Genotype resistance of hybrid corn varieties candidate against major corn diseases

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Abstract. The resistance level of several national superior corn varieties to the primary disease of corn is still varied and unstable. Therefore, evaluating the resistance of hybrid corn varieties candidate to the primary disease of corn is deemed necessary as an initial step in managing the disease. This study aimed to evaluate the resistance reaction of the hybrid corn varieties candidate against the primary disease of corn. This study was conducted at two different locations. The first location was at the Agricultural Technology Research and Assessment Installation (ATRAI) of the Indonesian Cereals Research Institute in Bajeng District, Gowa Regency, South Sulawesi first, where the test for *P. philippinensis* downy mildew, leaf blight, and leaf rust was conducted. The next location was at the ATRAI of Muneng of the Research Institute for Various Nuts and Tubers in Probolinggo, East Java, where the test for the *P. maydis* downy mildew was conducted. The tests were carried out using plants as the source of the test pathogen inoculum planted around the experimental block. The four corn genotypes tested were CHC1, CHC02, CHC3, CHC5, and CHC5. Meanwhile, the comparison varieties used were P36, Pertiwi-6, PAC339, BISI18, Anoman, and Pulut. The test results showed the two hybrid corn varieties candidate that were moderately resistant to *P. philippinensis*, namely CHC1 and CHC2, with attack intensities of 30.94% and 32.07%, respectively; one variety candidate resistant to *P. maydis*, namely CHC1 with an attack intensity of 15.92%. Meanwhile, the five hybrid corn varieties candidate, namely CHC1, CHC02, CHC3, CHC5, and CHC5, showed a moderately resistant reaction to *maydis* leaf blight and leaf rust with an attack intensity of 35%.

1. Introduction

Corn (*Zea mays* L.) is an essential requirement for human and animal life. The need for corn consumption from year to year continues to increase. Until now, efforts to increase production continue to be encouraged, but behind that, various inhibiting factors are still difficult to overcome. The production obtained by broad unity is still low. The high demand and need for domestic corn cause Indonesia to have to import. To meet national needs and reduce the volume of corn imports, the government has launched a program to increase production since 2007 with the goal of self-sufficiency [1]

One factor affecting the production and quality of corn yields is infection with plant pathogens, such as the attack of plant-pest organisms (PPO). PPO is one of the main limiting factors in developing corn in Indonesia. It was reported that there were three primary diseases of corn that could significantly reduce production, even in disease-prone varieties that could result in crop failure. The three primary diseases are downy mildew, leaf blight, and leaf rust. In Indonesia, downy mildew...
causes around 50-80% yield losses in East Java, South Sulawesi, and West Kalimantan [2]. In South Sulawesi, downy mildew can cause up to 90% [3-4]. Other diseases are *maydis* leaf blight caused by the pathogen *Bipolaris maydis* and leaf rust caused by *Puccinia* spp., these two pathogens cause significant diseases in corn which can be a factor in corn productivity. This disease attacks corn crops, with the most damage seen in the hot and humid tropics [5].

Efforts to control the disease are basically through maintaining the development of pathogens, utilizing the host and the environment to minimize the effects caused by the pathogen so that it reaches a point below the economic threshold with negligible losses. One of the main components in controlling significant corn diseases is resistant varieties; this is more profitable because its resistance is more stable, economical, and causes no side effects in poisoning and environmental pollution [6].

So far, the level of resistance of several national superior corn varieties to the primary diseases of corn is still varied, and many of them are infected or susceptible. Therefore, it is deemed necessary to evaluate the resistance screening of hybrid corn varieties candidate against the primary diseases of corn as the first step in managing these diseases.

2. Materials and Methods

2.1. Evaluation of resistance of hybrid corn varieties to downy mildew

The test of hybrid corn varieties candidate against downy mildew was carried out at two locations. First, for the downy mildew caused by *P. philipinensis*, the test was conducted at the Agricultural Technology Research and Assessment Installation (ATRAI) of the Indonesian Cereals Research Institute (ICERI) in Bajeng District, Gowa Regency, South Sulawesi. Second, for the downy mildew caused by *P. maydis*, the test was conducted at the ATRAI of the Indonesian Legumers and Tuber Crops Research Institute (ILETRI) in Probolinggo, East Java. The four corn genotypes tested were CHC1, CHC2, CHC3, CHC4, and CHC5. The comparison varieties used were P36, Pertiwi-6, PAC339, BISI18, and Anoman. The research implementation started by planting downy mildew inoculum source plants (Anoman variety), planted in three rows around the test plots and between the test genotype blocks. The inoculum source was planted four weeks before the test genotype was planted. Ten days after the inoculum source was planted, it was inoculated by conidia suspension of the fungus *P. philipinensis* in the morning or under high humidity conditions.

Three weeks after the inoculum source plant was inoculated by *P. philipinensis*, or when the downy mildew attack on the inoculum source plant reached 60%, the test genotype was planted without seed treatment. Each genotype was planted in two rows of 5-meter long with a spacing of 75 cm x 20, two seeds per hole. The plant fertilization was done twice using urea fertilizer at a dose of 300 kg/ha. The first fertilization was done when the plants were 10 DAP, using urea at a 300 kg/ha dose. The second was done at 30 DAP, using urea at a 150 kg/ha dose. The intensity of downy mildew was observed at 14, 21, 28, 35, and 42 DAP. This formula below calculated the percentage of downy mildew pathogen attack:

\[ I = \frac{(A/B) \times 100\%}{I = \text{Disease incidence (\%)}} \]
\[ A = \text{Total plant attacked by downy mildew} \]
\[ B = \text{Total plant observed in each genotype} \]

2.2. Evaluation of resistance of hybrid corn varieties to *maydis* leaf blight and leaf rust

The comparison varieties in this test were resistant comparisons (P36, Pertiwi-6, PAC339, BISI18) and susceptible comparisons (Anoman varieties for leaf blight, Pulut varieties for leaf rust). The unit for testing for corn leaf blight and leaf rust was made separately. The study was initiated by planting the inoculum sources for corn leaf blight (Anoman variety) and leaf rust (Pulut variety) in three rows, each around the test plots and between the experimental blocks. Three weeks after the inoculum source
plants were planted, the plants were sprayed with a conidia suspension of the fungus causing leaf blight (*B. maydis*) and the pathogen causing leaf rust (*P. polysora*) in the afternoon with a spore density of about 6 x 10^4 conidia/ml. The used isolate *B. maydis* was from the collection of the Balitcereal Diseases Laboratory. Meanwhile, the source of pathogenic *P. polysora* inoculum was collected directly from leaves with leaf rust. Furthermore, the test genotype was planted after the inoculum source plant was attacked by leaf blight by 60%. The test genotypes were planted in two rows and 5-meter long, with a spacing of 75 x 20 cm. Each hole was planted with two seeds and given Carbofuran 3G to prevent attacks by ants or leaf eaters. The plants were fertilized using urea of 300 kg/ha, Phonska of 200 kg/ha, a half dose of urea (150 kg/ha) at 10 DAP, and half urea (150 kg/ha) at 30 DAP.

The observation on corn leaf blight was carried out at the age of 45, 60, and 75 DAP using the modified [7] scale as follows:

### Table 1. Scoring of corn leaf blight disease

| Scale | Information |
|-------|-------------|
| 0     | No symptoms of disease |
| 1     | Very mild infection; there are symptoms of blight 1% - 5%; lesions are scattered on the lower leaves |
| 2     | Mild infection, blight symptoms in plants reach 6% - 20%; number of lesions < 25% on lower leaves |
| 3     | Moderate infection; blight symptoms 21% - 50%; number of lesions > 50% on lower leaves; some on middle leaf < 25% |
| 4     | Severe infection; blight attack reached > 50%; lower leaves die; lesions on the middle leaves > 50% and extends to the upper leaves with lesions < 25% |
| 5     | Very severe infection; abundant lesions on almost all leaves; the plant dries to death |

Source: Sharma, 1983.

Observations were made on the scoring of rust-infected plants at 50 DAP, 60 DAP, and 70 DAP. The method based on the Directorate of Maize Research India [8] was used to determine the scoring value of corn leaf rust disease as follows:

### Table 2. Scoring of corn leaf rust disease

| Scale | Information |
|-------|-------------|
| 0     | No symptoms of disease |
| 1     | The infection is minimal; one or two to several pustules scattered on the lower leaves only |
| 2     | The number of pustules is relatively low on the lower leaves only (mild infection) |
| 3     | Pustules are abundant on the lower leaves; some on the middle leaves |
| 4     | Abundant on the lower and middle leaves; extending to the top of the leaves in the middle |
| 5     | Pustules abound on all leaves; the plant may dry out prematurely or be killed by disease |

Source: Directorate of Maize Research India, 2012.

The disease scale was then transformed into the attack percentage formula as follows:

\[
DS = \frac{\sum (n \times v)}{Z \times N} \times 100\%
\]

**DS** : Disease Severity  
**n** : Number of affected plants in each category  
**v** : Scale value on each affected plant  
**Z** : Highest scale value  
**N** : Number of plants observed in each attack
The resistance criteria used for the three disease tests were based on the Food Crop Varieties Release Procedure (2019) as follows:

- **Very Resistant (VR)** = 0 – 5%,
- **Resistant (R)** = >5 - 20%,
- **Moderately Resistant (MR)** = >20 - 40%,
- **Susceptible (S)** = >40 - 60%, and
- **Very Susceptible (VS)** = >60%.

### 2.3. Experimental design and data analysis

This study was structured using a Randomized Block Design. A total of seven genotypes were treated, consisting of four test genotypes (CJH-01, CJH-02, CJH-03) and three comparison genotypes (varieties of P-36, PAC339, and Anoman). The study was organized into four experimental blocks. The observational data were analyzed statistically, followed by the Least Significant Difference (LSD) test at the 5% level.

### 3. Results and Discussion

#### 3.1. Resistance of hybrid corn varieties to downy mildew

The results of testing hybrid corn varieties candidate against downy mildew caused by *P. phillipinensis* showed an increase in infection every week. The downy mildew infection at 14 DAP had not been found in all entries. The downy mildew infection began to appear at 28 DAP with a relatively low attack intensity, ranging from 3.31% to 19.91%. A significant increase in the downy mildew infection was seen at 35 DAP until the last observation was 42 DAP. At 35 DAP, the comparison varieties of PAC339, BISI-8, and Anoman showed a relatively high attack intensity, namely 50.47%, 52.71%, and 67.98%, with the criteria Susceptible to Very Susceptible. The downy mildew infection at the last observation (42 DAT) showed the genotypes of hybrid corn varieties CHC1 and CHC2, which showed a moderately resistant reaction with a relatively low attack intensity of 30.94% and 32.07% (Table 3).

Observation of the test genotype resistance to the *P. maydis* pathogen at 14 DAP showed that the downy mildew was found in the test plants with a relatively low attack intensity. A significant increase in the downy mildew infection began to be seen at 28 DAP, where the susceptible comparison variety (Anoman) in all blocks had shown an incidence of downy mildew by 80%, with the criteria of being Very Susceptible. Downy mildew infection continued to increase until the plant was 42 DAP. At the last observation at 42 DAP, the susceptible comparison varieties of Anoman and PAC339 showed susceptible criteria with disease intensity of 100% and 92.38%, respectively, as well as resistant comparison varieties of P36, Pertiwi-6, and BISI18 that showed criteria of Resistant to Very Resistant, with intensity disease by 3.31%, 9.26%, and 11.59%, respectively. The test results showed one candidate genotype of hybrid corn varieties that showed resistance to the *P. maydis* pathogen with a disease intensity of 15.92%, namely the CHC1 genotype (Table 3).

The differences in the resistance reactions of the five genotypes of hybrid corn candidate to downy mildew at two different locations can be seen in Figure 1. Overall the intensity of downy mildew in the five genotypes of hybrid corn candidate in East Java was lower than in South Sulawesi. The occurrence of differences in resistance reactions of the five varieties candidate above at two different locations was thought to be caused by several factors, including differences in the species causing downy mildew in the two locations, and differences in plant genetics; some varieties were thought to have horizontal resistance genes or vertical resistance genes (major) [9,10].
### Table 3. Intensity of downy mildew in genotypes of hybrid corn variety in South Sulawesi

| Genotype       | Intensity of downy mildew (%) at age (DAP) | Resistance Criteria |
|----------------|------------------------------------------|---------------------|
|                | 14        | 21  | 28  | 35  | 42  |                    |
| CHC1           | 0.00      | 5.22| c   | e   | 15.83| cd | 24.89 | cde | 30.94| cde | MR   |
| CHC2           | 0.00      | 15.86| 22.73| d   | 27.57| cde | 32.07| cde | MR   |
| CHC3           | 0.00      | 17.30| 27.95| 46.27| e   | 60.13| e   | VS   |
| CHC4           | 0.00      | 17.51| 30.26| 51.43| e   | 60.86| e   | VS   |
| CHC5           | 0.00      | 15.47| 37.10| 56.97| 69.69| e   | VS   |
| P36 (a)        | 0.00      | 3.31 | 6.15 | 13.75| 20.73| MR   |
| Pertiwi-6 (b)  | 0.00      | 7.01 | 12.93| 23.99| 33.41| MR   |
| PAC339 (c)     | 0.00      | 20.68| 34.48| 50.47| 56.21| S    |
| BISI18 (d)     | 0.00      | 16.32| 34.88| 52.71| 71.06| VS   |
| Anoman (e)     | 0.00      | 19.91| 28.35| 67.98| 91.35| VS   |
| Means          | 0.00      | 13.86| 25.32| 41.60| 52.65|      |
| CV             | 0.00      | 48.06| 30.98| 20.36| 12.86|      |
| LSD 5%         | -         | 11.42| 13.46| 14.53| 11.61|      |

Information: (a) the significant infection rate was lower than the comparison variety P36 at the 5% LSD test level; (b) the significant infection rate was lower than the comparison variety Pertiwi-6 at the 5% LSD test level; (c) the significant infection rate was lower than the comparison variety PAC339 at the 5% LSD test level; (d) the significant infection rate was lower than the comparison variety Bisi-18 at the 5% LSD test level; (e) the significant infection rate was lower than the comparison variety ANOMAN at the 5% LSD test level; R: Resistant; MR: Moderately Resistant; S: Susceptible; VS: Very Susceptible.

### Table 4. Intensity of downy mildew in genotypes of hybrid corn variety in East Java

| Genotype       | Intensity of downy mildew (%) at age (DAP) | Resistance Criteria |
|----------------|------------------------------------------|---------------------|
|                | 14        | 21  | 28  | 35  | 42  |                    |
| CHC1           | 0         | 4.64 | c   | e   | 9.61 | c e | 15.56 | c e | 15.92| c e | R    |
| CHC2           | 0.82      | 38.85| c e | 84.24| e   | 89.47| e   | 89.47| e   | VS   |
| CHC3           | 0.00      | 49.40| c e | 83.41| c e | 86.80| e   | 89.16| e   | VS   |
| CHC4           | 0.00      | 34.47| c e | 85.31| e   | 82.97| e   | 87.15| e   | VS   |
| CHC5           | 1.81      | 78.65| 94.89| 95.98| 95.98| VS   |
| P36 (a)        | 0.00      | 0.42 | 2.24 | 2.67 | 3.31 | VR   |
| Pertiwi-6 (b)  | 0.00      | 4.16 | 3.43 | 8.69 | 9.26 | R    |
| PAC339 (c)     | 0.87      | 77.32| 89.96| 92.19| 92.38| VS   |
| BISI18 (d)     | 0.26      | 2.19 | 6.53 | 11.59| 11.59| R    |
| Anoman (e)     | 0.51      | 88.56| 100.00| 100.00| 100.00| VS   |
| Means          | 0.43      | 37.87| 55.96| 58.59| 59.42|      |
| CV             | 308.85    | 19.32| 7.94 | 11.40| 10.53|      |
| LSD 5%         | -         | 10.62| 6.45 | 9.69 | 9.08 |      |

Information: (a) the significant infection rate was lower than the comparison variety P36 at the 5% LSD test level; (b) the significant infection rate was lower than the comparison variety Pertiwi-6 at the 5% LSD test level; (c) the significant infection rate was lower than the comparison variety PAC339 at the 5% LSD test level; (d) the significant infection rate was lower than the comparison variety Bisi-18 at the 5% LSD test level; (e) the significant infection rate was lower than the comparison variety ANOMAN at the 5% LSD test level; VR: Very Resistant; R: Resistant; MR: Moderately Resistant; S: Susceptible; VS: Very Susceptible.
Figure 1. Reaction of the Resistance of Hybrid Corn Varieties to Downy Mildew in South Sulawesi and East Jawa; VR: Very Resistant, R: Resistant, MR: Moderately Resistant, S: Susceptible, VS: Very Susceptible.

3.2. Resistance of hybrid corn varieties to maydis leaf blight and leaf rust

The first observation (45 DAT) of the pathogenic infection that caused corn leaf blight showed that the whole plant had blight symptoms with attack intensity ranging from 18.79% - 34.5% (Table 3). In the first observation, generally, blight symptoms appeared on the lower leaves and some on the middle leaves but in a relatively low percentage. Wakman and Burhanuddin [11] and Latifahani et al. [9] explained that the symptoms of leaf blight at the beginning of infection were in the form of small spots, elongated oval in shape, then the spots became more elongated in the shape of an ellipse. The spots would expand, and some of the spots may coalesce, causing the leaf tissue to die (necrosis).

The observation of corn leaf blight at 75 DAP showed that the five genotypes of hybrid corn varieties candidate, namely CHC1, CHC2, CHC3, CHC4, and CHC5, experienced a relatively low increase in infection, respectively 26.67%, 30.91%, 30.91%, 26.06, and 329.70% with the category of Moderately Resistant (AT) reaction. The five candidate hybrid corn varieties showed a significantly lower infection rate than the comparison variety Anoman on the 5% LSD test, where the variety showed a Susceptible reaction (R) with an attack intensity of 60%. According to [12], corn leaf blight can develop rapidly in corn areas and cause significant yield losses of about 59% under optimum conditions.

Table 5. Intensity of corn leaf blight on genotypes of hybrid corn varieties in South Sulawesi

| Genotype   | 45 DAP | 60 DAP | 70 DAP | Resistance Reaction |
|------------|--------|--------|--------|---------------------|
| CHC1       | 20.00  | e      | 23.64  | e                   | MR      |
| CHC2       | 21.82  | e      | 26.36  | e                   | MR      |
| CHC3       | 22.42  | e      | 26.06  | e                   | MR      |
| CHC4       | 18.79  | e      | 23.03  | de                  | MR      |
| CHC5       | 21.82  | e      | 26.67  | e                   | MR      |
| P36 (a)    | 18.18  | e      | 25.45  | e                   | MR      |
| Pertiwi-6 (b) | 22.42  | e      | 25.45  | e                   | MR      |
| PAC 339 (c) | 24.24  | e      | 27.27  | e                   | MR      |
The test results of the resistance of hybrid corn varieties candidate against leaf rust showed that the leaf rust infection was found at 50 DAP with a relatively low intensity with an attack range of 1.21% – 30.30%. The attack illustrated that the severity of rust disease in all genotypes was still less than 35%.

The observation at 70 DAP showed that the severity of leaf rust in five hybrid corn varieties candidate, namely CHC1, CHC2, CHC3, CHC4, and CHC5, was significantly lower than the Pulut comparison variety. The severity of the disease in the Pulut comparison variety was very high at 69.70%, with the criteria of being Very Susceptible (SR). The leaf rust attack on hybrid corn varieties candidate was still low, below 30%, with the criteria of Moderately Resistant (MR) to Resistant (R) (Table 6).

### Table 6. Intensity of corn leaf rust on genotypes of hybrid corn varieties in South Sulawesi

| Genotype   | Intensity of leaf rust (%) at age (DAP) | Resistance Reaction |
|------------|----------------------------------------|---------------------|
|            | 50 HST | 60 HST | 70 HST |
| CHC1       | 1.82 e | 17.58 e | 27.27 a | MR     |
| CHC2       | 2.42 e | 17.58 e | 19.39 a | R      |
| CHC3       | 3.03 e | 15.15 e | 26.67 a | MR     |
| CHC4       | 5.45 e | 19.39 e | 25.45 a | MR     |
| CHC5       | 7.27 e | 20.61 e | 29.09 a | MR     |
| P36 (a)    | 3.03   | 18.79   | 37.58   | MR     |
| Pertiwi-6 (b) | 1.21   | 11.52   | 15.76   | R      |
| PAC 339 (c) | 2.42   | 15.76   | 20.00   | MR     |
| BISI18 (d) | 7.27   | 18.79   | 39.39   | MR     |
| Pulut (e)  | 30.30  | 43.64   | 69.70   | VS     |
| Means      | 6.42   | 19.88   | 31.03   |       |
| CV         | 89.53  | 16.52   | 18.20   |       |
| LSD 5%     | 9.86   | 5.63    | 9.69    |

Information: (a) the significant infection rate was lower than the comparison variety P36 at the 5% LSD test level; (b) the significant infection rate was lower than the comparison variety Pertiwi-6 at the 5% LSD test level; (c) the significant infection rate was lower than the comparison variety PAC339 at the 5% LSD test level; (d) the significant infection rate was lower than the comparison variety Bisi-18 at the 5% LSD test level; (e) the significant infection rate was lower than the comparison variety ANOMAN at the 5% LSD test level; R: Resistant; MR: Moderately Resistant; S: Susceptible; VS: Very Susceptible.
4. Conclusion
The CHC1 and CHC2 genotypes reacted moderately resistant to downy mildew (*P. philipinensis*) with attack intensities of 30.94% and 32.07%, respectively. The CHC1 genotype was resistant to downy mildew (*P. maydis*) with a relatively low attack intensity of 15.92%. The five candidate hybrid corn varieties, namely CHC1, CHC2, CHC3, CHC4, and CHC5, showed moderately resistant to resistant reactions to *maydis* leaf blight and leaf rust with attack intensity of 35%.

The CHC1 hybrid corn genotype consistently showed a relatively low resistance reaction to the primary corn disease, whereas this genotype reacted moderately resistant to resistant to downy mildew, corn leaf blight, and leaf rust, thus it could be recommended as a new high yielding variety.

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