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CORRELATION OF STOMATA DENSITY TO RUST SEVERITY ON SOME ACCESSIONS OF MAIZE GERMPLASM

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ABSTRACT

Correlation of stomata density to rust severity on some accessions of maize germplasm. Rust is an important disease on maize. Control of rust using resistant varieties is recommended because it is more practical and environmental friendly. This research aimed to study the correlation of stomata density to rust severity on the several maize germplasm accessions. The research was conducted in Bontobili Farm Experimental Station and ICERI Pathology Laboratory. As much as 30 corn germplasm accessions and 2 varieties for comparison (Bima 10 and Anoman) were planted by spacing 20 x 70 cm. The inoculation of *Puccinia* sp. was allowed to occur naturally. Stomata printing was conducted with painted the lower part of corn leaf with nail polish transparent. After drying, it was pasted with clear isolation and then it was pulled and saved on glass object. Observed parameters were rust severity on the age of 50, 60, 70 days after planting and density of stomata. Results of the experiment showed that disease severity of germplasm accession number 2, 218 and 243 were not significant with comparison (tolerant variety). Germplasm with accession number 234 was categorized tolerant to rust. The rust infection rate on all accession were categorized by mild to moderate. Density of stomata of each maize germplasm accession was significantly correlated with rust severity, the increase of stomata density could increase of rust severity at 0.73%.

Key words: correlation, stomata density, severity of rust disease

ABSTRAK

Korelasi kepadatan stomata dengan keparahan penyakit karat pada beberapa aksesi plasma nutfah jagung. Penyakit karat merupakan salah satu penyakit penting pada jagung. Pengendalian penyakit karat menggunakan varietas tahan dianjurkan karena lebih praktis dan ramah lingkungan. Penelitian ini bertujuan untuk mempelajari hubungan kerapatan stomata dengan tingkat keparahan penyakit karat pada beberapa aksesi plasma nutfah jagung. Penelitian ini dilakukan di Kebun Percobaan Bontobili dan Laboratorium Penyakit Balai Penelitian Tanaman Serealia. Sebanyak 30 aksesi plasma nutfah jagung dan 2 varietas untuk perbandingan (Bima 10 dan Anoman) ditanam dengan jarak 20 x 70 cm. Inokulasi *Puccinia* sp. terjadi secara alami. Pencetakan stomata dilakukan dengan mengecat daun bagian bawah menggunakan cat kuku transparan. Setelah kering ditempelkan isolasi bening kemudian ditarik dan disimpan pada objek gelas. Parameter yang diamati adalah tingkat keparahan penyakit karat pada umur 50, 60, 70 hari setelah tanam dan kepadatan stomata. Hasil penelitian menunjukkan bahwa aksesi plasma nutfah jagung 2, 218 dan 243 memiliki tingkat keparahan penyakit karat tidak berbeda nyata dengan varietas pembanding tahan. Plasma nutfah dengan nomor aksesi 243 dikategorikan tahan terhadap penyakit karat. Tingkat infeksi penyakit karat pada semua aksesi dikategorikan ringan hingga sedang. Kepadatan stomata dari setiap aksesi plasma nutfah jagung secara signifikan berkorelasi dengan keparahan penyakit karat, peningkatan kepadatan stomata dapat meningkatkan keparahan karat pada 0,73%.

Kata kunci: kerapatan penyakit karat, kerapatan stomata, korelasi

INTRODUCTION

Rust of maize is caused by fungus *Puccinia* sp. The pathogen was reported that it had spread broadly in maize production area at South Sulawesi, such as at Regency of Gowa, Soppeng and Maros (Muis *et al*., 2015; Suriani & Azrai, 2016; Takdir & Soenartiningsih, 2017). Physiologically, the maize suffering from rust has a reduced chlorophyll and hormone content, decreased photosynthetic rate, and increased respiration rate followed by increasing oxidase enzyme activity. Furthermore, the plant morphologically becomes dwarf with dried leaves, so then will negatively impact the yield (Pakki, 2017).

The rust disease can result in high loss of maize yield in tropical area (Brewbaker *et al*., 2011). In susceptible varieties, the yield loss can reach up to 50% (Shah & Dillard, 2006). The percentage of this yield loss caused by the disease depended on the time when the infection firstly occured. If the pathogen infects in the beginning of the plant growth, subsequently the yield loss will be greater (Takdir & Soenartiningsih, 2017).

The symptoms of rust disease on plant is indicated by the appearance of some yellowish and a bit protruding little spots (pustule). The first infection occur on lower surface of leaves. The pustule then becomes brown surrounded by yellowish color (Pataky, 1999). The pustule with approximately 1mm in size is called uredium, and there are some spores in the uredium. A mature uredium will be cracked and spread the flour of uredospores (Dillard & Zitter, 1987). The next symptom is indicated by forming spores on upper surface of leaves. The rust disease pathogen can also infect the midrib, stem, and corn cob. A severe symptom can cause the leaves dry out (Pataky, 1999).

The rust pathogen in the form of uredospores can be spread by means of wind, rain water, and insects. The uredospores cannot last longer on the remaining diseased plants, especially in warm days with temperature above 30 ^{o}C. The uredospore sprouts optimally in temperature range of 23–28 ^{o}C and can not survive in temperature below 20 ^{o}C (Puspawati & Sudarma, 2016). In extreme conditions, particularly in temperature below 20 ^{o}C, the rust fungi usually form sexual spores (teliospores) having thin wall that makes them to be resistant to unfavorable conditions (Dillard & Zitter, 1987).

The presence of teliospores produced by *Puccinia* spp. makes the pathogen is more difficult to control. The use of resistant varieties is one of the effective ways to control the maize rust disease epidemic (Chaves-Medina *et al*., 2007). The development of resistant varieties requires materials of superior lines resulted from crossing, introduced lines, or from germplasms having resistance to rust disease. Cultivation of the rust resistant maize can reduce uredospore production, therefore it can diminish the inoculum sources and therefore can inhibit its spreading in the fields (Pakki, 2016).

Resistance of plants is divided into two categories, horizontal and vertical resistances. A plant variety has vertical resistance if it is resistant to one or several pathogen races, or horizontal resistance if host plants have resistance to all pathogen races (Anonym, 2017). Furthermore, the case of horizontal resistance of soybean to rust can be identified which is based on several plant characteristics, such as quantity of stomata, and presence of leaf trichomes that can inhibit water entrance to the leaf, and consequently, can constrain infection of pathogen (Edington, 1994). Stomata are predicted to have influence on level of plant damages caused by pathogen. It is correlated to the pathogen infection that involving both formation of infection structure or toxin production (Grimmer *et al*., 2012).

The result of a research conducted by Djauhari (2008) showed that density of stomata had a significant positive correlation to intensity of rust disease on soybean leaves; while density of leaf hairs (trichoma) and wax thickness and cuticle has significant negative correlations to intensity of rust disease on soybean leaves. Furthermore, Groth & Urs (1982) discovered that plant response to infection of rust disease pathogen was correlated to quantity of stomata.

This study aimed to study on the correlation of stomata density on several accessions of maize germplasm to rust severity. The accessions with low severity (resistant accessions) with typical quantities of stomata can be selected to be genetic sources used by breeders to develop new superior varieties of rust resistant maize.

MATERIALS AND METHODS

Research Site. The research was conducted at the Bontobili Experimental Station and Plant Disease Laboratory of Cereal Research Center, Maros from March to August 2017.

Cultivation of Maize Germplasm Accessions. Soil processing was conducted thoroughly. Afterwards, 30 accessions of maize germplasm and two varieties as resistant (Bima 10) and susceptible (Anoman) controls were cultivated. Each accessions and varieties were planted along 5 m with distance 20 x 70 cm. The research used randomized block design with 2 replicates. The first fertilization was applied on the 7th day after planting (DAP) at a dosage of 135 kg N + 45 kg P_2O_5 + 45 kg K_2O ha^{-1}. The second fertilization was applied on the 30th DAP at a dosage of 200 kg N ha^{-1}. Plant rearing, including weeding, watering, and piling up were optimally.

Observation of Rust Disease Severity. The fungi of *Puccinia* were naturally inoculated, because when the test plants were cultivated, there were already some infected plants act as the inoculum sources. The rust disease severity was observed and scored from 1 to 5 when the plants reached 50 DAPs, 60 DAPs and 70 DAPs, then the severity of rust disease was calculated using the equation as follows:

$$I = \frac{\sum (ni \times vi)}{Z \times N} \times 100\%$$

I = rust severity
n = number of plants at a symptom category
v = score value at each symptom category
N = number of plants observed
Z = the highest score

The scoring value of rust symptom on maize leaves was determined by adopting a method used by Directorate of Maize Research of India (2012) as follows: Score 1, a very little infection, there are 1–2, or few pustules spread on only lower leaves; Score 2, several pustules on only lower leaves (mild infection); Score 3, abundant pustules on lower leaves, some on mid leaves; Score 4, abundant pustules on lower and mid leaves, and extended to upper mid leaves; Score 5, abundant pustules on all leaves, and the plant could be fast drying (dead) as a consequence of the disease.

The severity of the disease on each accession can be assessed and interpreted to characterize levels of plant resistance to rust. The levels of plant resistance to rust disease according to Cook, 1972 *in* Maman *et al.* (2014) are: Highly resistant (the intensity of disease development (I) = 0%); Resistant (0% < I < 25%); moderately resistant (25% < I < 50%); moderately susceptible (50% < I < 75%); Susceptible (75% < I < 100%).

Furthermore, the rate of infection was calculated by using the equation according to Van Der Plank (1963) as follows:

$$r = \frac{2.30259}{t2 - t1} \log 10 \frac{Xt}{Xo} \text{ (unit, per day)}$$

r = infection rate
X_0 = disease severity on first observation
X_t = disease severity on second observation
t1 = time of first observation
t2 = time of second observation

The criterion of rust infection rates was adopted from Van Der Plank (1963) as follows: Mild (r < 0.11); Moderate (0.11 < r < 0.50); Severe (r > 0.50).

Observation of Leaf Stomata Density. Two samples of mid leaves of maize from each accession at field were used as medium for stomata molding. The stomata molding was performed by staining 3 x 5 cm of lower leaf surface using transparent nail stain and left dried. A transparent tape was then sticked on the stained portion of leaf and was drawn immediately. The stomata molding on the transparent tape was then placed on an object glass and observed under microscope Olympus BX 21 with 40x magnification. The density of stomata was calculated by using equation as follows (Sulistyowati *et al*., 1997):

$$KS = \frac{JST}{LBP} \qquad LBP = \frac{1}{4}\pi d^2$$

KS = density of stomata
JST = number of stomata on each area of field of view
LBP = area of field of view (0.096 mm^2); ð = 3.14
d = diameter of field of view (35 x 0.01 mm)

Data analysis. The correlation of stomata density to rust severity was analyzed using Pearson's simple correlation at 5% significance level. The strength of correlation between variables was very weak, if the value of *r* was 0.00–0.199; weak, if the value of *r* was 0.20–0.399; adequately strong, if the value of *r* was 0.40–0.599; strong, if the value of *r* was 0.60–0.799; and highly strong, if the value of r was 0.80–0.100. The interpretation was also valid for a negative correlation (Sofyan, 2013).

RESULTS AND DISCUSSION

Severity of Rust Disease. Generally, the rust disease symptoms were found on all accessions of maize germplasm, including on the susceptible and resistant comparison varieties. The symptom was indicated by appearance of yellowish brown uredium of *Puccinia* sp. covering leaf surface. Uredium containing teliospores continued to develop, consequently the leaf surface became rough as rust (Figure 1).

The result indicated that there were some variations on rust severity development on every accession, and increment of rust severity at every time of observation at 50, 60, and 70 DAPs (Table 1). Data on Table 1 showed that rust severity on 50 DAPs plants was still low, in range of 0–26%, and there were 22 accessions had insignificantly higher percentage of disease severity than the resistant control variety (Bima 10). Furthermore, 3 accessions (103, 107, and 230) showed significantly higher rust severity than the susceptible control variety (Anoman).

Rust severity on 70 DAPs indicated that there were 3 accessions (2, 218, and 243) having insignificantly higher rust severity than Bima 10. The three accessions had insignificantly different rust severity than the resistant comparison variety (Bima 10) at three times of observation. A maize germplasm with accession number 243 showed a lower rust severity (18%) than Bima 10 and it was categorized as resistant to maize rust disease.

Infection Rate of Leaf Rust Disease. A lower rust severity at 70 DAPs can predict a very low yield loss, because the maize plant at 70 DAPs generally begin to come into generative phase. Rust disease infection on maize plants in generative phase resulted in lower economic potential lost, rather than the severe infection on plants in vegetative phase (phase V3 to V7).

The results on infection rate of disease is showed in Table 2. The average infection rate of rust disease on 30 accessions of maize germplasm was not significantly different, either compared to each other accessions nor to the resistant and susceptible comparison varieties. The average of infection rate of rust disease on all accessions was less than 0.5% per unit per day. This value indicated that the infection rate of disease can be categorized as mild to moderate. It is also indicated that the infection rate of rust disease was slow, because of the effect of resistance properties possessed by some accessions of maize germplasm. The resistance properties were predicted to be influenced by their own genes. According to Van der Plank (1963), there are 3 factors that can be lower the infection rate, those are a moderately non-aggressive pathogen, resistant host plant variety, and unfavorable environmental conditions for pathogen to develop.

Correlation of Stomata Density of Some Accessions of Maize Germplasm to Percentage of Leaf Rust Disease. Differences in outbreak levels incidence on all accessions of maize germplasm due to some factors, such as genetic properties, enzymatic (chemical) content, and form of plant morphology (Agrios, 2004). The resistant accession can be influenced by presence of plant metabolite in form of toxin that can neutralize phytoalexine produced by pathogens, so that they cannot develop. Additionally, the stomata density influenced the differences in outbreak level of rust disease on 30 accessions of maize germplasm (Table 3).

Accession of maize germplasm with highest density of stomata is the one with number 173, and it is significantly different higher than stomata density of both control varieties (Figure 2). Some other accessions have value of stomata density that are not significantly different to the same value of susceptible comparison variety (Table 3). Table 3 showed that rust severity of some accessions were significantly different to those on Bima 10 variety that had also significant difference of stomata density. It indicated that the factor of stomata density influenced the infection level of rust disease on maize plants. Baswarsiati (1994) stated that the higher density of stomata on leaves, the more susceptible plants

Figure 1. The symptom of rust on maize leaves.

Table 1. Rust severity on 30 accessions of maize germplasm and comparison varieties

Accession Number of Maize Germplasm	Rust severity at DAPs			Level of Resistance
	50	60	70	
2	2	6	30	Moderately resistant
9	14 a	26 a	42 a	Moderately resistant
102	12 a	36 ab	44 a	Moderately resistant
103	18 ab	18 a	50 a	Moderately resistant
104	6	26 a	46 a	Moderately resistant
107	18 ab	24 a	48 a	Moderately resistant
119	6	36 ab	50 a	Moderately resistant
124	0	20 a	44 a	Moderately resistant
131	2	12	50 a	Moderately resistant
151	10	32 a	54 a	Moderately susceptible
173	10	38 ab	68 ab	Moderately susceptible
183	10	38 ab	46 a	Moderately resistant
184	10	28 a	48 a	Moderately resistant
187	14 a	34 a	58 ab	Moderately susceptible
188	16 a	34 a	80 ab	Susceptible
193	4	30 a	54 a	Moderately susceptible
203	6	20 a	52 a	Moderately susceptible
218	8	38	58 ab	Moderately susceptible
218	6	12	28	Moderately resistant
230	26 ab	40 ab	54 a	Moderately susceptible
231	2	8	46 a	Moderately resistant
241	12 a	16	46 a	Moderately resistant
243	4	6	18	Resistant
248	6	38 ab	58 ab	Moderately susceptible
249	8	26 a	70 ab	Moderately susceptible
250	4	10	54 a	Moderately susceptible
252	6	26 a	54 a	Moderately susceptible
746	4	22 a	46 a	Moderately resistant
747	10	32 a	54 a	Moderately susceptible
748	4	48 ab	58 ab	Moderately susceptible
BIMA (a)	0	2	22	Resistant
ANOMAN (b)	6	20	42	Moderately resistant
LSD 5%	10.46	15.53	15.63	

Disease severity value in the same column followed by the same letter is not significantly different according to LSD test at significance level 5%.

Table 2. Average rate of rust disease development (%/day) on 30 accessions of maize germplasm and control varieties (Anoman and Bima 10)

Accession number of maize germplasm	Infection rate of disease development (unit/day) at DAPs			
	50 - 60 DAPs		60 – 70 DAPs	
2	0.11	Mild	0.17	Moderate
9	0.07	Mild	0.05	Mild
102	0.12	Moderate	0.03	Mild
103	0.00	Mild	0.11	Mild
104	0.15	Moderate	0.07	Mild
107	0.05	Mild	0.09	Mild
119	0.18	Moderate	0.04	Mild
124	0.30	Moderate	0.08	Mild
131	0.15	Moderate	0.17	Moderate
151	0.12	Moderate	0.06	Mild
173	0.14	Moderate	0.06	Mild
183	0.14	Moderate	0.02	Mild
184	0.11	Mild	0.06	Mild
187	0.09	Mild	0.06	Mild
188	0.08	Mild	0.09	Mild
193	0.20	Moderate	0.06	Mild
203	0.13	Moderate	0.10	Mild
218	0.17	Moderate	0.04	Mild
218	0.08	Mild	0.09	Mild
230	0.05	Moderate	0.03	Mild
231	0.13	Moderate	0.19	Moderate
241	0.03	Mild	0.11	Mild
243	0.07	Mild	0.12	Moderate
248	0.19	Moderate	0.05	Mild
249	0.13	Moderate	0.10	Mild
250	0.11	Mild	0.19	Moderate
252	0.20	Moderate	0.07	Mild
746	0.20	Moderate	0.09	Mild
747	0.20	Moderate	0.06	Mild
748	0.28	Moderate	0.03	Mild
BIMA 10	0.06	Mild	0.20	Moderate
ANOMAN	0.17	Moderate	0.12	Moderate

Table 3. Average number of leaf stomata density on 30 accessions of maize germplasm and comparison varieties (Anoman and Bima 10)

Accession number of Maize Germplasm	Stomata density (unit/mm^2)	Percentage of disease severity at 70 DAPs
2	59.375 a	30
9	46.875 a	42 a
102	56.25 a	44 a
103	42.500	50 a
104	49.375 a	46 a
107	44.375	48 a
119	38.750	50 a
124	36.250	44 a
131	50.625 a	50 a
151	39.375	54 a
173	63.125 ab	68 ab
183	49.375 a	46 a
184	45.000	48 a
187	41.250	58 ab
188	58.125 a	80 ab
193	43.125	54 a
203	47.500	52 a
218	56.875 a	58 ab
218	43.125	28
230	44.375	54 a
231	38.750	46 a
241	39.375	46 a
243	40.000	18
248	54.375 a	58 ab
249	49.375 a	70 ab
250	52.500 a	54 a
252	54.375 a	54 a
746	36.25	46 a
747	45	54 a
748	45	58 ab
BIMA 10 (a)	33.75 a	22 a
ANOMAN (b)	49.375 b	42 b
LSD 5%	11.53	15.627

Stomata density values followed by letter a and b are significantly higher than the resistant comparison variety (Bima 10) and the susceptible comparison variety (Anoman) based on LSD test with significant level 5%.

to be penetrated or infected by pathogen, because the pathogen can penetrate well through natural pores, such as stomata.

The Pearson's simple correlation test showed that the leaf stomata density was significantly correlated to rust severity on 30 accessions of maize germplasms. The correlation of both variables indicated an adequately strong correlation with *r* value 0.429. It means that the stomata density had a sufficiently strong correlation to rust severity. A similar result reported by Djauhari (2008) that the stomata density had a significant positive correlation to the rust severity on soybean leaves.

The correlation of stomata density to percentage of rust disease on soybean leaves at the 77th DAP was highly strong with *r* value 0.9727. Additionally, Chattopadhyay *et al.* (2011) found that the stomata and trichoma density of mulberry leaves had strong correlation to development of powdery mildew disease with r values 0.624 and -0.809, respectively. Pradana *et al.* (2017) specified that the intensity of scab disease on sweet potato leaves at generative phase was extended to 85.10% due to the length of stomata on the upper leaves on susceptible cultivars, and 62.97% of intensity of the disease incidence was influenced by stomata density.

An advanced test by linear regression test showed that every increment in stomata density causes increase in rust disease severity to 0.73% (Figure 3). It caused by stomata roles as a path of penetration of obligate pathogens, such as rust pathogen. The higher

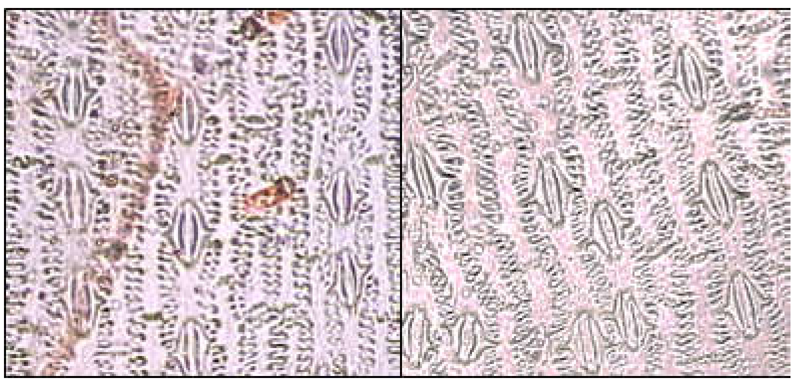

Figure 2. Appearance of stomata of accessions of maize germplasm (A) low density; (B) moderate density, observed under microscope.

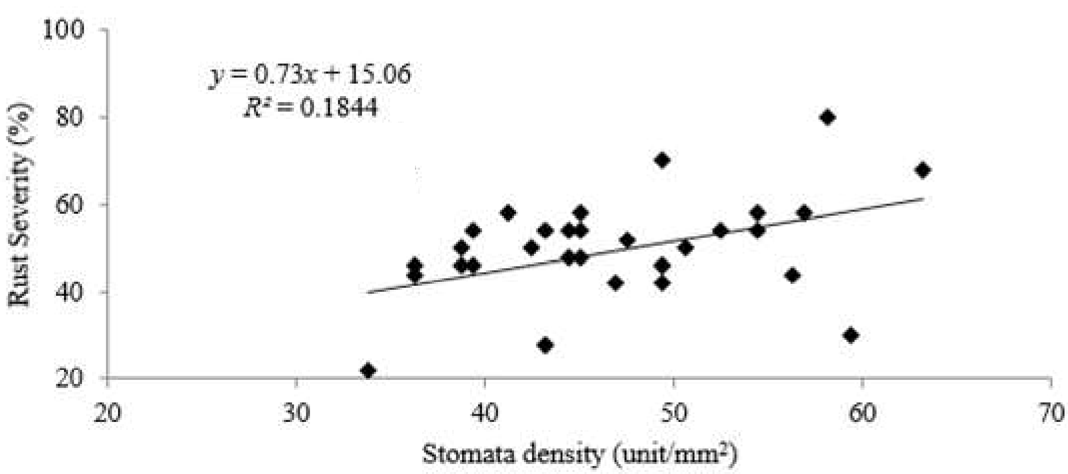

Figure 3. Rust severity correleted to stomata density.

stomata density, the higher probability of penetration by uredospore, and the more infections happened (Djauhari, 2008). However, the stomata density were subjected to be changed due to adaptation to local environment.

Figure 3 showed that the stomata density of maize leaves can be an indicator of plant resistance to rust disease. The accessions of maize germplasm with low stomata density can be considered as materials in maize plant breeding to construct new superior resistant varieties to rust disease. It is consistent with a statement that the stomata density can be an indicator of structural resistance to pathogen (Fahn, 1990).

CONCLUSION

There were 3 accessions of maize germplasm performing rust severity were insignificantly different to the resistant comparison variety (Bima 10), those were accession number 2, 218, and 243. The germplasm with accession number 243 was categorized as resistant to maize rust disease. The infection rate of disease found on all accessions was mild to moderate. The stomata density of each accession of maize germplasm had significant correlation to rust severity, and every increment in stomata density increased the rust severity to 0.73%.

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