Evaluation of maize hybrids resistance to northern corn leaf blight in Karo Highland

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Abstract. Around two-thirds of corn area in North Sumatera are in high altitude places, where northern corn leaf blight limits yield. Evaluation was performed to 125 new hybrids for their resistance to northern corn leaf blight and yield components in Garingging, District of Karo, North Sumatera, from October 2019 to March 2020. Experiments were arranged in Augmented RCBD with 4 checks, Artificial inoculation was applied to ensure high and uniform disease pressure. Eight new hybrids exceeded at least 3 checks regarding disease resistance and ear weight, namely N399, G163, N396, N374, D651, N020, D635, and N031. Hybrid N399 has ear weight above 4 checks and G163 has better AUDPC than the 4 checks, suggesting promising hybrids for developing varieties in areas with NCLB pressure. Significant association with AUDPC was seen in incubation period, ear diameter, ear length, and ear weight. High GCV was observed in disease resistance traits, while high heritability is estimated in incubation period, AUDPC, ear diameter, and ear length. Incubation period may be used in early selection.

1. Introduction

Maize in Indonesia is an important crop that mainly support the feed industry. The Province of North Sumatra plays an important role in the national maize production with 252,729 ha of planting area and 1.5 million mt of harvest [1]. Around two-thirds are planted in areas with altitude 300-1200 m asl in the District of Karo, Dairi, and Simalungun. Nevertheless, incidence of diseases limits the yield potential.

Northern corn leaf blight caused by *Exserohilum turcicum* (Pass.) Leonard et Suggs (anamorph *Setosphaeria turcica* (Luttrell) Leonard et Suggs) is a maize disease found in the highlands of Karo. It is a filamented hemibiotrophic fungi from class Dothideomycetes [2] First reported in 1917 [3], it has become a main disease along with the increase planting of maize in the region. Common symptoms be a form of cigar-shape lesions or necrotic green-gray lesions that are oblong in the leaves, with a length between 2 to 14 cm [4]. In susceptible hybrids, lesions can be found on the husk or sheath. In partially resistant hybrids, lesions tend to be reduced due to decreased formation of spores. In highly resistant hybrids, symptoms of the disease appear as small yellow patches [5]. Sporulation occurs in condition with wet periods that last more than six hours with temperature 18-27°C [6]. Insufficient humidity period prevents sporulation; hence sporulation occurs in night and cease in day [7]. Significant damage happens if infection appears before or during silking [8].

Chemical substance can be applied to control NCLB, for instance prothioconazole + trifloxystrobin [9] iprodione, fludioxonil, and thiram [10]. However, planting resistance varieties are more cost-efficient, environmental-friendly, and reduce cost of seed production [11]. Efforts were conducted to evaluate resistant varieties in Indonesia [3]. Yet, discovery of natural occurrence of sexual stage in *E.
*Turcicum*, first reported in Thailand, enhances pathogen virulence through combining diverse virulence and producing new races [12]. This mainly happens in the tropics although the reasons are still unknown [11]. Hence variety evaluation for resistance to NCLB is a continuous process, especially areas suffering from the disease.

Resistance breeding programs require an effective source of resistance, a testing system that can assess genetic differences in resistance, and adequate selection and breeding methods [11]. Source of resistance can be identified in areas having high disease pressure. Highlands with altitude of 900-1600 m asl, which has a long dew period, cool temperatures, and a short daylength is a hot-spot for the development of NCLB [13]. Resistance evaluation is usually performed to mature plants at growth stage of R1 (silking stage), R2 (early grain filling), R3 (milk stage), R4 (late grain filling), and R5 (dent stage) [14]. Artificial inoculation ensures high NCLB pressures and high disease spreads and in turn will maximize genetic differentiation and ensure high heritability and high potential selection [15].

Incubation period and Area Under Disease Progress Chart (AUDPC) are components of an important nature for quantitative NCLB resistance since both are highly correlated and highly heritable [13]. The most common method to estimate AUDPC is the trapezoid method, which averaged the time variable and calculates the average disease intensity between each pairs of adjacent time points [16]. Low AUDPC value illustrates the lower rate of disease progression and greater disease resistance, while higher AUDPC values depict higher rates of disease progression and higher disease susceptibility. Rating is performed with scores ranging from 1 to 9 or 1 to 5 with lowest score indicating plots without symptoms and highest score indicating plots with most severe disease symptoms [11].

The objective of this study is (1) to evaluate maize hybrids and select the best hybrids based on disease resistance and yield, (2) to examine the relationship between resistance, yield, and yield components, and (3) estimate the genetic variance and heritability of resistance and yield traits for further breeding progress.

2. Materials and Methods

2.1. Location and Period of Study
The study was conducted in the village of Garingging, Karo District with the altitude of 1,100 m asl and temperature 18-27°C, from October 2019 to March 2020.

2.2. Materials
Evaluation was performed to 129 maize hybrids from Corteva Agriscience. Four commercial hybrids are assigned as check entries, i.e. P33 (resistance check), P23, P25, and X576 (susceptible check), and 125 hybrids as new entries. Inoculum was prepared with source collected from previous planting season. About 45 g of sorghum grain is seeded with spores in a conical flask. After 2-3 days of incubation in an aseptic condition, the grains were air-dried and prepared for powdering with mixer grinder. The resulted powder was then applied for inoculation.

2.3. Methods
Experiment was performed using Augmented Randomized Complete Block Design [17]. Experiment was arranged to 5 blocks. Each block comprised of 25 new entries with 4 check entries. Hybrids are planted in 1 row with 4 m in length. Planting distance between rows were 70 cm and between plants in row were 20 cm. A highly susceptible variety, X576, was assigned as spreader plant to maintain uniformity of disease intensity in the experiment. One row of spreader was planted between every 4 rows of treatment. Inoculation was applied in 35, 45, and 55 days after planting. Around 3 g of powdered inoculum was spread into the whorl followed by spraying 10-12 ml of water to provide adequate moisture for the spores to germinate. Inoculation was done in the late afternoon to avoid maximum temperature during incubation period.
Observation of resistance traits were done for incubation period, disease intensity, and AUDPC. Incubation period was observed every day from the first inoculation. First observation of disease intensity was conducted post-silking and repeated every 7 days until susceptible check reached highest severity. Disease intensity was calculated with formula from [18]:

\[ I = \frac{\sum n_v}{Z N} \times 100\% \]  

Where \( I \) is disease intensity, \( n \) is number of infected plants, \( v \) is disease score of each plant, \( Z \) is the highest score, \( N \) is total number of plants. The scoring system was provided by [19] and resistance category by [20], presented in Table 1. The final score was used to determine the category for resistance.

| Score | Symptoms | Category |
|-------|----------|----------|
| 1     | Plants do not show disease symptoms | Highly Resistant |
| 2     | First small lesions appear on few plants per row and occupies less than 5% of leaf surface | Resistant |
| 3     | Many plants per row present in one leaf level lesions occupying 5–10% of the leaf | Resistant |
| 4     | Many plants per row present in several leaf level lesions occupying 10–20% of the leaf | Moderately Resistant |
| 5     | Lesions occupying 20–40% of the leaf and start to merge | Resistant |
| 6     | Lesions occupying 40–60% | Susceptible |
| 7     | Lesions occupying 60–80%. Half of the leaf is dry due to disease infection | Susceptible |
| 8     | Lesions occupying 80–90%. More than half of the leaf is dry due to disease infection | Highly Susceptible |
| 9     | Lesions occupying 90–100%. Nearly the whole plant is dry due to disease infection | Highly Susceptible |

AUDPC is calculated using formula from [16]:

\[ AUDPC = \sum_{i=1}^{n-1} \frac{(y_i+y_{i+1})(t_{i+1}-t_i)}{2} \]  

Where \( y_i \) is an assessment of a disease at the \( i \)th observation, \( t_i \) is time at the \( i \)th observation, and \( n \) is the total number of observations. Yield and yield component observation was performed by measuring plant height, ear diameter, ear length, and ear weight. Plant height of 4 plants was measured from the soil line to the base of the leaf flag at reproductive stage. Ten ears were selected for measurement of ear diameter, length, and weight.

2.4. Data analysis

Observed data were analyzed by ANOVA. Difference between each new entries and checks are further analyzed using least significant increase at 5% level [21]. Genetic variance was estimated with formula:

\[ \sigma_g^2 = \sigma_p^2 - \sigma_e^2 \]  

\[ \sigma_g^2 = MS_p - MS_e \]
Where $\sigma_g^2$ is genetic variance, $\sigma_p^2$ is phenotypic variance, $MS_p$ is mean square of new entries, $MS_e$ is error mean square. Genotypic coefficient of variance, GCV, were estimated according to [22]:

$$GCV = \sqrt{\frac{\sigma_g^2}{X}} \times 100\%$$  \hspace{1cm} (5)

GCV was high if more than 20%, medium if between 10 and 20%, low if less than 10% [23]. Heritability, $H^2$, is estimated by:

$$H^2 = \frac{\sigma_g^2}{\sigma_p^2}$$  \hspace{1cm} (6)

Heritability was categorized as high if more than 0.5, medium if between 0.2 and 0.5, and low if less than 0.2 [24]. ANOVA, $\sigma_g^2$, GCV, $H^2$ were analyzed with R and package from [25]. Simple correlation test was performed to observe relationship between traits.

3. Results and Discussion

3.1. NCLB resistance and yield evaluation

High and uniform disease pressure was observed in the susceptible check due to artificial inoculation, which enabled distinction between the resistant to the susceptible hybrid. Disease rating was done at 94, 101, 109, 116, and completed at 122 day after planting. Results from the analysis of variance showed significant difference of means in incubation period, AUDPC, ear diameter, ear length, and ear weight, while no significant difference was observed in plant height. Further analysis compared the means between new entries and checks by least significant increase. Hybrids with AUDPC and ear weight that exceeded at least 3 checks were selected (Table 2).

| Hybrid | Incubation period (dai)$^a$ | AUDPC$^b$ | Resistance Category$^c$ | Plant height (cm) | Ear diameter (cm)$^a$ | Ear Length (cm)$^a$ | Ear weight (cm)$^a$ |
|--------|-----------------------------|-----------|--------------------------|-------------------|----------------------|-------------------|-------------------|
| P33    | 13.00                       | 1154.04   | MR                       | 211.45            | 4.61                 | 17.24             | 2.43              |
| P23    | 11.40                       | 1511.72   | S                        | 218.85            | 4.14                 | 16.53             | 1.87              |
| P27    | 10.00                       | 1764.46   | HS                       | 219.30            | 4.20                 | 15.12             | 1.45              |
| X576   | 7.00                        | 2307.14   | HS                       | 233.20            | 3.81                 | 14.03             | 1.32              |
| N399   | 16.10 B                     | 953.00 b  | MR                       | 216.76            | 4.99 A               | 17.04 D           | 3.69 A            |
| G163   | 19.85 A                     | 501.87 a  | HR                       | 241.64            | 4.78 B               | 18.65 B           | 3.12 B            |
| N936   | 17.10 A                     | 889.95 b  | MR                       | 238.57            | 5.01 A               | 15.74             | 3.05 B            |
| N374   | 14.35 C                     | 1103.48 b | MR                       | 205.82            | 4.95 A               | 18.12 C           | 2.95 B            |
| D651   | 18.10 A                     | 975.69 b  | MR                       | 270.07            | 4.96 A               | 16.33 D           | 2.87 B            |
| N020   | 18.35 A                     | 1048.36 b | MR                       | 218.57            | 4.97 A               | 15.40             | 2.62 B            |
| D635   | 16.85 A                     | 992.98 b  | MR                       | 218.64            | 4.74 B               | 16.82 D           | 2.73 B            |
| N031   | 20.35 A                     | 627.61 a  | R                        | 229.82            | 4.60 B               | 18.60 B           | 2.70 B            |
| LSI    | 3.39                        | 347.51    |                          | 24.22             | 0.22                 | 1.96              | 0.80              |
| Average| 14.41                       | 1280      |                          | 224.70            | 4.47                 | 16.25             | 2.03              |

$^a$Means followed with letter A is significantly higher than 4 checks, B is higher than 3 checks, C is higher than 2 checks, D is higher than 1 check

$^b$Means followed with letter a is significantly lower than 4 checks, b is lower than 3 checks, c is lower than 2 checks, d is lower than 1 check

$^c$HR: highly resistant, R: resistant, MR: moderately resistant, S: susceptible, HS: highly susceptible
From 125 new hybrids, 8 hybrids, i.e. N399, G163, N396, N374, D651, N020, D635, and N031, were recognized to have the best performance both in disease resistance and yield. Hybrids N396, N374, D651, N020, and D635 has AUDPC and ear weight significantly exceed 3 checks with no significant difference from the best check, P33. Two hybrids, G163 and N031, significantly exceed the best check for AUDPC and 1 hybrid, N399, for ear weight. Two hybrids, N399 and G163, may be regarded as promising hybrids to be developed for areas with high NCLB pressure, especially in the highland of Karo.

3.2. Association between traits
Simple correlation was performed to observed association between traits from 129 hybrids (Table 3).

| Traits            | Incubation period | Plant height | Ear diameter | Ear length | Ear weight |
|-------------------|-------------------|--------------|--------------|------------|------------|
| Plant height      | -0.03             |              |              |            |            |
| Ear diameter      | 0.333 **          | 0.079        |              |            |            |
| Ear length        | 0.165 *           | 0.123        | 0.485 **     |            |            |
| Ear weight        | 0.425 **          | 0.218 **     | 0.729 **     | 0.559 **   |            |
| AUDPC             | -0.921 **         | 0.085        | -0.383 **    | -0.243 **  | -0.472 **  |

*significant at α 0.05 **significant at α 0.01

AUDPC and incubation period was highly correlated, as stated by [13]. Prolonged incubation period with fewer and smaller lesions suggesting a quantitative NCLB resistant [26]. AUDPC also correlated negatively with ear diameter, ear length, and ear weight. This implied that susceptible hybrids may have its yield suffer from high intensity of NCLB. Although no significant correlation between AUDPC and plant height was observed, plant height is significantly correlated with ear weight.

3.3. Genetic variance, GCV, and heritability estimates of traits
Results of genetic variance, GCV, and heritability estimates of traits from 129 hybrids were presented in Table 4.

| Trait             | $\sigma^2_g$ | GCV   | GCV category | $H^2$ | $H^2$ category |
|-------------------|--------------|-------|--------------|-------|---------------|
| Incubation period | 9.04         | 20.87 | High         | 0.80  | High          |
| AUDPC             | 104374.52    | 25.25 | High         | 0.81  | High          |
| Plant height      | 134.05       | 5.15  | Low          | 0.55  | High          |
| Ear diameter      | 0.05         | 4.96  | Low          | 0.77  | High          |
| Ear length        | 2.20         | 9.13  | Low          | 0.74  | High          |
| Ear weight        | 0.12         | 17.16 | Medium       | 0.48  | Medium        |

High GCV was observed in disease resistance traits. This presents opportunities for further selection in developing NCLB resistant varieties, since genetic diversity is adequate. However, intensive selection in plant height, ear diameter, and ear length might exhaust the genetic resource, hence the low GCV. High $H^2$ in incubation period, AUDPC, plant height, ear diameter, and ear length indicated that variations in the traits was largely due to genetic factor, while some degree of environmental factor affects the phenotypic variation in ear weight. Since incubation period is highly heritable and significantly correlated with yield and its component and, it may be considered as direct indicator for yield in early stage of selection.
4. Conclusions
Evaluation of disease resistance and yield components resulted in eight new hybrids that exceeded at least 3 checks regarding to disease resistance and ear weight, namely N399, G163, N396, N374, D651, N020, D635, and N031. Hybrid N399 has ear weight above 4 checks and G163 has better AUDPC than the 4 checks, suggesting these hybrids as promising for developing varieties in areas with NCLB pressure. Significant association with AUDPC was seen in incubation period, ear diameter, ear length, and ear weight. High GCV was observed in disease resistance traits, while high $H^2$ is estimated in incubation period, AUDPC, ear diameter, and ear length. Incubation period may be considered as direct indicator for yield in early stage of selection.

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