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Growth Characters, Physiology and Leaf Fall Disease Resistance in Immature Plants of Rubber (*Hevea brasiliensis*) IRR 400 Series Clones

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Abstract: Superior rubber clones have been used in Indonesia, but productivity achievements are low. The area and rubber production in 2020 was 3,316,047 ha with a productivity of 1,022 kg/ha. The low productivity is caused use of seedling materials. Adaptation testing is one of the stages for selecting superior rubber clones in various environmental conditions, the influencing factors are clone or environmental. The suitability of clones to grow in certain environments with special characteristics can be seen due to changes in growth and differences in physiological characteristics that support growth. This research is one of the test areas for adaptation to growing environmental conditions, the topography is flat, with an altitude of 34 m above sea level, rainfall is 2195 mm/year and rainy is 156 days/year. The aim of the research is to determine the growth characteristics, anatomy, physiology of plants and resistance to leaf fall diseases (*Colletotrichum*, *Oidium*, *Corynespora* and *Pestalotiopsis*) of immature plant IRR 400 series. This research was carried out at PT Socfindo Tanah Besih, physiology laboratory and protection laboratory of the unit research Sungei Putih. The IRR 400 series clones, namely IRR 425, IRR 428, IRR 434, IRR 440 and two comparison clones (PB 217 and PB 330), were planted in 2019 with a spacing of 7.5×2.5 m. The research was structured using a randomized block design (RAK) with three replications, wherein in each replication a number of 20 trees were planted. The research results showed that the IRR 434 clone had vigor growth with a girth of 40.99 cm and a girth increment of 9.06 cm/year. Resistance to leaffall disease of *Corynespora*, *Colletotrichum*, *Oidium* and *Pestalotiopsis* clone IRR 400 is moderate. The physiology analysis showed different values among clones, the highest total sugar and SOD (IRR 428), the highest proline and H_2O_2 (IRR 434), the highest chlorophyll and SOD (IRR 425), the highest APX (IRR 440).

Keywords: IRR 400 Series, Growth, Physiology, Resistance, Immature Plant

Introduction

Superior rubber clones that produce latex reduce basic costs (price) resulting in higher profits (Pasaribu *et al*., 2023). Rubber is an important plantation commodity on a global scale, especially in Indonesia (Arifin, 2005). The development of rubber commodities as part of the plantation subsector aims to increase the country's foreign exchange earnings by creating an efficient and competitive rubber plantation industry (Barlow, 1997). Therefore, the general aim of rubber research must be to be able to develop appropriate technology to support the performance and sustainability of the productivity and efficiency of the national rubber industry (Pasaribu and Woelan, 2017).

The specific aim of the rubber plant breeding program is to produce clones that have high productivity, vigor growth, resistance to leaf fall disease and wide adaptation.

Community rubber productivity is currently 1,022 kg/ha, still low compared to the productivity of large state plantations (area 138,594 ha and productivity 1,152 kg/ha), as well as private plantations (area 7,710 ha and productivity 1,225 kg/ha). The low level of adoption of superior clones by planters is one of the obstacles to improving Indonesian rubber plantations, especially smallholder plantations. The quality of planting materials have correlation characters of physiology analysis and management estate (Woelan *et al*., 2013).

Rubber plant breeding has experienced significant progress in character improvement over 5 (five) generations, namely Generation 1st (G1) in 1910 to Generation 4th (G4) in 2010 and is now in the 5th Generation (G5) which took place in 2010-2035. Production potential 5 (five) times, ranging from 500 kg/ha/year in G1 to 3,000 kg/ha/year in G5, as well as resistance to disease attacks. This output can still be increased to 7,000-12,000 kg/ha/year by assembling superior clones and carrying out progressive breeding measures. Another advantage is that the immature period of plants can be shortened from six years to four years. The production of new superior clones is one of the parameters used to measure the progress of the rubber plant cultivation program.

Superior clones are plant genotypes that exceed standard genotypes in terms of yield potential, agronomic traits and secondary characteristics (resistance to disease) in commercial plantings. New superior clones of the IRR series, including the 00, 100, 200, 300 and 400 series, have been produced since the fourth generation. The IRR 400 series rubber clone was selected from crossing activities in 1992. The IRR 400 series IRR clone was produced from crossing 31,120 genotypes which produced 828 genotypes of F1 offspring (Daslin, 2012).

Clone selection and testing activities are carried out in stages, starting from progeny testing on the seed population resulting from the cross and continuing with promotional clone trials, small-scale clone trials and further trials as well as adaptation trials. To explore the potential advantages of a clone, the breeding stages must be carried out systematically and continuously, so that the adaptation trial is the final stage of the selection cycle to determine which clone will be produced. This aims research to select IRR 400 series clones in the adaptation trial in the immature stage to growth characteristics, anatomy, plant physiology and resistance to leaf fall diseases (*Colletotrichum*, *Oidium*, *Corynespora* and *Pestalotiopsis*).

Materials and Methods

Experimental Site

This research was conducted at PT Socfindo Tanah Besih's plantation in Tebing Syahbandar District, Serdang Bedagai Regency, North Sumatra and the Sungei Putih research unit's physiology and Protection laboratory in Galang, Deli Serdang Regency, North Sumatera, Indonesia. The topography is flat, with an altitude of 34 m above sea level, rainfall is 2195 mm/year and rain is 156 days/year.

Experimental Design

The clones tested are four of the IRR 400 series, namely IRR 425, IRR 428, IRR 434 and IRR 440 with two control clones, namely PB 217 and PB 330. Field experiments are a random design group with three replications. The clone was planted with 60 trees/plot with a spacing of 7.5×2.5 m. The number of plants observed is 20 plants, so the total number of plants observed is 360 plants.

The chemical material used is KOH 3%, alcohol 96%, sudan III, TCA 2.5%, sulfosilicic acid, ninhydrin ($C_9H_6O_4$), glacial acetic acid (CH_3COOH), phosphoric acid (H_3PO_4), toluene (C_7H_8), potassium dihydrogen phosphate (KH_2PO_4), dipotassium hydrogen phosphate (K_2HPO_4), EDTA ($C_{10}H_{14}N_2Na_2O_8 2H_2O$), L-methionine ($C_5H_{11}NO_2S$), nitro blue tetrazolium chloride ($C_{40}H_{30}Cl_2N_{10}O_6$), riboflavin ($C_{17}H_{20}N_4O_6$), hydrogen peroxide ($H_2O_2$), potassium iodide (KI), TCA, calcium chloride ($CaCl_2$), HEPES buffer, Mess buffer, Sodium Hydroxide (NaOH), Hydrochloric Acid (HCl), pVPP, liquid nitrogen, L-proline, CTAB buffer, Tris HCL buffer and EDTA. The tools used are an autoclave, beaker glass, Erlenmeyer, object glass, deck glass, measuring cup, scissors, hand sprayer, hot plate, analytical balance, water bath, vortex, UV spectrophotometer, gloves, mask, tissue, distilled water, mortar, test tube, goblet, micropipette 1 mL and 100 Mirko, stirrer, 15-w lamp, centrifuge and others.

Growth Characters

Growth was observed six times every four weeks. Girth observations were measured at a height of 130 cm above growth level using a cloth meter (100 cm size). Each clone was calculated based on plant height as well as the height of the main branch and the number of primary branches. Plant height was measured from the base stem to the growing point, while the height first branch was measured from the base stem to the main branch using a scaled wooden meter. Primary branches were counted by the number of branches that grow from the main stem. Wood production potential was calculated using the formula developed by Aditya *et al*. (2015).

Leaf Disease Resistance

Leaf fall disease resistance was observed two times in early January 2023 and late June 2023. Field observations were carried out directly in the experimental garden and were like taking samples of sick plants. Plant samples taken from each clone were 5 leaves/plant clones.

Assessment of the scale of leaf spots and defects for the four leaf fall diseases was carried out using the formula developed by Purwantara and Pawirosoemardjo (1991). Four types of leaf fall diseases were observed in this study, namely *Oidium*, *Colletotrichum*, *Corynespora* and *Pestalotiopsis*.

Physiological Characters

Leaf sugar (mm) was measured using the Dische method (1962), Samples were taken 150 microliters plus TCA 2.5% to a total of 500 microliters, 3 mL of Anthrone reagent and vortex, heated by immersing in boiling water for 15 min, cooled by immersing in water and absorbance is measured at a wavelength of 627 nm.

Chlorophyll a, chlorophyll b and total chlorophyll using the Hendry and Grime method, total chlorophyll and carotenoid contents were measured using spectrophotometric methods. The leaves of aquatic plants were crushed with a mortar, then the weight was measured as 1 g. The crushed sample (slurry) was then extracted with 100 mL of 80% acetone and stirred until the chlorophyll and carotenoids dissolved. The extract was filtered with filter paper. The filtrate was placed in a cuvette and then the absorbance value was measured using a UV VIS spectrophotometer at wavelengths of 480, 645, 646 and 663 nm to test the chlorophyll and carotenoid content (Hendry and Grime, 1990). After obtaining the absorbance value, the chlorophyll content is calculated using the following formula:

$$Chlorophylla\left(mg/\mathrm{m}\right)=\left(\left(12.7\times A663\right)-\left(2.69\times A645\right)\right)\times 10\text{-}1 \quad (1)$$

$$Chlorophyllb\left(mg/m\right)=\left(\left(22.9\times \mathrm{A645}\right)-\left(4.68\times \mathrm{A663}\right)\right)\times 10\text{-}1 \quad (2)$$

$$Total\,chlorophyll\left(mg/m\right)=\left(\left(8.02\times \mathrm{A663}\right)-\left(20.2\times \mathrm{A645}\right)\right)\times 10\text{-}1 \quad (3)$$

Notes:

A480 = Absorbance at a wavelength of 480 nm
A645 = Absorbance at a wavelength of 645 nm

Proline was measured using the method (Bates, 1973) leaf pieces were taken at 0.2 g, then ground with liquid nitrogen and the frozen plant material was homogenized in 3% aqueous sulfosalicylic acid (0.01 g/0.5 mL) and the residue was removed by centrifugation at 12000 rpm for 10 min. Proline levels were analyzed based on the method of Bates *et al*. (1973) using a spectrophotometer with pure proline as a standard. Ninhydrin acid was prepared as a reagent by dissolving 1 g of ninhydrin in 30 mL of glacial acetic acid in a test tube for 1 h at 100°C and the reaction was stopped in an ice bath. The reaction mixture was extracted with 2 mL of toluene, mixed vigorously and left at room temperature for 30 min until separation of the two phases. The solution was then cooled on ice for 5 min, the solution was extracted with 4 mL of toluene until chromo form was formed. Meanwhile, as a standard, D-proline (sigma) 0.1-3.0 mm was used dissolved in 3% sulfosalicylic acid in the same way as was done for the sample plants. The absorbance of the solution was read at a wavelength of 520 nm. Proline concentration was determined from the D-proline standard.

Super peroxidase Dismutase (SOD) enzyme was assessed using the (Bradford, 1976) method, the leaves were crushed 0.2 g with PVP and liquid nitrogen until they became powder and then put into a tube containing 1 mL $CaCl_2$. Then centrifuge at 10,000 rpm and 4°C for 10 min. $CaCl_2$ 0.5 m solution was made by dissolving $CaCl_2$ as much as 5.55 g into 100 mL of distilled water. Solution A was prepared by dissolving 1.458 g of phenol and 0.045 g of 4-dimethylaminoantipyrine into 90 mL of distilled water. MES buffer was prepared by dissolving 0.293 g MES into 75 mL distilled water and then divided into three parts of 25 mL each. The solution was optimized at pH 5.5; 6.0; 6.5 by adding 1 m NaOH. Then tested for peroxidase activity with a UV/VIS spectrophotometer. HEPES buffer was prepared by dissolving 0.357 g HEPES into 75 mL distilled water and then divided into three parts of 25 mL each. The solution was optimized at pH 7.0; 7.5; 8.0 by adding 1 m NaOH. Then tested for peroxidase activity with a UV/VIS spectrophotometer. Peroxidase activity test of HEPES and MES obtained the highest result in MES buffer solution pH 5.5 with peroxidase activity 0.79. Solution B was made by means of MES solution pH 5.5 mixed with 30% H_2O_2 solution of as much as 1.5 mL with a final level of 0.01 m. Prepared test tubes and then entered solution A as much as 1.4 mL and solution B as much as 1.5 mL. 200 µL of sample was entered. In the blank solution, no enzyme extract (sample) was added. The solution was read using a UV/VIS spectrophotometer at a wavelength of 510 nm at 0 and 2 min.

Peroxidase Dismutase (POD) enzyme using the SOP, plant peroxidase activity determination, 1994 method, Extract buffer was made by mixing 10 mL of 1 mm EDTA solution with 50 mL of phosphate buffer pH 7.6. Then distilled water was added to 100 mL. Leaves were crushed 0.2 with 0.1 g PVP and liquid nitrogen until they became powder and then put into a tube containing 1 mL of extract buffer. Then centrifuge at 10,000 rpm and 4°C for 10 min. A methionine 13 mm solution was made by weighing methionine as much as 19.3973 mg and dissolving it into 10 mL distilled water. EDTA 0.1 mm solution was made by weighing EDTA as much as 1.1167 mg and dissolved in 30 mL distilled water. NBT 75 mm solution was made by weighing NBT 0.61323 mg and dissolved in distilled water 10 mL. A riboflavin 2 mm solution was made by weighing riboflavin as much as 0.00752 mg and dissolving it in distilled water 10 mL. A test tube was prepared and 750 µL extract buffer, 50 µL methionine solution, 50 NBT, 150 EDTA and 100 µL enzyme extract were added. In the blank solution, no enzyme extract (sample) was added. Then brought to the light condition of 15 w. Added 50 µL riboflavin and allowed to stand for

15 min. Added 50 µL riboflavin and allowed to stand for 15 min. 350 µL sterile distilled water was added. The solution was stirred and then read with a UV/VIS spectrophotometer at a wavelength of 510 nm.

Ascorbate Peroxidase Enzyme (APX) was assessed by Nakano and Asada (1981). The leaf samples used were fully developed leaves. Analysis was observed based on the method conducted by Nakano and Asada (1981). Extract buffer was prepared by putting 1 mL of 0.5 mm ascorbic acid into a tube. Leaves were crushed 0.2 with 0.1 g PVP and liquid nitrogen until they became a powder and then put into a tube containing 1 mL of extract buffer. Then centrifuge at 12000 rpm and 4°C for 15 min and take 100 µL of enzyme extract. Added 300 µL 50 mm phosphate buffer pH 7 and 400 µL 0.5 mM ascorbic acid, 300 µL 0.1 mm EDTA and 400 µL H_2O_2. No enzyme extract (sample) was added to the blank solution. APX activity measurements were read with a UV/VIS spectrophotometer every 10 sec for 1 min at a wavelength of 290 nm. APX activity is expressed in units/mg protein.

Hydrogen peroxide (H_2O_2) using the Bradford (1976) method. H_2O_2 analysis was performed by Bradford (1976) by mixing 0.2 g sample extract with 1 mL Trichloroacetic Acid (TCA), then centrifuged at 12,000 rpm for 15 min. The enzyme extract was taken 200 µL and added with 0.5 mL of 10 mm potassium phosphate buffer pH 7 and 1 mL of KI. The blank solution used was 1.5 mL H_2O_2 and 200 µL extract buffer. The measurement of hydrogen peroxide activity was calculated with a UV/VIS spectrophotometer at a wavelength of 390 nm.

Data Analysis

The data for all the parameters collected in the study were subjected to ANOVA. Means were separated using the Tukey test at 5% while to see the relationship between traits observed, correlation analysis was carried out using SPSS 29 software. Results were presented in tables and graphs.

Results

Growth Characters

The mean of girth (cm) and girth increment (cm/year) of IRR 400 series dan clone control are presented in Table 1. Based on Table 1, no clones had significantly different girth growth during the 1st, 2nd and 3rd months of observation, but in 4th and 5th months observation was significant among IRR 400 series and clone control. IRR 434 has vigor girth (39.30 cm), then followed by IRR 440 (40.6 cm), IRR 425 (39.8 cm), PB 330 (39.6 cm), PB 217 (35.8 cm) and IRR 428 (35.3 cm). The girth increment showed IRR 434 have higher (4.54 cm/6) per month, furthermore PB 330 (4.23 cm/6 month), IRR 440 (3.90 cm/6 month), IRR 425 (3.75 cm/6 month), PB 217 (2.91 cm/6 month) and IRR 428 (2.71 cm/ 6 month).

Plant height characteristics were observed to assess prospective wood production (m^3/tree). In this study, the plants' heights were measured twice: Once at the beginning and once at the conclusion. The IRR 400 series clones' plant heights are detailed in Table 2.

Table 2 shows the plant height (m) differs significantly. Clone IRR 425 had the greatest initial plant height (8.44 m), then followed by IRR 440 (7.14 m), IRR 434 (7.12 m), IRR 424 (6.80 m), PB 330 (6.78 m) and PB 217 (6.70 m). Similarly, IRR 425 had the highest final plant height (9.97 m), followed by IRR 440 (9.15 m), IRR 434 (8.87 m), PB 330 (8.80 m), IRR 428 (8.67 m) and PB 217 (8.64 m). The average plant height increase after 6 months ranged between 1.530 and 2.023 m. During 6 months of observation, the fastest plant height is two clones, namely PB 330 (2.023 m) and IRR 440 (2.010 m).

Table 1: The girth (cm) and girth increment (cm/6 month) of IRR 400 series and clone control in the immature period

	Girth						
	Month-						Girth increment
Clones	1	2	3	4	5	6	(cm/6 month)
IRR series 400							
IRR 425	36.00±2.65^{a}	36.8±2.43^{a}	37.4±2.34^{a}	38.1±2.47^{ab}	38.7±2.51^{ab}	39.8±2.41^{a}	3.75±0.24^{a}
IRR 428	32.06±1.95^{a}	33.1±2.12^{a}	33.5±2.07^{a}	33.9±2.009^{b}	34.3±2.026^{a}	35.3±2.43^{a}	2.71±0.51^{a}
IRR 434	36.04±2.42^{a}	37.2±2.43^{a}	38.2±2.16^{a}	39.3±1.099^{a}	39.5±2.025^{a}	40.9±2.40^{a}	4.53±1.01^{a}
IRR 440	36.07±0.61^{a}	37.2±0.71^{a}	38.0±0.97^{a}	38.6±0.39^{ab}	39.3±0.32^{ab}	40.6±0.10^{a}	3.90±0.57^{a}
Control clones							
PB 217	32.09±1.71^{a}	33.3±2.11^{a}	33.7±2.11^{a}	34.0±2.36^{ab}	34.2±1.082^{a}	35.8±2.81^{a}	2.91±1.10^{a}
PB 330	35.38±2.33^{a}	36.0±1.70^{a}	37.2±1.64^{a}	37.9±1.82^{ab}	38.4±1.84^{ab}	39.6±1.67^{a}	4.23±1.52^{a}

Mean values are significantly different from control at p≤0.05

Table 2: Plant height (m) and height increment (m/6 month) of IRR 400 series and clone control in the immature period

Clones	Initial plant height (m)	Final plant height (m)	Height increment (m/6 month)
IRR series 400			
IRR 425	8.443±0.52^{a}	9.970±0.69^{a}	1.530±0.021^{c}
IRR 428	6.800±0.31^{b}	8.677±0.30^{b}	1.887±0.01^{ab}
IRR 434	7.127±0.05^{b}	8.877±0.09^{b}	1.757±0.08^{bc}
IRR 440	7.147±0.25^{b}	9.153±0.27^{b}	2.010±0.005^{a}
Control clones			
PB 217	6.703±0.23^{b}	8.640±0.31^{b}	1.937±0.10^{ab}
PB 330	6.780±0.05^{b}	8.800±0.08^{b}	2.023±0.004^{a}

*Mean values are significantly different from control at p≤0.05

Table 3: Potential timber production of IRR 400 series, clones control in immature period and estimated in 20 years

	Potential timber (m^3/tree)		
Clones	Volume total log	Volume total wood	Estimate timber production (20 years) (m^3/tree)
IRR series 400			
IRR 425	0.04±0.04	0.13±0.002	2.08
IRR 428	0.03±0.04	0.09±0.011	1.44
IRR 434	0.04±0.03	0.12±0.014	1.92
IRR 440	0.04±0.03	0.12±0.004	1.92
Control clones			
PB 217	0.03±0.03	0.09±0.003	1.44
PB 330	0.04±0.04	0.11±0.004	1.76

Table 4: The intensity resistance of leaf fall disease (*Crynespora*, *Colletotrichum*, *Pestalotiopsis*, *Oidium*) of IRR 400 series and clone control in the immature period

Clones	Diseases	Intensity attack (%)	Resistance value
IRR 425	Corynespora	0.00	Resistant
	Colletotrichum	15.33	Resistant
	Pestalotiopsis	18.33	Resistant
	Oidium	13.50	Resistant
IRR 428	Corynespora	0.00	Resistant
	Colletotrichum	15.00	Resistant
	Pestalotiopsis	6.67	Resistant
	Oidium	20.00	Resistant
IRR 434	Corynespora	0.00	Resistant
	Colletotrichum	25.00	Moderate resistant
	Pestalotiopsis	15.00	Resistant
	Oidium	28.33	Moderate resistant
IRR 440	Corynespora	1.67	Resistant
	Colletotrichum	10.00	Resistant
	Pestalotiopsis	20.83	Moderate resistant
	Oidium	8.33	Resistant
PB 217	Corynespora	0.00	Resistant
	Colletotrichum	8.33	Resistant
	Pestalotiopsis	15.00	Resistant
	Oidium	14.33	Resistant
PB 330	Corynespora	0.00	Resistant
	Colletotrichum	23.33	Moderate resistant
	Pestalotiopsis	8.33	Resistant
	Oidium	26.67	Moderate resistant

The potential timber production of IRR 400 series clones in 4 years (planted in April 2019) is in Table 3, based on the formula proposed by Aditya *et al*. (2015). Table 3 shows that the average volume of logs produced at 4 years is 0.04 m^3/tree and the average timber total volume is 0.11 m^3/tree. The highest timber potential was produced by the IRR 425 (0.13 m^3/tree) while the lowest is IRR 428 (0.09 m^3/tree). Based on the type of rubber clone when viewed volume timber, IRR 400 series planted if rejuvenated (20 years) in this study are latex-timber clones and timber-producing rubber is medium timber production (>1.5 m^3/tree).

Leaf Disease Resistance

The leaf fall disease resistance in the field of IRR 400 seria and clones control *(Corynespora, Colletotrichum, Pestalotiopsis and Oidium)* showed varies, with the majority being resistant to all leaf fall diseases (Table 4). Based on the intensity resistance in the field, all clones classified are resistant to *Corynespora* leaf fall disease. This is indicated by the percentage of disease intensity <20%. *Colletotrichum* leaf fall disease, all clones are resistant except IRR 434 (25%) and PB 330 (23.33%). *Oidium* leaf fall disease, IRR 425 and IRR 428 are classified as resistant with a percentage of 20.83%. IRR 440 and PB 217, IRR 434 and PB 330 were classified as moderate resistant with percentages 26.67-28.33%. *Pestalotiopsis* leaf fall disease is classified as resistant to IRR 425, IRR 428, IRR 434, PB 217 and PB 330 with a percentage <20% and IRR 440 is classified as moderate resistant with a percentage 20.83%.

Table 5: Physiological characters of IRR 400 series and control clone in immature period

Clones	Chlorophyll a (µg/mL)	Chlorophyll b (µg/mL)	Chlorophyll total (µg/mL)	Sugar total (µm)	SOD (unit/mg)	POD (unit/mg)	APX (unit/mg)	Prolin (mg/L)	H_2O_2 (µmoL/g)
IRR 400 series									
IRR 425	0.626±0.15^{a}	1.141±0.28^{ab}	1.767±0.43^{ab}	130.693±19.57^{a}	1.998±0.32^{a}	1.354±0.38^{a}	0.460±0.28^{a}	8.278±0.81^{abc}	1.013±1.01^{a}
IRR 428	0.559±0.16^{ab}	1.016±0.29^{ab}	1.575±0.44^{ab}	131.303±18.42^{a}	1.590±0.29^{a}	1.502±0.52^{a}	0.403±0.15^{a}	11.980±2.05^{ab}	1.058±1.06
IRR 434	0.344±0.03^{b}	0.629±0.06^{b}	0.973±0.09^{b}	72.910±08.74^{bc}	1.830±0.35^{a}	1.326±0.45^{a}	0.413±0.18^{a}	14.111±4.88^{a}	0.922±0.92
IRR 440	0.425±0.08^{ab}	0.776±0.15^{ab}	1.201±0.23^{ab}	49.127±08.78^{c}	1.946±0.41^{a}	1.369±0.59^{a}	0.480±0.15^{a}	6.358±1.18^{bc}	1.061±1.00
Control clones									
PB 217	0.569±0.14^{ab}	1.037±0.26^{ab}	1.607±0.40^{ab}	112.037±14.02^{ab}	1.401±0.54^{a}	1.441±0.61^{a}	0.500±0.31^{a}	6.448±0.55^{bc}	1.203±1.20
PB	3300.635±0.15^{a}	1.151±0.28^{a}	1.786±0.43^{a}	95.657±07.98^{bc}	1.485±0.67^{a}	1.470±0.66^{a}	0.540±0.40^{a}	5.729±0.69^{c}	1.147±1.15

*Mean values are significantly different from control at p≤0.05

Physiological Characters

The research studies the physiological characteristics of the IRR 400 series and clon control. The observations are shown in Table 5, which identifies initial physiological characteristics in the immature period. Based on the physiological analysis, the chlorophyll content analysis revealed the significance of each clone. PB 330 (0.63 µg/mL) has a high chlorophyll content, followed by IRR 425 (0.62 µg/mL), PB 217 (0.56 µg/mL), IRR 428 (0.55 µg/mL), IRR 440 (0.42 µg/mL), IRR 434 (0.34 µg/mL). Chlorophyll b content analysis is significantly different for each clone. PB 330 (1.15 µg/mL) has a high chlorophyll b content, followed by IRR 434 clone (0.62 µg/mL), IRR 425 (1.14 µg/mL), PB 217 (1.03 µg/mL), IRR 428 (1.01 µg/mL) and IRR 440 (0.77 µg/mL). Total chlorophyll was also significantly different for each clone. PB 330 (1.786 µg/mL) has a high chlorophyll total content, followed by IRR 434 (0.973 µg/mL), IRR 425 (1.76 µg/mL), PB 217 (1.60 µg/mL), IRR 428 (1.57 µg/mL) and IRR 440 (1.20 µg/mL). The total sugar content analysis is significantly different for each clone. IRR 428 (131.3 µm) and IRR 425 (130.6 µm) had the highest total sugar content, than PB 217 (112.037 µm), PB 330 (95.65 µm), IRR 440 (72.9 µm) and IRR 434 (49.1 µm).

The antioxidant content of the IRR 400 series clone was examined, including Superoxide Dismutase (SOD), Peroxide Dismutase (POD), Ascorbate Peroxidase (APX) enzymes, Hydrogen Peroxidase (H_2O_2) enzyme and proline content. The SOD content is not significantly different for each clone. The highest SOD was observed in RR 425 (1.99 unit/mg), followed by IRR 440 (1.94), IRR 434 (1.83), IRR 428 (1.59), PB 330 (1.48) and PB 217 (1.40 unit/mg). the POD content is not significantly different for each clone, IRR 428 has the highest (1.50 unit/mg), followed by PB 330 (1.47), PB 217 (1.44), IRR 440 (1.36), IRR 425 (1.35) and IRR 434 (1.32 unit/mg). The APX content is not significantly different for each clone, the highest APX content is PB 330 (0.54 unit/mg), followed by PB 217 (0.50 unit/mg), IRR 440 (0.48 unit/mg), IRR 425 (0.46 unit/mg), IRR 434 (0.41 unit/mg) and IRR 428 (0.40 unit/mg). The hydrogen peroxidase (H_2O_2) analysis is not significantly different in all clones. The highest H_2O_2 content is PB 217 (1.203 µmoL/g), followed by PB 330 (1.147 µmoL/g), IRR 440 (1.061 µmoL/g), IRR 428 (1.058 µmoL/g), IRR 425 (1.013 µmoL/g) and IRR 434 (0.922 µmoL/g). The proline levels are significantly different of each clone significantly different for each clone. IRR 434 has the highest proline content (14.11 mg/L), followed by IRR 428 (11.98 mg/L), IRR 425 (8.27 mg/L), PB 217 (6.44 mg/L), IRR 440 (6.35 mg/L) and PB 330 (5.72 mg/L).

Discussion

Growth Character

The growth characteristics observed are girth, girth increment, plant height, plant height increment and timber potency. The standard growth criteria in the immature period based on the size girth growth normally is 37-40 cm (4 years). De Castro Sant'Anna *et al*. (2020), the character of the girth at the time of the immature plant period has a positive correlation with latex yield. Siagian and Siregar (2014), girth growth is one of the important characteristics in the selection of superior rubber clones. Rubber clones have vigor growth and are expected to shorten the immature period to open tapping. The increase in the trunk circumference can be expected to produce higher production. This happens because the size of girth that increases with the increasing age of the plant causes the slice of the tapping field to get longer which affects the yield.

Aidi-Daslin (2005) said that vigor growth has fast initial growth during the immature period with an average increase in girth of more than 11 cm/year. In this study, the average girth is estimated to be tapped at the age of 4.5-5 years. The girth increment of rubber plants for tapping maturity is influenced by rate growth during the immature period. Measurement of girth is a parameter that is often used in evaluating immature period growth. The growth rate of girth will be illustrated by plant growth which will ultimately affect the immature period. Clones with fast growth rates will also affect the potential for timber production during rejuvenation (Daslin, 2012).

The increment girth in the immature period is influenced by certain agro-climatic/regional conditions. The IRR 400 series clones tested in this study have improved when compared to the research of Suhendry and Azwar (1998), who reported that areas with moderate rainfall of 1,500-2,500 mm/year accompanied by 2-3 firm dry months have an immature period of 5.5-6.0 years.

In general, Table 1 shows that there is a significant difference between clones in the observation of girth from 1st month until 6th month. From 1st month until 3rd did not show a real difference in each clone. This is due to the plant canopy experiencing leaf fall during the dry season. Rainfall affects the availability of soil water. During the dry season, rainfall decreases so that water becomes a limiting factor for plant growth. With the limited water during the dry season, rubber plants adapt to reduce transpiration by shedding their leaves following the statement of Junaidi, (2015), natural leaf fall/leaf shedding is a physiological process of rubber trees responding to insufficient water in the dry months each year. The condition of rubber trees in the field can be seen in Fig. 1.

Leaf Disease Resistance

There were differences in intensity resistant at the beginning and end of the observation. The average intensity resistance of *Colletotrichum, Pestalotiopsis and Oidium* leaf fall diseases at the end of the observation (July 2023 with 170-200 mm rainfall) was greater than at the beginning of the observation (February 2023 with 50-100 mm rainfall). Disease development is greatly assisted by air humidity and rainfall. In very humid weather the fungus forms many spores on diseased plant parts because infection is assisted by high humidity.

Febbiyanti and Fairuza (2019) said that the leaf fall disease will develop rapidly during the rainy season and when the plant is under pressure from other diseases. Overall the IRR 400 series is resistant to *Corynespora, Colletotrichum, Oidium and Pestalotiopsis* leaf fall diseases. Rubber plant productivity is strongly influenced by genetic factors, physical environment and biological environmental conditions. Biological environmental factors in the form of leaf fall disease caused by the fungi *Colletotrichum* sp., *Corynespora* sp., *Oidium* sp. *and Pestalotiopsis* sp. greatly affect the productivity of rubber plants (Purwantara and Pawirosoemardjo, 1991).

Information on disease resistance is critical to completing the clone recommendation. Previous research has shown that disease resistance can reduce productivity by 7-40% (Nasution *et al*., 1990). Long-term leaf fall disease resistance can reduce productivity by up to 40% and cause plant death (Nasution *et al*., 1990). Based on observations in this study and grouping by based on the magnitude of the disease resistant, the IRR 400 series has a moderate resistance to *Corynespora, Colletotrichum, Oidium and Pestalotiopsis* leaf fall diseases. This is also consistent with Woelan (2009) research on the performance of rubber clones in the IRR 400 series. Figure 2 shows the field conditions of plants affected by *Colletotrichum, Pestalotiopsis* and *Oidium* leaf spot.

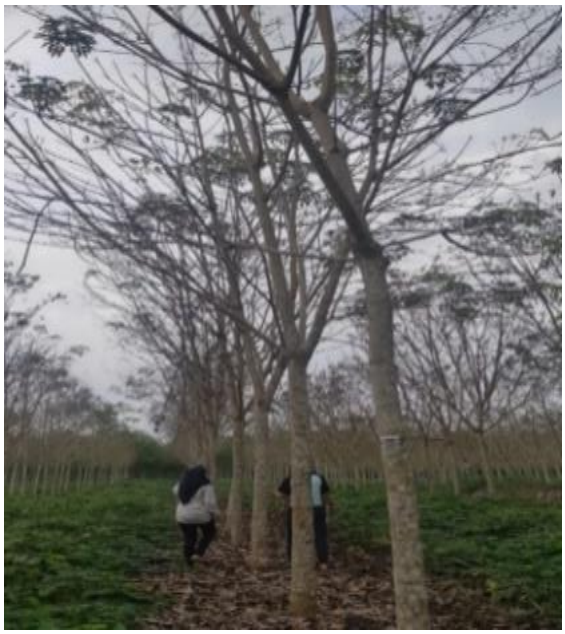

Fig. 1: Rubber trees experience natural leaf fall during the dry season

Fig. 2: Leaves affected by *Pestalotiopsis*, *Colletotrichum* and *Oidium* in the field

Physiological Characters

This study observed chlorophyll a, chlorophyll b and total chlorophyll. Chlorophyll is very important in the photosynthesis process because it supports the production of carbohydrates needed for latex formation. Photosynthesis is an important process in theformation of food (carbohydrates) for plants assisted by chlorophyll a and b. High lightintensity tends to increase chlorophyll production in plants. This is because chlorophyllis needed to capture the light energy needed in the photosynthesis process. Plants receive sufficient light, chlorophyll production can increase to support this process. An increase in total chlorophyll in plants, which is a

combination of chlorophyll a and chlorophyll b, is often associated with high light intensity. Plants exposed to sufficient light can produce more chlorophyll to support a more efficient photosynthesis process. Healthy plants with efficient photosynthesis rates tend to have better yields.

Total sugar content is also important to observe because sugar content in rubber plants can affect latex production. Sugar is one of the results photosynthesis process which is very important to provide energy and raw materials for latex formation. The higher of total sugar content has better energy availability for latex production and plant growth (Anasrullah *et al*., 2023). Total sugar content also affects disease resistance as it can influence the response of the plant's defense system to pathogen attack. Plants have sufficient sugar availability is can increase disease resistance. Plants experience stress the sugar content can change. Morkunas and Ratajczak (2014), someplants increased when facing certain stresses. Plants are exposed to abiotic stress,total sugar content and metabolic enzyme changes (De Souza *et al*., 2018). Various solutes will be recycled and metabolized into sugar accumulation which is considered an important energy source in growth recovery. Plants often experience an increase intotal sugars in reaction to stress conditions or certain growth phases, as well as nutrientavailability and growth phases.

The observation of antioxidant enzymes needs to be observed because antioxidant enzymes such as Superoxide Dismutase (SOD), Peroxidase (POD), Ascorbate Peroxidase (APX), Hydrogen Peroxidase (H_2O_2) and proline have an important role in the plant defense system against oxidativestress. Oxidative stress can occur due to an imbalance between the production of free radicals (such as superoxide radicals and hydrogen peroxide) and the plant's ability to remove free radicals using antioxidant enzymes, so plant cells activate various sensors due to stress which will then activate various signal pathways. Antioxidants are molecules that can inhibit the oxidation of other molecules. Oxidation reactions can produce achain of free radicals that can cause damage or cell death. Antioxidants stop this chain reaction by removing free radical intermediates and inhibiting other oxidation reactions (Arifin, 2005).

Antioxidant enzymes such as SOD, POD and APX play a role in enhancing latex production as they can provide protection against oxidative stress SOD is responsible for converting superoxide into hydrogen peroxide which is then removed by other enzymes such as POD and APX. By reducing oxidative stress levels, these enzymes can help maintain the metabolic processes necessary for latex production.

Antioxidant enzymes can also have a direct impact on several metabolic pathways involved in latex production. Excessive oxidative stress can disrupt these pathways and antioxidant enzymes can help maintain the balance. The adaptation mechanism of plants under stressful conditions is by an increase in the activity of Catalase (CAT), Superoxide Dismutase (SOD) and Peroxidase (POD) enzymes (Tang *et al*., 2019) that suppress oxidative damage caused by ROS. Antioxidant enzymes also play an important role in overcoming oxidative stresscaused by pathogen attacks, with the presence of these antioxidant enzymes, plant resistance to leaf fall disease also involves a variety of good defense mechanisms (Khompatara *et al*., 2019).

The H_2O_2 content of the IRR 400 series is observed in this study. This is because, in plants, the accumulation of Reactive Oxygen Species (ROS) such as hydrogen peroxide (H_2O_2) is one of the initial responses of plants to environmental stress and causes damage to various biological processes. Sharma and Dubey (2005), increased SOD activity is associated with decreased levels of H_2O_2 by increasing antioxidant activity and several non-enzymatic compounds. By looking at the levels of H_2O_2 and antioxidant compounds SOD is expected to determine the extent to which plants adapt to overcome stress. The important activity of metabolic protection when plants experience stress is due to oxidative stress and antioxidant abundance.

The proline content is observed because the tolerance mechanism is described by the value of proline content, which allows to maintenance of the growth and productivity of a plant. Plants experience environmental stress, proline production often increases to help plants survive unfavorable conditions. Proline is an osmolyte that plays a role in maintaining osmotic balance and response to environmental stress, such as drought or extreme temperatures. A high proline content in rubber plants can help reduce the impact of certain environmental stresses and thus provide better growth. High proline content has been associated with better plant response to pathogenattack increasing disease resistance. Proline may play a role in enhancing the plant's defense system against pathogens and provide protection against oxidative stress associated with pathogen attack.

Conclusion

IRR 434 has vigorous growth at four years with a girth of 40.99 with a girth increase of 9.06 cm/year. IRR 400 series are categorized as moderately resistant to leaf fall disease (*Corynespora, Colletotrichum, Oidium* and *Pestalotiopsis*). In future research, the exact mechanism of resistance needs to be further investigated. Early physiological characters were identified in the IRR 400 series during in immature period, the highest total sugar IRR 428, the highest proline by IRR 434, the highest chlorophyll a, chlorophyll b and total chlorophyll content IRR 425,

the highest SOD IRR 425, the highest POD IRR 428, the highest APX content IRR 440 and the lowest H_2O_2 IRR 434.

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Author's Contributions

Rahayu Novrina Rosa: Conceive and designed the analysis. Collected the data. Written the manuscript.

Mohammad Basyuni: Designing research on growth characteristics and leaf fall disease resistance. Written and reviewed a drafted manuscript with the research team and submitted the manuscript to Biodiversity.

Luthfi Aziz Mahmud Siregar: Designed a physiological character study. Reviewed the article.

Syarifah Aini Pasaribu: Assisted implementation in the field and coordinated the data-analysis.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and that no ethical issues are involved.

Competing Interests

The authors report no conflicts of interest regarding this study.

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