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Genotypic and Phenotypic Correlations between Leaf-Rust Disease and Leaf Morphology and its Ratio in Arabica Coffee

(Korelasi Genotipik dan Fenotipik antara Penyakit Karat Daun dan Morfologi Daun dan Rasionya pada Kopi Arabica)

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ABSTRACT

Selection parameter in coffee breeding for leaf rust (*Hemeleia vastatrix*) resistance is very important. Breeders used leaf-rust severity and leaf-rust incidence as parameters of direct selection. However, scientific proof is not yet available whether leaf morphology can be used as a parameter of indirect selection. The objective of this research was to seek the possibility of leaf morphology parameter and its ratio to be used as selection criteria through analyses of genotypic and phenotypic correlations of parameter of rust disease and parameter of leaf morphology and its ratio. The result revealed that genotypes showed significant variations in leaf-rust severity (5.21–25.84%), leaf morphology, and leaf-morphology ratio. Leaf length to leaf width ratio, leaf length to leaf area ratio, and leaf width to leaf weight ratio were not affected by the environment. Leaf-rust severity performed highly significant positive genotypic and phenotypic correlations the ratio of with leaf length to leaf area. For selection criteria, leaf-rust severity could be better used rather than leaf-rust incidence and branch-rust incidence. The ratio of leaf length to leaf area could also be used as an indirect selection criterion because the ratio showed a highly significant genotypic correlation with leaf-rust severity (r_{Gab} = 0.254). However, the ratio of leaf length to leaf area is even better chosen for selection criteria rather than leaf-rust severity because the ratio was not affected by the environment.**

Keywords: fungus, *Hemeleia vastatrix,* indirect selection

ABSTRAK

Parameter seleksi dalam pemuliaan kopi untuk ketahanan karat daun (*Hemeleia vastatrix*) sangat penting. Pemulia menggunakan keparahan karat daun dan insiden karat daun sebagai parameter seleksi langsung. Akan tetapi, bukti ilmiah belum tersedia apakah morfologi daun dapat digunakan sebagai parameter seleksi secara tidak langsung. Tujuan penelitian ini adalah untuk mencari kemungkinan apakah parameter morfologi daun dan rasionya dapat digunakan sebagai kriteria seleksi melalui analisis korelasi genotipik dan fenotipik parameter penyakit karat dan parameter morfologi daun dan rasionya. Hasil penelitian menunjukkan bahwa genotipe menunjukkan variasi yang signifikan dalam tingkat keparahan karat daun (5,21–25,84%), morfologi daun, dan rasio morfologi daun. Rasio antara panjang daun dan lebar daun, rasio antara panjang daun dan luas daun, dan rasio antara lebar daun dan bobot daun tidak dipengaruhi oleh lingkungan. Keparahan karat daun menunjukkan korelasi genotipik dan fenotipik yang positif signifikan dan tinggi dengan rasio antara panjang daun dan luas daun. Untuk kriteria seleksi, tingkat keparahan karat daun lebih baik digunakan daripada insiden karat daun dan insiden karat cabang. Rasio antara panjang daun dan luas daun juga dapat digunakan sebagai kriteria seleksi tidak langsung karena rasio tersebut menunjukkan korelasi genotipe yang sangat signifikan dengan tingkat keparahan karat daun (r_{Gab} = 0,254). Akan tetapi, rasio antara panjang daun dan luas daun lebih baik dipilih untuk kriteria seleksi daripada keparahan karat daun karena rasio itu tidak dipengaruhi oleh lingkungan.**

Kata kunci: *Hemeleia vastatrix*, jamur, seleksi tidak langsung

INTRODUCTION

Coffee is very important for Indonesia to generate income and jobs. In 2016, Indonesia produced around 190 thousand tons of Arabica coffee and 474 thousand tons of Robusta coffee (DGEC 2017). This country has1.25 million ha of coffee farms and 1.78 million of coffee farmers (households). As one of the main production area of Arabica coffee in Indonesia, North Sumatra Province produced 53.2 thousand tons of green beans on a total of 63.3 thousand ha of coffee growing area which was income source for 110.6 thousand farmers (households).

Fungus *Hemileia vastatrix* cause rust disease on leaf of coffee plant. The fungus lives in the underside of the leaf. Fungi that live on the bottom surface of the leaves (Figure 1) can cover up to 100% of the surface. The leaves are then damaged (Figure 2) and eventually fall off.

This fungus severely destructed coffee plants in many countries in Asia, Central America, and Africa

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Figure 1 Fungi *Hemileia vastatrix* live on the bottom surface of the leaf.

Figure 2 The leaf is damaged by fungi *Hemileia vastatrix* and eventually falls off.

(McCook 2006; Zeru *et al*. 2009; Jeffuka *et al*. 2010; Avelino *et al*. 2015; Talhinhas *et al.* 2017). In North Sumatra Province of Indonesia, this fungus covered some farms with leaf-rust severity from 1% to 45% (average 15.8%) (Siska *et al*. 2018).

Resistant genotypes of coffee could be found through crossbreeding (Jeffuka *et al.* 2010; Sera *et al.* (2010); Hindorf & Omondi 2011; Rodrigues *et al.* 2012; Caicedo *et al.* 2013, Andreazi *et al.* 2015) or in existing genotypes having genotypic variation and phenotypic variation (Carvalho *et al.* 2011; Harni *et al.* 2015). In existing genotypes of Arabica coffee in North Sumatera Province, genotypes are rich in variabilities in many important traits such as rust resistance (Malau 2019a; Malau *et al.* 2019a), morphological traits (Malau & Pandiangan 2018), seed-germination ability (Malau *et al.* 2018a), water-stress tolerance (Malau *et al*. 2018b), adaptability to climate changing (Malau *et al.* 2018c), and taste (Malau *et al.* 2017; Malau *et al.* 2018d; Malau *et al.* 2019b; Malau 2019b).

So far, leaf-rust severity and leaf-rust incidence were used by coffee breeder as direct selection parameters. It seems to be important to study the correlation between rust disease with leaf morphology because spores of this fungus only live on coffee leaf, and infect through the stomata on the underside of the leaf (López-Bravo *et al.* 2012; Boudrot *et al.* 2016). The objective of this research was to genotypically and phenotypically correlate parameter of rust disease with leaf morphology and the ratios of leaf-morphology parameters. It was hypothesized that rust disease had significant genotypic and phenotypic correlations with leaf morphology and its ratios. The result of this research was expected to contribute to a method of coffee breeding for rust resistance.

MATERIAL AND METHODS

The research was conducted in Regencies of Dairi, Pakpak Bharat, Simalungun, Samosir, Humbang Hasundutan, Tapanuli Utara, and Toba Samosir of North Sumatra Province in June 2017. According to Sudrajat (2009), the climate zones of the research locations were A1, B1, C1, D1, D2, E1, and E2 for Humbang Hasundutan, Simalungun, Pakpak Bharat, Samosir, Dairi, North Tapanuli, and Toba Samosir Regencies, respectively (Table 1). The length of rainy seasons and rainfall was different enough. Wet season and dry season have rainfall in the amount of more than 200 mm and less than 100 mm per month, respectively. In the period of 1970–2017, the average range of temperature was 21.8–23.6°C (BMKG 2017).

The nested design with three factors (regency, sub regency, and genotype) was used (Quinn & Keough, 2002). In each regency, two sub regencies nested in regency were selected. In each sub regency, six farms nested in sub regency were chosen. Therefore, 7 x 2 x 6 = 84 farms were selected. The farms were treated as genotypes. Ten plants with age 6-7 years of each farms were selected randomly which were treated as genotype. Leaf length (L) and leaf width (W) were measured while estimated leaf area (ELA) was calculated using formula ELA = 0.99927*(L*(–0.14757

Table 1 Length of rainy season, length of dry season, minimum rainfall, maximum rainfall, average rainfall, and temperature of climate zones

	Regency						
	Humbang Hasundutan	Simalungun	Pakpak Bharat	Samosir	Dairi	North Tapanuli	Toba Samosir
CZ	A1	B1	C1	D1	D2	E1	E2
LoRS	0	0	1	1	2	1	2
MinR	3108	2595	1750	1705	1749	1615	1172
MaxR	4388	3104	3957	3085	2409	2145	2233
AvR	3822	2933	2729	2274	1911	1922	1685
AvT	23.3	23.1	22.9	22.5	21.8	23.6	23.6

Source: Sudrajat (2009); BMKG (2017).
Description: CZ = Climate zone, LoRS = Length of rainy season (months), MinR = minimum rainfall (mm per year), MaxR = maximum rainfall (mm per year), AvR = average rainfall (mm per year), and AvT = average temperature (°C).

+ 0.60986*W)) (Unigarro-Muñoz *et al.* 2015). Branch-rust incidence (BRI), leaf-rust incidence (LRI), and leaf-rust severity (LRS) were calculated. The proportion of rust-infected branches from total branches (%) is branch-rust incidence. A branch with at least one leaf with powdery lesion orange-yellow color due to sporulation on the underside of leaf was defined as a rust-infected branch. All branches were checked. BRI of a plant (BRI_P) was calculated with the formula:

$$\mathrm{BRI_p} = \frac{\text{number of rust infected branches}}{\text{total branches}} \text{ x } 100\%.$$

Averaged BRI per plant of a genotype in a farm (BRI_g) was calculated with the formula:

$$\mathrm{BRI_g} = \frac{\sum_{p=1}^{q} \mathrm{BRI_p}}{q}$$

Whereby:
BRI_p = BRI per plant of p^{th} plant (p = 1, 2, ...q)
q = number of plants

The proportion of rust-infected leaves from total leaves (%) of a rust-infected branch is leaf-rust incidence (LRI). A leaf with powdery lesion orange-yellow color due to sporulation on the underside of leaf was treated as a rust-infected leaf. To determine LRI per plant, one rust-infected branch from the lowest part of plant, one rust-infected branch from the middle part of plant, and one rust-infected branch from the most upper part of plant were selected. LRI of all sample plants were checked. LRI of a rust-infected branch (LRI_{rib}) of a plant is calculated with the formula:

$$\mathrm{LRI_{ribp}} = \frac{\text{number of rust} - \text{infected leaves of the selected branches rusted}}{\text{total leaves of the selected branches rusted}} \text{ x } 100\%$$

Averaged LRI_{rib} per rust-infected branch of a genotype in an experimental plot (LRI_{ribg}) was counted with the formula:

$$\mathrm{LRI_{ribg}} = \frac{\sum_{p=1}^{q} \mathrm{LRI_{ribp}}}{q}$$

Whereby:
LRI_{ribp} = LRI_{rib} of p^{th} plant (p = 1, 2, ...q)
q = Number of plants

The proportion of leaf-area rusted (%) was defined as leaf-rust severity (LRS). To calculate LRS, all rust-infected leaves used in determining the LRI were used. Assessment of LRS was determined using a diagrammatic scale (Jeffuka et al 2010). Leaf-rust severity (LRS_{rilp}) of a plant was determined with the formula:

$$LRS_{rilp} = \frac{\sum_{w=1}^{z} LRS_w}{z} x\ 100\%$$

Whereby:
LRS_w = LRS of w^{th} leaf (w = 1, 2, ... z)
z = Number of rust infected leaves

Averaged LRS_{ril} of a genotype in a farm (LRS_{rilg}) was counted with the formula:

$$\mathrm{LRS_{rilg}} = \frac{\sum_{p=1}^{q} \mathrm{LRS_{rilp}}}{q}$$

Whereby:
LRS_{rilp} = LRS_{ril} of p^{th} plant (p = 1, 2, ...q)
q = Number of plants

Genotypic and phenotypic correlation coefficients were then calculated. The first step was to create a dummy phenotype (= a + b). Therefore, $\sigma^2_{ab} = \sigma^2_a + \sigma^2_b + 2\ cov_{ab}$ where σ^2_{ab} = variance of the dummy phenotype, σ^2_a = variance of phenotype a, σ^2_b = variance of phenotype b and cov_{ab} = covariance of the dummy phenotype. The second step was to analyze the variance of the dummy phenotype by using the nested design method, then cov_{Gab}, cov_{Pab}, σ^2_{Gab}, σ^2_{Pab}, r_{Gab} and r_{Pab} could be calculated. Genetic correlation coefficient r_{Gab} between two phenotypes (a and b) was calculated as $r_{Gab} = cov_{Gab}/(\sigma^2_{Ga} \text{ x } \sigma^2_{Gb})^{0.5}$ and phenotypic correlation coefficient r_{Pab} between two phenotypes was calculate as $r_{Pab} = cov_{pab}/(\sigma^2_{Pa} \text{ x } \sigma^2_{Pb})^{0.5}$ where cov_{Gab} was genetic covariance between phenotypes a and b, and cov_{Pab} was phenotypic covariance between phenotypes a and b (Mayo 1987). The significant magnitude of r_{Gab} and r_{Pab} were tested by using critical r tabular value at α = 0.05 and α = 0.01 using the degree of freedom of the error (Quinn & Keough 2002).

RESULT AND DISCUSSION

The regencies and sub regencies were significantly different in rust disease as well as in some leaf morphologies (Table 2). Leaf morphology is generally significantly affected by regency or sub regency or both. This indicates that environmental factors for growing coffee in general significantly affected the morphology of the leaves. Environmental components that influence the growth and development of coffee are climate zones, length of dry seasons, high elevation, temperature levels, and soil pH levels (Malau *et al.* 2018c).

Genotypes were highly significantly different in leaf-rust severity, leaf-rust incidence, branch-rust incidence, leaf length, leaf width, leaf weight, leaf area, and the ratios (Table 2). It indicated that there were variations among genotypes in rust disease and leaf morphology. Genotypes had a variation in leaf rust such as leaf-rust severity (5.2–25.84%) (Table 3, Figure 3) of which G56 genotype derived from Dairi Regency performed the lowest leaf-rust severity (5.21%). Genotypes showed also variation in leaf morphology. The results of this study were in line with Malau *et al.* (2018c); Malau & Pandiangan (2018) who found variations in leaf morphology in genotypes that grew in North Sumatra. In contrast to leaf morphology, the ratio of leaf morphology (Table 4) such as the ratio of leaf length to leaf width, ratio of leaf length to leaf area, and the ratio of leaf width to leaf weight were not affected by the regency or sub regency or both (Table 2). This showed that these three ratios were not influenced by the growing environment. The expressivity stability of these ratios occurred because these ratios had high coefficients of heritability (Malau, 2019b).

Leaf-rust severity had highly significant negative genotypic correlation with leaf-rust incidence (r_{Gab} = -0.230*) (Table 5). Leaf-rust severity did not have significant genotypic correlation with branch-leaf rust. Leaf-rust incidence had significant negative correlation with branch-leaf rust (r_{Gab} = -0.096*).

Leaf-rust severity had highly significant positive genotypic and phenotypic correlations with ratios of leaf length and leaf area (r_{Gab} = 0.254** and r_{Pab} = 0.132**, respectively). Leaf-rust incidence did not correlate genotypically with any ratios of leaf morphology. Branch-leaf rust showed significant positive genotypic and phenotypic correlations with ratios of leaf length and leaf area.

In compared to leaf-rust incidence and branch-rust incidence, leaf-rust severity should be better used asmain selection criteria in coffee breeding for leaf-rust resistance because leaf-rust severity had the highest significant genotypic correlation (r_{Gab} = 0.254**). Let alone leaf-rust severity explains the level of damage caused by fungus *Hemeleia vastatrix*. The more the leaf-rust severity, the higher the level of damage. This fungus absorbs nutrients from the leaf on the underside of the leaf of the host plant (Ameson 2000). The more spores of this fungus germinate, the more nutrients will be absorbed by this fungus so the leaf becomes more nutrient deficiencies and then the leaf falls early. This leaf abscission significantly reduces the photosynthesis and then reduces production. Production of coffee fruit could be reduced up to 99% depending on the leaf-rust severity and the number of abscission leaves caused by this fungus. The results of this research supported Sera *et al*. (2010); Lima *et al.* (2018) who directly used rust-leaf severity to evaluate resistance of coffee plant in breeding process to search resistant genotype. The result of this research showed that rust-leaf incidence had no phenotypic correlation with rust-leaf severity (r_{Pab} = -0,073ns) (Table 5). However, this result was in contrary with the results of research conducted by Silva-Acuña *et al.* (1999) who concluded that leaf-rust incidence could be used to estimate rust severity.

Ratio of leaf length to leaf area, however, might be used as an indirect selection criterion for leaf-rust severity because the ratio had significant positive genotypic correlation with leaf-rust severity (r_{Gab} = 0.254**) (Table 5). Regarding the correlation numbers,

Table 2 Analysis of variance and genetic components of leaf rust and leaf traits

Parameter	MS regency (p = 7; df = 6)	MS subdistict (q = 2; df = 7)	MS genotype (r = 2; df = 14)	MS error (df = 84)	F-ratio for regency	F-ratio for sub regency	F-ratio for genotype	*s2G*	*s2E*	*s2P*
Rust disease										
LRS	1970.66875	65.48090	63.94922	12.26887	30.10**	1.02ns	5.21**	5.168035	12.268868	17.436903
LRI	10367.86316	346.74569	164.60851	9.49763	29.90**	2.11ns	17.33**	15.511088	9.497630	25.008718
BRI	9250.94989	241.53762	199.94726	13.71423	38.30**	1.21ns	14.58**	18.623302	13.714233	32.337535
Leaf morphology										
Leaf length (LL)	208.02766	19.10252	12.20260	0.96886	10.89**	1.57ns	12.59**	1.123373	0.968864	2.092237
Leaf width (LWi)	14.08990	9.75618	4.95895	0.18515	1.44ns	1.97ns	26.78**	0.477379	0.185154	0.662533
Leaf weight (LWe)	3.35942	0.04172	0.05307	0.01453	80.53**	0.79ns	3.65**	0.003854	0.014532	0.018386
Leaf area (LA)	5009.23085	1222.43084	504.48282	23.57652	4.10*	2.42*	21.40**	48.090629	23.576524	71.667153
Ratio of LL/LWi	5.95689	1.69598	2.00919	0.08481	3.51ns	0.84ns	23.69**	0.192438	0.084813	0.277251
Ratio of LL/LWe	43.05727	8.16200	7.12434	0.88252	5.28*	1.15ns	8.07**	0.624182	0.882521	1.506703
Ratio of LL/LA	0.05940	0.04128	0.02078	0.00083	1.44ns	1.99ns	24.95**	0.001995	0.000833	0.002828
Ratio of LWi/LWe	7.61022	3.59845	2.01103	0.13785	2.11ns	1.79ns	14.59**	0.187317	0.137855	0.325172
Ratio of LWi/LA	0.01697	0.00059	0.00193	0.00008	28.67**	0.31ns	24.79**	0.000186	0.000078	0.000264
Ratio of LWe/LA	0.00071	0.00085	0.00032	0.00002	0.84ns	2.67*	13.52**	0.000029	0.000024	0.000053

Description: MS = mean square, df = degree of freedom, LRS = leaf-rust severity, LRI = leaf-rust incidence, RBI = branch-rust incidence, LL = leaf length, LWi = leaf width, LWe = leaf weight, LA = leaf area, F-table for regency at α 0.05 = 3.87 and α 0.01 = 7.19, F-table for sub regency at α 0.05 = 2.17 and α 0.01 = 2.91. F-table for genotype at α 0.05 = 1.30 and α 0.01 = 1.44. s^2_G = estimated variance of genotype, s^2_E = estimated variance of error (mean square of error), and s^2_P = estimated variance of phenotype.

Table 3 Leaf-rust disease and leaf morphology of 84 Arabica coffee genotypes

Regency	Genotype	LRS (%)	LRI (%)	BRI (%)	LL (cm)	LWi (cm)	LWe (g)	LA (cm^2)	LL/ LWi	LL/ LWe	LL/ LA	LWi/ LWe	LWi/ LA	LWe/ LA
Humbang	G1	5.90	46.38	48.94	16.42	6.62	1.81	63.65	2.48	9.07	0.258	3.66	0.106	0.028
Hasundutan	G2	6.39	46.84	35.91	15.49	6.63	1.82	60.38	2.33	8.51	0.257	3.65	0.113	0.030
	G3	5.99	24.69	39.06	16.24	4.58	1.82	43.26	3.55	8.91	0.377	2.52	0.106	0.042
	G4	6.41	47.21	40.93	15.98	6.43	1.81	60.53	2.49	8.82	0.264	3.55	0.106	0.030
	G5	6.03	45.16	38.20	17.06	6.39	1.73	63.92	2.68	9.91	0.268	3.72	0.105	0.027
	G6	6.16	44.84	42.72	17.34	6.38	1.82	64.86	2.73	9.58	0.269	3.52	0.098	0.028
	G7	5.78	50.00	36.47	17.38	4.61	1.77	46.22	3.78	9.88	0.377	2.62	0.100	0.038
	G8	6.14	45.85	37.01	17.34	4.56	1.78	45.66	3.82	9.76	0.381	2.57	0.100	0.039
	G9	6.74	44.78	39.53	17.35	6.58	1.78	67.08	2.64	9.76	0.259	3.70	0.098	0.027
	G10	6.75	49.57	32.58	14.38	6.46	1.79	54.44	2.24	8.06	0.265	3.63	0.101	0.033
	G11	6.64	46.96	38.23	16.53	6.59	1.80	63.93	2.51	9.21	0.259	3.67	0.127	0.028
	G12	5.78	44.14	37.83	16.53	6.54	1.63	63.44	2.54	10.19	0.261	4.03	0.140	0.026
Simalungun	G13	9.24	65.50	54.54	13.90	4.65	1.81	39.35	2.99	7.67	0.368	2.57	0.129	0.048
	G14	9.18	65.85	54.29	16.53	4.54	1.81	59.93	3.65	8.79	0.383	2.41	0.130	0.044
	G15	9.57	56.24	55.18	16.40	4.54	1.83	42.89	3.62	8.98	0.383	2.49	0.072	0.043
	G16	9.91	66.38	44.58	16.43	6.48	1.81	54.61	2.54	9.11	0.263	3.59	0.104	0.029
	G17	9.66	62.72	45.98	15.12	4.54	1.83	39.58	3.34	8.28	0.383	2.48	0.081	0.047
	G18	9.88	61.99	49.33	16.53	6.28	1.81	60.84	2.64	9.16	0.273	3.47	0.103	0.030
	G19	10.91	61.78	55.42	14.61	4.54	1.78	37.64	3.23	8.22	0.391	2.55	0.082	0.048
	G20	10.51	65.51	30.20	14.90	5.91	1.78	51.42	2.53	8.39	0.291	3.33	0.115	0.035
	G21	9.19	59.85	54.37	15.25	6.68	1.77	60.80	2.29	8.64	0.252	3.78	0.110	0.029
	G22	10.18	63.93	53.52	14.97	4.36	1.77	36.87	3.45	8.49	0.410	2.47	0.119	0.049
	G23	9.24	55.69	52.48	14.83	6.51	1.82	56.16	2.28	8.16	0.264	3.58	0.116	0.033
	G24	10.17	63.98	58.20	14.89	6.46	1.85	56.30	2.31	8.06	0.265	3.49	0.115	0.033
Pakpak Bharat	G25	11.93	50.83	57.31	14.59	4.37	1.57	36.48	3.36	9.42	0.404	2.82	0.120	0.043
	G26	12.48	53.53	58.34	15.34	5.21	1.73	46.16	2.99	8.94	0.338	3.02	0.113	0.038
	G27	11.33	49.92	64.18	13.90	5.41	1.56	44.44	2.58	8.99	0.316	3.51	0.122	0.035
	G28	12.79	50.68	66.08	14.38	5.74	1.74	48.08	2.53	8.28	0.301	3.30	0.119	0.037
	G29	12.98	55.14	59.97	14.83	5.08	1.66	43.87	2.97	9.05	0.345	3.09	0.116	0.039
	G30	12.79	53.74	66.04	14.84	5.64	1.59	48.99	2.65	9.52	0.304	3.63	0.115	0.033
	G31	12.99	46.23	65.30	14.78	5.44	1.60	46.95	2.73	9.31	0.317	3.43	0.116	0.034
	G32	12.22	50.13	60.56	14.78	5.47	1.57	47.07	2.72	9.53	0.316	3.54	0.116	0.034
	G33	12.76	49.58	60.79	14.59	5.70	1.64	48.50	2.57	9.00	0.303	3.53	0.118	0.034
	G34	12.39	48.01	59.67	14.30	5.63	1.68	47.01	2.55	8.67	0.306	3.39	0.120	0.036
	G35	12.91	46.58	60.89	14.58	5.46	1.63	45.99	2.69	9.05	0.319	3.40	0.119	0.036
	G36	12.44	48.04	64.43	14.41	5.58	1.55	46.86	2.60	9.49	0.310	3.68	0.119	0.033
Samosir	G37	12.03	36.90	55.46	15.63	5.62	1.75	51.23	2.81	8.95	0.308	3.23	0.110	0.035
	G38	11.97	43.92	47.41	14.08	5.73	1.72	47.57	2.47	8.22	0.300	3.35	0.121	0.037
	G39	10.29	37.42	45.97	14.76	5.95	1.59	50.97	2.50	9.42	0.292	3.80	0.117	0.031
	G40	10.78	43.09	44.67	13.26	5.59	1.77	43.99	2.40	7.58	0.307	3.17	0.130	0.042
	G41	12.64	43.88	45.68	14.48	5.89	1.77	49.70	2.49	8.22	0.295	3.34	0.119	0.036
	G42	11.72	44.98	46.02	15.15	5.89	1.78	52.15	2.60	8.55	0.294	3.32	0.113	0.035
	G43	11.64	44.95	51.20	14.25	5.64	1.77	46.56	2.55	8.10	0.309	3.20	0.121	0.038
	G44	11.89	45.60	50.24	14.23	5.92	1.76	49.14	2.44	8.14	0.293	3.39	0.121	0.037
	G45	11.44	45.05	53.89	14.81	5.69	1.78	48.96	2.63	8.37	0.306	3.20	0.116	0.037
	G46	10.32	45.10	49.58	14.55	5.91	1.77	50.16	2.50	8.23	0.294	3.34	0.118	0.036
	G47	11.47	44.92	53.81	14.48	5.73	1.70	48.69	2.56	8.64	0.301	3.40	0.118	0.035
	G48	11.82	45.35	46.19	14.59	5.68	1.79	48.35	2.60	8.16	0.306	3.18	0.118	0.038
Dairi	G49	7.54	65.80	55.15	11.27	4.44	1.45	28.85	2.55	7.80	0.393	3.07	0.090	0.050
	G50	7.12	64.81	50.92	11.51	4.43	1.45	29.39	2.61	7.96	0.394	3.06	0.151	0.050
	G51	8.17	60.41	44.37	12.33	5.60	1.28	40.64	2.24	9.84	0.309	4.44	0.138	0.032
	G52	8.78	64.18	54.80	12.66	4.33	1.29	31.80	2.94	9.99	0.400	3.42	0.137	0.041
	G53	8.09	66.21	54.32	15.25	6.47	1.48	57.86	2.36	10.34	0.264	4.39	0.112	0.026
	G54	10.27	66.18	54.85	15.77	4.41	1.43	39.97	3.59	11.15	0.395	3.11	0.110	0.036
	G55	7.35	63.33	45.40	12.63	6.53	1.44	48.96	1.94	8.82	0.259	4.55	0.135	0.030
	G56	5.21	60.30	55.90	13.80	6.37	1.51	52.30	2.17	9.18	0.265	4.25	0.123	0.029
	G57	7.32	51.70	52.48	11.38	6.51	1.43	43.60	1.75	7.96	0.261	4.56	0.150	0.033
	G58	8.17	62.08	44.59	12.12	6.06	1.48	43.49	2.01	8.20	0.281	4.10	0.141	0.035
	G59	10.31	61.47	55.14	15.16	4.46	1.48	38.83	3.42	10.32	0.392	3.04	0.115	0.039
	G60	8.32	59.81	55.78	16.62	4.37	1.34	41.93	3.81	12.51	0.398	3.29	0.104	0.032
Tapanuli Utara	G61	11.68	42.18	45.65	12.60	4.53	1.40	32.85	2.80	9.03	0.385	3.25	0.138	0.043
	G62	13.84	44.00	44.76	12.10	5.63	1.47	39.65	2.16	8.26	0.305	3.84	0.144	0.038
	G63	13.21	41.26	46.14	12.39	5.84	1.38	42.72	2.14	9.07	0.294	4.24	0.138	0.033
	G64	14.91	43.85	45.57	12.60	5.15	1.46	37.71	2.48	8.68	0.340	3.54	0.137	0.039
	G65	14.11	39.16	45.12	12.81	5.58	1.41	41.46	2.34	9.12	0.315	3.95	0.135	0.035
	G66	12.00	42.22	46.07	13.33	5.47	1.46	42.44	2.50	9.19	0.322	3.78	0.129	0.035
	G67	11.92	37.53	53.37	13.81	5.60	1.50	45.13	2.47	9.24	0.306	3.75	0.124	0.033
	G68	13.38	35.68	42.55	12.22	5.63	1.49	12.17	2.18	8.27	0.305	3.81	0.140	0.037
	G69	12.12	42.11	44.10	12.11	5.71	1.48	40.29	2.13	8.20	0.301	3.88	0.144	0.037
	G70	25.84	34.34	55.70	11.41	4.63	1.48	30.45	2.47	7.71	0.377	3.12	0.152	0.049
	G71	12.30	42.99	54.92	13.09	4.73	1.44	35.63	2.78	9.12	0.367	3.29	0.134	0.041
	G72	14.41	44.56	54.66	10.55	5.74	1.43	35.02	1.88	7.42	0.307	4.01	0.163	0.041

Table 3 Leaf-rust disease and leaf morphology of 84 Arabica coffee genotypes (advanced)

Regency	Genotype	LRS (%)	LRI (%)	BRI (%)	LL (cm)	LWi (cm)	LWe (g)	LA (cm^2)	LL/ LWi	LL/ LWe	LL/ LA	LWi/ LWe	LWi/ LA	LWe/ LA
Toba Samosir	G73	13.72	36.20	32.91	15.29	6.27	1.85	55.94	2.44	8.27	0.274	3.39	0.116	0.034
	G74	12.15	42.55	35.03	17.00	6.18	1.41	61.39	2.75	12.09	0.277	4.39	0.102	0.023
	G75	13.43	44.94	35.95	14.78	6.26	1.40	53.87	2.36	10.58	0.275	4.47	0.116	0.026
	G76	13.55	46.80	35.55	16.07	4.46	1.41	41.40	3.62	11.43	0.392	3.18	0.109	0.035
	G77	23.49	31.55	35.42	16.45	5.34	1.45	51.13	3.09	11.39	0.322	3.70	0.130	0.028
	G78	23.93	48.65	35.37	13.76	4.52	1.41	35.34	3.06	9.73	0.392	3.20	0.088	0.041
	G79	13.34	48.13	35.51	14.43	4.42	1.44	37.36	3.28	10.02	0.389	3.08	0.123	0.040
	G80	16.36	45.77	35.29	14.67	4.47	1.49	37.71	3.30	9.86	0.391	3.02	0.121	0.040
	G81	24.48	38.55	36.14	14.63	4.39	1.44	36.23	3.34	10.18	0.405	3.06	0.122	0.040
	G82	18.52	40.26	35.16	10.96	4.39	1.40	27.43	2.52	7.88	0.400	3.14	0.109	0.052
	G83	24.63	41.15	35.67	12.85	4.44	1.40	32.98	2.91	9.23	0.393	3.18	0.092	0.043
	G84	23.52	41.26	35.59	15.14	4.40	1.61	38.50	3.45	9.51	0.396	2.77	0.080	0.043
	Minimum	5.21	24.39	30.20	10.55	4.33	1.28	12.17	1.75	7.42	0.252	2.41	0.072	0.023
	Maximum	25.84	66.38	66.06	17.38	6.68	1.85	67.08	3.82	12.51	0.410	4.56	0.163	0.052
	Median	11.44	46.58	46.19	14.63	5.63	1.60	46.16	2.57	8. 99	0.307	3.39	0.118	0.036
	Mean	11.52	49.42	48.04	14.49	5.47	1.62	46.33	2.71	9.06	0.306	3.42	0.118	0.036
	sd	0.64	0.56	0.68	0.18	0.08	0.02	0.89	0.05	0.17	0.005	0.07	0.002	0.001

Description: G = genotype, LRS = leaf rust severity, LRI = leaf-rust incidence, BRI = branch-rust incidence, LL = leaf length, LWi = leaf width, LWe = leaf weight, LA = leaf area, and S_d = standard deviation.

Figure 3 Leaf surface rusted by fungi *Hemileia vastatrix* with 15% leaf-rust severity.

happened because the number of data units in this study was quite large (Gomez & Gomez 1984). Furthermore, by squaring the correlation coefficient number, the coefficient of determination is obtained at 6.5%. That is, as much as 6.5% of variation in leaf-rust severity is explained by variations in the ratio of leaf length to area, the rest (93.5%) is explained by the other unknown factors. In this such condition, namely the large number of data units that causes a small coefficient of determination, the focus of attention should be directed primarily to the significance of the relationship between parameters. The highly significant positive genotypic association between the ratio of leaf length to leaf area and leaf-rust severity was the decrease in the ratio of leaf length to leaf area that caused both characters to decrease simultaneously. The less the ratio is, the less the severity. For selection criteria, the ratio of leaf length to leaf area is even better chosen than leaf-rust severity because the first is more able to guarantee success than the latter. This result is because this ratio will be the same in different environments (Table 2). Besides, the ratio of leaf length to leaf area had a high coefficient of heritability (H^2_b = 70.54%) while leaf-rust severity showed a low one (H^2_b = 29.64%) (Malau 2019b). Also, because the ratio of leaf length to leaf area showed a higher genetic advance in percentage of mean (GAM= 23.95%) than leaf-rust severity (GAM = 22.16%), selection using the ratio as selection criteria can then produce a genotype performing lower leaf-rust severity in compare to selection using leaf-rust severity as criteria.

CONCLUSION

The regencies and sub regencies showed significant difference in rust disease and some leaf morphology. Genotypes had high significant difference in all rust-disease parameters, leaf morphology and the ratio of leaf morphology. Genotypes showed variation in leaf-rust severity (5.21–25.84%) of which genotype G56 had the lowest leaf-rust severity. Genotypes had also variation in leaf morphology. Leaf length to leaf width ratio, leaf length to leaf area ratio, and leaf width to leaf weight ratio were not affected by the regency or sub regency or both. Leaf-rust severity showed highly significant positive genotypic and phenotypic correlation with leaf-length and leaf-area ratio.

In coffee breeding for leaf-rust resistance, leaf-rust severity could be better used as selection criteria rather than leaf-rust incidence and branch-rust incidence. Ratio of leaf length to leaf area could also be used as indirect selection criteria because the ratio showed

Table 4 Ratio of leaf morphology of 84 Arabica coffee genotypes

Regency	Genotype	LL/ LWi	LL/ LWe	LL/ LA	LWi/ LWe	LWi/ LA	LWe/ LA
Humbang	G1	2.48	9.07	0.258	3.66	0.106	0.028
Hasundutan	G2	2.33	8.51	0.257	3.65	0.113	0.030
	G3	3.55	8.91	0.377	2.52	0.106	0.042
	G4	2.49	8.82	0.264	3.55	0.106	0.030
	G5	2.68	9.91	0.268	3.72	0.105	0.027
	G6	2.73	9.58	0.269	3.52	0.098	0.028
	G7	3.78	9.88	0.377	2.62	0.100	0.038
	G8	3.82	9.76	0.381	2.57	0.100	0.039
	G9	2.64	9.76	0.259	3.70	0.098	0.027
	G10	2.24	8.06	0.265	3.63	0.101	0.033
	G11	2.51	9.21	0.259	3.67	0.127	0.028
	G12	2.54	10.19	0.261	4.03	0.140	0.026
Simalungun	G13	2.99	7.67	0.368	2.57	0.129	0.048
	G14	3.65	8.79	0.383	2.41	0.130	0.044
	G15	3.62	8.98	0.383	2.49	0.072	0.043
	G16	2.54	9.11	0.263	3.59	0.104	0.029
	G17	3.34	8.28	0.383	2.48	0.081	0.047
	G18	2.64	9.16	0.273	3.47	0.103	0.030
	G19	3.23	8.22	0.391	2.55	0.082	0.048
	G20	2.53	8.39	0.291	3.33	0.115	0.035
	G21	2.29	8.64	0.252	3.78	0.110	0.029
	G22	3.45	8.49	0.410	2.47	0.119	0.049
	G23	2.28	8.16	0.264	3.58	0.116	0.033
	G24	2.31	8.06	0.265	3.49	0.115	0.033
Pakpak Bharat	G25	3.36	9.42	0.404	2.82	0.120	0.043
	G26	2.99	8.94	0.338	3.02	0.113	0.038
	G27	2.58	8.99	0.316	3.51	0.122	0.035
	G28	2.53	8.28	0.301	3.30	0.119	0.037
	G29	2.97	9.05	0.345	3.09	0.116	0.039
	G30	2.65	9.52	0.304	3.63	0.115	0.033
	G31	2.73	9.31	0.317	3.43	0.116	0.034
	G32	2.72	9.53	0.316	3.54	0.116	0.034
	G33	2.57	9.00	0.303	3.53	0.118	0.034
	G34	2.55	8.67	0.306	3.39	0.120	0.036
	G35	2.69	9.05	0.319	3.40	0.119	0.036
	G36	2.60	9.49	0.310	3.68	0.119	0.033
Samosir	G37	2.81	8.95	0.308	3.23	0.110	0.035
	G38	2.47	8.22	0.300	3.35	0.121	0.037
	G39	2.50	9.42	0.292	3.80	0.117	0.031
	G40	2.40	7.58	0.307	3.17	0.130	0.042
	G41	2.49	8.22	0.295	3.34	0.119	0.036
	G41	2.49	8.22	0.295	3.34	0.119	0.036
	G42	2.60	8.55	0.294	3.32	0.113	0.035
	G43	2.55	8.10	0.309	3.20	0.121	0.038
	G44	2.44	8.14	0.293	3.39	0.121	0.037
	G45	2.63	8.37	0.306	3.20	0.116	0.037
	G46	2.50	8.23	0.294	3.34	0.118	0.036
	G47	2.56	8.64	0.301	3.40	0.118	0.035
	G48	2.60	8.16	0.306	3.18	0.118	0.038
Dairi	G49	2.55	7.80	0.393	3.07	0.090	0.050
	G50	2.61	7.96	0.394	3.06	0.151	0.050
	G51	2.24	9.84	0.309	4.44	0.138	0.032
	G52	2.94	9.99	0.400	3.42	0.137	0.041
	G53	2.36	10.34	0.264	4.39	0.112	0.026
	G54	3.59	11.15	0.395	3.11	0.110	0.036
	G55	1.94	8.82	0.259	4.55	0.135	0.030
	G56	2.17	9.18	0.265	4.25	0.123	0.029
	G57	1.75	7.96	0.261	4.56	0.150	0.033
	G58	2.01	8.20	0.281	4.10	0.141	0.035
	G59	3.42	10.32	0.392	3.04	0.115	0.039
	G60	3.81	12.51	0.398	3.29	0.104	0.032

Table 4 Ratio of leaf morphology of 84 Arabica coffee genotypes

Regency	Genotype	LL/ LWi	LL/ LWe	LL/ LA	LWi/ LWe	LWi/ LA	LWe/ LA
Tapanuli Utara	G61	2.80	9.03	0.385	3.25	0.138	0.043
	G62	2.16	8.26	0.305	3.84	0.144	0.038
	G63	2.14	9.07	0.294	4.24	0.138	0.033
	G64	2.48	8.68	0.340	3.54	0.137	0.039
	G65	2.34	9.12	0.315	3.95	0.135	0.035
	G66	2.50	9.19	0.322	3.78	0.129	0.035
	G67	2.47	9.24	0.306	3.75	0.124	0.033
	G68	2.18	8.27	0.305	3.81	0.140	0.037
	G69	2.13	8.20	0.301	3.88	0.144	0.037
	G70	2.47	7.71	0.377	3.12	0.152	0.049
	G71	2.78	9.12	0.367	3.29	0.134	0.041
	G72	1.88	7.42	0.307	4.01	0.163	0.041
Toba Samosir	G73	2.44	8.27	0.274	3.39	0.116	0.034
	G74	2.75	12.09	0.277	4.39	0.102	0.023
	G75	2.36	10.58	0.275	4.47	0.116	0.026
	G76	3.62	11.43	0.392	3.18	0.109	0.035
	G77	3.09	11.39	0.322	3.70	0.130	0.028
	G78	3.06	9.73	0.392	3.20	0.088	0.041
	G79	3.28	10.02	0.389	3.08	0.123	0.040
	G80	3.30	9.86	0.391	3.02	0.121	0.040
	G81	3.34	10.18	0.405	3.06	0.122	0.040
	G82	2.52	7.88	0.400	3.14	0.109	0.052
	G83	2.91	9.23	0.393	3.18	0.092	0.043
	G84	3.45	9.51	0.396	2.77	0.080	0.043
	Minimum	1.75	7.42	0.252	2.41	0.072	0.023
	Maximum	3.82	12.51	0.410	4.56	0.163	0.052
	Median	2.57	8.99	0.307	3.39	0.118	0.036
	Mean	2.71	9.06	0.323	3.42	0.118	0.036
	sd	0.49	1.08	0.077	0.72	0.009	0.011

Description: G = genotype, LRS = leaf-rust severity, LRI = leaf-rust incidence, BRI = branch-rust incidence, LL = leaf length, LWi = leaf width, LWe = leaf weight, LA = leaf area, and S_d = standard deviation.

Table 5 s^2_{Gab}, s^2_{Eab}, s^2_{Pa}, cov_{Gab}, cov_{Pab}, r_{Gab} and r_{Pab} between genotypes

		LRS	LRI	BRI	LL	LWi	LWe	LA	Ratio LL/LWi	Ratio LL/LWe	Ratio LL/LA	Ratio LWi/LWe	Ratio LWi/LA	Ratio LWe/LA
			1	2	3	4	5	6	7	9	8	10	11	12
LRS	s^2_{Gab}	x	16.5622	25.5096	5.9426	4.8968	5.1621	45.5043	5.6560	5.6041	5.2215	4.8582	5.1699	5.1741
	s^2_{Eab}	x	22.8399	29.1927	13.2752	12.2753	12.2790	34.3900	12.4342	13.2096	12.2756	12.3072	12.2681	12.2695
	s^2_{Pab}	x	39.4021	54.7022	19.2178	17.1721	17.4411	79.8944	18.0902	18.8136	17.4972	17.1653	17.4380	17.4436
	cov_{Gab}	x	-2.0585	0.8591	-0.1744	-0.3743	-0.0049	-3.8772	0.1478	-0.0941	0.0258	-0.2486	0.0008	0.0030
	cov_{Pab}	x	-1.5218	2.4639	-0.1557	-0.4637	-0.0071	-4.6048	0.1880	-0.0650	0.0287	-0.2984	0.0004	0.0033
	r_{Gab}	x	-0.230**	0.088ns	-0.072ns	-0.238**	-0.035ns	-0.246**	0.148**	-0.052ns	0.254**	-0.253**	0.027ns	0.243**
	r_{Pab}	x	-0.073ns	0.104*	-0.026ns	-0.136**	-0.013ns	-0.130**	0.086ns	-0.013ns	0.129**	-0.125**	0.006ns	0.109*
LRI	s^2_{Gab}		x	30.8614	16.4837	15.4912	15.4889	67.1009	15.4912	16.2157	15.4959	15.8758	15.5178	15.5088
	s^2_{Eab}		x	24.0966	10.1845	9.5517	9.4884	32.4458	9.5517	10.3601	9.4981	9.7066	9.4986	9.4978
	s^2_{Pab}		x	54.9579	26.6682	25.0429	24.9773	99.5467	25.0429	26.5758	24.9939	25.5824	25.0164	25.0066
	cov_{Gab}		x	-1.6365	-0.0754	-0.2486	-0.0130	1.7496	-0.1062	0.0402	-0.0086	0.0887	0.0033	-0.0012
	cov_{Pab}		x	-1.1942	-0.2164	-0.3142	-0.0249	1.4354	-0.1215	0.0302	-0.0088	0.1242	0.0037	-0.0011
	r_G		x	-0.096*	-0.018ns	-0.091*	-0.053ns	0.064ns	-0.061ns	0.013ns	-0.049ns	0.052ns	0.061ns	-0.055ns
	r_P		x	0.099*	-0.030ns	-0.077ns	-0.037ns	0.034ns	-0.046ns	0.005ns	-0.033ns	0.044ns	0.046ns	-0.030ns
BRI	s^2_{Gab}			x	21.6223	18.2200	18.6160	64.4995	19.6916	20.5315	18.6971	18.1669	18.6114	18.6268
	s^2_{Eab}			x	14.8330	13.6848	13.7652	35.8281	13.9157	14.5138	13.7228	13.6641	13.7131	13.7160
	s^2_{Pab}			x	36.4553	31.9047	32.3812	100.3277	33.6073	35.0453	32.4200	31.8310	32.3245	32.3427
	cov_{Gab}			x	0.9378	-0.4404	-0.0056	-1.1072	0.4380	0.6420	0.0359	-0.3219	-0.0060	0.0017
	cov_{Pab}			x	1.0128	-0.5477	0.0126	-1.8385	0.4963	0.6005	0.0398	-0.4159	-0.0067	0.0026
	r_{Gab}			x	0.205**	-0.148**	-0.021ns	-0.037ns	0.231**	0.188**	0.186**	-0.172**	-0.103*	0.073ns
	r_{Pab}			x	0.123**	-0.118**	0.016ns	-0.038ns	0.166**	0.086ns	0.132**	-0.128**	-0.072ns	0.062ns

Description: n = 84, LRS = leaf-rust severity, LRI = leaf-rust incidence, RBI = branch-rust incidence, LL = leaf length, LWi = leaf width, LWe = leaf weight, LA = leaf area, s^2_{Gab} = estimated variance of genotype of dummy phenotypes (a + b), s^2_{Eab} = estimated variance of error (mean square of error) of dummy phenotypes (a + b), s^2_{Pab} = estimated variance of phenotype of dummy phenotypes (a + b), cov_{Gab} = genetic covariance between phenotypes a and b, cov_{Pab} = phenotypic covariance between phenotypes a and b, r_{Gab} = genotypic correlation between phenotype a and b, r_{Pab} = phenotypic correlation between phenotype a and b, r table at α 0.05 = 0.088, r table at α 0.01 = 0.115, ns = not significant, * = significance at α = 0.05, and ** = significance at α = 0.01.

significant genotypic correlation with leaf-rust severity (r_{Gab} = 0.254**). However, the ratio of leaf length to leaf area is even better chosen for selection criteria rather than leaf-rust severity because the ratio was not affected by the environments.

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