Variations of cob rot infection caused by Fusarium verticillioides in the Filial 1(F1) hybrid maize line

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Abstract. The study of infection variations of the cob rot disease caused by Fusarium verticillioides on the hybrid maize line Filial 1(F1) aimed to determine the variation of infection of corn cob rot disease caused by F. verticillioides. This research was carried out in Sinoa District, Bantaeng Regency from November 2020 to February 2021. Research design used was randomized Block Design with 3 replications. The treatment were Filial line (F1) of 6 lines (D71, D72, D73, D74, BMD75, D76) and 4 commercial varieties Indonesia (Pioner 36, BISI 2, BISI 18, P36 , and NK22). Bisi 2 was used as susceptible check variety and NK 22 as a resistant check. The response of lines D71, D72, D74 and D75, were classified moderately resistant to cob rot disease F. verticillioides, infected with 30.66%, 32.66%, 33.33% and 28.00%, respectively. Strains D73 and D76, classified as susceptible (47.33% and 46.66%). BISI 2 was very susceptible (62.33% and 63.66%) and NK 22 was resistant (13.33%-14.00%). The variable of infected cobs showed D71, D72, D74 and D75 (27.53%-32.13%) significantly lower compared D73 and D76 (52.56%-44.76%). Susceptible varieties Bisi 2 (63.06%-62.43%). Asymptomatic seeds (asymptomatic) can achieve internal infection of F. Verticillioides seeds, ranging from 14.00%-16.33%.

1. Introduction
Cob rot disease in maize is one of the important disease in maize caused by the fungal pathogen Fusarium spp. The main species that infects corn cobs is Gibbrella monoliformis (Fusarium verticillioides). Taxonomist’s state that the F. verticillioides is a synonym with G. monoliformis, with other names F. moniloformae, F. F. moniloforme, F. moniloforme J. Sheld. 1904 and Fusarium verticillioides (Sacc) [1]. F. verticillioides is known to predominantly infect maize [2-4]. In Indonesia, six species of Fusarium spp. have been identified and one of them is F. verticillioides [5]. G. Zeae is a synonym for F. gramineaum caused by Fusarium Head Blight (FHB) [6]. That fungus predominantly infecting wheat and barley crops, and generally found to thrive at temperatures of 15-25°C [7].

The F. verticillioides infects corn stalks, cobs, and seeds. The infection can occur in the field or in the storage place/ warehouse. Cob rot is the main pathogen found in corn producing centers in South Sulawesi, Gorontalo and East Nusa Tenggara, as well as in Java and Sumatra [8]. Initial infection occurs in the cob and continue to warehouse. Conidia that infect corn kernels from the field, then develop and infect other seeds in storage [9]. The dynamics of infection in the warehouse is influenced by the humidity and moisture content of the seeds. The higher the moisture content, the greater chance of its spread in storage warehouses.

Cob rot disease (F. verticillioides) can survive in the soil, the infested soil will be the main source of infection through the wounds, and the propagules of fungi will develop in the plant tissue vessels.
and become the initial source of infection that spreads to all parts of the corn plant. Similarly, spores from corncobs that are infected early will be a source of spread to other corncobs [10].

Physically, infection in field crops causes yield loss around 1.8 tons/ha. It is dominantly find in high-altitude planting areas that have high humidity [11]. Mycotoxin also cause the quality loss of corn kernels. Because of that, it can be the limiting factor in determining the price at the producers’ level, farmers, also as animal feeding.

Efforts to reduce high infection rate on corncobs caused by *F. verticillioiides* is through prevention of early infection in the field. This can be carried out by using resistant varieties. In Indonesia, varieties that are moderate to resistant to *F. verticillioiides* have not been widely reported. The aim of this study was to determine the resistance response of Filial 1 (F1) maize hybrid lines to the *F. verticillioiides* and the dynamics of *F. verticillioiides* infection on asymptomatic cobs and seeds.

2. **Materials and Methods**

The research was carried out at an altitude of 780 meters above sea level (asl), in Sinoa Village, Sinoa District, Bantaeng Regency, from November 2020 to February 2021. Field testing was carried out with a Randomized Block Design with 3 replications. The treatments were six lines of F1 hybrid maize (D71, D72, D73, D74, BMD75, D76) and five commercial varieties in Indonesia (Pioner 36, BISI 2, BISI 18, P36, and NK22). Bisi 2 used as susceptible comparison, and NK 22 used as the resistant comparison. to avoid the uneven distribution of spores, the susceptible comparison (BISI 2) and resistant comparison (NK 22) were planted side by side in every 4 test lines and planted in 2 plots in each replication.

2.1. **Preparation of inoculum source plants**

Natural sources of inoculum (local varieties) were planted in 3 rows around the test plot. After 50 days old planting, the treatment material was planted. To validate the fungi species, isolation, identification and propagation of isolates were carried out from endemic areas to laboratory of Indonesian Cereal Research Institute. After the identification, the isolates of *Fusarium* inoculated in suspension on the cob, the same time with the release of the cob crest.

2.2. **Planting**

Each treatment planted in 4 rows along 5 m, spacing of 70 x 20 cm with 3 replications. The seed coated with pre-plant insecticides to avoid the pest attack while planting. After 10 days after planting (DAP), the fertilization carried out with Phonska and Urea 150 kg/ha and 200 kg/ha, respectively. The second fertilization was carried out at the age of 30 DAP by giving Urea 150 kg/ha.

2.3 **Variables**

2.3.1 **Infection Scoring**

By following the attack intensity scoring system (Reid et al. 1993), this variable was observed at harvest day (126 DAP) by observing 10 samples of cobs at per treatment unit. The results of the scoring of cob damage were converted to the formula for the severity of the disease.

The score for cob damage due to *F. verticillioiides* infection is as follows:

Score 1 = 1-3% of maize seeds infected with *Fusarium* spp
Score 2 = 4-10% corn kernels infected with *Fusarium* spp
Score 3 = 11-25% of maize seeds infected with *Fusarium* spp
Score 4 = 26-50% of maize seeds infected with *Fusarium* spp
Score 5 = > 75% corn kernels infected with *Fusarium* spp

The disease scoring scale transformed into the following attack intensity formula:

\[ I = \frac{\Sigma (n \times v)}{ZN} \times 100\% \]
I = Attack intensity  
\( n \) = Number of affected plants in each category  
\( v \) = Scale value for each affected plant  
\( Z \) = The highest scale value  
\( N \) = Number of plants observed in each attack

The resistance criteria used are as follows:
- Very resistant (ST): disease infection < 5%
- Resistant (T): disease infection > 5% - 20%
- Moderately Resistant (AT): disease infection > 20% - 40%
- Susceptible (R): infectious disease > 40% - 60%
- Very susceptible (SR): disease infection > 60%

2.3.2 Percentage of infected cob
Calculating the intensity of the attack of cob rot (F. verticillioides) was carried out at harvest, age 126 (DAT). The percentage of attacks is calculated by the formula:

\[
I = \left( \frac{A}{B} \right) \times 100\%
\]

\( I \) = Percentage of F. verticillioides presence  
\( A \) = Number of cobs attacked by F. verticillioides  
\( B \) = Number of cobs observed in each corn line

2.3.3 Number of infected seeds from asymptomatic seeds (Symptomless)
This parameter used 20 samples of cobs from each treatment line and randomly choose 100 asymptomatic seeds each treatment line. Furthermore, the 100 seeds were grown in sterile Petridix dishes in the laboratory for 4 replications. Observations were made, 7 days after growing by calculating the percentage of seed infected of Fusarium spp and continued with validation of identification of morphological characters.

3 Results and Discussion
Fusarium spp. reported consist of 31 species (Glenn et al. 2004) classify as the fungi imperfecti because of the absence of the sexual phase. The prominent organs presence in the form of conidia as reproductive structures. Some of this fungi group belong to the phylum Ascomycota, family Hypocreaceae. Cob rot caused by F. verticillioides produces asexual spores with mycelia consisting of 3-7 bulkheads, measuring 2.4-4.9 x 150 x 160 µm.

In this research, adequate source of inoculum distributed evenly in each experimental plant. This showed in the field while all plant in the plot was highly infected by cob rot. This also supported by the high topography (780 m ASL) with low temperatures (14-28ºC). In the vegetative phase, disease development is influenced by moderate temperatures and higher humidity. The spores of F. verticillioides spread through the wind and vectors such as herbivores insects (stem borers). Infection progresses rapidly when maize conditions are under stress (10,12).
Table 1. Percentage of *F. Verticillioides* infection, Sinoa, Sinoa District, Bantaeng Regency (780 dpl) 2021.

| No | Line  | Replication | Mean (%)* | Resistance reaction** |
|----|-------|-------------|-----------|-----------------------|
| 1  | D71   | 24          | 38        | 30,66                 | Moderately resistant |
| 2  | D72   | 32          | 34        | 32,66                 | Moderately resistant |
| 3  | D73   | 52          | 46        | 47,33                 | Susceptible         |
| 4  | D74   | 30          | 38        | 33,33                 | Moderately resistant |
| 5  | D75   | 28          | 28        | 28,00                 | Moderately resistant |
| 6  | D76   | 50          | 44        | 46,66                 | Susceptible         |
| 7  | BISI 18 | 46          | 36        | 41,33                 | Susceptible         |
| 8  | P 36  | 28          | 28        | 26,66                 | Moderately resistant |
| 9  | NK 22 | 12          | 18        | 14,00                 | Susceptible         |
| 11 | NK 22 | 14          | 12        | 13,33                 | Susceptible         |
| 12 | BISI 2| 64          | 62        | 63,33                 | Very susceptible    |
| 13 | BISI 2| 66          | 58        | 62,66                 | Very susceptible    |
| 14 | NK 6172 | 64          | 66        | 65,33                 | Very susceptible    |

*) Percentage of infection after transformation  
**) Criteria for resistance to *Fusarium* spp (Reid 1993)

The observation about infected cob showed that D71, D72, D74, D75 categorized as moderately resistant to *F. verticillioides* by infection percentage between 28.00%-33.33%. The percentage of D73 and D76 was 47.33% and 46.66% that classify as susceptible to *F. verticillioides*. Other than that, Bisi 2 and NK 6172 categorized as very susceptible (62.66%-65.33%) and NK 22 was resistant (13.33%-14.00%) (Table 1). According to [13-15] explained that there were differences in resistance between maize varieties to *F. Verticillioides*. The use of resistant varieties plays an important role in suppressing the accumulation of pine in the field. The more susceptible a variety to *F. verticillioides*, the higher the attack intensity found and positively correlated with the fumonisins content (16). Fumonisins is mycotoxin produced by *F. Verticillioides* that contaminate the maize product. It was found that *F. proliferatum* and *F. verticillioides* the main source of fumonisins that harm the agricultural production. The experiment about maize variety resistance to cob rot by [17] conclude that the best husk cover on the cob was found affect the cob rot infection. Based on the experiment, the variety where the husk covered well all the cob has less infection than poor husk (the tip of the cob is visible).

Under ideal conditions without disturbance of the microenvironment, the conidia will germinate, start the infection process and enter the corn seed cell tissue and cause specific symptoms limited to the tip of the corncob. Severe infection in corn kernels, usually causes the cobs to rot. This is because the infecting *F. verticillioides* will release a toxin, which can change the permeability of the cell wall membrane of the host seed, causing the seeds to rot.

The resistance of a variety to pathogens is controlled by the genes of each variety. In susceptible varieties, spore-host compatibility is high, allowing spore mycelia to thrive in plant cell tissues. This results in optimal spore production and high infection intensity. In contrast to resistant varieties, physical and genetic traits were found that were able to prevent the infection process, so that the pathogen was unable to develop properly, and infection did not occur. Thus the intensity of attack is relatively low and plants are able to produce optimally.
Table 2. Percentage of infected cob, *F. verticillioides*, Sinoa, Sinoa District, Bantaeng Regency (780 dp1) 2021.

| No | Line/variety | Mean |
|----|--------------|------|
| 1  | D71          | 29.27<sup>c</sup> |
| 2  | D72          | 32.13<sup>de</sup> |
| 3  | D73          | 52.57<sup>bc</sup> |
| 4  | D74          | 27.70<sup>c</sup> |
| 5  | D75          | 27.53<sup>c</sup> |
| 6  | D76          | 44.77<sup>cd</sup> |
| 7  | BISI 18      | 48.53<sup>c</sup> |
| 8  | P 36         | 28.83<sup>c</sup> |
| 9  | NK 22        | 14.03<sup>f</sup> |
| 11 | NK 22        | 14.77<sup>f</sup> |
| 12 | BISI 2       | 63.07<sup>ab</sup> |
| 13 | BISI 2       | 62.43<sup>ab</sup> |
| 14 | NK 6172      | 69.63<sup>a</sup> |

CV (%) 19.01
BNT 5% 12.70

Numbers followed by the same letter in the same column and/or in the same row are not significantly different at the 5% level of the DMRT test.

Analysis of variance in the number of infected cobs (Table 2) showed that *F. verticillioides* in strains (F1) D71, D72, D74, D75, infected 27.53% - 32.13%, or significantly lower than D73 (52, 56%) and D76 (44.76) and the susceptible comparison of BISI 2 varieties (62.43% - 63.06%). These data indicate that (F1) lines D71, D72, D74, D75, were more resistant than strains D73 and D76. Symptoms of infection on the cobs showed a collection of mycelia pink, whitish, more dominant symptoms were found at the tip of the cob and some showed symptoms in the seeds located in the center of the cob (Picture 1). The result between the numbers of infected cobs was relate with infection percentage in D71, D72, D74, and D75. There were several research mentioned that there were relation between the concentration of mycotoxin from *F. verticillioides* with the damage level of corn cob in cob rot disease (18–20). Under the experiment, Genotypes which belong to the flint, dent or Lancaster group, and were characterized as moderately resistant were classified separately as the same susceptible one (19).
The symptomless seed infection test of each strain showed a different intensity reaction (Table 3). The initial symptoms of all strains, which were grown on sterile paper media, were seen together with the appearance of seed germination from each strain sample (Figure 2). Asymptomatic infection or internal seed infection in lines D71, D72, D73, D74, D75, and D76 ranged from 14.00% to 16.33%. In the susceptible comparison, it was significantly higher and reached 23.66%-29.66%, in addition to the resistant comparator it reached 3.00%-8.67%. Oren et al., (2003) mentioned that the infection of *F. verticilliodies* depends on the virulence level. The less virulence, the respond of the plants will slower. Moreover, the plants health will also has line with the symptoms. The weakened the plants, the faster infection grow in plants (2).

![Figure 2. Symptoms of *F. verticilliodies* from asymptomatic seeds (Symptomless B: microscopic, mi: micro conidia; ma: macro conidia)](image)

| No | Galur/Varietas               | Rerata   |
|----|------------------------------|----------|
| 1  | D71                          | 16.33 c  |
| 2  | D72                          | 16.33 c  |
| 3  | D73                          | 14.00 cd |
| 4  | D74                          | 15.33 c  |
| 5  | D75                          | 15.33 c  |
| 6  | D76                          | 15.00 cd |
| 7  | BISI 18                      | 19.67 bc |
| 8  | P 36                         | 6.00 e   |
| 9  | NK 22 (resistant)            | 3.00 e   |
| 11 | NK 22 (resistant)            | 8.67 de  |
| 12 | BISI 2 (susceptible)         | 29.67 a  |
| 13 | BISI 2 (susceptible)         | 23.67 ab |
| 14 | NK 6172 (susceptible)        | 18.00 bc |
|    | CV (%)                       | 25.32    |
|    | BNT 5%                       | 6.60     |

Numbers followed by the same letter in the same column and/or in the same row are not significantly different at the 5% level of the DMRT test.

The data on variation in infection intensity showed that the more susceptible a variety, the higher the chance of *F. verticilliodies* infection in the seed internals. According to (William and Munkvold 2008), *F. Verticilliodies* infection on corn cobs was significantly affected by macro and micro
humidity, but systemic infection from plant to seed was not much influenced by temperature and humidity factors. However, the higher the moisture content of corn kernels during harvest and storage, the greater the chance that the asymptomatic seeds will become a source of spread of *F. verticilliodies* in storage in warehouses.

The initial natural infection of corn kernels comes from conidia on the soil surface, crop residues or plant infection in the vegetative growth phase. The conidia are then deposited on the corn hair at the tip of the cob, then through the corn hair, the pathogen enters the cob and infects the seeds (Duncan and Richard 2010). *F. verticillioides* infection, is often symptomless or asymptomatic in seeds but damages the inside of corn seed cell tissue (Bacon et al. 2008; Thomas et al. 2014).

4. **Conclusion**

Resistance response of lines D71, D72, D74 and D75, were classified as lower and somewhat resistant to cob rot disease *F. Verticilliodies*, infected with 30.66%, 32.66%, 33.33% and 28.00%, respectively. Strains D73 and D76, have a resistance response classified as vulnerable (47.33% and 46.66%). In a state of infection in the BISI 2 variety (62.33% and 63.66%) or very susceptible. This situation was also corrected for resistant varieties (NK 22) infected with 13.33%-14.00% or classified as resistant.

In the variable number of infected cobs, the variation in reaction was significantly lower, namely strains (F1), D71, D72, D74 and D75 (27.53%-32.13%) compared to lines D73 and D76 (52.56%-44.76%).% and Bisi 2 susceptible varieties (63.06%- 62.43%). Asymptomatic seeds (Symtonless) can achieve internal infection of *F. verticilliodies* seeds, ranging from 14.00%-16.33%.

**References**

[1] National Center for Biotechnology Information. Taxonomy browser (Fusarium verticillioides 7600) [Internet]. NCBI. [cited 2021 Jul 12]. Available from: https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=334819&lvl=3&keep=1&srchmode=1&unlock&mod=1&log_op=modifier_toggle#modifier

[2] Oren L, Ezrati S, Cohen D, Sharon A. 2003. Early events in the Fusarium verticillioides-maize interaction characterized by using a green fluorescent protein-expressing transgenic isolate. *Appl Environ Microbiol* 69(3).

[3] Ono EYS, Fungaro MHP, Sofia SH, de Miguel TÁ, Sugiura Y, Hirooka EY. 2010. Fusarium verticillioides strains isolated from corn feed: Characterization by fumonisin production and RAPD fingerprinting. *Brazilian Arch Biol Technol* 53(4).

[4] Stumpf R, dos Santos J, Gomes LB, Silva CN, Tessmann DJ, Ferreira FD, et al. 2013. Fusarium species and fumonisins associated with maize kernels produced in Rio Grande do Sul State for the 2008/09 and 2009/10 growing seasons. *Brazilian J Microbiol* 44(1).

[5] Bahri S, Maryam R, Widiastuti R. 2005. Cemaran aflatoksin pada bahan pakan dan pakan di beberapa daerah Propinsi Lampung dan Jawa Timur. *Jitv* 10 (1994).

[6] Gilbert J, Brülé-Babel A, Guerrieri AT, Clear RM, Patrick S, Slusarenko K, et al. 2014. Ratio of 3-ADON and 15-ADON isolates of Fusarium graminearum recovered from wheat kernels in Manitoba from 2008 to 2012. *Can J Plant Pathol* 36(1).

[7] Guenther JC, Trail F. 2005. The development and differentiation of Gibberella zeae (anamorph: Fusarium graminearum) during colonization of wheat. *Mycologia* 97(1).

[8] Pakki S. 2016. Mycotoxin contamination, bioecology of Fusarium verticillioides pathogen and its control on maize. / Cemaran mikotoksin, bioekologi patogen Fusarium verticillioides dan upaya pengendaliannya pada jagung. *J Penelit dan Pengemb Pertan* 35(11–16).

[9] Pakki S, Haris Talanca. Pengelolaan Penyakit Pascapanen Jagung. Balai Penelit Tanam Sereal.

[10] Ncube E, Flett BC, Van den Berg J, Erasmus A, Viljoen A. 2018. Fusarium ear rot and fumonisins in maize kernels when comparing a Bt hybrid with its non-Bt isohybrid and under conventional insecticide control of Busseola fusca infestations. *Crop Prot* 110.

[11] Eller MS, Robertson-Hoyt LA, Payne GA, Holland JB. 2008. Grain yield and fusarium ear rot
of maize hybrids developed from lines with varying levels of resistance. *Maydica* 53(1–4).

[12] Schulthess F, Cardwell KF, Gounou S. 2002. The effect of endophytic Fusarium verticillioides on infestation of two maize varieties by lepidopterous stem borers and coleopteran grain feeders. *Phytopathology* 92(2).

[13] Schjøth JE, Tronsmq AM, Sundheim L. 2008. Resistance to Fusarium verticillioides in 20 zambian maize hybrids. *J Phytopathol* 156(7–8).

[14] Balconi C, Berardo N, Locatelli S, Lanzanova C, Torri A, Redaelli R. 2014. Evaluation of ear rot (Fusarium verticillioides) resistance and fumonisin accumulation in Italian maize inbred lines. *Phytopathol Mediterr* 53(1).

[15] Venturini G, Assante G, Toffolatti SL, Vercesi A. 2013. Pathogenicity variation in Fusarium verticillioides populations isolated from maize in northern Italy. *Mycoscience* 54(4).

[16] Nayaka SC, Udaya Shankar AC, Reddy MS, Niranjana SR, Prakash HS, Shetty HS, et al. 2009. Control of Fusarium verticillioides, cause of ear rot of maize, by Pseudomonas fluorescens. *Pest Manag Sci* 65(7).

[17] Girsang W, Girsang R. 2020. The Tolerance of Several Hybrid Corn (Zea mays L.) Varieties Type on Cob Rot in the Plateau of Simalungun Regency. *Int J Sci Res Manag* 8(03).

[18] Mukanga M, Derera J, Tongoona P, Laing MD. 2010. A survey of pre-harvest ear rot diseases of maize and associated mycotoxins in south and central Zambia. *Int J Food Microbio* 141(3).

[19] Czembor E, Waśkiewicz A, Piechota U, Puchta M, Czembor JH, Stepień Ł. 2019. Differences in ear rot resistance and fusarium verticillioides-produced fumonisin contamination between polish currently and historically used maize inbred lines. *Front Microbiol* 10.

[20] COOK RJ. 1978. The incidence of stalk rot (Fusarium spp.) on maize hybrids and its effect on yield of maize in Britain. *Ann Appl Biol* 88(1).