Selection of Superior Genotypes at Early Stage of the Rubber (*Hevea brasiliensis*) Breeding Cycle

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ABSTRACT

Hybridization and selection procedure of *Hevea brasiliensis* plays an important role in genetic improvement of planting material with a wide range of genetic diversity to aim at developing superior *Hevea* clones. Time is the critical factor of the conventional breeding program and as it needs around 20-25 years to complete a one cycle. Early selection is very important to shorten the breeding program. However, the selections need to be strengthened through thorough analysis of all possible yield parameters. This study mainly aimed to precise selection of genetically superior genotype(s) at early stage of the *Hevea* breeding cycle (first evaluation stage) to reduce the evaluation period of breeding cycle. Preliminary four outstanding genotypes which were already selected from 2011 hand pollination progeny were taken to the study. Performances were monitored using more yield parameters as girth, bark thickness and bark anatomical parameters such as number of latex vessels per unit area (density), number of latex vessel rows and diameter of latex vessels. Each parameter was analyzed with their yield performance. These parameters analyzed to verify the previous preliminary selections as well to develop the correlation of these parameters with high yield. Regression analysis, cluster analysis and two sample t-test in Minitab 17 version were used to analyze data. It has observed around 39.3% positive correlation between yield and diameter of latex vessels and around 4.6% negative correlation between yield and bark thickness. Strong positive correlations between yield and latex vessels’ density and between yield and number of latex vessel rows were observed with 81.9% and 91.7% respectively. In addition to 57.4% negative correlation was observed between yield and diameter of latex vessels. In the cluster analysis with yield, girth and all the bark anatomical parameters, three clusters showed significant difference. Out of four preliminary selected outstanding genotypes only the 2011HP42 clearly separated from other three genotypes showing higher performances than other three genotypes. All four low yielding genotypes separated into cluster three showed the poor performances. Results clearly showed that the precise early selections can be done by taking more yield parameters and their correlations will be helpful to develop a yield index in the future. However, further studies need to be carried out with more number of genotypes to strengthen the precise early selection procedure.

Keywords: *Hevea brasiliensis*, *Hevea* breeding, Early selection, Bark anatomy.

1. INTRODUCTION

The para rubber tree, (*Hevea brasiliensis* (Willd. ex Adr. de Juss.) Muell.-Arg.) is the primary source of natural rubber which belongs to family Euphorbiaceae (Priyadarshan & Goncalves, 2003). *Hevea brasiliensis* is belonging to the deciduous perennial tree (Jain & Priyadarshan, 2009) with a straight trunk. It is a quick growing tree and grow up to about 25m – 30m in height (Names & Description, 2009). The trunk is conical or cylindrical. The bark is introduced as most important part of rubber tree due to the harvested part consists with lactiferous system (Verheyne, 2010). It is usually grey and fairly smooth.

The rubber tree which is native from Brazil was introduced by Sir Henry Wickham in 1876. Now it is become to the third largest plantation crop in Sri Lanka according to the land extent (Ranasinghe et al., n.d and Ranasinghe et al 2020). Main economically important part of the rubber tree is latex. And also timber and biomass are given economical value for the rubber with 30 years of economical lifespan.

The rubber plantation in Sri Lanka has raised with a narrowed Wickhams genetic base (Liyanage et al., 2014). Beginning with these unselected seedlings which yielded around 300 – 400 kg/ha/year, now it has been increased to 3000 – 3500 kg/ha/year with a genetically improved planting materials. This success was achieved by *Hevea*’s breeding initiative over the past 100 years (Withanage et al., 2014). The primary objective of the *Hevea* breeding is the development of genetically superior genotypes (Liyanage, 2016).

*Hevea* breeding is a challenging activity due to its perennial nature. The traditional breeding program of *Hevea* carried out every year with the annual hand pollination developing new genotypes. Completing a breeding cycle takes at least 20-25 years. It takes at least 4 years, from hand pollination to the first stage of selection at the mother plant nursery. The selected genotypes are then taken to the Small Scale Clonal (SSCTs) evaluation stage. The genetics potential of genotypes is assessed around 12-15 years. Then the genotypes with high genetic potential are selected to evaluate their performances under commercial level in

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Estate Collaborative Trials (ECTs) further 10-12 years. After the ECTs, the selected superior genotypes are recommended to group III level of clone recommendation (Withanage et al., 2014).

Time is a critical factor in Hevea breeding programs. Tree breeders have to wait for test and selection results for a long time (Karkiet et al., 1998). Not only the time, cost and resources also waste due to wait for many years. Therefore early selection is very important in tree breeding process.

To enrich the breeding Hevea pool, annual breeding programs are carried out by Genetics and Plant Breeding department of RRISL. The year 2011 hand pollination progeny which was established at mother plant nursery as single tree basis is used for this study. If superior outstanding genotypes selected from this stage, it can be skipped SSCTs level, and reduce around 10-12 years of evaluation period, cost and resources.

As we know, single tree selection is critical issue, because it is difficult to overcome the influence of micro environment. However, usually selections are carried out according to the yield and girth of 3-5 years old plants. Therefore more yield parameters should be incorporated to precise selection of superior genotypes at the early stage of breeding cycle.

The goal of this study was strengthen and shorten the Hevea breeding cycle with precise selections based on bark anatomical characteristics at early stage of the Hevea breeding cycle. This study was provide the relationships of bark anatomical features such as numbered latex cells per unit area, diameter of latex vessels, number of latex vessels and girth and bark thickness of high yielding and low yielding selected accessions and how these parameters correlated with the latex yield.

II. MATERIALS AND METHODS

The study assessed the progeny which was resulted from 2011 annual Hand Pollination (HP) program. 2011 hand pollinated genotypes were established at the mother plant nursery located in Niwithgalakele substation and was managed according to the RRISL recommendations. Each genotype is represented by a single seedling tree. According to their previous selection based on yield data, eight genotypes were selected with representing the highest yield genotypes which were already selected as outstanding genotypes ($2011\text{HP} - 42$, $2011\text{HP} - 202$, $2011\text{HP} - 297$, $2011\text{HP} - 300$) and the lowest yield genotypes ($2011\text{HP} - 256$, $2011\text{HP} - 53$, $2011\text{HP} - 291$, $2011\text{HP} - 183$).

Girth was measured from each genotype at 18 inches (45 cm) height above the ground level using a measuring tape. Bark thickness (BT) was measured around 12 inches (30 cm), 18 inches (45 cm) and 24 inches (60 cm) above the ground level from each accession using the bark gauge. Three bark thickness measurements were taken at each height of each genotype. Yield data for each selected genotypes were taken from past record which were taken from test tapping as gram per tree per tapping ($\text{g/t/t}$). Each trial had been carried out at the Genetics and Plant Breeding Department. Growth data were taken from past record. That data had been taken as diameter using the venire caliper.

Bark Anatomy

The specialized bark characters which are number of latex vessels per unit area (density of latex vessels), diameter of latex vessels and the number of latex vessels rows were studied using the previously used protocol which was optimized with some modification (Mihiran, 2018).

For the preliminary preparation, three blocks of bark in size (2 cm × 1 cm) (three replicates) from each accession were obtained and three slides from each block were prepared. Just after remove the bark samples from the tree, bark sample were fixed into falcon tube which are filled with FAA solution (100 ml of FAA contains; 5 ml of Formaldehyde, 5 ml of 100% Acetic acid and 90 ml of 50% Alcohol) for 10 – 30 minutes. The samples were taken into the laboratory and latex was wiped using mosses which were in bark samples were removed using a scalper and forceps. Then the samples were added into the labeled falcon tubes filled with 50% alcohol.

For the paraffin embedding paraffin were peeled using a knife and were melted on hot plate with 60°C - 65°C melting point. Then the melted paraffin was filled into labeled blocks and remains few minutes. For the molding purpose, plastic ice cube molds were used for get perfect small paraffin blocks. After the bark samples which were in 50% alcohol were taken out and were wiped using tissues. Thereafter bark samples were dipped in the liquid paraffin block using forceps with the soft bark facing to the downward with ensuring bark sample will not be lost during sectioning and were kept at room temperature for one hour to solidify paraffin. Then the paraffin block container was transferred to the refrigerator for 2 – 3 hours to hardened further and easy to remove the paraffin block from the container.

The paraffin blocks were removed and were sharpened using a knife to fit the rotary microtome. A few sections of paraffin were cut off to expose the bark surface and the bark sample blocks were labeled accordingly the accessions. Then the blocks were place in a beaker with cold water and transferred to the refrigerator (4°C) overnight.

Bark sections were prepared of the order of 10 – 20 µm in thickness tangential longitudinal sections (TLS) using rotary microtome (LAB-KITS SF-22580) and the sections with soft bark were separated carefully using smooth brush. Just after separate the sections were put into cold water to prevent drying. Then the sections were transferred in to staining protocol. First the sections were transferred into 50% alcohol for 5 minutes and then were stained with 1% safranin for 15 minutes. Then the stained sections were

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carried out through alcohol series to wash out excesses staining with 70% alcohol for 10 minutes, 90% alcohol for 5 minutes and 100% alcohol for 5 minutes respectively. Finally sections were fixed with xylene for 5 minutes. The stained samples were mounted with mounting medium (DPX MOUNTANT) and covered with glass. Then the samples were visualized in light microscopes and microscopic images were analyzed using the Image Focus 4 (version 2.9) software.

III. RESULTS AND DISCUSSION

Figure 1: Average test tapping data as gram per tree per tapping (g/t/t).

Red color rectangular; High yielding genotypes. Other four genotypes- the lowest yielding genotypes

Out of 337 genotypes, 2011HP-42, 2011HP-202, 2011HP-297, 2011HP-300 were already selected as outstanding as they showed the highest yield. And 2011HP-183, 2011HP-291, 2011HP-53 and 2011HP-256 showed lowest yield. 2011HP-42 showed the highest yield than other genotypes (Figure 1).

Preliminary selection was done based on yield and their girth only. But with incorporation of more yield parameters preciseness of these preliminary selections can be further confirmed.
Cluster analysis for yield and girth

Genotypes which were considered as outstanding genotype are in the red rectangle. Cluster 1: 2011HP-42, 2011HP-202, 2011HP-300. Cluster 2; 2011HP297, 2011HP-256, 2011HP-183, 2011HP-53, 2011HP-291.

According to the cluster analysis with yield and girth (Figure 2), two clusters can be identified clearly. Out of four outstanding genotypes, three genotypes (2011HP-42, 2011HP-202, and 2011HP-300) belong to cluster one and one genotype (2011HP297) belong to cluster two which had low yield and girth. But when consider about 2011HP291, it was deviated from low yielding groups (2011HP-256, 2011HP-183, 2011HP-53, and 2011HP-291). Therefore it shows best performances in yield and girth than other low yielding genotypes. 2011HP-42 genotype showed better performances when compared to other outstanding genotypes.

Relationship between yield and girth

The correspondence regression equation is Yield = - 161.2 + 4.157 Girth. Black arrow: Best fitted line. Red, purple, green, and yellow circles shows cluster 1, cluster 2, cluster 3 and cluster 4 respectively. Cluster 1; 2011HP-42. Cluster 2; 2011HP-202 and 2011HP-300. Cluster 3; 2011HP297. Cluster 4; 2011HP-256, 2011HP-183, 2011HP-53, and 2011HP-291.

The best fitted line which is in regression analysis between yield and latex vessels density showed, there is a positive correlation between yield and girth with $R^2$ 39.3% (0.393) value ($R^2$ = Square of regression / coefficient of determination). That means plant girth is affected to the yield increment.

According to Karim, (2007), there was a positive correlation between latex yield and plant girth. Regression curve showed four clusters based on high yield and high girth (Cluster 1: 2011HP-42), Moderate yield and high girth Cluster 2; 2011HP-202 and
2011HP-300), moderate yield (Cluster 3; 2011HP297) and low girth and low yield and low, moderate girth (Cluster 4; 2011HP-256, 2011HP-183, 2011HP-53, and 2011HP-291) (Figure 3). It proved the result of cluster analysis with yield and girth (Figure 2). Out of four outstanding genotypes 2011HP-42 is showed higher performances than others.

There was a poor correlation between yield and bark thickness as 4.6% (0.046) R square value. According to Sankarimal et al., (2009), some clones were showed negative correlation between yield and bark thickness.

Figure 4 showed five clusters based on yield and girth. Out of outstanding genotypes one is belong to cluster one and other three are belong to cluster two and cluster three. Though that genotypes showed high yield with low bark thickness. Although 2011HP-42 was showed highest yield and girth with low bark thickness. Low yielding genotypes belong to cluster four and cluster five. Out of low yielding genotypes, two genotypes had highest bark thickness (2011HP-256, 2011HP-53).

According to Sankarimal et al., (2009) significant clonal difference was observed for bark thickness and there was 5.23mm to 8.30mm.

Two sample t-test was carried out and showed significant difference among clusters at 0.05 level (α) (Figure 3).

**Relationship between yield and bark thickness**

![Figure 4: Variation of yield with bark thickness.](image)

![Figure 5: Variation of yield with latex vessels density (number of latex vessels per unit area).](image)
The correspondence regression equation is Yield = -37.75 + 6,999 Latex vessels density. Black arrow: Best fitted line. Red circle: cluster 1 (2011HP-42), green circle: cluster 2 (2011HP-202, 2011HP-297 and 2011HP-300) and yellow circle: cluster 3 (2011HP-256, 2011HP-53, 2011HP-183 and 2011HP-291).

The best fitted line which was in regression analysis between yield and latex vessels density showed, there is a positive correlation between yield and latex vessels density with having $R^2 = 81.9\%$ (0.819) ($R^2 =$ Square of regression / coefficient of determination) (Figure 5). $R^2$ value is closed to the one (100%). That means, there was higher correlation between yield and latex vessels density.

Wylerley, (1969) and Gomez, (1982), verified that latex vessels density showed positive correlation with the latex yield. Frey-Wyssling, (1930), observed higher correlation between yield and number of latex vessels.

It was clearly showed three clusters (Figure 5). 2011HP-42 showed high yield with high latex vessels density. It comprises in to cluster one. Other high yielding genotypes (2011HP-202, 2011HP-297 and 2011HP-300) comprise in to cluster two. All the low yield genotypes (2011HP-256, 2011HP-53, 2011HP-291 and 2011HP-183) grouped in to cluster three. Although, four outstanding genotypes were selected earlier based on yield, three of them are grouping as moderate yielders. Only one (2011HP-42) was come as outstanding performances with following yield and latex vessels density.

Two sample t-test was carried out and showed significant difference among clusters at 0.05 level ($\alpha$). According to it there is a significant difference between each cluster with verifying the clusters which are in the figure 5 based on yield and latex vessels density.

Relationship between yield and latex vessel rows

The correspondence regression equation is Yield = -52.75 + 14.25 No. of latex vessel rows. Black arrow; best fitted line. Red circle: cluster 1 (2011HP-42), green circle: cluster 2 (2011HP-202, 2011HP-297 and 2011HP-300) and yellow circle: cluster 3 (2011HP-256, 2011HP-53, 2011HP-183 and 2011HP-291).

The best fitted line which is in regression analysis between yield and number of latex vessel rows is showed positive correlation between yield and latex vessels density with having $R^2 = 91.7\%$ (0.917). The positive correlation is high, due to the higher $R^2$ value.

According to Gomez, (1982), when the latex yield is determined, number of latex vessel rows plays an important role.

There were three clusters in the graph based on yield and number of latex vessel rows (Figure 6). 2011HP-42 which was in cluster one acts as the genotype with high yield and higher number of latex vessel rows. Though 2011HP-202, 2011HP-297 and 2011HP-300 genotypes were selected as outstanding genotypes, now that genotypes are grouping the moderate (cluster 2). 2011HP-256, 2011HP-53, 2011HP-291 and 2011HP-183 genotypes which were categorized as low yielding genotypes have grouped in to cluster three with having low number of latex vessel rows. Out of four outstanding genotypes 2011HP-42 showed outstanding performances in yield and number of latex vessel rows.

According to two sample t-test, there is a significant difference between cluster one and cluster two, between cluster one and three and between cluster two and cluster three at $\alpha = 0.05$ significant level with verifying the clusters which are in the graph based on yield and number of latex vessel rows.
The correspondence regression equation is Yield = 364.1 – 28.14 Diameter of latex vessels. Black arrow: Best fitted line. Red circle: cluster 1 (2011HP-42), Purple circle: cluster 2 (2011HP-202 and 2011HP-297), green circle: cluster 3 (2011HP-300) and yellow circle: cluster 4 (2011HP-256, 2011HP-53, 2011HP-183 and 2011HP-291).

According to the regression analysis between yield and diameter of latex vessels, diameter of latex vessels negatively correlated with the latex yield with 57.4% (0.574) R-square value. That means, diameter of latex vessels is not affected to the latex yield increment.

Mihiran, (2018) showed that, there is a negative correlation between yield and diameter of latex vessels.

There were four clusters can be identified clearly in the Figure 7. Four outstanding genotypes were belong to cluster one, cluster two and cluster three.2011HP-42 which was in cluster one showed highest yield with lower diameter of latex vessels. But that genotype showed high characters with density of latex vessels (Figure 5) and number of latex vessel rows (figure 6). 2011HP-202 and 2011HP-297 which were in cluster two were already considered as outstanding genotypes due to the high yield, were showed lowest diameter of latex vessels. Though other four genotypes (2011HP-256, 2011HP-53, 2011HP-183 and 2011HP-291) were considered as lowest yielded genotypes, show higher diameter of latex vessels.

Two sample t-test was carried out and showed significant difference among clusters at 0.05 level (α). According to it there is a significant difference between each cluster with verifying the clusters which are in the graph based on yield and diameter of latex vessels.

**Cluster analysis for the bark characters**

![Figure 8: Cluster analysis with yield and bark anatomical characters (latex vessels density, number of latex vessel rows and diameter of latex vessels).](image-url)
Three clusters show as cluster 1, cluster 2 and cluster 3. Outstanding genotypes with high yielding are in the red color rectangular. Black arrow: Extremely outstanding genotype based on yield and all three bark anatomical characters.

Three clusters were identified clearly in above Figure 8. Although four outstanding genotypes were selected based on only latex yield, performances of those genotypes were differed when considered yield with bark anatomical parameters. Out of four outstanding genotypes, one was belong to cluster one (2011HP-42) and showed outstanding performances with yield and bark anatomical parameters. Two outstanding genotypes were belong to cluster two (2011HP-202 and 2011HP-297) with showing moderate performances with yield and bark anatomical parameter. 2011HP-300 which was showed high yield was belong to cluster three with all the low yielding genotypes. But 2011HP-300 genotype has deviated with higher distance than low yielding genotypes.

Cluster analysis for yield, girth and bark characters

Figure 9: Cluster analysis with yield, girth and bark anatomical parameters (latex vessels density, number of latex vessel rows and diameter of latex vessels).

Three clusters show as cluster 1, cluster 2 and cluster 3. Outstanding genotypes with high yielding are in the red color rectangular. Black arrow: Extremely outstanding genotype based on yield and all three bark anatomical characters.

There were three clusters in the above Figure 9. Out of four outstanding genotypes only 2011HP-42 separated from other three genotypes with genetically superior outstanding performances based on yield, girth and all bark anatomical parameters. Other three outstanding genotypes were belong to cluster two (2011HP-202, 2011HP-297 and 2011HP-300) with showing moderate performances base on yield, girth and all bark anatomical parameters. Low yielding genotypes belong to cluster three (2011HP-256, 2011HP-183, 2011HP-53 and 2011HP-291) showing lowest characters.

IV. CONCLUSIONS

Preliminary selected outstanding genotypes can be confirmed as precise selections. Among those outstanding genotypes the genotype 2011HP-42 can be categorized as most outstanding genotype.

Adding more yield parameters to the evaluation process, supported and confirm the precise selection at early stage.

Out of all bark anatomical characters, latex vessels density and number of latex vessel rows are more important foe evaluation.

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