *- Eucalypt is known as the fastest growing tree species in the world. Some advantages such as fast growing, hight yielding, short rotation, less desease, lots of purposes and good economic benefit have made Eucalypt as the most important forest tree product for industrial raw material in Indonesia. However, those advantages of Eucalypt including fast growing, high yielding, short rotation and less desease can only be achieved through selecting a good clone. Cutting propagation is considered as one of the main methode to produce good seedling. Sprouts of two clones of Eucalyptus pellita F. Muell (E. 13 and E. 77) were collected from mini hedge. Total of 1080 of each clone was planted in greenhouse. The result showned that Clone E. 77 was better than E. 13 in term of growth of rooting, the increment of height, stem diameter and number of leaves at 60 and 75 days after planting.*

Keywords*: Clonal, propagation, eucalyptus, mini-cutting (;)*

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I. INTRODUCTION

Currently, eucalypts and pines are the most commonly used species in tropical timber plantations together, they are accounted by 43 percent of all tropical plantation area (FAO, 1999). Pines dominate temperate and boreal plantations accounted by 54 percent of the total area (FAO, 1999).

Eucalyptus pellita is indigenous species of eucalypt are found in Irian Jaya, Indonesia are planted in Kalimantan and Sumatera. There are widely planted for developing the timber estate in Indonesia.

Australian eucalypts have been the most widely used and successful plantation trees. Only a few of 500 species is potential in industrial plantations (FAO, 2000). Jacobs (1981) suggested that a MAI of 10-15 m^3 ha/y was commonly obtained from the large scale plantations, but more recent experience suggested that on many sites faster growth rates are possible. Eucalypts are recognized as one of the fastest growing trees. The dominant trees on fertile sites at Gogol, near Madang, Papua New Guinea reached 38 m in height and 39 cm in diameter breast height over bark (DBHOB), at three years of age; this was equivalent to MAI of 80-90 m^3 ha/y (Eldridge *et al.*, 1993).

The rate growth is resulted from using careful site selection, intensive cultural practices, selection of the best species and provenances, and genetic improvement. In the other hand not all of the material easy to propagate by cutting.

In most of forestry companies that have adopted clonal forestry, vegetative propagation of Eucalyptus genus is carried out to a greater or lesser degree of sophistication in regard to improve the yield and quality. As yields from Eucalyptus forestry will continue to increase as more improved clones are developed, matched with improvements silvicultural methods for growing on these plants for which they are well adapted (Zobel, 1993),

Cutting method is the most widely used technique for Eucalypt propagation due to easier handling compared to micro propagation method). However, this method presents some difficulties in the production process, such as poor rooting and plants formation in certain clones, which consequently affects their deployment during planting (Xavier *et al.*, 1997).

The poor rooting of certain clones which using conventional cutting is the major constraint to cloning, and has been attributed to the maturation degree of the plant material (Hackett, 1987), leading to the adoption of various techniques for "rejuvenating" the mature plants

material into the "juvenile" stage (Bonga, 1982), among of them is technique (Struve and Lineberger, 1988; Gupta and Durzan; 1987; George and Sherrington, 1993; Hartmann, *et al*., 2002). The development of micro-cutting technique (Assis *et al*., 1992; Xavier and Comerio, 1996) and mini-cutting technique (Xavier and Wendling, 1998; Wendling *et al*., 2000) led to considerable gains which mainly derived from increasing the proportion of plants that rooted and shortening the production time.

Clonal propagation of *Eucalyptus is* very important in intensive forestry. Mini-cutting technique has been developed and implemented in practice, but the advantages and/or disadvantages of this method has not been extensively evaluated. Once identified, high-yielding and/or high quality clones can be mass propagated and deployed for practical forestry purposes, where a continuous and reliable supply of stock plantation is highly demanded.

The objective of this study was to compare the efficiencies of mini-cutting technique for clonal propagation of two *Eucalyptus pellita* clones.

II. Research Method

The experiment was conducted from March to July 2012 in *a* the greenhouse of PT Surya Hutani Jaya, Sebulu, Kutai Kartanegara, East Kalimantan, Indonesia.

The clonal hedges under two years old that derived from micro-propagation of plus trees using tissue culture method were set up using micro cutting plant material. Two clones of 13 and 77 were used in this experiment. Before sprout was pruned, the plant material was sterilized with a 70% water ethanol solution (vv^{-1}) and the sprout of two clones of *Eucalyptus pellita* were collected. The sprout was stored in styrofoam boxes containing water to prevent the loss of cellular turgor in the tissues and reduce oxidation (Figure 3A). After the sprout was collected, a pair of leaves under shoot tip from the mini-cutting were reduced by 75 % of the total leaf area (Figure 3B).

The basal region of each mini-cutting was immersed in a solution containing 2,000 mg L^{-1} IBA. The mini-cutting was placed in polypropylene plastic containers with 2 cm of the basal region inserted in the substrate. The substrate was prepared with roots decomposition. The plastic containers/tubes were sterilized before the mini-cuttings were inserted. Total of 54 cutting was) inserted per tray, and then placed into bench trays. The experiment used 540 cuttings for each clone, so the total cutting used for experiment was 1 080 cuttings.

Data in this experiment was analyzed using descriptive statistic method which presented in tables and graphics.

The mini-cutting was stored in a greenhouse under intermittent mist system. The plants were fertilized with composed of phosphate, ammonium sulphate, and potassium 5 g L^{-1} (24 days old), 7 g L^{-1}g (31, 38, 45, 52 days old). The mini-cutting was *keep* stored in a greenhouse for 30 days *before* prior transferring to shaded house for 20 days and finally placed under full sun light/open area.

The observed parameters are as follow: a). rooting condition at 60 and 75 days after planting ; b) the increment of height, number of leaves, and stem diameter of mini-cutting at 60 and 75 days after planting.

III. Result and Discussion

The big problem of vegetative propagation by cuttings was rooting ability. The age of mother plant used for cuttings material affected rooting ability. Using mini clonal hedge in green house treatment, having many advantages compare with tree as mother plant. Moreover, application plant growth regulators at cutting material for example IBA can promote rooting ability.

Rooting percentage of two clones was observed show that both of clones easy for rooting. That results because of using 6 months old mother plant that from tissue culture showed performance with high regeneration rate. Similar with Si-tong, *et al*. (2006) compare to the use of seed tree of cutting stocks, the output of cuttings by using seed tree of tube ramet increased 100–216.6%, and that this way the average 28.5%. When the tube ramet was more than three years old showed the output of cuttings and the average of annual total growth was high yield of forest decreased evidently. Hartmann, *et al*. (2002), rooting ability decreased linier with the addition of material age.

Shoots and roots was observed at 30 days after planting, that vary depend on the genetic material. Clone E77 better than E13 at rooting and sprout growth. Number of root at day 30 only one root with white color. Little leaves was found at material that have root it was 1-2 cm long and 0.5-1 cm wide. Around 20%-30% material having no root. Qun-ying, *et al*. (2008), using plant growth regulator decrease rooting time and increase root quality of Eucalyptus seedling.

The clones had different speeds of adventitious rooting. The optimum time for permanence of the mini-cuttings inside the greenhouse for rooting was between 35 and 42 days, and varied depending on the genetic material (Brondani, *et al*., 2012).

Mass propagation using mini cutting technique was started with production material for mini cutting in clonal hedge garden using hydroponic system then mini cutting material was planted and incubate in a green house for 30 days. The mini cutting was kept from pest and diseases. Watering and fertilizing regularly was gave to the material to promote cutting growth better.

Cutting plant was incubated treatment in shading net after 30 days in green house, and then be transferred to open area (45 days old seedling). The result shown that rooting ability was 100% for both clone E13 and E77 at 60 days old.

Percentage of loose, medium, and compact rooting at 60 days old for E77 clone (24%, 39%, 37%) higher than E13 clone (32%; 48%; 20%). Loose percentage of root at 75 days old at E13 clone (25%) or 5 % decreased for 15 days on the other hand at E77 clone (10%) or decreased 14 % for 15 days. Root growth of the two clones (E. 13 and E.77) at days 60th and days 75th shown in Figure 1 and

Figure 2 presented that root growth or root formation clone E. 77 was better than E.13.

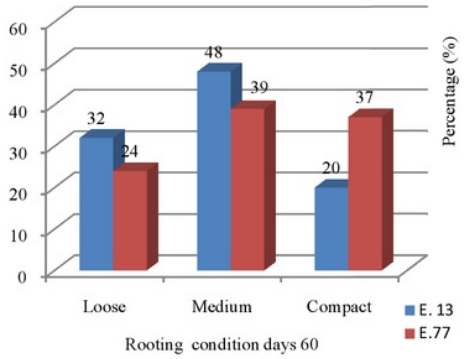

Figure 1. Percentage of rooting growth condition of two clones *E. pellita* (E. 3 and E. 77) at 60 days after planting

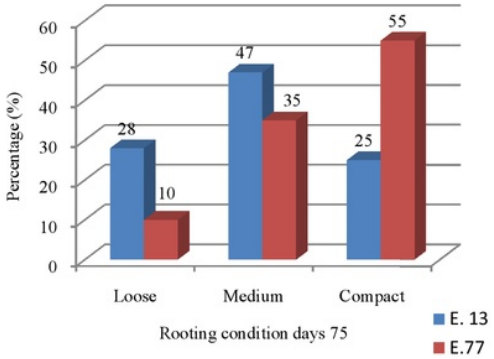

Figure 2. Percentage of rooting growth condition of two clones *E.pellita* (E. 13 and E. 77) at 75 days after planting

The rooting formation at 75 days after planting was seen at *numerous* many samples of two clones of mini cutting. Visual observation on root growth based on the medium distribution showned three categories i.e. loose, medium and compact. Loose means that root formation in the medium was rarely found so the percentage of root in the medium was considered low. Medium means that root formation in the medium was nearly good which represented by the percentage of root almost covered half of the medium. Compact means that root formation grew very well. Root covered all the medium that caused the medium did not experience breakage when seedling pull out of the container (Figure 3).

The research shown that rooting growth depend on genotype. Shaoman and Gan (1996), the different species and clones their rooting rate are different. Many factors could be influence differences rooting ability of plant material such as enzymes, genes, proteins, temperature, mother plant age, and plant growth regulator (Andres, *et al.*, 2002; Correa and Fett-Neto, 2004; Dai, *et al.*, 2004; Fogaca and Fett-Neto, 2005; Husen and Pai., 2007; Li, *et al.*, 2009; Papp and Platt, 2011; Rasmussen *et al.*, 2009; Risopatron, *et al.*, 2010; Thorpe, 2004; Trueman and Richardson, 2008).

Figure 3. Material for mini-cutting (sprout from clonal hedge (A); cutting ready for planting (B), compact rooting (C)

The adventitious root growth of mini cutting related to juvenile cells which influence by internal and external factors (Dettmer, *et al.*, 2009; Papp and Plath, 2011; Risopatron, *et al.*, 2010; Smet and Beeckman, 2011; Smet, *at al.*, 2009). Many rooting ability of cutting was influenced by inhibitor. Weidong, *et al.* (1998), rooting growth inhibited by phenol and tannin, it also influence cutting survival rate.

The factors that affecting rooting in cutting propagation of Eucalyptus include: (1) The source of mother plants. It is better to using tissue culture plantlets as mother plants than using cutting plantlets. (2) The age of mother plants. The cutting have high rooting rate when mother plants are between 1 to 3 years old. (3) The age of cuttings. Cuttings with 3 to 4 pairs leaves can take more roots. (4) Reserve area of leaves on cuttings. In seasons favoring cutting propagation, leaves of cutting cut by 2/3 are better for rooting, but in seasons disadvantaging to cutting propagation, leaves of cutting cut by 1/2 are better in rooting. (5) Operation seasons. The suitable seasons for eucalyptus cuttage are from November to the next April. (6) Plant growth regulators. It is better to using IBA as plant growth regulator than using NAA in cutting propagation of eucalyptus (Fanghong, *et al.*, 2003). Application plant regulator substance IBA at cutting material increase polyphenol oxidase and protein at many level of rooting growth (Lie, *et al.*,2003). This phenomena shown that any relationships between polyphenol oxidase activity and rooting ability of cutting material. Ming, *et al.*, (2000[a]) the activities of polyphenol oxidase and indole acetic acid and the is coenzymes of polyphenol oxidase were specific to organ. Ming, *et al.*, (2000[b]) peroxidase activity was higher in a difficult-to-root clone than in easy-to-root clone of the same species, and peroxidase activity increased with organ maturity.

Shoot growth started at 30 days old linier with root growing. The average of leaves increment at E13 clone (2.7703) less than E77 clone (6.0000) at 60 days old, and also at 75 days old 5.9578 (E13 clone), 7.2297 (E77 clone) (Figure 4). The increment of leaves linier with cutting root growth rate. Because of E77 clone has better root growth rate than E13 clone so the increment of leaves E77 clone better than E13 clone. Primer root growing well in length

and number of roots. It cover the medium and influence plant ability for absorb the water and nutrient from the medium. Better root make seedling growing well in height and number of leaves. High number of leaves increasing photosynthesis rate and sink results. The results shown that there were any relation between rooting growth rate and number of leaves at 60 and 75 days old seedling.

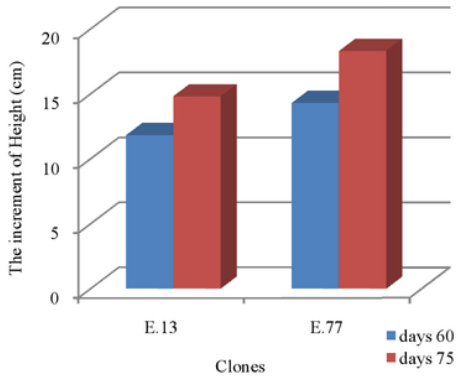

Figure 4. The increment of leaves at days 60th and days 75th at two observation clones.

The average of increment plant high at 60 and 75 days old seedling shown that E77 better than E13. The increment of plant height at 60 days old 11.94 cm (E13) and 14.97 cm (E77) and 14.74 cm (E13) and 18.73 cm (E77) at 75 days old seedling (Figure 5). Rooting ability, number of leaves and plant height are physiology process that influence each others. High rate of photosynthesis process depend on root and leaves condition. Rooting rate ability, leaves growing and plant height E77 clone better than E13 clone.

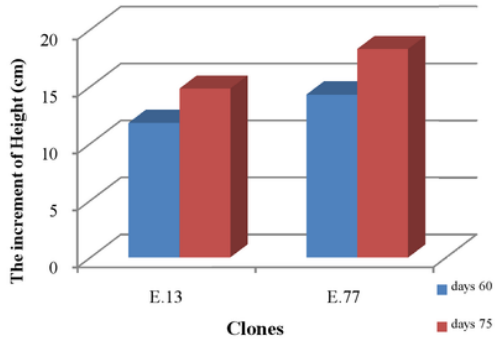

Figure 5. The increment of height at days 60th and days 75th at two observation clones.

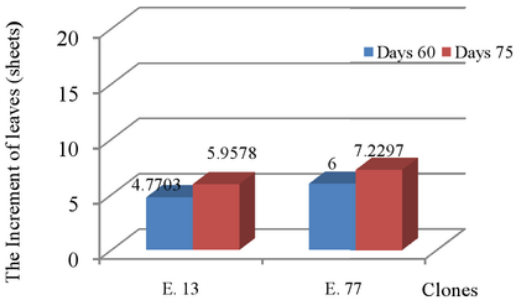

Figure 6. The increment of diameter at days 60th and days 75th at two observation clones.

The stem diameter average of E13 clone (0.95 cm) lower than E77 (1.20 cm) at 60 days old and 1.75 cm (E13 clone) and 1.32 cm (E77 clone) at 75 days old (Figure 6). Seedling with good high growth was follow by diameter growth. Plant growth process is complex process in each cell which not only depend on water and mineral absorption but also genotype material.

The increment of height, stem diameter, and number of leaves at 60 and 75 days after planting showed insignificant number (Figure 4, 5, 6). However, E. 77 clone had better growth than E.13).

Mini-cutting is important vegetative propagation method which mainly used to produce selected trees as it can maintain the genetic characteristic. Hartmann, *et al.* (2002), despite advances in plant biotechnology, rhizogenesis of adventitious root formation is poorly understood, including the different factors (chemical and physical) that influence their induction and formation. Many factors may be related to the behavior differences of rooting, including enzymes, genes, proteins, the temperature of the rooting substrate, the ontogenetic age of the plant tissue and the application of growth regulators (Truemans, *et al.*, 2008).

CONCLUSION

Rooting ability and root formation at substrate of E.13 clone was better than E.77 clone. The increment of height, stem diameter and number of leaves was also better at Clone E. 77.

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