Genotypic and phenotypic correlations among coffee leaf rust symptoms of Arabica coffee genotypes in North Sumatra, Indonesia

S Malau*, M R Sihotang
Agriculture Faculty, Universitas HKBP Nommensen, Jalan Sutomo 4-A, Medan 20234, Indonesia

*Email: sabam.malau@uhn.ac.id

Abstract. Coffee leaf rust (CLR) is a pandemic and a serious threat for coffee sustainability in many coffee producing countries. To overcome this CLR, the world’s consensus is to use resistant cultivars which can be created through coffee breeding program. This research aimed to study genotypic and phenotypic correlations between CLR symptoms of seven arabica coffee (Coffea arabica L.) genotypes that were selected from different districts of North Sumatra Province, Indonesia. This experimental research using a randomized complete block design with three replications was conducted at the experimental garden of the Faculty of Agriculture, Universitas HKBP Nommensen in Medan. The result of this study indicate that leaf rust severity (LRS) had a genotypic component variance of 86.8% which indicated that LRS was controlled more dominantly by plant genetics than other unknown factors. Severity indicated by LRS showed no genotypic and phenotypic correlation with dispersal indicated by branch rust incidence (BRI) and leaf rust incidence (LRI). BRI genotypically correlated with LRI. The results of this study could contribute to resistance coffee breeding for CLR.

1. Introduction
In Indonesia, coffee is a very important commodity because it is a source of income for around 1.9 million coffee farmers (households) of which around 116 thousand of them were living in North Sumatra Province in 2018 [1]. Aceh Province and North Sumatra Province are of the main production areas of arabica coffee in Indonesia.

CLR caused by fungus Hemileia vastatrix is the most destructive disease of arabica coffee. The global CLR epidemic has caused 1.7 million workers to lose their jobs in addition to losing $3.2 billion in income [2]. Although comprehensive data on the effects of CLR in Indonesia and North Sumatra are not yet available, some researchers informed that this fungus caused LRS in amount of 1-45% (averaged around 14%) in North Sumatra [3][4]. Indonesia and North Sumatra need to comprehensively anticipate this situation early on.

The biological life cycle of H. vastatrix was described as following [5][6]. The life cycle of the fungus H. vastatrix begins with the dispersal of urediniospores from infected plants to uninfected plants. Urediniospores attach to the lower surface of the leaves of the new host plant then germinate and enter the cell through the stomata. By absorbing nutrients from the host plant, these fungi then reproduce to produce urediniospores. This urediniospores then repeat its cycle meaning that the
urediospores can spread on the same leaf or spread to other leaves on the same plant or to other plants assisted by wind or splashing water or coffee harvesters. This fungus causes chlorotic spots and then turns orange according to the level of development (Figure 1). Because this fungus absorbs nutrients from the host plant, the larger the leaf area attacked by these fungi, the more nutrients they absorb [7]. Leaves also become unable to carry out photosynthesis. This collection of fungi can cover the entire leaf surface, and the leaf is damaged and eventually fall prematurely. The result of this whole process is a decrease in the level of fruit production. The dispersal of urediniospores was measured by BRI and LRI, while the level of damage was measured by LRS [5][8].

Figure 1. Symptom of *H. vastratix* on the underside of the leaf of coffee plant.

CLR disease can be controlled through using chemical, biological, agronomic treatment and resistant cultivars [6][8]. The best way is the use of resistant cultivar. However, such cultivar is not easy to create. Selection breeding through the selection of resistant plants from among coffee populations that grow in an area is one alternative way.

The purpose of this experimental research is to study genotypic and phenotypic correlations between CLR symptoms of coffee genotypes taken from the coffee populations growing in several districts of North Sumatra Province. The results of this experiment are expected to contribute to the efforts to find resistant coffee plants.

2. Materials and methods

Arabica coffee genotypes used in this research originated from seven Districts in North Sumatra Province (Indonesia). One coffee farm in each District was selected. Around 200-300 plants with age of 6-7 years were growing in each farm. The plants had a shot of bronze-coloured leaves and frequency of harvest of once per two weeks. Full ripped red fruits of ten best plants were harvested, treated as genotype (G). Therefore, genotype G1, G2, G3, G4, G5, G6 and G7 originated from Districts Tapanuli Utara, Toba Samosir, Humbang Hasundutan, Samosir, Simalungun, Pakpak Bharat and Dairi, respectively. Semi-circular shaped seeds were used to produce seedlings [9].

The experiment was carried out at the experimental garden of the Faculty of Agriculture, Universitas HKBP Nommensen in Medan (Indonesia). The experiment was conducted using
randomized complete block design with three replications whereby genotype was the treatment [10]. Hence, there were seven levels of the treatment namely G1, G2, G3, G4, G5, G6 and G7. Five seedlings per genotype were planted in a row in December 2014. The distance between the rows and plants was 3.0 and 2.5 m, respectively. Fungicides, pesticides, shading trees and insecticides were not applied. The experimental plants were inoculated twice (in January and February 2017) using urediniospores of *H. vastatrix*. Urediniospores were taken from the infected leaves of coffee plants growing in a farm in District Karo (around 60 km from the experimental plots). The urediniospores collected were immediately brought to the experimental plots and directly applied using cotton buds to the abaxial face of one leaf in the middle part of all branches of all plants.

CLR symptoms were observed in July and December 2018 and 2019. CLR symptoms were observed on the coffee leaf to determine branch rust incidence (BRI), leaf rust incidence (LRI) and leaf rust severity (LRS). To determine BRI, the following method was used. A branch was defined as a rust infected branch if it had at least one leaf with powdery lesion orange-yellow colour due to sporulation on the underside of a leaf. All branches of the plants were observed. BRI (%) was determined as the proportion of rust infected branches from total branches. LRI was calculated using the following method. One rust infected branch from the most upper, one from middle and one rust infected branch from the lowest part of a plant were selected to be sample to determine LRI. All leaves of these sample branches were observed. LRI (%) was determined as the proportion of the rust infected leaves from total leaves. LRS was determined with the following method. All infected leaves used for counting the LRI were observed to determine LRS. LRS (%) was determined as proportion of rusted area of an infected leaf [11].

Averaged BRI, LRI and LRS were used in analysis of variance and variance component based on the randomized complete block design [10][12] (Table 1).

### Table 1. Expected mean squares, F-ratios and variance component for randomized complete blocks experiments.

| SV            | df | EMS | MS  | F-ratio | EVC | EVC (%) |
|---------------|----|-----|-----|---------|-----|---------|
| Replication (r) | r-1 | M1/M3 | M1 |   | EVC for replication, r = number of replication (block), t = number of blocks experiment |   |
| Genotype (t)   | t-1 | σ²_E + rσ²_G | M2 | M2/M3 | 100 | (σ²_E/σ²_P) x 100 |
| Error          | (r-1)(t-1) | σ²_E | M3 | σ²_E = (M2 - M3)/r | σ²_E = EVC of genotype, σ²_E = EVC for error, σ²_P = EVC for phenotype, r = number of replication (block), t = number of blocks experiment |
| Total          | (r-1) | σ²_E | M3 | σ²_G = EMS for genotype, σ²_E = EMS for error, EVC = estimated variance component, σ²_E = EVC for error, σ²_P = EVC for phenotype, r = number of replication (block), t = number of blocks experiment |
| CV (%)         | 100 | 100 | 100 | 100 | 100 |

SV = source of variation, df = degree of freedom, EMS = expected mean squares, MS = mean squares; M1 = MS for replication, M2 = MS for genotype, M3 = MS for error, EVC = estimated variance component, σ²_G = EMS for genotype, σ²_E = EMS for error, σ²_E = EVC of genotype, σ²_E = EVC for error, σ²_P = EVC for phenotype, r = number of replication (block), t = number of blocks experiment, CV = coefficient of variation.

Genotypic correlation coefficient (r_G) and phenotypic correlation coefficient (r_P) among BRI, LRI and LRS were then determined after counting the estimated variance components (EVC) [13]. The first step was to create three dummy phenotypes as the sum of two observed CLR symptoms (BRI + LRI, BRI + LRS, LRI + LRS). The second step was to determine variance of dummy phenotype using randomized complete block experiment. BRI and LRI were used here as examples for an explanation of how r_G and r_P were determined. Variance of the dummy phenotype BRI + LRI (σ²_BRI+LRI) was determined with equation σ²_BRI+LRI = σ²_BRI + σ²_LRI + 2cov_BRI+LRI where cov_BRI+LRI was covariance of the dummy phenotype, σ²_BRI was variance of phenotype BRI and σ²_LRI was variance of phenotype LRI. Genotypic covariance (cov_G(BRI+LRI)), genotypic variance (σ²_G(BRI+LRI)) phenotypic covariance (cov_P(BRI+LRI)) and phenotypic variance (σ²_P(BRI+LRI)) of dummy phenotype were then determined. Genetic correlation coefficient (r_G) between BRI and LRI was determined with equation r_G = cov_G(BRI+LRI)/(σ²_G(BRI) x σ²_G(LRI))⁰.⁵,
and phenotypic correlation coefficient ($r_P$) between BRI and LRI was calculated with equation $r_P = \frac{\text{cov}_{\text{BRI},\text{LRI}}}{\sqrt{s^2_{\text{BRI}} \times s^2_{\text{LRI}}}}$. The significant magnitude of $r_G$ and $r_P$ were tested by using critical value of simple linear correlation coefficient ($r$) for the 5% and 1% level of significance with the degree of freedom of the error [12].

3. Results and discussion
The result of analysis of variance showed that genotype difference in was significant in BRI and LRI and highly significant in LRS (Table 2).

### Table 2. Analysis of variance and genetic components of CLR symptoms.

|                  | CLR symptom |      |      |
|------------------|-------------|------|------|
|                  | BRI         | LRI  | LRS  |
| MS replication   | 90.3748     | 2.0119 | 0.1671 |
| MS genotype      | 212.6933*   | 87.8260* | 173.9160** |
| MS error         | 46.5731     | 25.4541 | 8.4127  |
| F-ratio for genotype | 4.57       | 3.45    | 20.67   |
| $s^2_G$          | 55.3734     | 20.7906 | 55.1678 |
| $s^2_E$          | 46.5731     | 25.4541 | 8.4127  |
| $s^2_P$          | 101.9465    | 46.2448 | 63.5805 |
| CV (%)           | 15.64       | 15.90   | 14.97   |

CLR = coffee leaf rust, BRI = branch rust incidence, LRI = leaf rust incidence, LRS = leaf rust severity, MS = mean square, df = degree of freedom, $s^2_G$ = estimated variance of genotype, $s^2_E$ = estimated variance of error (mean square of error), $s^2_P$ = estimated variance of phenotype, ns = not significant, * = significance at the 5% level of significance, ** = significance at the 1% level of significance. CV = coefficient of variation.

The result revealed that the genotype had higher variance component on BRI and LRS (54.3% and 86.8%, respectively) than error while LRI contributed variance less than error to total variance (Table 3). It indicated that LRS was controlled mainly by plant genetics and BRI was slightly more controlled by plant genetics while LRI was slightly more controlled by unknown factors that accumulated in the error.

### Table 3. Variance component for CLR symptoms.

| SV            | BRI     | LRI     | LRS     |
|---------------|---------|---------|---------|
| Genotype      | 54.3    | 45.0    | 86.8    |
| Error         | 45.7    | 55.0    | 13.2    |
| Total         | 100     | 100     | 100     |

SV = source of variation, EVC = estimated variance component, BRI = branch rust incidence, LRI = leaf rust incidence, LRS = leaf rust severity

The result showed that BRI had highly significant genotypic correlation with LRI (Table 4). The decrease in BRI that caused both characters to decrease simultaneously. This result was contrary with the result of other research that showed a negative significant correlation between BRI and LRI [4]. BRI and LRI had no genotypic correlation with LRS. It indicated that no correlation between dispersal (BRI and LRI) with severity (LRS). A decrease in BRI or LRI does not necessarily result in a decrease in LRS. However, the results of this research are different from the results of other research which proved that dispersal (LRI) has a negative genotypic correlation with severity (LRS).
[4]. The results of this research indicated that selection for resistance against *H. vastatrix* would better to be carried out based on LRS. Since the LRS indicates the extent of damage to the coffee crop, a decrease in the LRS will also result in a decrease in losses in coffee fruit production.

| Table 4. Genotypic and phenotypic correlation coefficient among CLR symptoms. |
|---------------------------------|-----------------|
| CLR symptoms | LRI | LRS |
|----------------|-----------------|
| BRI | $r_G$ | 0.792** | -0.058ns |
| | $r_P$ | 0.559ns | -0.064ns |
| LRI | $r_G$ | x | 0.510ns |
| | $r_P$ | x | 0.360ns |

$n = 12$, CLR = coffee leaf rust, BRI = branch rust incidence, LRI = leaf rust incidence, LRS = leaf rust severity, $r_G$ = genotypic correlation coefficient between two CLR symptoms, $r_P$ = phenotypic correlation coefficient between two CLR symptoms, $r_{0.05} = 0.576$ = simple linear correlation coefficient for the 5% level of significance, $r_{0.01} = 0.708$ = simple linear correlation coefficient for the 1% level of significance, ns = not significant, * = significance at the 5% level of significance, ** = significance at the 1% level of significance.

4. Conclusions
LRS was controlled more dominantly by plant genetics than other unknown factors. LRS showed no genotypic and phenotypic correlation with BRI and LRI. BRI showed genotypic correlation with LRI. Selection for resistance to *H. vastatrix* seemed to be better carried out on the basis of LRS.

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