The resistance of various maize germplasms collected from several regions in Indonesia to downy mildew 
(*Peronosclerospora philippinensis*)

S H Kalqutny*, S Pakki
Indonesian Cereals Research Institute, South Sulawesi, Indonesia

*Email: septianharykalqutny@gmail.com

Abstract. Downy mildew is one of the limiting factors of corn production in Indonesia. Several efforts have been needed to control pathogens, one of them is the development of resistant varieties. New resistant varieties must be developed to prevent a decrease in genetic diversity in a region due to dependence on one variety. Germplasms collected from a variety of different environmental conditions is a potential source of genetic diversity. This study aims to determine the resistance of maize germplasm accession to downy mildew caused by *P. philippinensis*. In this study, the total number of 96 accessions of maize germplasm were tested. The experiment was arranged in a group design with 2 replications. The intensity of downy mildew infection was observed based on the incidence of the disease in 25, and 35 days after planting (DAP). The value of Area under Disease Progress Curve (AUDPC) is also calculated based on the intensity of downy mildew infection in a particular observation period. One accession which showed high resistance to downy mildew (*Peronosclerospora philippinensis*) was obtained, namely accession number 808 with 0 per cent infection and 0 AUDPC value, lower than the resistant varieties (Bima 3) used as a comparison, which had an average 5-10% infection and 103.5 AUDPC value. The accession obtained can be used as the source of the downy mildew resistance trait for the development of the new highly resistant variety.

1. Introduction

Cereals such as maize are one of the major commodities that have good prospects, due to the growing domestic food industry and the export opportunities. National maize self-sufficiency efforts have been carried out with both intensification, extensification. One major limiting factor in efforts to reach these national targets is pests and diseases. Some important diseases that might cause a decrease in yields on maize are including leaf blight, stem rot, cob rot, bacterial and viral infections [1] and also leaf rust and downy mildew. Downy mildew may cause up to 100% yield losses if the infection occurs at the beginning of the growth phase [2]. Early infection and the use of susceptible varieties will cause greater loss of the yields compared to the later infection and more resistant varieties.

Several efforts have been made to control pathogens such as quarantine actions, crop cultivation strategies, use of chemicals, and the development of resistant plant genotypes [3]. Although the process of development of resistant varieties is quite long and laborious, the use of resistant varieties to control the disease is environmentally friendly, easy, and inexpensive to farmers.

New resistant varieties must be developed to prevent a decrease in genetic diversity in a region due to dependence on one variety. Varieties that are genotypically similar are also at risk of having the
same susceptibility to a disease and may cause an outbreak to a certain disease [4]. Germplasms collected from a variety of different environmental conditions is a potential source of genetic diversity. Germplasms collected from several regions in Indonesia is a good source of genes for the development of new superior varieties, such as resistance to biotic and abiotic stresses, and high yields. To obtain a variety that is resistant, the breeding plant material needs to be screened for its resistance to disease. Based on these conditions, the selection was conducted to obtain the sources of resistance originating from maize germplasm from various regions in Indonesia. This study aims to determine the resistance of maize germplasm accession to downy mildew caused by P. philippinensis.

2. Material and Methods

The research was conducted at the Bajeng Experimental Farm of Indonesian Cereal Research institute. The total number of 96 accessions of maize germplasm were tested. The experiment was arranged in a randomized block design with 2 replications. Each accession in one replication was planted in one row for 5 m, with plant spacing of 75 x 20 cm. Carbofuran 3G was used to prevent ants or other pests at the beginning of plant growth. The fertilizer was applied at 10 DAP (urea and ponska, 100 kg and 200 kg/ha respectively), 30 DAP and 45 DAP (100 kg urea/ha).

As a source of inoculum for downy mildew, four rows of susceptible varieties (Anoman) were planted three weeks before the test material around the field and between replications as spreader row. The spreader row was inoculated twice by spraying the conidial suspension of the Peronosclerospora philippinensis. The conidial suspension was obtained from infected leave dissolved in a sterile water solution. A resistant variety was also planted as a comparison.

The intensity of downy mildew infection was observed in 25, and 35 days after planting(DAP). The intensity of the disease was based on the incidence of the disease observed according to Pakki and Mappaganggang [5], namely: 0 - 10% = Resistant; 11-25% = Moderately Resistant; 26-50% = Susceptible; 50 % = Highly Susceptible.

The value of Area under Disease Progress Curve (AUDPC) is calculated based on the intensity of downy mildew infection in a particular observation period. AUDPC describes the rate of disease progression over time in a period of observation. The value of AUDPC is evaluated using the following equation [6]:

$$AUDPC = \sum_{i=1}^{n} \left( \frac{X_i + X_{i+1}}{2} \right) (t_{i+1} - t_i)$$

n = number of observations; x = disease intensity; (ti +1 -ti) = time interval between observations

Accessions that show high resistance, are self-fertilized and the seeds are stored as germplasm material (Peronosclerospora philippinensis).

3. Results And Discussion

The downy mildew Inoculant collected from the location (KP Bajeng) belongs to the species of P. philippinensis [5, 7, 8, 9, 10]. The observation showed that the artificial inoculation of the spreader row around the accession plants was showing initial symptoms about 10 days after inoculation and then spread evenly in the spreader row plant population with downy mildew infection around > 90% at the end of the study.

Observation at 25 days after planting (DAP) showed that the susceptible varieties (Anoman) were infected with downy mildew about 55% - 100%. Initial infection was observed at the base of the leaf, followed by symptoms of white stripes in the direction of the leaf vein.Unlike the resistant variety (Bima 3), downy mildew only infected around 0-5% of the population. The conidia produced by the pathogen infects and develops well in susceptible varieties, but in resistant varieties, its development is hampered. This result indicates that the spread of the disease from the spreader row is sufficient and assumed to be able to infect evenly across the test field.

In this study, the dewdrops were observed at the base of the leaves, this might be the source of the moisture needed for the conidia of the pathogen to germinate and infects the tissue. Wakman and S.
Kontong [11] found that conidia began to germinate at around midnight with the help of the dewdrops at the base of the leaves of the maize plant. The conidia of downy mildew usually produced at night, with temperatures around 70-79 ° F, then spread across the surrounding crops by wind. Conidia attached to the leaves takes an hour to germinate, then infects through stomata [12].

The observation of disease incidence at the age of 25 DAP (Table 1), shows that in comparison to the susceptible varieties (74% - 100%) about 57% of accession infected with > 50%, only about 3% were infected with 0-10% ie accession number 290 and 808. The resistance of a variety is influenced by several factors, one of them is the ability of varieties/lines to protect themselves from pathogenic stresses caused by the genetic properties of the variety [13] or the nutrient content of the host plant [14].

| Accession | Incidence (%) of DM at 25 DAP | Accession | Incidence (%) of DM at 25 DAP | Accession | Incidence (%) of DM at 25 DAP |
|-----------|-------------------------------|-----------|-------------------------------|-----------|-------------------------------|
| 305       | 55                            | 712       | 26                            | 733       | 38                            |
| 710       | 32                            | 755       | 20                            | 719       | 47                            |
| 722       | 18                            | 302       | 50                            | 835       | 31                            |
| 109       | 28                            | 211       | 44                            | 812       | 33                            |
| 340       | 42                            | 699       | 32                            | 742       | 26                            |
| 785       | 52                            | 94        | 13                            | 818       | 27                            |
| 693       | 51                            | 725       | 10                            | 724       | 11                            |
| 59        | 58                            | 694       | 18                            | 306       | 27                            |
| 282       | 19                            | 685       | 12                            | 736       | 45                            |
| 286       | 36                            | 740       | 39                            | 839       | 47                            |
| 690       | 32                            | 101       | 34                            | 228       | 46                            |
| 769       | 45                            | 764       | 15                            | 820       | 92                            |
| 687       | 40                            | 239       | 18                            | 762       | 42                            |
| 773       | 45                            | 724-A     | 43                            | 263       | 33                            |
| 686       | 37                            | 731       | 33                            | 737       | 28                            |
| 696       | 40                            | 705-A     | 46                            | 763       | 43                            |
| 765       | 40                            | 291       | 32                            | 762       | 42                            |
| 744       | 45                            | 735       | 22                            | 809       | 13                            |
| 285       | 38                            | 275       | 15                            | 226       | 24                            |
| 149       | 29                            | 290       | 7                             | 808       | 0                             |
| 308       | 32                            | 734       | 80                            | 810       | 18                            |
| 691       | 45                            | 271       | 60                            | 814       | 16                            |
| 761       | 40                            | 695       | 24                            | 813       | 27                            |
| 137-13    | 27                            | 732       | 50                            | 271-A     | 23                            |
| 765       | 47                            | 723       | 18                            | 817       | 15                            |
| 301       | 87                            | 718       | 21                            | 730       | 21                            |
| 774       | 48                            | 743       | 20                            | 273       | 14                            |
| 207       | 37                            | 771       | 15                            | 771       | 45                            |
| 414       | 32                            | 741       | 20                            | 738       | 23                            |
| 688-A     | 31                            | 307       | 50                            | 811       | 38                            |
| 339       | 17                            | 717       | 22                            | 245       | 12                            |
| 772       | 15                            | 739       | 38                            | 50±278    | 37                            |

Table 1. The average percentage of downy mildew infection (P. philipinensis) at 25 and 35 DAP.

Description: 0 - 10% = Resistant; 11-25% = Moderately Resistant; 26-50% = Susceptible; 50% = Highly Susceptible [5].

The different intensity of the disease across the test material may indicate the effect of the level of resistance of each test material on the disease. The resistance of plants may reduce the production of
conidia, reducing the source of the inoculum, thus slowing the spread of downy mildew [15]. Plants that have resistance to downy mildew are known to inhibit the conidial germination, penetration, and pathogenic infection processes [10]. Subsequent observations at 35 DAP (Table 1), show that the susceptible varieties (Anoman) have been 100% infected. A large number of the plant in susceptible varieties are dead as well as some of the test material. In contrast, different things were found in the resistant varieties (Bima-3), only maximum 10% of the population were infected. This fact indicates that the spread of conidia in the field is still ideal for infecting plants, thus minimize the possibilities of the test plant survives simply because it escapes the infection.

Table 2. The distribution of the intensity of downy mildew (P. philippinensis) at 25 and 35 DAP.

| Disease Incidence (%) | 25 DAP (96 accession) | 35 DAP (96 accession) |
|-----------------------|-----------------------|-----------------------|
| 0-10                  | 3                     | 1                     |
| >10-25                | 30                    | 0                     |
| >25-50                | 55                    | 15                    |
| >50                   | 8                     | 80                    |
| Average percentage    | 32.94%                | 73.86%                |
| Minimum               | 0%                    | 0%                    |
| Maximum               | 92%                   | 100%                  |
| Susceptible var. (Anoman) | 93.5%               | 100%                  |
| Resistant var. (Bima 3) | 3.8%                | 7.4%                  |
| Sd                    | 21.79                 | 16.19                 |

In table 2, it can be seen that at 25 DAP the disease incidence of susceptible varieties (Anoman) reaches around 93.5 per cent, and most of the accession infected around 25-50%, there are 3 accessions observed with the low disease percentage (0-10%). Furthermore, at 35 DAP, the development of the disease continued, all the population of susceptible varieties (anoman) are infected and most of them were killed, the majority of the accession was observed with more than 50% disease incidence, but one accession, ie accession 808, still showed a high resistance reaction with a disease incidence of 0%. This fact indicates that the accession has a genetic trait that is able to limit the initial infection of downy mildew.

The intensity of downy mildew caused by P. philippinensis is determined by the ability of the plant to repress the pathogen and its host-pathogen compatibility. In susceptible varieties, the production of conidia is high, this may causing the wide spreading of conidia resulting in the death of the plants. In the process of plant growth, pathogens will interfere with the nutrients of the cells of host plant tissues this condition causes the plants to die. Whereas in resistant varieties, plants have the ability to inhibit the development of conidial germination and colonization of mycelium in plant tissues so that the development of the disease is slower and shows a resistant reaction to downy mildew disease.
Figure 1. The distribution of the reaction of accession at 25 and 35 DAP.

Accession number 808 shows a high level of resistance (0%) to the disease, and consistent until the age of 35 DAP. This is different from most other accessions that are infected with fairly high intensity at 35 DAP as seen in Figure 1. Therefore this may give an indication that the conidia which usually infects after germinating in gut water at the whorl, is unable to invade the cell surface and infecting it, or its development is inhibited by phytoalexin released by plants so that the intensity of the attack is lower. In some cases, the resistance response of plants to the pathogen is influenced by the production of phytoalexin from the host [16,17].

In the high-infected accessions, mycelia are thought to successfully infect the plant tissue cells, utilize plant nutrients for its development, resulting in stunted growth or death. Furthermore, most of the obligate parasitic pathogen extract the nutrients from its host cell tissues with haustoria, therefore the host plant growth is inhibited.

As seen in Table 3, out of the 96 accessions tested, 80 accession numbers that included in the highly susceptible category had higher AUDPC scores compared to other accessions that had higher resistance. The value of AUDPC in accession with the resistant category even has a value of 0, lower than the value of AUDPC of the known resistant varieties (Bima-3), which is 103.5. The Anoman variety which is known to be a susceptible variety has the highest AUDPC average value of 2136.25. The difference in the value of AUDPC illustrates the difference in the rate of disease progression in each test material over a period of time observation.

Genetic resistance to common pathogens in plants is divided into two, qualitative resistance derived from a single resistance gene and quantitative resistance based on a combination of several genes (multigenic). Qualitative resistance generally provides a high level of resistance to certain pathogenic races, in contrast to quantitative resilience that is not specific to a particular pathogen race and has a broader spectrum of resistance, but the resistance provided is not as high as qualitative resistance. Qualitative resistance is known to be very effective in controlling biotroph pathogens (pathogens that receive nutrients from living cells) rather than quantitative resistance which is often associated with resistance to necrotrophic pathogens (pathogens that receive nutrients from dead cells) [19].
Table 3. The mean value of Area Under Disease Progress Curve (AUDPC) of downy mildew \((P.\text{philipinensis})\).

| Response                  | Mean value of AUDPC |
|---------------------------|---------------------|
| Resistant (1)             | 0                   |
| Moderately Resistant (0)  | -                   |
| Susceptible (15)          | 627.33              |
| Highly Susceptible (80)   | 1019.09             |
| Anoman (Susceptible Var)  | 2136.25             |
| Bima-3 (Resistant Var)    | 103.5               |

Generally, the genetic trait used by the breeders is quantitative resistance, but biotrophic pathogens such as downy mildew are known to be a major threat in Indonesia. Therefore the development of qualitative resistance from downy mildew needs to be concerned. The discovery of genes responsible for resistance to downy mildew may facilitate the discovery of new varieties that are more resistant to downy mildew. There have not been many reports on certain compounds that regulate the resistance of maize to downy mildew, in the future in-depth studies of the dominant factors that affect the characteristics of resistance to downy mildew are needed.

4. Conclusion

Majority of the accessions tested have low resistance to downy mildew, only one percent of total accessions tested have good resistance to downy mildew. One accession that showed high resistance to downy mildew \((Peronosclerospora \text{philipinensis})\) was obtained namely accession number 808 with 0 per cent infection and 0 AUDPC value, lower than the resistant varieties (Bima 3) used as a comparison, which had an average 5-10% infection and 103.5 AUDPC value. The accession obtained that have a good resistance to downy mildew can be used as breeding materials, as a source of downy mildew resistance trait for the development of the new highly resistant variety.

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