Response of Entres Age and Clone to Growth of Rubber Seedling (*Hevea brasiliensis* Muell. Arg)

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Abstract
Effort to increase rubber plant production should be done, especially rejuvenation for old rubber plant which in turn require high numbers of seedling within short time period. One of the method to provide rubber plant seedlings within short time period is through grafting technique which combine plant having strong rootas base stem and high production plant as upper stem (entres). The objective of this research was to study response of entres age and clone to growth of rubber seedling (*Hevea brasiliensis* Muell, Arg.) produced from green and brown grafting. This study was conducted from February 2019 to Febuarti 2020. This study used Factorial Randomized Block Design with entres age as the first factor consisting of 3, 6, 9, 12 and 15 months as well as clone as the second factor consisting of PB 260 and BPM 24. Data was analyzed by using analysis of variance and followed bywith Honestly Significant Different (HSD) test and regression analysis. The study results showed that the best treatment was entres having 9 months of age as indicated by grafting success, the speed of stum out, stumlength, stumdry weight, root length androot dry weight which had the highest values than that of other treatments. Clone of PB 260 was the best entres that was very compatible with base stem of GT1 clone than that of BPM 24 clone because it gave the highest yield in term of all observed parametes. The interaction between 9 month entres age and PB 260 clone was the best treatment

Keywords: entres, clone, grafting, rubber

A. Introduction
Rubber plant (*Hevea brasiliensis* Muell.Arg) is one of foreign exchagesource for Indonesia so that area and cultivation technique of rubber plant are continues to be developed (Setiawan & Andoko, 2008). The problem for community rubber plantation was low productivity because superior rubber plant variety was not yet applied in this plantation (Bourhendy, 2006; Marsono & Sigit, 2005). One of the effort to increase plant productivity can be done through genetic improvement by using new clones (Sembawa Research Council, 2010). The technology and
Effort to produce superior seedling are through grafting technique. Grafting is one of vegetative propagation technique by sticking entres eye of one plant into other similar plants with objective to produce superior characteristics, better genetic combination indicated by high productivity, with stand to leaf and root diseases as well as with stand to poor environment (Toruan-Mathius, Lizawati, Aswidinnoor, 2002). Grafting seedling that can be used were in forms of sleeping eye stum, mini stum and grafting seedling within polybag. However, seedlings that most frequently used are sleeping eye stum and grafting seedling within polybag (Ampyalupi, 2010).

Recommendation for the fifth generation of rubber clone is arranged by considering consumer interests to develop rubber agrobusiness in term of latex needs and rubber wood needs. Rubber clone which is recommended to be used for commercial business covering extended planting area is called seed development with the recommended composition as follows: latex producing clones are consisted of IRR 104, IRR 112, IRR 118, IRR 220, BPM 24, PB 260, PB 330 and PB 340. Latex-wood producing clones are consisted of IRR 5, IRR 39, IRR 42, IRR107, IRR 119 and RRIC 100. The recommended base stems are consisted of seedlings from mono clonal plants of AVROS 2037, GT 1, BPM 24, PB 260, PB 330 and RRIC 100 (Sembawa Research Council, 2010).

According to (Goncalves, Silva, & Scalovi, 2006), the use of PB 260 clone with base stem of GT 1 clone showed the highest percentage of grafting success than that of other entres. According to recommendation from (Sembawa Research Council, 2010), the recommended clones for entres eye were BPM 24 and PB 260. Rubber clone of BPM 24 was results of crosses from clones of GT1 x AVROS 1734 which have good growth, high production and tolerant to Corynespora. PB 260 was results of crosses from PB5/51 x PB 49 which have very good growth, high production and tolerant to Corynespora.

There are three grafting types which consisted of early grafting, green grafting and brown grafting. These grafting techniques are relatively similar and their differences are resides on ages of base stem and upper stem. Green and dini graftings are not widely developed because of low success level and require high skill and accuracy. Grafting technique that widely used in rubber plantation is brown grafting. The ages of base stem used in brown grafting are in the range of 8 to 16 months in nursery or have diameter in the range of 1.8 to 2 cm as well as have brown color. Upper stems used in brown grafting technique are originated from entres farm having colors of brownish green to brown, straight stem and stump eye is in sleep position at cutting period. Upper stem (entres) used in brown grafting have age of 6 to 12 months and brown color (Kamil, 1986; Kuswanhadi, 1992). Conformity test between GT1 clone as base stem as well as PB 260 and BPM 24 clones as upper stem having different ages should be done in order to produce the best rubber seedling from brown grafting. The research objective was to study the response of entres and clone ages towards rubber seedling growth (Hevea brasiliensis Muell, Arg.) produced from grafting.

B. Methodology

Research Method

This research was conducted at rubber nursery farmer, Sembawa Village Km 29 Palembang, from February 2019 to February 2020. This study used Factorial Randomized Block Design with two treatment factors of entres age and clone, consisting of 10 treatments combination and three replicatons with 10 crops for each treatment. Entres ages were consisted of $E_1 = 3$ months, $E_2 = 6$ months, $E_3 = 9$ months, $E_4 = 12$ months $E_5 = 15$ months, whereas clones were consisted of $K_1 = PB 260$ and $K_2 = BPM 24$.

Research Implementation

Grafting materials are consisted of base stem of GT 1 clone and upper stem of PB 260 and BPM 24 clones from entres farm of Rubber Research Council of Sembawa, Palembang. Upper stems are consisted of 2 clones, i.e. PB 260 and BPM 24 clones having green and brown color and straight stem. These upper stems were cut 100 cm in length and transported to the land where base stem of GT 1 clone was available. Grafting was done at 1 year of age base stem by making the grafting window with length of 7 cm and width of 1/3 stem circumference. The position of grafting window was 10 cm from soil surface. Entres ages used in this study were 3 months, 6 months, 9 months, 12 months and 15 months. Grafting eyes were taken from leaf armpit by cutting with dimension 1 cm in width and 5 cm in length accompanied with small part of wood. This grafting eye was then put on grafting window of base stem which was already available. Subsequently, dressing was done by using plastics ribbon with dimension 30 cm in
length and 3 cm in width to prevent grafting eye from dirt and rainfall water. Grafting was opened at the fourth week to determine grafting success and marked by making scratches on grafting shield eye. If scratches color was green, then grafting was considered successful.

Data Analysis

Data was analyzed by using program of Microsoft Excell for Windows ver.7 and SAS ver. 9.13 to determine error variance and significance test amongst treatments (Anova). Data was also analyzed by using Honestly Significantly Different test and regression analysis (Steel & Torrie, 1993) to determine the effect of treatments on rubber seedling growth.

C. Result and Discussion

D. Table 1. Analysis of Variance Results Related to The Effect of Entres Age, Clone and Their Interaction on Observed Parameters

| Parameters          | Entres Age | Clone | Interaction | CV (%) |
|---------------------|------------|-------|-------------|--------|
| Grafting success (%)| 1.632 **   | 8.42 * | 0.55 ns     | 6.09   |
| Speeds of stump out | 49.26 **   | 7.41 * | 1.88 ns     | 7.54   |
| Stum length         | 375.23 **  | 18.68 ** | 3.44 *    | 3.80   |
| Stum dry weight     | 160.07 **  | 27.27 ** | 1.78 ns    | 3.01   |
| Root length         | 9.54 **    | 76.87 ** | 2.12 ns    | 6.82   |
| Root dry weight     | 0.09 ns    | 1.38 ns | 0.24 ns    | 8.33   |
| F- Table 5 %        | 3.24       | 4.49   | 3.24        |        |
| F- Table 1 %        | 5.29       | 8.53   | 5.29        |        |

Remarks: *= Significant; ** = Highly Significant; ns = Not Significant; CV = Coefficient of Variation

E. Table 2. Results of HSD Test Related to The Effect of Entres Age on Rubber Seedling Growth of Green and Brown Grafting Produce

| Entres age | Grafting success (%) | Speeds of stump out (days) | Stum length (cm) | Stum dry weight (g) | Root length (cm) | Root dry weight (g) |
|------------|----------------------|---------------------------|------------------|---------------------|------------------|--------------------|
| 3 months   | 75.00 d              | 19.69 a                   | 21.38 e          | 6.35 d              | 22.49 c          | 0.82 d             |
| 6 months   | 88.33 ab             | 13.68 bc                  | 30.48 c          | 8.48 b              | 25.38 a          | 1.04 cb            |
| 9 months   | 95 a                 | 11.57 d                   | 34.82 a          | 10.04 a             | 26.05 a          | 1.79 a             |
| 12 months  | 85 bc                | 12.57 cd                  | 31.99 b          | 8.92 b              | 25.48 a          | 1.20 b             |
| 15 months  | 76.76 cd             | 14.95 b                   | 28.89 d          | 7.78 c              | 24.55 b          | 0.92 cd            |

Remarks: Numbers followed by the same letters at the same column are not significantly different at 5% HSD test

F. Table 3. Results of HSD Test Related to The Effect of Clones on Rubber Seedling Growth of Green and Brown Grafting Produce

| Clones | Grafting success (%) | Speeds of stump out (days) | Stum length (cm) | Stum dry weight (g) | Root length (cm) | Root dry weight (g) |
|--------|----------------------|---------------------------|------------------|---------------------|------------------|--------------------|
| PB 260 | 86.67 a              | 15.04 a                   | 32.70 a          | 8.86 a              | 25.01 a          | 1.18 a             |
| BPM 24 | 81.33 b              | 13.94 b                   | 29.01 b          | 8.05 b              | 24.57 b          | 1.11 a             |

Remarks: Numbers followed by the same letters at the same column are not significantly different at 5% HSD test

Grafting Success (%)

Results of HSD test showed that 9 month age entres had the highest grafting success with magnitude of 95%, followed by 6 month age entres with magnitude of 82%. But it was not significantly different than that of 12 month age entres with magnitude of 85%. On the other hand, 15 month age entres had the lowest grafting success with magnitude of 75% and it was highly significantly different than that of other treatments. Clone of PB 260 showed higher grafting success than that of PBM 24 clone with difference level of 5-6% such as shown in Figure 1.
Figure 1. Grafting success of rubber seedling by using treatment of clone and entres age

GT1 clone and PB 260 clone have genetical similarity with magnitude of 82%. This genetical factor results in high compatibility between base stem of GT1 clone and upper stem of PB 260 clone (Toruan et al., 2002; Kuswanhadi, 1992). Grafting success is determined by linking between upper stem and base stem. Proper linking of grafting is begin by cell division and followed by callus formation process into vascular tissues consisting of xylem and phloem (Hadi, 2010; Gardener, Pearce, & Mitchell, 2006).

Determination of branchas upper stem is requirement for entres eye taking on plant having superior characteristics. Entres eye is located at leaf armpit which have big leaf and better characteristics than that of leaf armpit having smaller leaf. Entres eye from very young twigs will require relatively longer time to grow. Proper entres eye to be used in grafting is located at center and close to base, whereas entres eye located at tip can not be used because it has angular shape resulting in difficulty of skin peeling (Anindiawati, 2011; Hartman, Kester, Davis, & Geneve, 2011).

Maintenance of grafting success is also affected by compatibility between upper stem and base stem, age, capability of entres eyeto crack and grow, climate and technical skill of grafter. The best time to conduct grafting is between 07.00 to 10.00 in the morning, between 16.00 to 18.00 in the afternoon, plant is not in wet condition and rainfall water is not penetrate into grafting. If entres eye is rocking and rainfall water penetrating into grafting, then grafting process will unsuccessful (Kuswanhadi, 1992; Tambing, Adelina, Budiarti, & Murniati, 2008).

**Speeds of Stum Out (Day)**

Results of HSD test showed that 9 month age entres had the fastest time in term of stum eye appearance with magnitude of 11.57 days, followed 12 month age entres with magnitude of 12.57 days and 6 month age entres with magnitude of 13.67 days. On the other hand, 3 month age entres had the slowest time in term of stum eye appearance with magnitude of 19.69 days which was highly significantly different than that of other treatmens. The effect of clone on speeds of stum eye appearance was not significantly different between PB 260 clone and PBM 24 clone such as shown in Figure 2.
Stumon upper stem which start cracking and growing on the second week after grafting was planted in polybag. The speeds of stum eye appearance is highly affected by upper stem age used in grafting. The fastest time in term of stum eye appearance was found on upper stem having 9 month age. This was due to tissue similarity with base stem of GT 1 clone having 1 year age than other upper stem ages (Hidayat, Poerwanto, Latifat, Darusman, & Purwoko, 2005). Cracking of stum eye was start to occur at ages of 10 to 40 days after planting depending on entres age and clone (Indraty, 2007).

**Stum Length (cm)**

Results of HSD test showed that 9 month age entres had the highest stum length with magnitude of 34.82 cm followed by 12 month age entres with magnitude of 31.99 cm and 6 month age entres with magnitude of 30.57 cm. On the other hand, 3 month age entres had the lowest stum length with magnitude of 21.38 cm and it was highly significant different than that of other treatments. The effect of clone on stum length was highly significantly different between PB 260 clone and PBM 24 clone such as shown in Figure 3.

![Figure 3. Stum length of rubber seedling using treatment of clone and entres age](image)

The highest stum length was found on PB 260 clone with magnitude of 30.02 cm, whereas PBM 24 clone had produced stum length with magnitude of 29.01 cm after 12 weeks of grafting.

Tissue unification between GT1 and PB 260 was better so that metabolisms process can be easily distributed either through phloem or xylem. This metabolisms product will provide raw material for growth and development of stum. Good unification between base stem and upper stem will facilitate transport process of nutrients and minerals which in turn affect stum length. Stum growth rate is affected by base stem capability as facilitator for distribution of nutrients and water into all parts of plant. Stum activity at initial growth period is depended on carbohydrate accumulation within plant and keep moving into meristem direction resulting in increase of stum growth rate (Hartawan, 2012). Upper stem is the site for stum formation process on grafting, started with plant hormonal process where cytokinin has role in cell division within callus resulting in translocation of food supply up to the growing point.

**Stum Dry Weight (g)**

Results of HSD test showed that 9 month age entres had the highest stum dry weight with magnitude of 10.04 g followed by 12 month age entres with magnitude of 8.92 g and 6 month age entres with magnitude of 8.48 g. On the other hand, 3 month age entres had the lowest stum dry weight with magnitude of 6.35 g and it was highly significantly different than that of other treatments such as shown in Figure 4.

The effect of clone on stum dry weight was highly significantly different between PB 260 clone and PBM 24 clone. The highest value of stum dry weight was found on PB 260 clone with magnitude of 9.08 g, whereas PBM 24 clone had produced stum dry weight of 8.53 g after 12 weeks of grafting. Dry weight is indication of net photosynthesis quantity that can be utilized by rubber seedling to support its growth. Higher dry weight is indication of better growth for rubber seedling (Bourhendy, 2006).

Stum dry weight is consisted of all stum materials which originated from photosynthesis product, nutrients absorption and water that are processed through photosynthesis process.
Stem dry weight is affected by stem length and leaf numbers which experience photosynthesis. The increase of dry weight indicates protoplasm increment in term of its size or cell numbers. Nutrients are required by plant to stimulate its growth; if plant can properly grow, then nutrient absorption will run smoothly. This activity results in better plant growth and development as well as parts of plant become better in which turn produce high dry weight. According to (Chanana & Gill, 2008), plant development is a combination of complex processes, i.e. growth and differentiation processes which lead to dry weight accumulation. Nutrients availability has significant effect on plant growth and development resulting in increase of dry weight.

![Figure 4. Stem dry weight using treatment of clone and entres age](image)

**Root Length (cm)**

Results of HSD test showed that 9 month age entres had the highest root length with magnitude of 26.05 cm followed by 12 month age entres with magnitude of 25.49 cm and 6 month age entres with magnitude of 25.40 cm which are not significantly different. On the other hand, 3 month age entres had the lowest root length with magnitude of 22.49 cm and it was highly significantly different than that of other treatments. The effect of clone on root length was highly significantly different between PB 260 clone and BPM 24 clone. The highest root length for PB 260 clone was 25.01 cm, whereas BPM 24 clone had root length of 24.57 cm after 12 weeks of grafting such as shown in Figure 5.

![Figure 5. Root length of rubber seedling using treatment of clone and entres age](image)
obstruction of crop growth. The longer the plant roots, the higher the nutrients that can be absorbed by plant (Junaidi, Atminingsih, & Nurhawati, 2014).

**Root Dry Weight (g)**

Results of HSD test showed that 9 month age entres had the highest root dry weight with magnitude of 1.79 g followed by 12 month age entres with magnitude of 1.20 g and 6 month age entres with magnitude of 1.04 g. On the other hand, 3 month age entres had the lowest root dry weight with magnitude of 0.82 g and it was highly significantly different than that of other treatments. The effect of clone on root dry weight was highly significantly different between PB 260 clone and PBM 24 clone. The highest root dry weight for PB 260 clone was 1.18 g, whereas PBM 24 clone had root dry weight 1.11 g after 12 weeks of grafting such as shown in Figure 6.

![Root dry weight using treatment of clone and entres age](Image)

Figure 6. Root dry weight using treatment of clone and entres age

Root dry weight is affected by root length, wide range of root and nutrients that can be absorbed by plant. Root dry weight is also indicates plant capability to absorb water because plant which have high value of root dry weight also has higher tolerance level to drought than that of plant having low value of root dry weight (Hartmann et al, 2011). Dry weight is closely related to the ratio of metabolics and nutrients of plant tissue constituent as well as water. The higher the dry weight, the denser the plant tissue which results in lower water content (Manalu, 2014). The magnitude of dry weight is depended on magnitude of available dry matter. Dry matter usually is consisted of carbohydrate, lipid and protein raw materials (Hartman et al, 2002).

**D. Conclusion**

Nine months is the best age for entres because it had the highest values in term of grafting success percentage, speeds of stem eye out, stem length, stem dry weight, root length, root dry weight and gave the highest yield than that of other treatments. Clone of PB 260 was the best entres and most suitable with base stem of GT1 clone. Interaction between 9 month entres age and PB 260 clone was the best treatment.

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