Identification of Super and Ultra Early Maturity Maize Lines Resistance Against Leaf Blight Disease (*Bipolaris maydis*)

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ABSTRACT. The leaf blight disease is one of the major maize diseases and causes yield losses up to 70% particularly when it infects maize planting on generative phase. Therefore, The maize variety which is resistant to this one is needed. The research was aimed to identify the maize variety resistance against leaf blight disease caused by *Bipolaris maydis*. This study was carried out at Maros Experimental Farm from May to July 2012. A randomized complete block design with maize lines as block and three replications was utilized. Natural infection was treated on maize lines. The result revealed that from 20 BLPT lines, only BPLT 004 showed highly resistant, five lines i.e. BPLT 001, BPLT 003, BPLT 007, BPLT 008 and BPLT 012 were resistant, seven lines indicated moderately resistant reaction namely BPLT 002, 005, 009, 010, 017, 019 and 020, while BPLT 006, 013, 014 and 016 were susceptible and BPLT 011, 015 and 018 were highly susceptible. From other 20 BJMN lines, BJMN 008, 010, 012 and 013 showed resistant to leaf blight with varied intensity from 10% to 20%. Nine lines namely BJMN 001, 002, 006, 007, 014, 015, 016, 018 and 020 indicated moderately resistant, while BJMN 003, 005 and 019 were reacted susceptible to leaf blight disease and the highly susceptible lines were BJMN 003, 005 and 019. The resistance genotypes will be used as donor parents in the varietal improvement to obtain maize varieties with high yield and resistant to leaf blight disease.

Key word: leaf blight disease, *bipolaris maydis*, sweet corn.

Introduction

Leaf blight disease caused by *Bipolaris maydis* was reported distributed and infected on almost all of maize growing region of the world. The yield losses caused by this disease could be up to 70% particularly when it infected maize planting on generative phase (Pal and Kaiser, 2001). B. *maydis* has been known having three physiological races i.e Race T, Race O and Race C and these races influenced the symptom and severity of one. Race O produces lesions that are initially small then expand. These lesions elongate, although growth of lesions is restricted by leaf veins. Final lesions are rectangular (2-6 × 3-22 mm) and tan in color. Lesions caused by isolates of Race O are limited to leaves. Symptoms of Maydis leaf blight caused by Race T are oval and slightly larger (6-12 × 6-27 mm) than those caused by Race O. Lesion borders are usually characterized by dark, brown borders.

Race T causes lesions on all above ground parts of the plant (including stems, sheaths and ears) and can also cause ear rots. Seedlings from seeds infected with Race T often wilt and die within 3 to 4 weeks. While Race C is pathogenic only for maize germplasm with T cytoplasm sterile. Race C are found in China, while many Race T infected corn planting in Texas (CAB International 2003).

The spread of the disease is through the wind and splash water of rain around the new corn crop planted. The germinated conidia infect plants through the stomata, so lesions or spots will appear in the plant tissue. The environmental condition with temperature about 20-32^{o}C and 80-90% of humidity is favorable one to this disease to grow. If the temperature and humidity encourage for the development of *B. maydis* conidia, this disease can complete its life cycle in just 72 hours, so it can be said that the development is very fast (CIMMYT 2012). The damage caused by this disease is because of the patches, thus reducing leaf area for photosynthesis, furthermore it can diminish photosynthate in seed filling. High infection can occur the death of plants.

This disease can be controlled by using fungicides but their residues are not compatible to the conservation of agriculture and environmental unfriendly. The host resistance is the most cost effective and practical to pathogen management and does not negatively affect the environment.

Materials and Method

A total of 40 maize genotypes/ lines consisting of 20 BPLT lines and 20 BJMN lines were tested at Maros

Experimental Farm from May to July 2012. A randomized block design with maize lines as block and three replications was utilized. Natural infection was treated on maize lines. Each maize line was planted in a five meter of long row with planting distance 75 cm x 20 cm. Application of fertilizer such as Ponska 400 kg and 200 kg Urea/ha was performed at 10 and 30 days after planting. Furadan 3 g dose of 10 kg/ha was applied to prevent infestation of insect pests.

In term of isolation and identification of pathogens in the Laboratory, samples of maize leaves infected leaf blight were collected from the experiment field. Each leave sample was cut about 2 mm as many as 5 pieces and these one were taken by sterile forceps and dipped a few socond in a solution of 70% alcohol to sterilize the surface of the leave pieces, then they were put into distilled water for 10 minutes so that the alcohol on the surface of the leaf dissolved in d-H_2O. The leaves pieces were taken by sterile forceps and placed in Petri dishes that had already been put filter paper about 2-3 hours. Furthermore, the leaves was grown on PDA and incubated for 7 days. The fungi that grow from leaf pieces were observed every day. Once the growth of the hyphae had reached 2-3 cm, the outskirts of the colony were taken by scalpel and transferred to another sterile PDA medium to obtain pure cultures. From the pure culture, their spore was put on object glass to observe and inditify under microscope.

Observation of leaf blight disease was done at 2, 3, 4 and 5 weeks after planting (WAP). The intensity of leaf blight disease was based on formula used Meyee and Datar (1986):

$$I = \frac{\Sigma (n \times V)}{ZN} \times 100\%$$

Remarks:
I = intensity of severity
N = number of affected leaves in each category for each plant
V = value score on any affected leaves
Z = value of the highest score
N = number of leaves were observed in each attack

Table 1. Scoring levels of lef blight disease and resistance category varieties.

No	Percentage of severity (%)	Reaction
1	0 – 10	Highly resistance (HS)
2	>10 – 20	Resistance (R)
3	> 20 – 40	Moderately resistance (MR)
4	> 40 – 60	Susceptible (S)
5	> 60 – 100	Highly susceptible (HS)

Value scoring leaf blight disease on maize:

Score 0 = no symptoms
Score 1 = spots on the leaves at the bottom with very small lesions and little
Score 2 = spots on the lower leaf surface and middle
Score 3 = spotting occurs in all of the leaves and the leaves dead
Score 4 = patches occur on all leaves, the leaves die down and the middle
Score 5 = spots on all the leaves and the plant begins to dry or plants dead

Results and Discussions

The results of the pathogen isolation and identification showed that the morphology of the conidia had measurement of 15-25 × 60-150 ìm, brown conidia and spindle-shaped or elliptical and tapered edges and sprout at the end or at the bipolar (Figure. 1). Conidia generally consisted of 5 to 9 septate, if developed in a black PDA. According to Carson (1999), conidia having that shape and size were *Bipolaris maydis.*

The spread of the disease occur rapidly and the death of maize crops was happened at 3-4 WAP on susceptible varieties, so it can be said that the virulence was similar to Race T but the symptoms of this form looked like Race O. According to CIMMYT (2004), race T pathogen attacked not only the leaves, but also the stem and cobs of corn.

The symptom type was almost similar to Race O, because the early infection seemed small brown spots, then they were increasingly the size together along with the maize age. The spot was measure of 2-4 x 4-17 mm and brown color (Figure 2). White (1999) reported that Race O had patches of rectangular shape and size (2-6 × 3-22 mm),

Figure 1. Conidia of *Bipolaris maydis.*

Figure 2. Symptom of *Bipolaris maydis*.

brown spots. Spotting caused by Race O isolates confined to the leaves and it was also occurred on this research in which infection was only happened in leaves and it did not infect the other parts.

Table 2 showed the susceptible lines had already dead at 4-5 WAP, it indicated that intensity of leaf blight disease was high meaning the pathogen virulence was high. In addition, it was known that *Bipolaris maydis* Race O was pathogen which infected the studied lines where the virulence of this race was little bit low compared to Race T. The Race O virulence showed high on this research, it was probably due to the infection had already been happened in the seed.

40 maize genotypes/linesof super and ultra early maturity maize evaluated at Maros showed a difference of the leaf blight disease ((*Bipolaris maydis*) intensity. From 20 BLPT lines, only BPLT 004 showed highly resistant, five lines i.e. BPLT 001, BPLT 003, BPLT 007, BPLT 008 and BPLT 012 were resistant which their percentage were varied from > 10%-20%, seven lines indicated moderately resistant reaction namely BPLT 002, 005, 009, 010, 017, 019 and 020, while BPLT 006, 013, 014 and 016 were susceptible and BPLT 011, 015 and 018 were highly susceptible (Table 2).

From other 20 BJMN lines, BJMN 008, 010, 012 and 013 showed resistant to leaf blight with varied intensity from 10% to 20%. Nine lines namely BJMN 001, 002, 006, 007, 014, 015, 016, 018 and 020 indicated moderately resistant, while BJMN 003, 005 and 019 were reacted susceptible to leaf blight disease and the highly susceptible lines were BJMN 003, 005 and 019 (Table 2).

Chang (2002) said that the exsistence of the differences of maize crop characters caused less the symptoms on resistant varieties than on the susceptible varieties. Meanwhile, Zhinhuan *et al*. (2000) reported that the resistant varieties containing enzyme on the cell wall can reduce the aggressiveness of the fungal spores.

Table 2. The intensity average of leaf blight disease on 40 varieties/lines, observation 2, 3, 4 and 5 WAP.

Genotypes/Lines	Intensity of *B. maydis* (%)				Reaction
	2 WAP	3 WAP	4 WAP	5WAP	
BPLT 001	0,00	6,75	10,50	15,65	R
BPLT 002	12,56	22,40	28,20	35,20	MR
BPLT 003	0,00	7,50	12,25	15,80	R
BPLT 004	0,00	5,20	6,70	9,57	HR
BPLT 005	5,50	10,47	19,70	23,80	MR
BPLT 006	8,90	20,50	32,80	40,20	S
BPLT 007	0,00	5,25	7,80	12,90	R
BPLT 008	0,00	9,70	14,50	19,25	R
BPLT 009	6,45	12,25	16,50	21,70	MR
BPLT 010	4,27	10,50	14,70	20,50	MR
BPLT 011	20,38	40,70	70,50	Plant of dead	HS
BPLT 012	0,00	6,50	11,65	16,53	R
BPLT 013	10,25	27,80	38,40	56,85	S
BPLT 014	12,76	20,50	32,57	44,40	S
BPLT 015	30,52	57,20	Plant of dead	Plant of dead	HS
BPLT 016	11,92	22,80	35,70	40,90	S
BPLT 017	7,26	14,50	29,60	35,70	MR
BPLT 018	25,34	50,75	Plant of dead	Plant of dead	HS
BPLT 019	11,67	17,80	23,50	35,40	MR
BPLT 020	8,43	12,70	15,76	25,30	MR
BJMN 001	7,24	12,40	19,40	22,56	MR
BJMN 002	8,75	14,25	25,30	37,48	MR
BJMN 003	9,56	18,30	31,20	45,25	S
BJMN 004	20,45	60,30	Plant of dead	Plant of dead	HS
BJMN 005	10,29	17,50	25,58	42,65	S
BJMN 006	10,52	12,70	20,80	29,52	MR
BJMN 007	6,93	13,45	21,70	30,75	MR
BJMN 008	0,00	6,25	11,75	15,40	R
BJMN 009	22,50	59,70	Plant of dead	Plant of dead	HS
BJMN 010	5,27	10,75	14,46	19,58	R
BJMN 011	20,80	50,72	Plant of dead	Plant of dead	HS
BJMN 012	0	4,75	10,67	14,00	R
BJMN 013	0,00	6,25	12,72	18,70	R
BJMN 014	8,25	14,56	21,45	32,67	MR
BJMN 015	7,86	11,25	19,72	24,50	MR
BJMN 016	12,50	12,50	24,80	32,25	MR
BJMN 017	27,52	73,56	Plant of dead 15,42	Plant of dead	HS
BJMN 018	0,00	8,85	32,50	28,40	MR
BJMN 019	12,50	25,30	20,72	40,75	S
BJMN 020	8,50	13,43		31,50	MR

HS = highly resistance (HS), R = resistance , MR = moderately resistance
S = susceptible and HS = highly susceptible

Conclusion

From 20 BLPT lines, only BPLT 004 showed highly resistant, five lines i.e. BPLT 001, BPLT 003, BPLT 007, BPLT 008 and BPLT 012 were resistant which their percentage were varied from > 10%–20%, seven lines indicated moderately resistant reaction namely BPLT 002, 005, 009, 010, 017, 019 and 020, while BPLT 006, 013, 014 and 016 were susceptible and BPLT 011, 015 and 018 were highly susceptible. From other 20 BJMN lines, BJMN 008, 010, 012 and 013 showed resistant to leaf blight with varied intensity from 10-20%. Nine lines namely BJMN 001, 002, 006, 007, 014, 015, 016, 018 and 020 indicated moderately resistant, while BJMN 003, 005 and 019 were reacted susceptible to leaf blight disease and the highly susceptible lines were BJMN 003, 005 and 019 .

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