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Effect of *Bipolaris maydis* (Y. Nisik & C. Miyake) shoemaker at various growth stages of different maize cultivars

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Abstract

Three local maize cultivars, namely Jalal, Azam and Iqbal were inoculated with *Bipolaris maydis* at 6, 10, 14 leaf stages and tasseling stages. Cultivars were allotted to the main plots and growth stages to the subplots, each consisting of six rows, 3m long and 75cm apart. The central two rows of each sub-plot were inoculated with 10^4 conidia/ml concentration of the pathogens during summer 2013 at New Developmental Farm of The University of Agriculture, Peshawar. Overall, Jalal registered the lowest disease severity (1.53) on a 0-5 scale while Iqbal was observed to have the highest disease severity (2.40). Among the growth stages inoculation at the 6-leaf stage resulted in the highest disease severity (2.78) and lowest yield (5328.2kg/ha) and yield components while inoculation at the tasseling stage was found to be the least effective in terms of disease severity (1.44) and reduction in yield and yield components. Similarly, a significant interaction between the cultivars and growth stages was observed ($P \leq 0.05$).

Keywords: Maize, *Bipolaris maydis*, Cultivars, Disease severity, Inoculation

Introduction

One of the most important cereal crops of the world is maize (*Zea mays* L.) which is grown in rainfed as well as irrigated areas. It grows best in well aerated, warm, loamy soil which is rich in organic matter and has high nitrogen, potassium and phosphorus contents. Good crop performance is gained under moderately high summer temperature having warm nights and adequate rainfall which is evenly distributed during growing season.

Maize is one of the world's most widely grown crop, ranking third in cereals after wheat and rice. Its annual worldwide production is 822 million tons [1]. Pakistan is also a major maize producing country, contributing an area of 974200 ha with a total annual production of 3.707 million tons. While Khyber Pakhtunkhwa (KPK) shares an area of 422900 ha with a total production of 740500 tons [2]. It has been estimated that maize farmers and consumers will produce more than half of the increased demand in the world food in terms of cereals as a whole [3]. By 2050, almost 70% more food than today's population will be required by the predicted 9 billion people in the world and a huge proportion of the increased demand will definitely come from the developing countries [1]. For molecular studies like genetics, functional genomics, cytogenetics and genomics, maize is a vital model plant [4]. Thus, there is a good opportunity for maize breeders to utilize their attempts to increase significantly the production of maize in an environmentally sensitive way [3].

The total production of maize is affected by several factors, like cultivation of hybrids, favorable weather conditions, balanced use of inputs as well as economical management of insects and diseases. Maize crop is in general exposed to a great number of adverse biotic and abiotic factors. For the biotic stresses, emphasis is given to leaf diseases, which affect both yield and quality of the crop product.

In maize, worldwide losses due to diseases not including viruses and insects were estimated to be about 9% in 2001-3 [5]. It varies significantly by region which is estimated 4% in Northern Europe and 14% in West Africa and South Asia. Losses have tended to be effectively controlled in high-intensity agricultural systems where it has been economical to invest in resistant germplasm and in some cases pesticide applications.

Instead of its high yielding potential, its sensitivity to diseases is one of the main limiting factors to maize grain yield. Maize is infected by as many as 365 pathogens [6]. One of the most important diseases of maize caused by the fungus *Bipolaris maydis* (*Cochliobolus heterostrophus*) is maydis leaf blight (MLB) or southern leaf blight (SLB) which poses a great threat to production of maize worldwide [7]. Under epiphytotic conditions this fungus reduces crop stand and yield substantially. Several factors such as crop growth stage, susceptible varieties and planting time added to high disease intensities and ultimate yield losses [8]. The severity and extent of this disease varies from season to season. In warm (20-32°C) and moderately humid environment of the world, MLB is potentially damaging and may cause significant losses [9]. The blight spreads from the lower leaves to developing ears and then flag leaf to whole plant. MLB is also prevalent in the maize growing areas of KPK and results in more than 20% yield losses to the crop in Pakistan [10].

MLB is most dangerous in wet, warm temperate and tropical areas, where yield losses are reported very close to 70% due to this disease. Many races of *B. maydis* are pathogenic to maize crop. Severity and symptoms of *B. maydis* mainly depends on the host germplasm and pathogen race. Three physiological races of *B. maydis* have been reported: Race C, Race O and Race T. Race C and Race T are only pathogenic to maize germplasm having cytoplasm male-sterile C and cytoplasm male-sterile T respectively.

MLB symptoms vary according to host germplasm and the causal race. Symptoms of maydis leaf blight which is caused by Race T are slightly larger than those caused by Race O (6-12 × 6-27 mm). Borders of lesions are usually dark brown. Race T causes lesions on all above ground parts of plant including ears and sheaths and can also cause ear rots. Seeds infected seedlings with Race T often wilt and die within 3 to 4 weeks. Under severe disease pressure, lesions may merge or coalesce, blighting the entire leaf, usually when infection occurs before silking. In such situation, sugars may be diverted from stalk to grain filling and thus predisposing the plant to lodging. Diamond-shaped and initially small lesions are produced by Race O. These lesions elongate as they mature and also growth of lesions is restricted by leaf veins. Final lesions are rectangular (2-6 × 3-22 mm) and tan in color. Race O causes lesions which are restricted to leaves only. Race C produce necrotic lesions which are about 5 mm long and they may cause wilt [8]. The severity and extent of MLB varies from season to season and considerable losses in grain yield of

maize occur when infection initiated late in the season. These losses were recorded 9.7% and 11.7% in 1975 and 1976 respectively [11]. It is reported that temperature, relative humidity and rainfall are main factors in spreading of *B. maydis* [12]. In addition to host resistance, host age plays an important role in determining the severity of a plant disease [13]. Therefore, this study was conducted with the objectives to evaluate the response of maize cultivars for resistance against maydis leaf blight (MLB) under field conditions, to test the effect of the crop age on the severity of MLB and extent of yield losses and to determine the most suitable growth stage for screening maize cultivars against MLB.

2. Materials and Methods

This study was carried out at New Developmental Research Farm of The University of Agriculture Peshawar during summer 2013.

Source of Maize cultivars and culture of *B. maydis*

Maize cultivars (Jalal, Azam and Iqbal) and culture of *B. maydis* were collected from New Developmental Research Farm of The University of Agriculture Peshawar.

Isolation of pathogen

Maize crop was surveyed in order to obtain leaves with maydis leaf blight symptoms. These infected leaves were brought to the Plant Pathology laboratory of The University of Agriculture Peshawar. Infected portion along with healthy areas were cut out into small (about 1cm² pieces) and dipped in 0.1% HgCl₂ solution for about 15-30 seconds in order to remove superficial microbes. The pieces were then rinsed three times in sterile distilled water, blotted dry with sterile tissue paper and plated aseptically on fresh PDA medium (4-5 pieces/ petri dish). The petri dishes were sealed with parafilm and incubated at 25°C for culture development and regularly checked for the growth of *B. maydis*. Pathogen was identified on the basis of morphological characteristics and pure culture was produced for the preparation of inoculum suspension, using identification key [14].

Field experiment and experimental design

Three maize cultivars were inoculated with *B. maydis* at different vegetative growth stages in a split plot RCB Design as shown in the lay out. Cultivars were allotted to the main plots and growth stages of the crop to the sub plots. Each sub plot consisted of six rows 3m long and 75cm apart. Each treatment was replicated three times.

Block-1

C1					C3					C2				
C1S0	C1S2	C1S1	C1S3	C1S4	C3S1	C3S3	C3S2	C3S0	C3S4	C2S2	C2S3	C2S1	C2S0	C2S4

Block-2

C2					C1					C3				
C2S1	C2S3	C2S2	C2S4	C2S0	C1S4	C1S3	C1S1	C1S2	C1S0	C3S2	C3S3	C3S1	C3S4	C3S0

Block-3

C3					C2					C1				
C3S3	C3S4	C3S1	C3S2	C3S0	C2S4	C2S2	C2S0	C2S3	C2S1	C1S2	C1S4	C1S0	C1S3	C1S1

C (cultivars) S (Growth Stages)

C₁= Iqbal
C₂= Azam
C₃= Jalal

S₀= Control (No inoculation)
S₁= 6-leaf stage
S₂= 10-leaf stage
S₃= 14-leaf stage
S₄= Tasseling stage

Field layout of the trial

Inoculum preparation and method of inoculation

The isolated pathogen was sub-cultured on PDA medium to obtain enough inoculum (figure 1). Conidia from the fully grown culture were washed with sterile water and collected in a beaker. To prepare the required concentration of inoculum

i.e. 10⁴ spores/ml fresh culture of the pathogen was flooded with distilled water and scraped with the help of sterile glass rod to get maximum numbers of spores. One ml of the prepared concentration was taken with the help of Pasteur's pipette and put on the chamber of hemocytometer. After placing the cover slip the spores were counted under compound microscope. The procedure was repeated before each inoculation.

The central two rows of the relevant sub-plots were inoculated with a hand sprayer. Control sub plots were sprayed with sterile water only. The uninoculated two rows on both sides of the inoculated rows served as buffer rows to prevent the spread of the disease between treatments. Inoculation was done in late afternoon to avoid desiccation of conidia in the warm weather.

Data collection and statistical analysis

Data on disease severity were recorded on a 0-5 scale [15] as follows:

Scale	Description
0	No disease
1	One or two to few scattered lesions on lower leaves.
2	Moderate number of lesions on lower leaves only.
3	Abundant number of lesions on lower leaves, few on middle leaves.
4	Lesions abundant on lower leaves and middle leaves, extending to upper leaves.
5	Lesions abundant on almost all the leaves, plants prematurely dried or killed by the disease.

Data were also recorded on plant height, fresh ear weight, number of ears/plant, cob length, number of grain rows/cob, 200-grains weight and yield/plot. The data were subjected to ANOVA to determine the significance of variation. LSD (5%) was used for mean separation in case of significant differences between the treatments.

3. Results

Disease severity

Infected maize plants showed characteristic symptoms. The main effects of cultivars as well as growth stages differed significantly ($P=0.0064$ & $P=0.0000$ respectively) (Table 1). The highest disease severity of 2.40 was recorded on cultivar Iqbal while the lowest (1.53) was observed on Jalal. Similarly, plants inoculated at 6-leaf stage registered the highest disease severity of 2.78 while those left uninoculated (control) showed the lowest (1.00) (figure 2).

The interaction between maize cultivars and growth stages was also found to be significant ($P=0.0375$). Cultivar Iqbal inoculated at 6-leaf stage resulted in the highest disease severity (3.33) while cultivar Jalal inoculated at tasseling stage gave the lowest (1.00) disease severity. In the control plots, all the three cultivars gave the same level of disease severity (1.00). Generally, all the three cultivars were found to be the least affected when inoculated at tasseling stage. Iqbal variety exhibited more severe disease when inoculated at this stage. On the contrary, all the cultivars inoculated at 6-leaf stage resulted in the most severe disease as compared with inoculation at the other growth stages. However, the disease severity (2.00) on cultivar Jalal inoculated at 6-leaf stage was at par with the disease severity on cultivar Iqbal inoculated at the tasseling stage.

Plant height

Plant height differed significantly (Table 2) for cultivars ($P=0.0001$) as well as growth stages ($P=0.0000$). Cultivar Jalal was found to be the tallest (169.42cm) while Iqbal was

observed to be the shortest (136.63cm). Similarly, plants in the un-inoculated control plots were observed to be the tallest (157.90cm) followed by those inoculated at tasseling stage (155.48cm) while inoculation at the 6-leaf stage resulted in the shortest plants (147.72cm).

Interaction between cultivars and growth stages was also found to be significant ($P=0.0104$). Un-inoculated plots of cultivar Jalal registered the tallest plants (175.26cm) followed by plants of the same cultivar inoculated at tasseling stage (172.29cm) while plants of cultivar Iqbal inoculated at 6-leaf stage were observed to be the shortest (131.12cm). Although, plants of cultivar Jalal inoculated at 6-leaf stage were found to be the shortest (162.64cm) as compared with the plants of the same cultivar inoculated at other growth stages. These were taller than even the uninoculated plants of cultivar Azam (156.10cm) and Iqbal (142.34cm).



Fig 1: Maize leaves showing symptoms of *Bipolaris maydis*. Leaf streaks which are rectangular in shape and tan in colour was taken at six leaf stage of the crop.



Fig 2: Uninoculated control maize plant

Table 1: Maydis leaf blight disease severity recorded on different maize cultivars inoculated with *Bipolaris maydis* at various growth stages

Growth stages	Cultivars			Mean
	Iqbal	Azam	Jalal	
Uninoculated control	1.00 g	1.00 g	1.00 g	1.00 e
6- leaf stage	3.33 a	3.00 ab	2.00 de	2.78 a
10-leaf stage	3.00 ab	2.33 cd	2.00 de	2.4 b
14-leaf stage	2.67 bc	2.00 de	1.67 ef	2.11 c
Tesseling stage	2.00 de	1.33 fg	1.00 g	1.44 d
Mean	2.40 a	1.93 b	1.53 c	

LSD value for cultivars 0.3544

LSD value for growth stages 0.2990

LSD value for cultivars x growth stages 0.5779

Means for each category followed by the same letter do not differ from each other at

$P \leq 0.05$

Table 2: Plant height (cm) of different maize cultivars inoculated with *Bipolaris maydis* at various growth stages

Growth stages	Cultivars			Mean
	Iqbal	Azam	Jalal	
Uninoculated control	142.34 j	156.10 f	175.26 a	157.90 a
6- leaf stage	131.12 n	149.40 i	162.64 e	147.72 e
10-leaf stage	133.94 m	151.34 h	167.28 d	150.85 d
14-leaf stage	136.93 l	153.19 g	169.64 c	153.25 c
Tesseling stage	138.84 k	155.30 f	172.29 b	155.48 b
Mean	136.63 c	153.07 b	169.42 a	

LSD value for cultivars 4.1101
 LSD value for growth stages 1.0651
 LSD value for cultivars x growth stages 1.8448
 Means for each category followed by the same letter do not differ from each other at $P \leq 0.05$

Fresh ear weight

Fresh ear weight also differed significantly (Table 3) for cultivars ($P=0.0001$) as well as growth stages ($P=0.0000$). Cultivar Jalal was found to have highest fresh ear weight (343.90g) while Iqbal was observed to have lowest fresh ear weight (338.17g). Similarly, plants in the un-inoculated control plots were observed to have the highest fresh ear weight (351.85g) followed by those inoculated at tasseling stage (341.97g) while inoculation at the 6-leaf stage resulted in the lowest fresh ear weight (335.32g). Interaction between cultivars and growth stages was also found to be significant ($P=0.0204$). Un-inoculated plots of cultivar Jalal registered the highest fresh ear weight (355.17g) followed by plants of uninoculated plots of cultivar Azam (351.46g) while plants of cultivar Iqbal inoculated at 6-leaf stage were observed to have the lowest fresh ear weight (332.62g). Although, fresh ear weight of cultivar Jalal inoculated at 6-leaf stage was found to be the lowest (338.12g) as compared with the fresh ear weight of the same cultivar inoculated at other growth stages, this was higher than the fresh ear weight of cultivar Azam inoculated at 10-leaf stage or cultivar Iqbal inoculated at 14-leaf stage.

Table 3: Fresh ear weight (g) of different maize cultivars inoculated with *Bipolaris maydis* at various growth stages

Growth stages	Cultivars			Mean
	Iqbal	Azam	Jalal	
Uninoculated control	348.92 c	351.46 b	355.17 a	351.85 a
6- leaf stage	332.62 m	335.21 kl	338.12 gh	335.32 e
10-leaf stage	334.75 l	336.75 ij	339.16 f	336.89 d
14-leaf stage	335.87 jk	337.54 hi	341.29 e	338.23 c
Tesseling stage	338.70 fg	341.46 e	345.75 d	341.97 b
Mean	338.17 c	340.48 b	343.90 a	

LSD value for cultivars 0.7610
 LSD value for growth stages 0.5313
 LSD value for cultivars x growth stages 1.1094
 Means for each category followed by the same letter do not differ from each other at $P \leq 0.05$

Cob length

Cob length also differed significantly (Table 4) for cultivars ($P=0.0000$) as well as growth stages ($P=0.0000$). Cultivar Jalal was found to have maximum cob length (16.8cm) while Iqbal was observed to have minimum cob length (13.4cm). Similarly, plants in the un-inoculated control plots were

observed to have the maximum cob length (18.2cm) followed by those inoculated at tasseling stage (15.8) while inoculation at the 6-leaf stage resulted in the minimum cob length (12.7cm).

Interaction between cultivars and growth stages was also found to be significant ($P=0.0000$). Un-inoculated plots of cultivar Jalal registered the maximum cob length (20cm) followed by uninoculated plants of cultivar Azam (18.5cm) while plants of cultivar Iqbal inoculated at 6-leaf stage were observed to have minimum cob length (11cm). Although, the cob length of cultivar Jalal inoculated at 6-leaf stage was found to be the less (14cm) as compared with the plants of the same cultivar inoculated at other growth stages, the cob length of Iqbal was even less when inoculated at 14, 10 or 6-leaf stages.

Table 4: Cob length (cm) of different maize cultivars inoculated with *Bipolaris maydis* at various growth stages

Growth stages	Cultivars			Mean
	Iqbal	Azam	Jalal	
Uninoculated control	16 d	18.5 b	20 a	18.2 a
6- leaf stage	11 l	13 j	14 h	12.7 e
10-leaf stage	12 k	14 h	16 d	14 d
14-leaf stage	13.4 i	14.6 f	16 d	14.6 c
Tesseling stage	14.5 g	15 e	18 c	15.8 b
Mean	13.4 c	15 b	16.8 a	

LSD value for cultivars 0.0151
 LSD value for growth stages 0.0145
 LSD value for cultivars x growth stages 0.0269
 Means for each category followed by the same letter do not differ from each other at $P \leq 0.05$

Yield

Yield also differed significantly (Table 5) for cultivars ($P=0.0000$) as well as growth stages ($P=0.0000$). Cultivar Jalal was observed to have maximum yield (5813.8kg/ha) while Iqbal was observed to have minimum yield (5367.8kg/ha). Similarly, plants in the un-inoculated control plots were observed to have the maximum yield (5843.3kg/ha) followed by those inoculated at tasseling stage (5701.6kg/ha) while inoculation at the 6-leaf stage resulted in the minimum yield (5328.2kg/ha). Interaction between cultivars and growth stages was also found to be significant ($P=0.0003$). Un-inoculated plots of cultivar Jalal registered the maximum yield (6070.3kg/ha) followed by plants of the same cultivar inoculated at tasseling stage (5930.5kg/ha) while plants of cultivar Iqbal inoculated at 6-leaf stage were observed to have minimum yield (5093.9kg/ha). Although, yield of cultivar Jalal inoculated at 6-leaf stage was found to be the minimum (5507.0kg/ha) as compared with the plants of the same cultivar inoculated at other growth stages, it was greater than the yield of cultivar Azam inoculated at 6-leaf stage or cultivar Iqbal inoculated at 6, 10 or 14-leaf stages.

Table 5: Yield (kg/ha) of different maize cultivars inoculated with *Bipolaris maydis* at various growth stages

Growth stages	Cultivars			Mean
	Iqbal	Azam	Jalal	
Uninoculated control	5640.6 fg	5819.0 d	6070.3 a	5843.3 a
6- leaf stage	5093.9 l	5383.9 j	5507.0 hi	5328.2 e
10-leaf stage	5253.9 k	5549.0 h	5687.2 ef	5496.7 d
14-leaf stage	5377.1 j	5629.2 g	5873.8 c	5626.7 c
Tesseling stage	5473.8 i	5700.5 e	5930.5 b	5701.6 b
Mean	5367.8 c	5616.3 b	5813.8 a	

LSD value for cultivars 33.973

LSD value for growth stages 23.782

LSD value for cultivars x growth stages 49.597

Means for each category followed by the same letter do not differ from each other at $P \leq 0.05$

4. Discussion

The response of three local maize cultivars viz. Jalal, Azam and Iqbal was studied against a uniform inoculum load (10^4 conidia/ml) of *Bipolaris maydis* applied at different growth stages under field conditions. Significant differences ($P \leq 0.05$) were found in their response regarding disease severity and yield parameters. Cultivar Jalal registered the minimum disease severity when inoculated at tassel stage. Likewise, cultivar Azam was second in resistance to the disease. However, it was not very much tolerant when exposed to southern corn leaf blight at earlier stages of development. Conversely, cultivar Iqbal was found to be the most susceptible at all its developmental stages against the disease. Many researchers studied response of different maize cultivars, lines and hybrids to inoculum load of southern corn leaf blight under various environmental conditions. Tahir *et al.* [16] studied comparative response of various maize hybrids under natural field conditions. The data on all the agronomic characteristics for hybrid HG-3740 was significantly higher as compared to all other varieties. Shah *et al.* [17] also reported significant differences between two maize varieties in terms of disease severity and yield under inoculum stress of MLB. In another study carried out by Junaid *et al.* [18], cultivar Jalal was proved to be resistant against MLB, amongst four varieties when it was applied with fertilizer doses of 180, 60 and 60 kg ha⁻¹ of nitrogen, phosphorus and potassium rather than their recommended doses of 120, 60 and 60 kg ha⁻¹ respectively. Similarly, agronomic characteristics of all maize varieties were enhanced when fertilizers applied in two split doses.

Disease severity is closely correlated with yield of maize as well as other parameters such as cob length and numbers of grains per cob. In this study cultivar Jalal was the least affected by southern corn leaf blight and resulted in maximum yield followed by cultivar Azam. Conversely, cultivar Iqbal resulted in lowest yield as it was highly sensitive to inoculum load at earlier stages of development. These results were according to the finding of Pataky *et al.* [19] who carried out his study on maize hybrid under northern leaf blight stressed conditions. Similarly, growth stages of maize are very important in terms of disease severity. Early growth stages are more sensitive towards inoculum as compared to later stages. Researchers studied this phenomenon for various crops. Headrick *et al.* [20] studied the effect of common maize rust in six sweet corn hybrids under inoculum stress of *Puccinia sorghi*. They found adult plant resistance in all hybrids when inoculated at later stages rather than earlier stages of development. Moreover, number of leaf spots was fewer at the onset of reproductive stage when it was compared to earlier stage of five to six leaves stages. This was also confirmed by Hooker [21], who studied Dent corn under stressed conditions. Different names such as generalized resistance, mature plant resistance and adult plant resistance were proposed for this type of resistance [22].

Adult plant resistance is a universal phenomenon, increases from seedling to harvesting during the plant growth. Conversely, partial resistance in maize is concern with genotype specific trait present at growth stages [19]. However, both types of resistance are not dependent upon each other. In this study cultivar Iqbal was more sensitive even during

anthesis as compared to cultivar Jalal and Azam at similar growth stages. Similarly, response of all three maize cultivars was significantly different at 10 and 14-leaf stages against a uniform inoculum load of southern corn leaf blight. Variation in environmental condition, appearance of disease and fitness of the pathogen is also an important factor in disease development and yield reduction of maize crop. Abebe *et al.* [23] reported that disease severity was higher in maize crop in Bako where environmental conditions were favorable for northern corn leaf blight even though disease occurrence was lower because of the dry weather during planting. Conversely, in Gambella northern leaf blight severity was minimum due to low raining and unfavorable conditions. Similarly, higher relative humidity optimum temperature is also helpful for the pathogen to cause infection more readily.

It is clear from the present study that both cultivars as well as growth stages of maize are important for the development of southern corn leaf blight. A susceptible cultivar can perform better when it is not affected by the pathogen during earlier stages of development. Similarly, at tassel stage of maize cultivars generalized resistance is at its peak, reducing the infectivity of the pathogen and ultimately reducing the need of late fungicides application even for susceptible cultivars. Furthermore, prominent output is not possible even from a high productive maize cultivar when it is exposed to inoculum during earlier stages of development.

5. Conclusion and Recommendations

Cultivar Jalal registered lowest disease severity than cultivars Azam and Iqbal. Inoculation of the maize cultivars with *B. maydis* at the early growth stages resulted in more severe disease and more reduction in yield parameters. Resistant maize cultivars such as Jalal should be sown in order to get the maximum yield. For effective screening of maize cultivars against maydis leaf blight, inoculation with *B. maydis* should be carried out at early growth stages (6-leaf stage).

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