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REACTION OF SELECTED CORN CULTIVARS TO *STENOCARPELLA MAYDIS*, INOCULATED USING DIFFERENT INOCULATION METHODS

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ABSTRACT

Field and laboratory experiments were conducted to determine the effects of cultivar and inoculation method on the incidence of corn stalk and kernel rots caused by *Stenocarpella maydis* (Berk.) Sutton. Seven high-yielding corn cultivars were evaluated in the laboratory and in the field for reaction to the diseases. Bisi 226 had the lowest incidences of stalk rot. Bisi 2 had the lowest incidence of ear rot but not significantly different from Bisi 321 and NK 6172. Thus, the cultivars tested reacted differently to stalk rot and ear rot. Bisi 226 reacted strongly resistant to stalk rot and resistant to ear rot. Bisi 321 was resistant to both the stalk rot and ear rot diseases. Bisi 2, NK 6172, and JH 29 were moderately resistant and resistant against the stalk rot and ear rot diseases, respectively. Nasa 29 reacted resistant and moderately resistant to stalk rot and ear rot diseases, respectively. While JH 37 was moderately resistant to both diseases. For the incidence of kernel rot and the percentage of yield loss, again, Bisi 226 exhibited the lowest among the tested cultivars. Based on the percentage of the rot kernels, all cultivars reacted similarly to the kernel rot disease in both artificial inoculation and natural inoculation experiments. Therefore, artificial inoculation in the laboratory could be used to separate resistant cultivars from susceptible ones under a homogeneous environment.

Keywords: Corn, stalk rot, ear rot, kernel rot, fungal disease, artificial inoculation.

INTRODUCTION

Corn (*Zea mays* L.) is the second most important food crop globally, in terms of cultivation area and economic value (Mario *et al*., 2017). The majority of corn is utilized as food, animal feed, and industrial raw materials (Ranum *et al*., 2014). Indonesia's corn production is 30 million tons, harvested from 5.7 million ha annually, or an average of 5.2 tons per ha (Ministry of Agriculture, 2018). This yield is substantially low compared to the crop's genetic potential of up to 13.7 tons per ha (cultivars Nakula and Sadewa 29) (Aryan and Aqil, 2020). One of the main limiting factors of corn production is plant disease infections. Fungal diseases, such as downy mildew caused by several Peronosclerospora spp. and leaf blight caused by *Rhizoctonia solani* are major limiting factors of corn cultivation in the country (Wakman and Burhanuddin, 2007).

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The fungus *Stenocarpella maydis* (Berk.) Sutton, the causal agent of the corn stalk rot and corn ear rot diseases, has been reported to cause severe corn yield losses in many countries, including South Africa, China, and USA (CABI, 2022). In recent years, the fungal pathogen has been detected in North Sumatra Province of Indonesia, (Soenartiningsih, 2015 and Syahriani *et al*., 2021). In 2020, however, the pathogen was discovered causing severe damage to corn crops in the East Luwu Regency of South Sulawesi Province in the central part of the country. Natural infection of the disease in the field caused stalk rot disease incidence of 5 to 35% and kernel rot incidence of 8.5 to 45.5%, depending on the plant cultivar (Sumarianto, 2022). However, the pathogen is still classified as Quarantine Pest A1 based on the pest classification of the National Agency for Plant Quarantine (Kementerian Pertanian, 2020).

In the affected regions, farmers used fungicides to control the stalk and ear rot diseases. Previous studies have shown that fungicides that are used as seed treatment provide effective control of the disease (Marley and Gbenga, 2006) but Romero and Wise (2015), Kleinschmidt and White (2003), and Lee *et al*. (2008) reported that the chemical control tool is not consistently effective against the diseases in the field. Thus, the field use of fungicides is not only impractical and uneconomical but also potentially harmful to the environment and non-target organisms. Therefore, more effective and safer alternative control measures must be sought, including the use of resistant cultivars against *S. maydis*. The most promising and efficient way to control the pathogen is through genetic resistance (Klapproth and Hawk, 1991). Soenartiningsih (2015) reported that corn cultivars cv Kenia 2 and Bima 3 are resistant to the pathogen, but no cultivars tested in that particular study and other studies (EPPO, 2017) are completely resistant to the diseases. Nevertheless, cultivars with partial resistance to the pathogen could help reduce fungicide use.

Since *S. maydis* is a relatively new pathogen in Indonesia, limited number of commercially available corn cultivars are known resistant to the pathogen. At this time, it is necessary to conduct a study on resistance of a number of high-yielding corn cultivars that are widely cultivated by local farmers against the diseases. Existing cultivars with some degree of resistance to the pathogen could be recommended to local farmers as a stopgap measure against the pathogen until more cultivars resistant to the disease become available.

In screening corn cultivars for stalk and ear rot diseases, artificial inoculation in the field is typically used (Klapproth and Hawk, 1991; Bensch, 1995; Mário *et al*., 2012). Five milliliters of the conidial suspension are injected using a syringe into the base of the cob. However, it is difficult to standardize the amount of inoculum used for artificial inoculation in the field because naturally occurring inoculum could interfere with the inoculation process. Standardization of the homogeneous environment and inoculum concentration is possible during laboratory inoculation (no interference from natural inoculum and environmental heterogeneity), thus the result should be more consistent and reliable in separating the susceptible and resistant genotypes (Del Rio and Melara, 1991). In addition, the inoculation method should produce infection similar to the natural inoculation in the field. The objectives of the present study were to determine the effects of: 1) corn cultivar on the incidence of the corn stalk and ear rot diseases and crop yield; and 2) inoculation methods (natural inoculation in the field and artificial inoculation in the laboratory) on the incidences of the corn stalk and ear rot diseases.

MATERIALS AND METHODS

Field Experiment: Field experiment was conducted to assess the effect of corn cultivar on the infection rates of stalk rot and ear rot diseases, caused by *S. maydis*. The study was carried out in a local farmer's corn field in Luwu Timur Regency, South Sulawesi Province, Indonesia. The location was chosen because infection of the pathogen was first found in that particular site. Treatment consisted of seven high-yielding corn cultivars: Bisi 2, Bisi 226, Bisi 321, NK 6172, Nasa 29, JH 29, and JH 37 that were arranged in a randomized complete block design with three replications each. Each replication consisted of one plot of 2 x 18 m and each plot contained two plant rows and 42 plants in each row. Planting space used was 0.7 m between rows and 0.4 m between plants in a row. There was a 0.75 m space between replication plots.

Plants maintenance followed the local recommendations except no fungicide applications were administered throughout the season. Plants were fertilized with chicken manure of 0.5 ton per ha and applied one day prior to the planting. Urea and NPK of 175 and 300 kg/ha, respectively, were applied 15 and 35 days after planting with a half of the dosage for each application. Weeds were mechanically controlled using a hoe as needed.

Incidence of stalk rot and ear rot diseases and percentage of rotted kernels per ear were determined by using the method of Flett and McLeron (1994); Nowell (1997). Stalk rot incidence was assessed by calculating the percentage of infected plant in each plot and it was observed every seven days, starting from 21 days after planting. Similarly, the ear rot incidence was determined by calculating the percentage of infected ear in each plot and it was also observed every seven days, starting 50 days after planting. Cultivar response to

S. maydis was assessed using the categories described by Soenartiningsih, (2015) (Table 1).

Table 1. Scoring for plant response against stalk or ear rot diseases, caused by *S. maydis*.

Incidence (%)	Response category
0 – 10	Strongly resistant (SR)
>10 – 20	Resistant (R)
> 20 – 40	Moderately resistant (MR)
> 40 – 60	Susceptible (S)
> 60	Strongly susceptible (SS)

Incidence of infected kernels was determined by threshing all kernels from all ears harvested per plot. The kernels were then mixed well before 250 g of the kernels were sampled randomly. The incidence was the percentage of infected kernels per 250 g sample (Mario *et al*., 2017). In addition, the percentage of weight loss of the yield due to the ear rot disease was also assessed in this experiment.

Laboratory Experiment: The laboratory experiment using artificial inoculation on soft dough stage of ears was conducted to determine the incidence of ear rot and infected kernel per ear. The results of this experiment were compared with the results of the natural inoculation in the field. Inoculum of *S. maydis* was isolated from infected corn kernels in the field where our field experiment was conducted. The isolate of the fungus was grown on potato dextrose agar (PDA) medium and the isolate was purified by transferring it twice onto fresh PDA media.

To produce inoculum in large quantity, corn kernels were used as the substrate for *S. maydis* culture. Two hundred and fifty grams of corn kernels was placed into an Erlenmeyer flask containing 500 mL of sterile distilled water for 12 h. The water was then discarded before the kernels were put inside of zip-lock bags. After that, the bags were placed inside of an autoclave at 125ºC for 20 minutes. The bags were then allowed to cool down before one dish of the fungal colony was poured into each bag. To assure the colony was evenly distributed throughout the kernels, the bag was shaken thoroughly and the shaking was repeated every 24 h. The bags were placed in room temperature of 27 ± 2^{o}C, photoperiod of 12 h light : 12 h dark, and humidity of about 90% for 14 days to let the fungus grow.

To harvest the conidia, the kernels covered by the fungal colony were poured into an Erlenmeyer, 250 mL sterile distilled water was added and then the container was shaken and stirred for 30 minutes to separate the colony from the kernels. The inoculum suspension was filtered through double layers of cheesecloth. Conidial concentration was assessed using hemocytometer and then adjusted to 10^4 conidia per mL of sterile distilled water.

The experiment consisted of seven corn cultivars as treatments, namely: Bisi 2, Bisi 226, Bisi 321, NK6172, Nasa 29, JH29, and JH 37, arranged in a completely randomized design with seven replications of one ear each. Corn ears were picked up from the field at soft dough stage (R4) (Matiello *et al*., 2015). Each of the ear was inoculated with 5 mL of the conidial suspension (10^4 conida per mL distilled water) by injecting on the base of the ear using a medical syringe. The inoculated ears were individually wrapped with wet paper towel then placed inside of a plastic bag and then incubated in room temperature of 27 ± 2^{o}C for ten days. The incidence of the infected ear was expressed by the percentage of ears showing ear rot symptom (kernels with white mycelia and dark pycnidia). While the incidence of infected kernel was determined by threshing all kernels from a cob and then the healthy and infected kernels were visually separated and counted. The incidence of infected kernel was expressed as percentage of infected kernel per cob.

STATISTICAL ANALYSIS

Data were subjected to analysis of variance after the data were transformed using an arcsin square root transformation. If significant differences were detected among the treatments, the means were separated using Tukey's test at 5% probability level, SPSS 2020 version 27.0.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Field Experiment: Incidences of stalk rot and ear rot diseases on seven different corn cultivars are shown in Figure 1. There were significant differences among the cultivars in the incidence of stalk rot disease. The lowest incidence was found on Bisi 226, which was significantly different from those incidences in Bisi 2, NK6172, Nasa 29, and JH 37. However, for incidences of ear rot disease, the lowest incidences were found on Bisi 2, Bisi 321, and NK 6172, which was significantly different from those incidences in Bisi 226, Nasa 29, JH 29, and JH 37. In Bisi 2, stalk rot incidence was significantly higher than the ear rot incidence. Similarly, in NK 6172 and JH 37, the stalk rot incidences were higher than the ear incidences but no significant difference was detected in each cultivar. In cultivars Bisi 2, NK 6172, and JH 37, the stalk rot incidence was higher than the ear rot incidence, however, a significant difference was only found in Bisi 2. On the other hand, in cultivars Bisi 226, Bisi 321, Nasa 29, and JH 29, the stalk rot incidences were lower than the ear rot incidences, but a significant difference was only found in Bisi 226.

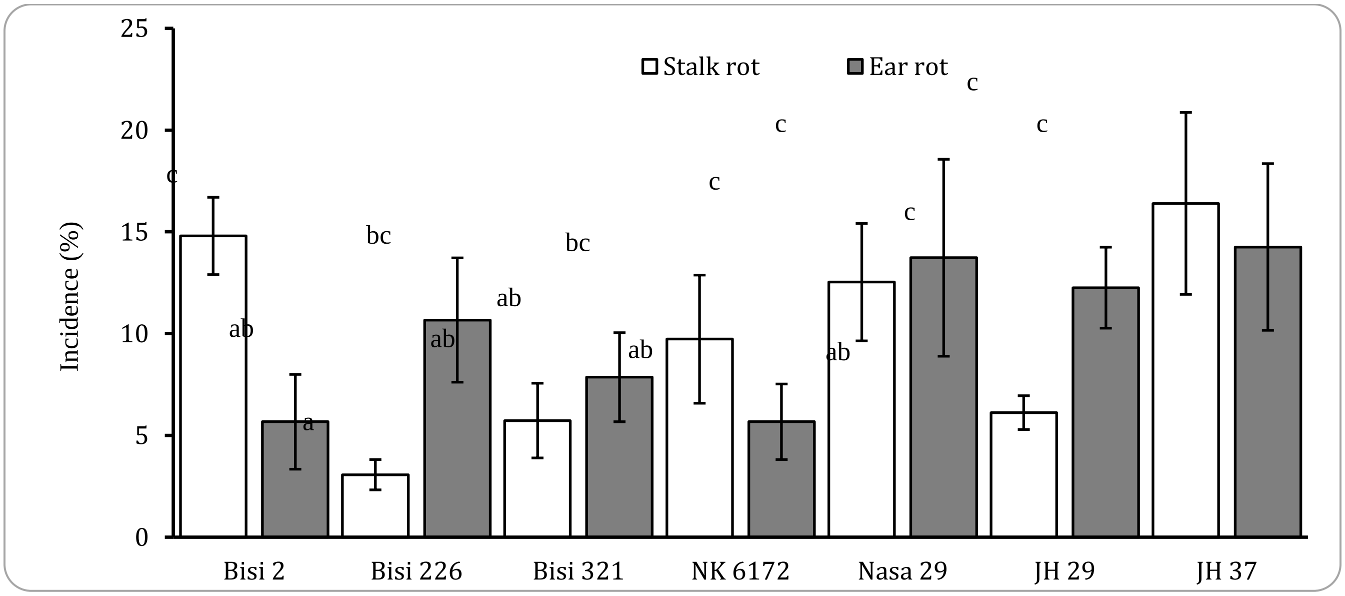

Figure 1. Average incidence of stalk rot and kernel rot caused by *S. maydis* on seven corn varieties tested in the field. Bars = standard errors (SE).

Significant differences in the average percent of kernels infected by *S. maydis* were detected on seven corn cultivars tested in the field (Figure 2). The lowest incidence was found on Bisi 226, which was significantly different from the other cultivars tested in this experiment. This was followed by Bisi 2, which was significantly different from Bisi 226 and JH 37 but it was not significantly different from the other cultivars.

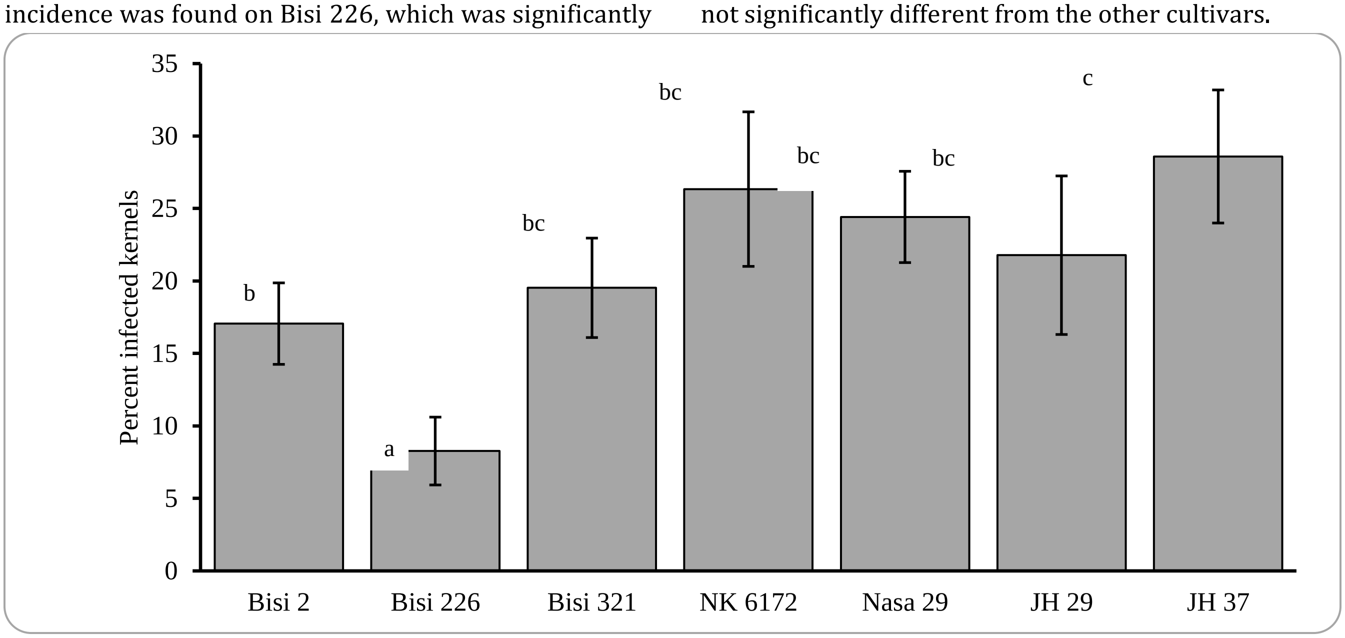

Figure 2. Average percent of kernels infected by *S. maydis* on seven corn varieties tested in the field. Bars = standard errors (SE).

Figure 3 shows percent of weight loss of the yield due to *S. maydis* infection on seven corn cultivars in the field. The lowest yield loss of about 18% was found on Bisi 226, which was significantly lower than the other tested cultivars but no significant differences among the other treatments. The highest yield loss of about 43% was found on cultivar NK 6172.

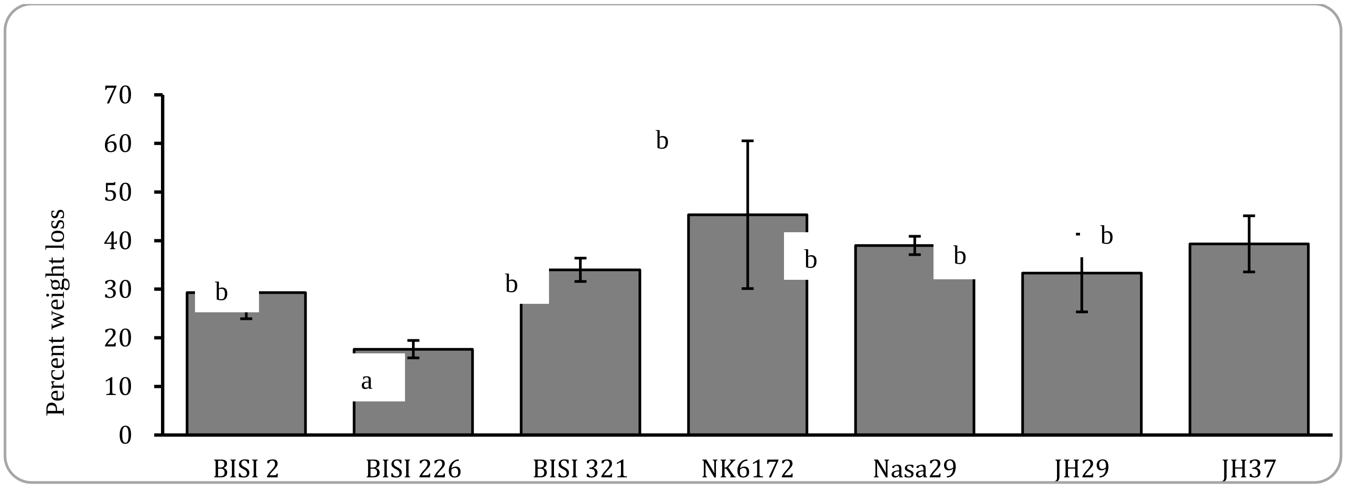

Figure 3. Percent of weight loss of the yield due to *S. maydis* infection on seven corn cultivars in the field. Bars = standard errors (SE).

Tabel 1. Average incidence of stalk rot and ear rot diseases caused by *S. maydis*, and cultivar reaction to the pathogen

Cultivar	Stalk Rot		Ear rot	
	Incidence	Cultivar reaction	Incidence	Cultivar Reaction
Bisi 2	28.00	Moderately resistant	12.67	Resistant
Bisi 226	5.33	Strongly resistant	17.33	Resistant
Bisi 321	12,00	Resistant	14,67	Resistant
NK 6172	34.67	Moderately resistant	11,33	Resistant
Nasa 29	19.33	Resistant	25.33	Moderately resistant
JH 29	24.00	Moderately resistant	15,33	Resistant
JH 37	30.00	Moderately resistant	23,33	Moderately resistant

Cultivar reaction was assessed based on Soenartingsih, (2015)

Laboratory Experiment: The average percent of infected ears and percent of infected kernels per ear on seven cultivars tested in the laboratory are presented in Figure 4. The lowest ear rot incidence was found in Bisi 226 and it is not significantly different from those in Bisi 2, Bisi 321, and NK 6172 but it was significantly different from those in Nasa 29, JH 29 and JH 37. Similarly, the lowest kernel rot incidence was found in Bisi 226 but it was not significantly different from Bisi 2 but significantly different from the other cultivars. Ear rot incidences in Bisi 2, Bisi 226, Bisi 321, and NK 6172 were higher than the kernel rot incidences but significant differences were found only in Bisi 2 and Bisi 226.

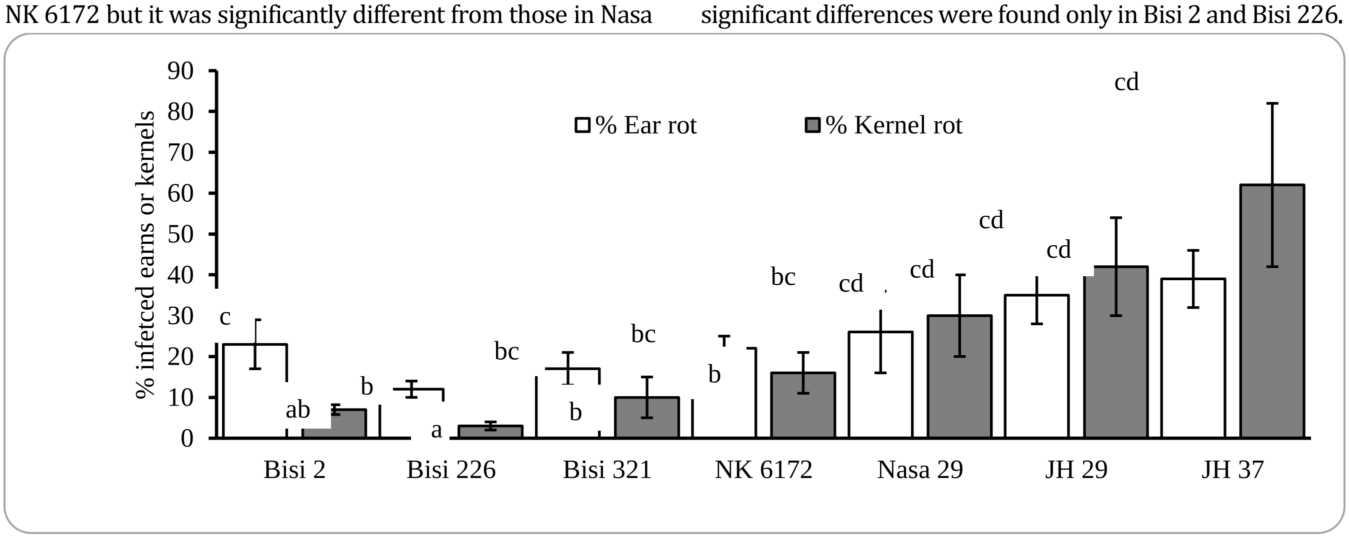

Figure 4. Average percent of infected ears and percent of infected kernels per ear on seven cultivars tested in the laboratory. Bars = standard errors (SE).

Figure 5 shows the average percent of infected kernels on seven corn cultivars, artificially inoculated in the laboratory or naturally inoculated in the field. The lowest incidences of the kernel rot for both inoculation methods occurred in Bisi 226 and they are significantly different from the other cultivar treatments. In each cultivar, no differences in percent infected kernels between artificial inoculation and natural inoculation.

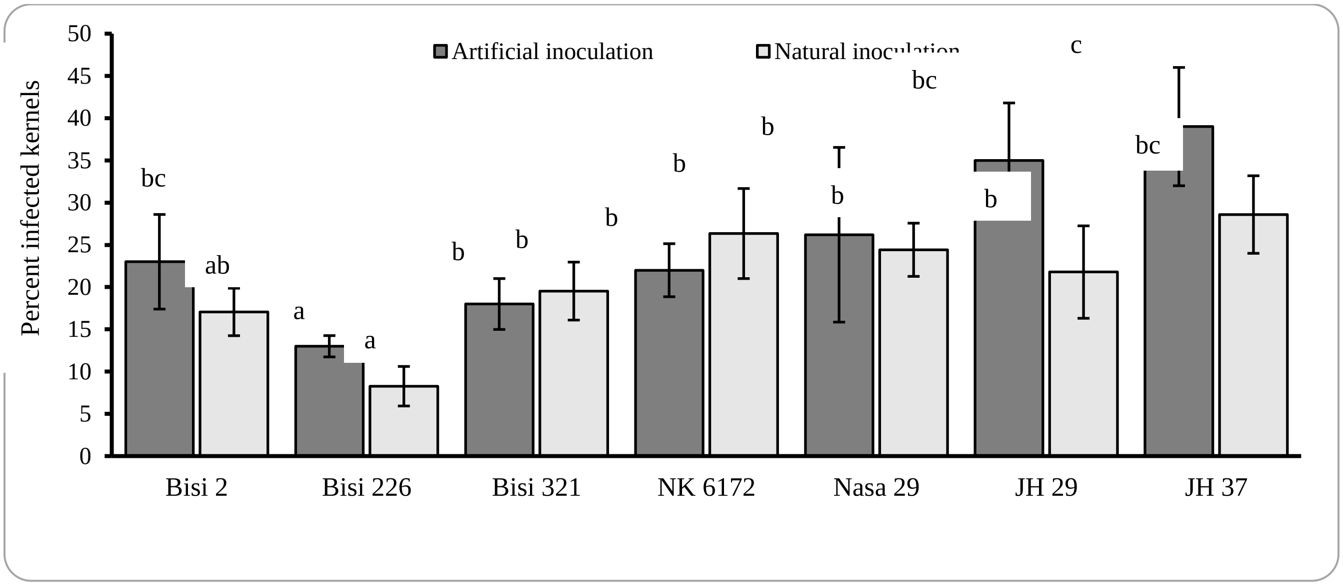

Figure 5. Average percent of infected kernels on seven corn cultivars, artificially inoculated in the laboratory or naturally inoculated in the field. Bars = standard errors (SE).

Pearson's correlation analysis showed a strong positive correlation between percent of infected kernels of the artificially inoculated ears in the laboratory and naturally inoculated ears in the field (r = 0.79, P < 0.001) (Figure 6).

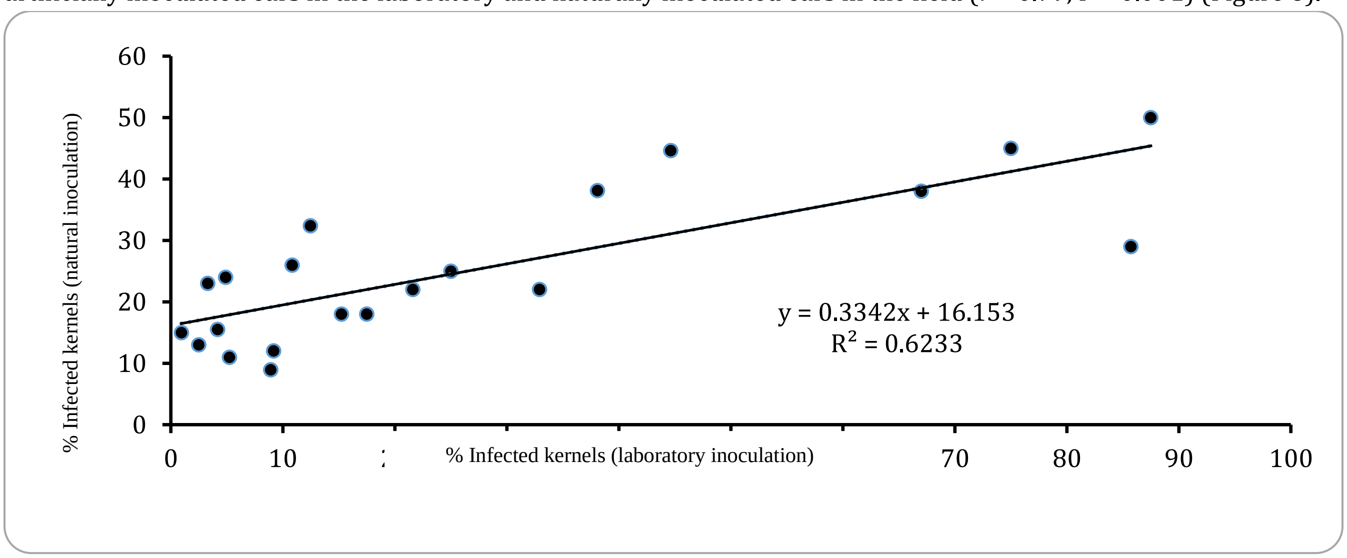

Figure 6. Relationship between percents of infected kernels of the artificially inoculated ears in the laboratory and of naturally inoculated ears in the field

DISCUSSION

The current study attempted to determine reaction of seven high-yielding corn cultivars that are widely cultivated in the area against *S. maydis,* namely: Bisi 2, Bisi 226, Bisi 321, NK6172, Nasa 29, JH29, and JH 37. Besides that, in this study, the effects of natural inoculation in the field and artificial inoculation in the laboratory on the ear rot and kernel rot incidences were also compared.

The results of the field experiment showed that all cultivars tested were infected by *S. maydis,* causing stalk rot and ear rot diseases with varying levels of incidence (Figure 1). This indicated that natural inoculum was present with sufficient quantity to infect the plants in the field. The planting pattern applied in the study site was corn monoculture cultivated year around, enabling the pathogen inoculum to accumulate through time. Flett and Mclaren (1994) reported that corn monoculture interrupted with soybean monoculture in a plant rotation scheme effectively reduces the *S. maydis* inoculum pressure.

All cultivars that were tested reacted at least moderately resistant to the stalk rot and ear rot diseases but none reacted completely resistant to the diseases. This is similar to the finding of Soenartiningsih (2015). Our results indicated that Bisi 226 reacted strongly resistant to stalk rot and resistant to ear rot disease. Bisi 321 was resistant to both the stalk rot and ear rot diseases. Bisi 2, NK 6172, and JH 29 were moderately resistant and resistant against the stalk rot and ear rot diseases, respectively. Nasa 29 reacted resistant and moderately resistant to stalk rot and ear rot diseases, respectively. While JH 37 was moderately resistant to both diseases.

The lowest incidence of stalk rot was on Bisi 226, while the lowest incidences of ear rot disease were on Bisi 2, Bisi 321, and NK 6172. The data suggested that the cultivars reacted differently to the stalk rot and ear rot diseases. There is a tendency that in cultivars Bisi 2, NK 6172, and JH 37, the stalk rot incidence was higher than the ear rot incidence, On the other hand, in cultivars Bisi 226, Bisi 321, Nasa 29, and JH 29, the stalk rot incidences were lower than the ear rot incidences. Cultivar Bisi 226 also had the lowest kernel rot incidence and yield loss among the cultivars tested.

The artificial inoculation employed in this study was effective for screening large numbers of entries with high efficiencies in time and resources. Besides that, artificial inoculation of harvested ears at soft dough stage (R4) in the laboratory enabled the study to be conducted in a homogeneous and controlled environment. Thus, any differences among the cultivars in terms of disease incidence are due to the genotypes of the cultivars. Genotype screening in the field could allow the interaction between genotype and environment to hamper the results (Santos *et al*., 2016). Ears are most vulnerable to *S. maydis* infection at the soft dough stage (R4) and it is ideal for distinguishing susceptible from resistant genotypes (Chambers, 1988; Flett and Mclaren, 1994). Artificial inoculation also uses precise inoculum concentration that otherwise can hardly be achieved in the natural inoculation due to the possible interference from the inoculums already exist in the field (Bensch, 1995).

In this study, artificial inoculation method of injecting conidial suspension of *S. maydis* on the base of the ear effectively developed infection on the ear. Inoculation at the base and center of the ear inflicts the highest disease incidence and yield reduction (Matiello *et al*., 2015). In addition, the inoculation method was capable of differentiating resistant and susceptible corn genotypes and the kernel rot incidences were comparable to those found in the natural inoculation in the field. This is reflected by the Pearson's correlation analysis showing a strong positive correlation between percent of infected kernels of the artificially inoculated ears in the laboratory and naturally inoculated ears in the field (r = 0.79, $P < 0.001$). Disease evaluation based on kernel rot incidence is an accurate indicator of *S. maydis* incidence (Rossouw *et al*., 2002). We agree with Rossouw *et al*. (2002) that some cultivars do not express clear symptom of kernel rot while the kernels are still on the ear, hence in this experiment, the kernels were removed from the ear for incidence evaluation.

CONCLUSION

All cultivars were infected by stalk and ear rot diseases with varying degrees of incidence. However, all cultivars expressed some resistance against *S. maydis* with reactions of at least a moderate resistance. Bisi 226 was superior to other cultivars because it had the lowest incidences of the stalk rot, kernel rot, and yield loss. The cultivar reacted strongly resistant and resistant to stalk and ear rot diseases, respectively. While, the highest incidences of the stalk rot, ear rot, kernel rot, and yield loss occurred on JH 37. Artificial inoculation of harvested ears at soft dough stage (R4) in the laboratory enabled the study to be conducted in a homogeneous and controlled environment. The inoculation method was ideal for distinguishing susceptible from resistant cultivars and can be used for screening a large number of plant genotypes.

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Contribution of Authors:

Sumarianto	:	Conduct research and analyzed the data
Elkawakib Syam'un	:	Supervised the study and wrote early version of manuscript.
Andi Nasruddin	:	Supervised the study, prepare the tables and figures, wrote the final draft of the manuscript and acted as the corresponding author