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Virulence of Four *Cercospora zeae-maydis* Isolates on Tolerant and Susceptible Maize Varieties under Greenhouse Condition

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Abstract **– Maize (*Zea mays* L.) is one of the most important food security crops mainly due to its high productivity and wider adaptability in Ethiopia. In recent years, grey leaf spot (GLS) has become a serious disease challenging maize production across the different parts of the country. Empirical study that evaluated the virulence variation of *Cercospora zeae-maydis* isolates and their reaction to maize varieties in Ethiopia is lacking. Therefore, this study was conducted to evaluate the virulence variation of four *C. zeae-maydis* isolates of different locations on six maize varieties under greenhouse conditions during 2015 cropping season. Data were recorded on latent period, lesion length, initial and final percent severity index (PSI), disease infection rate, area under disease progress curve (AUDPC) and dry biomass yield. Highly significant difference were observed among tested *C. zeae-maydis* isolates, varieties and their interaction on latent period, lesion length, initial and final GLS severity, disease infection rate and AUDPC. Isolate Huru-I had the shortest mean latent period (21 days), highest initial (45.3PSI) and final (81.3PSI) GLS severity, highest lesion length (3.3cm), fastest disease progress rate (0.0297 disease day^{-1}), highest AUDPC (2188.67%-day) and lowest dry biomass yield (0.08kg) on tested maize varieties as compared to the other isolates. Huru-I was found to be the most aggressive isolate followed by Bor-S. In conclusion, this study revealed an extensive virulence variation among the isolates as well as variable responses of maize varieties tested. Results could be useful for maize breeder and producers in order to effectively manage maize GLS in Ethiopia.**

Keywords **– AUDPC, Grey Leaf Spot, Latent Period, Lesion Length, Maize Disease.**

I. INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereal grains grown worldwide under a wider range of environments because of its greater adaptability [1]. In Ethiopia, maize (*Zea mays* L.) is the most essential staple food crop standing first in total production among the cereals [2]. It grows in 13 agro-ecological zones on 1.99million ha of land [3]. Several million people in developing countries derive their protein and caloric requirements from maize [4]. The calorie contributions of maize to the Ethiopian diet has doubled to around 20% while, its protein contribution to the country's diet has been doubled to 16% in the same period [3]. Maize is also being used as important raw material for animal feed [5].

The productivity of maize in Ethiopia remains low due to a number of biotic and abiotic constraints. Among the biotic stresses diseases such as maize grey leaf spot is one. Grey leaf spot caused by the fungus *C. zeae-maydis* Tehon and Daniels, is one of the devastating disease of maize (*Zea mays* L.) in several countries, causing severe losses of up to 65% [6]; [7]; [8]. Dagne *et al*. [9] reported yield losses up to 37% in Ethiopia.

Methods to manage grey leaf spot include conventional tillage that buries crop residues, crop rotation, fungicides, and utilization of resistant varieties [8]. Fungicides are widely used in maize production [10] but are too expensive for low income-resource poor farmers in the tropics [11]. Host resistance is the most efficient and cost-effective means of managing grey leaf spot and preventing leaf blighting [12]. However, no commercial

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hybrids with sufficient resistance presently exist in Ethiopia, as they were not improved for resistance to this specific disease [13].

Although isolates of the pathogen have been found to vary in aggressiveness, little races/pathotypes so far have been reported to occur [14]. Carson *et al*. [14] also reported that, varying degrees of virulence demonstrated by grey leaf spot on hybrid maize in Kenya. Many 'hot spot' sites should be considered for effective screening programmes to capture the various pathogen populations available. Therefore, to maximize gain from selection for grey leaf spot resistance, locations with favorable environment for pathogens and with the most highly aggressive type should be considered as test sites [14].

In Ethiopia, Dagne *et al.* [13] reported that, differentiating the importance and potential destructiveness of grey leaf spot disease, the Ethiopian national maize research program has been conducting a series of experiments on evaluation and selection of maize genotypes from local and exotic sources. In these tests, some of the entries exhibited fleck type lesions (resistance reaction) when artificially inoculated with *C. zea-maydis* [13]. On the contrary, some of the sophisticated and commercial inbreds with good agronomic characters show susceptible reaction to the disease. Hence, understanding the virulence variation of *C. zeae-maydis* isolates collected from different maize growing areas of the country leads to successful management of the disease through selection and breeding in Ethiopia. However, no information on the diversity of *Cercospora zeae-maydis* isolates interms of virulence on different maize varieties. Thus, the objective of this piece of work was to evaluate the virulence variation of *C. zeae-maydis* isolates of different locations on tolerant and susceptible maize varieties under greenhouse conditions in Jimma, southwestern Ethiopia.

II. Materials and Methods

Greenhouse experiment was conducted at Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) to evaluate the virulence of four *C. zeae-maydis* isolates collected from different areas of southern and southwestern Ethiopia by artificial inoculation of maize plant seedling.

2.1. *Tested Maize Varieties*

For this study, six maize varieties with different reaction to grey leaf spot were used (Table 2).

Table 1. Description of maize varieties with different grey leaf spot (GLS) reaction.

Varieties	Reaction to GLS	Released by
BH-660	T	BARC
BH-670	T	BARC
BH-140	MT	BARC
BH-543	MT	BARC
BH-540	MT	BARC
Local-K	S	-

Source: Mandefro *et al*. [15]. T = Tolerant; MT = Moderately tolerant; S = Susceptible. BARC = Bako Agricultural Research Center.

2.2. *Source and Description of Cercosporazeae-maydis Isolates*

Four representative *C. zeae-maydis* isolates, each from Jimma, Illuababora, Sidama and Wolaita zones were

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selected based on the altitude gradient, cultural and morphological variations of the isolates. The detail descriptions of the collection sites and characteristics of the isolates are shown in Table 2.

Table 2. Description of *C. zeae-maydis* isolates used for the study.

No.	Isolate	Zone	Altitude (m.a.s.l.)	Colony color		Colony elevation	Culture growth rate (mm/day)	Mycelial growth diameter (mm/7 days)	Conidial size (µm)	
				Upper side	Reverse side				Length	Width
1	Bor-S	Sidama	2000	white	corn silk	Raised	8.30	59.67	49.5	9.0
2	BS-W	Wolaita	1800	brown	Indian red	Raised	7.29	48.49	34.5	7.9
3	Huru-I	Illuababora	1817	gray	light gray	Raised	8.49	62.19	31.8	7.5
4	SK-J	Jimma	1780	pink	light pink	Raised	8.88	58.12	38.3	7.6

Source: Adapted from preliminary study result. Where, SK-J = Isolate from Seka (Jimma), Bor-S = Isolate from Boricha (Sidama), Huru-I = Isolate from Hurumu (Illuababora) and BS-W = Isolate from Boloso sore (Wolaita).

2.3. *Experimental Procedures and Design*

To remove seed-borne inoculums seeds were surfaced-sterilized by soaking in 5% sodium hypochlorite (NaOCl) for 5 minutes, and washed thoroughly three times with sterile distilled water, and dried at room temperature (25^{o}C). Finally, seeds were sown in 30cm diameter plastic pots filled with autoclaved (at 105^{o}C for 30 minutes) sterile soil. The total experiment were contained 4 isolates, 6 varieties and one control/check with entire treatment combination of 5x6x3 (90) pots/ treatments and laid out factorial arrangement in a Complete Randomized Design (CRD) with three replications. The seedlings in each plastic pot were thinned to two plants per pot. The seedlings were grown in the greenhouse and inoculation was started at the fourth leaf stage (the stage at which the leaf of tested maize varieties were fully expanded for inoculation). Irrigation was applied to maintain optimum moisture for seed germination, emergence and growth of seedling in the greenhouse. The experiment was repeated once parallel in the same greenhouse.

2.4. *Inoculum Preparation*

Cultures of *C. zeae-maydis* were isolated from infected leaf-samples collected from the four locations (Jimma, Illuababora, Sidama, and Wolaita) of south and southwest Ethiopia. Cultures were incubated at 25^{o}C and exposed to fluorescent light until conidia formation in laboratory bench. Mycelia plugs of 15-27 day old sporulating colonies were aseptically transferred from PDA agar to a test tube containing 10ml of sterile, 0.1% solution of Tween 20 and distilled water. Test tubes were mixed to dislodge conidia with a touch mixer, and approximately 0.3ml of the resulting conidial suspension was transferred and spread on the surface of PDA agar in Petri dishes. After 10-15 days at room temperature (25^{o}C), 5ml of distilled water was added to the Petri dishes and conidia were dislodged with a rubber detective. Resulting conidial suspensions were strained through two layers of cheesecloth. Conidial concentration was estimated using a haemocytometer by determining the average number of conidia counted in subsamples. Subsequently, conidial concentrations were adjusted to $5x10^{4}$/ml with sterile distilled water. The suspensions from plates of the isolates were combined to create a conidial suspension used to inoculate plants. Conidial suspensions were prepared fresh before each inoculation as suggested by [16].

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2.5. *Inoculation*

The seedling was inoculated 25 days after planting at V_4 growth stage (fourth leaf vegetative stage) [17] when the plant had four expanded leaves. After inoculation and during the subsequent four days, pots were covered with a big plastic for 24 hours per day for 4 day to have enough humidity. The experiment was conducted under high humidity (95%) conditions achieved by daily watering of the greenhouse. Inoculation was carried out with a conidial suspension of $5x10^4$/ml of distilled water through wounding the plants. About 1ml of conidial suspension was placed in the whorl with a disposable 10ml hypodermic syringe. The syringe needle was inserted vertically down into the leaf whorl and the needle tip was used to puncture the leaf surface at several locations in the whorl [16]. Control plants of each variety were wounded at the same time and growth stage, without adding inoculums.

2.6. *Disease Assessment*

One week after inoculation, plants were evaluated every day for the presence of symptoms. Latent period was taken as time in days from inoculation to appearance of first fleck/chlorotic/necrotic lesion for each isolate-variety combination. The top healthy leaves were examined for the appearance of chlorotic or necrotic lesion every day after inoculation. Disease reactions were evaluated at 21 days after inoculation when lesions were easily visible. The disease severity on a whole plant basis was visually estimated to account for lesion development on multiple leaves. After visual symptom development, one leaf per plant was marked with a non-phytotoxic permanent marker and lesion length was measured in centimeter (cm). Disease severity was assessed in a weekly interval starting from 21 days after inoculation by using 1-5 disease rating scale of Maroof *et al*. [18], where: 1 = no symptoms; 2 = moderate lesion development below the leaf subtending the ear; 3 = heavy lesion development on and below the leaf subtending the ear with a few lesions above it; 4 = severe lesion development on all but the uppermost leaves, which may have a few lesions; and 5 = all leaves dead. Moreover, the dry biomass yield (kg) was recorded from tagged one plant per pot at the end of final severity rating before physiological maturity, immature ears were removed and fresh weight were measured immediately after harvest and dry biomass yield were weighed after plants in forced air circulated hot air oven at 105^{o}C for 72 hours.

The disease progress rate (r) was calculated basing on the linearized model [19]; [20] and the calculated value were analyzed by using SAS software: $r = \frac{\left(Ln\frac{X}{1-X}\right)-\left(Ln\frac{X_o}{1-X_0}\right)}{t}$

Where: r = disease progress rate, X_o = initial disease severity, X = final disease severity, t = the duration of the epidemic and Ln = Natural logarithm.

Area under disease progress curve (AUDPC) was computed from severity data using the formula suggested by Shaner and Finney [21]: $AUDPC = \sum_{i=1}^{n}[0.5\ (x_i\ +\ x_{i+1})]\ [t_{i+1} - t_i]$

Where, x_i is the disease severity expressed in percentage at i^{th} observation, t_i is time (days after planting) at the i^{th} observation and n is total number of days disease was assessed.

2.7. *Data Analysis*

All data including latent period, lesion length, mean initial and final percent severity index, disease progress rate (r) and AUDPC and dry biomass yield were analyzed using SAS software version 9.2 SAS [22]. LSD test at the 5% probability level was used for mean comparison among treatment. Correlation analysis was also performed

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using SASV 9.2 software to determine the relationship among disease assessment parameters such as latent period, lesion length, initial and final percent severity index, disease progress rate (r) and AUDPC and dry biomass yield under greenhouse conditions.

III. RESULTS

3.1. *Virulence Variation of Cercospora zeae-maydis Isolates*

3.1.1. *Latent Period*

The mean latent period of *C. zeae-maydis* varied from 21 to 29 days and showed highly significant ($P \leq 0.01$) differences among isolates, within maize varieties and their interaction (Table 3). The longest latent period was recorded when isolate BS-W inoculated with BH-660. Whereas, the shortest latent period was recorded when the susceptible maize variety, Local-K, was inoculated with isolates Huru-I. Overall, among the tested isolates, inoculation of Huru-I had caused shortest latent period on all tested maize varieties, while isolates BS-W produced the longest latent period on all the tested maize varieties.

Table 3. Effect of maize varieties and *C. zeae-maydis* isolates on latent period of GLS under greenhouse condition.

Variety	Isolate			
	SK-J	**Bor-S**	**Huru-I**	**BS-W**
BH-140	$23.33 \pm 1.52^{i-l}$	$24.00 \pm 0.00^{h-k}$	$25.00 \pm 1.00^{f-i}$	$25.33 \pm 1.15^{e-h}$
BH-540	$24.67 \pm 0.57^{g-j}$	$24.00 \pm 0.00^{h-k}$	$25.33 \pm 0.57^{e-h}$	$25.33 \pm 0.57^{e-h}$
BH-543	$22.00 \pm 1.00^{l-n}$	$23.00 \pm 0.00^{j-m}$	$23.33 \pm 0.57^{i-l}$	$23.00 \pm 1.00^{j-m}$
BH-660	$26.67 \pm 1.53^{c-f}$	$28.33 \pm 2.30^{a-c}$	28.67 ± 1.15^{ab}	29.33 ± 1.15^{a}
BH-670	$26.33 \pm 2.08^{d-g}$	$26.67 \pm 2.08^{c-f}$	$27.00 \pm 1.00^{b-e}$	$28.00 \pm 0.00^{a-d}$
Local-K	21.33 ± 0.57^{nm}	$21.67 \pm 0.57^{l-n}$	21.00 ± 0.00^{n}	$22.67 \pm 0.57^{k-n}$
CV (%)	3.91			
LSD (0.05)	1.80			

Means followed by the same letters are not significantly different according to LSD at 5% probability level. CV = Coefficient of Variation, LSD = Least Significant Difference. Where, SK-J = Isolate from Seka (Jimma), Bor-S = Isolate from Boricha (Sidama), Huru-I = Isolate from Hurumu (Illuababora) and BS-W = Isolate from Boloso sore (Wolaita).

3.1.2. *Lesion Length*

The interaction of selected four *C. zeae-maydis* isolates and six maize varieties showed highly significant ($P \leq 0.01$) difference in terms of lesion length (Table 4). Inoculation of both Bor-S and Huru-I isolates on the susceptible maize variety, Local-K, gave the highest mean lesion length while inoculation of BH-660 with BS-W gave the lowest mean lesion length. On the other hand, but no lesion symptom on check were observed and plants remained completely disease free through the final date of disease assessment. In general, between the tested isolates, inoculation of Huru-I had caused higher lesion length on all tested maize varieties, while isolates BS-W produced the lowest lesion length on all the tested maize varieties.

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Table 4. Effect of maize varieties and *C. zeae-maydis* isolates on lesion length of GLS under greenhouse condition.

Variety	Isolate			
	SK-J	Bor-S	Huru-I	BS-W
BH-140	1.36 ± 0.57^{g}	1.38 ± 0.0^{fg}	1.38 ± 1.38^{fg}	1.27 ± 1.15^{g}
BH-540	1.36 ± 0.57^{g}	1.55 ± 0.00^{de}	1.34 ± 0.57^{g}	1.26 ± 0.57^{g}
BH-543	1.60 ± 1.00^{de}	1.67 ± 0.00^{d}	1.69 ± 0.57^{d}	1.52 ± 1.00^{ef}
BH-660	0.66 ± 1.52^{hi}	0.52 ± 2.30^{ij}	0.61 ± 1.15^{hi}	0.37 ± 1.15^{k}
BH-670	0.74 ± 2.08^{h}	0.63 ± 2.08^{hi}	0.67 ± 1.00^{hi}	0.45 ± 0.00^{jk}
Local-K	3.06 ± 0.57^{b}	3.23 ± 0.57^{a}	3.33 ± 0.00^{a}	2.71 ± 0.57^{c}
CV (%)	6.44			
LSD (0.05)	0.14			

Means followed by the same letters are not significantly different according to LSD at 5% probability level. CV = Coefficient of Variation, LSD = Least Significant Difference. Where, SK-J = Isolate from Seka (Jimma), Bor-S = Isolate from Boricha (Sidama), Huru-I = Isolate from Hurumu (Illuababora) and BS-W = Isolate from Boloso sore (Wolaita).

3.1.3. *Initial Disease Severity*

The initial disease severity assessment of *C. zeae-maydis* isolates was recorded 21 days after inoculation and showed a highly significant ($P \leq 0.01$) difference among the tested maize variety, *C. zeae-maydis* isolate and their interactions (Table 5). Inoculation of susceptible maize variety, Local-K, with Huru-I isolate gave significantly higher initial disease severity which was statistically at par when the susceptible maize variety, Local-K, was inoculated with both SK-J and Bor-S isolates. BH-660 inoculated with BS-W gave the lowest initial disease severity. On the other hand, no symptoms on check were observed and plants remained completely disease free through the final date of disease assessment. Overall, between the tested isolates, inoculation of Huru-I had caused higher initial disease severity on all tested maize varieties. Whereas, isolates BS-W produced the lowest initial disease severity on all the tested maize varieties.

Table 5. Effect of maize varieties and *C. zeae-maydis* isolates on initial GLS severity assessment 21 days after inoculation under greenhouse condition.

Variety	Isolate			
	SK-J	Bor-S	Huru-I	BS-W
BH-140	29.33 ± 3.78^{fg}	35.33 ± 0.57^{b-d}	33.33 ± 3.78^{c-e}	26.33 ± 2.30^{g}
BH-540	33.67 ± 0.57^{c-e}	31.67 ± 3.78^{ef}	32.67 ± 1.15^{c-f}	26.67 ± 1.15^{g}
BH-543	29.33 ± 2.08^{fg}	36.00 ± 1.75^{bc}	32.00 ± 3.00^{d-f}	27.33 ± 2.08^{g}
BH-660	17.67 ± 2.08^{jk}	16.00 ± 0.00^{k}	18.00 ± 1.00^{h-k}	15.33 ± 1.52^{k}
BH-670	20.67 ± 0.57^{h}	20.33 ± 2.30^{hi}	19.67 ± 1.52^{h-j}	17.00 ± 1.73^{i-k}
Local-K	42.33 ± 1.52^{a}	44.00 ± 1.00^{a}	45.33 ± 3.05^{a}	38.00 ± 1.73^{b}

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Variety	Isolate			
	SK-J	Bor-S	Huru-I	BS-W
CV (%)	7.4			
LSD (0.05)	3.46			

Means followed by the same letters are not significantly different according to LSD at 5% probability level. CV = Coefficient of Variation, LSD = Least Significant Difference. Where, SK-J = Isolate from Seka (Jimma), Bor-S = Isolate from Boricha (Sidama), Huru-I = Isolate from Hurumu (Illuababora) and BS-W = Isolate from Boloso sore (Wolaita).

3.1.4. *Final Disease Severity*

Both isolate and maize variety significantly influenced the final disease severity. Local- K maize variety gave the highest final grey leaf spot severity when inoculated with Huru-I but there was no significant difference between the susceptible maize variety, Local-K, inoculated with both Bor-S and SK-J isolates (Table 6). Whereas, the lowest final disease severity was recorded when BH-660 maize variety inoculated with BS-W isolate. On the other hand, no symptoms on check were observed and plants remained completely disease free through the final date of disease assessment. Overall, between the tested isolates, inoculation of Huru-I had caused higher final disease severity on all tested maize varieties, whereas isolates BS-W produced the lowest final disease severity on all the tested maize varieties.

Table 6. Effect of maize varieties and *C. zeae-maydis* isolates on final GLS severity assessment (PSI) after 56 days of inoculation under greenhouse condition.

Variety	Isolate			
	SK-J	Bor-S	Huru-I	BS-W
BH-140	65.33 ± 3.78^{ef}	71.00 ± 0.57^{bc}	69.33 ± 3.78^{cd}	62.33 ± 2.30^{f}
BH-540	69.67 ± 0.57^{cd}	67.67 ± 3.78^{de}	$68.67 \pm 1.15^{c-e}$	62.67 ± 1.15^{f}
BH-543	65.33 ± 2.08^{ef}	73.00 ± 1.73^{bc}	69.00 ± 4.58^{cd}	63.33 ± 2.08^{f}
BH-660	27.33 ± 0.57^{g}	28.67 ± 1.15^{g}	31.00 ± 1.00^{g}	26.33 ± 0.57^{g}
BH-670	28.33 ± 1.52^{g}	30.00 ± 1.73^{g}	33.00 ± 1.00^{g}	27.67 ± 0.57^{g}
Local-K	78.33 ± 1.52^{a}	80.00 ± 1.00^{a}	81.33 ± 3.05^{a}	74.00 ± 1.73^{b}
CV (%)	8.4			
LSD (0.05)	7.76			

Means followed by the same letters are not significantly different according to LSD at 5% probability level. CV = Coefficient of Variation, LSD = Least Significant Difference. Where, SK-J = Isolate from Seka (Jimma), Bor-S = Isolate from Boricha (Sidama), Huru-I = Isolate from Hurumu (Illuababora) and BS-W = Isolate from Boloso sore (Wolaita).

3.1.5. *Disease Progress Rate (r)*

Disease progress rate of all *C. zeae-maydis* isolates tested under greenhouse condition was calculated and highly significant ($P \leq 0.01$) differences were showed among maize varieties, *C. zeae-maydis* isolates and their interactions (Table 7). Inoculation of BH-140 maize variety with Bor-S (0.022 disease day^{-1}) and Local-K maize

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variety with Huru-I (0.029 disease day^{-1}) isolates gave significantly fastest disease progress rate and which was statistically at the same level when BH-140 and the susceptible maize variety, Local-K, was inoculated with SK-J and BS-W isolates. Whereas, lower disease progress rates was observed when BH-660 maize variety inoculated with all isolates tested. Overall, among the tested isolates, inoculation of Huru-I had caused higher disease progress rate on all tested maize varieties, while isolates BS-W produced the lowest disease progress rate on all the tested maize varieties.

Table 7. Effect of maize varieties *and C .zeae-maydis* isolates on disease progress rate (r) under greenhouse condition.

Variety	Isolate			
	SK-J	Bor-S	Huru-I	BS-W
BH-140	0.0202 ± 0.007^{ef}	0.0221 ± 0.0002^{cd}	0.0214 ± 0.0013^{d-f}	0.0216 ± 0.0008^{de}
BH-540	0.0213 ± 0.0002^{d-f}	0.0208 ± 0.0014^{d-f}	0.0214 ± 0.0006^{d-f}	0.0212 ± 0.0018^{d-f}
BH-543	0.0200 ± 0.0004^{f}	0.0214 ± 0.0016^{d-f}	0.0204 ± 0.0005^{d-f}	0.0196 ± 0.0002^{f}
BH-660	0.0203 ± 0.0013^{d-f}	0.0210 ± 0.0015^{d-f}	0.0203 ± 0.0003^{d-f}	0.0195 ± 0.0003^{f}
BH-670	0.0201 ± 0.0009^{ef}	0.0209 ± 0.0014^{d-f}	0.0203 ± 0.0012^{d-f}	0.0194 ± 0.0001^{f}
Local-K	0.0267 ± 0.0013^{b}	0.0282 ± 0.0009^{ab}	0.0297 ± 0.0031^{a}	0.0236 ± 0.0011^{c}
CV (%)	5.16			
LSD (0.05)	0.002			

Means followed by the same letters are not significantly different according to LSD at 5% probability level. CV = Coefficient of Variation, LSD = Least Significant Difference. Where, SK-J = Isolate from Seka (Jimma), Bor-S = Isolate from Boricha (Sidama), Huru-I = Isolate from Hurumu (Illuababora) and BS-W = Isolate from Boloso sore (Wolaita).

3.1.6. *Area under Disease Progress Curve (AUDPC)*

AUDPC of all *C. zeae-maydis* calculated for each maize varieties, *C. zeae-maydis* isolates and their interaction of maize varieties with isolates, and showed highly significant ($P \leq 0.01$) difference among the tested maize varieties, within isolates and their interactions (Table 8). The highest AUDPC value (2188.67%-day) was calculated on the inoculation of susceptible maize variety, Local-K, with Huru-I isolate. Whereas, the lowest AUDPC value was calculated from the inoculation of BH-660 and BH-670 maize varieties with all tested *C. zeae-maydis* isolates. In general, between the tested isolates, inoculation of Huru-I had caused higher AUDPC on all tested maize varieties, while isolates BS-W produced the lowest AUDPC on all the tested maize varieties.

Table 8.Effect of maize varieties and *C. zeae-maydis* isolates on AUDPC under greenhouse condition.

Variety	Isolate			
	SK-J	Bor-S	Huru-I	BS-W
BH-140	1628.67 ± 132.50^{d-f}	1838.67 ± 20.20^{bc}	1768.67 ± 132.50^{cd}	1523.67 ± 80.82^{f}
BH-540	1780.33 ± 20.20^{c}	1710.33 ± 132.50^{cd}	1773.33 ± 45.00^{c}	1535.67 ± 40.41^{f}
BH-543	1628.67 ± 72.85^{d-f}	1778.00 ± 126.58^{c}	1697.50 ± 71.47^{c-e}	1558.67 ± 72.85^{ef}
BH-660	1188.83 ± 105.40^{g}	1146.33 ± 120.50^{g}	1188.83 ± 35.00^{g}	1099.00 ± 50.03^{g}

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Variety	Isolate			
	SK-J	Bor-S	Huru-I	BS-W
BH-670	1162.00 ± 86.96^{g}	1117.67 ± 143.47^{g}	1151.50 ± 98.56^{g}	1051.12 ± 95.91^{g}
Local-K	2083.67 ± 53.46^{a}	2142.00 ± 35.00^{a}	2188.67 ± 106.92^{a}	1932.00 ± 60.62^{b}
CV (%)	5.35			
LSD (0.05)	143.94			

Means in a column followed by the same letters are not significantly different according to LSD at 5% probability level. CV = Coefficient of Variation, LSD = Least Significant Difference. Where, SK-J = Isolate from Seka (Jimma), Bor-S = Isolate from Boricha (Sidama), Huru-I = Isolate from Hurumu (Illuababora) and BS-W = Isolate from Boloso sore (Wolaita).

3.1.7. *Dry Biomass Yield (DBY)*

Inoculation of maize varieties with different isolates result influenced dry biomass yield of maize tested under greenhouse conditions (Table 9). The highest dry biomass yield was measured when BH-660 maize variety inoculated with Huru-I and BS-W isolates. Whereas, the lowest dry biomass yield was measured when the susceptible maize variety, Local-K, inoculated with SK-J and Huru-I isolates. Overall, among the tested isolates, inoculation of Huru-I had caused lower dry biomass yield on all tested maize varieties, while isolates BS-W produced the highest on all the tested maize varieties.

Table 9. Effect of maize varieties and *C. zeae-maydis* isolates on dry biomass yield under greenhouse condition.

Variety	Isolate			
	SK-J	Bor-S	Huru-I	BS-W
BH-140	0.12 ± 0.026^{e-g}	0.11 ± 0.015^{e-g}	0.14 ± 0.017^{b-e}	0.11 ± 0.023^{e-g}
BH-540	0.11 ± 0.040^{e-g}	0.12 ± 0.026^{e-g}	0.11 ± 0.037^{e-h}	0.13 ± 0.005^{d-g}
BH-543	0.12 ± 0.005^{d-g}	0.12 ± 0.015^{d-g}	0.11 ± 0.015^{e-g}	0.10 ± 0.020^{gh}
BH-660	0.17 ± 0.010^{ab}	0.13 ± 0.023^{c-f}	0.18 ± 0.010^{a}	0.18 ± 0.010^{a}
BH-670	0.15 ± 0.005^{a-d}	0.13 ± 0.015^{d-g}	0.15 ± 0.005^{a-d}	0.16 ± 0.010^{a-c}
Local-K	0.08 ± 0.011^{h}	0.10 ± 0.005^{f-h}	0.08 ± 0.020^{h}	0.09 ± 0.005^{gh}
CV (%)	12.26			
LSD (0.05)	0.03			

Means followed by the same letters are not significantly different according to LSD at 5% probability level. CV = Coefficient of Variation, LSD = Least Significant Difference. Where, SK-J = Isolate from Seka (Jimma), Bor-S = Isolate from Boricha (Sidama), Huru-I = Isolate from Hurumu (Illuababora) and BS-W = Isolate from Boloso sore (Wolaita).

3.1.8. *Relationship between Disease Variables and Dry Biomass Yield*

Latent period showed significant and strong negative ($P \leq 0.01$) associations with lesion length, initial and final percent severity index, disease infection rate and AUDPC of grey leaf spot disease. On the other hand, significant and strong positive ($P \leq 0.01$) association was observed among all disease variables.

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There was also highly significant (P≤0.01) positive association between latent period and dry biomass yield of maize varieties grown under the greenhouse. However, we observed highly significant (P≤0.01) negative association between dry biomass yield with lesion length (disease reaction), initial and final percent severity index, disease infection rate and AUDPC of grey leaf spot disease. All disease parameters had significant negative (P≤0.01) effect on dry biomass yield of tested maize varieties.

Table 10. Correlation analysis among disease variables and dry biomass yield of maize varieties with *C. zeae-maydis* isolates.

Disease assessment	LL	PSI_i	PSI_f	r	AUDPC	DBY
LP	-0.83***	-0.83***	-0.83***	-0.45**	-0.83**	0.75***
LL		0.91***	0.90***	0.76**	0.90***	-0.70***
PSI_i			0.99***	0.67***	0.98***	-0.71***
PSI_f				0.67***	0.97***	-0.71***
r					0.66***	-0.39**
AUDPC						-0.71***

Where: AUDPC = Area under Disease Progress Curve, LP = Latent period, LL = Lesion Length, PSI_i= Initial Percent Severity Index (after 21 days), PSI_f = Final Percent Severity Index (after 56 days), r = disease progress rate and DBY = Dry biomass yield. ***Significant at P<0.001, **Significant at P<0.01, *Significant at P<0.05 and ns = no significant difference.

IV. DISCUSSION

In this study, the four *C. zeae-maydis* isolates collected from major maize growing areas of south and southwestern Ethiopia, exhibited variable disease severity on the six maize varieties tested under greenhouse conditions. These isolates showed considerable variation in pathogenic characteristics such as lesion length and disease severity on all tested maize varieties; and similar observation was reported by Freppon *et al.* [23]. Latent period had also showed a strong negative correlation with all disease variables; which means, as the latent period of the pathogen (isolates) increased, severity as well as other disease variables decreased. This strong negative association clearly indicated that these disease variables are interrelated to each other and it may be possible to use any one of the variables as evaluation criteria. While the negative association between latent period and disease variables showed that the impact of latent period on the development of *C. zeae-maydis* isolates on tested maize varieties. As the latent period becoming longer (delaying in disease development) resulted in reduced disease severity as well as other disease variables of *C. zeae-maydis. C. zeae-maydis* isolate having long latent period are less pathogenic than thus having short latent period to develop on the host and cause grey leaf spot disease on maize varieties. Compared to other foliar pathogens, grey leaf spot had longer latent period (14 to 28 days). Forrest and Nutter [24] also reported similar observation. Reportedly, maize grey leaf spot lesion sizes varied greatly among genotypes having different resistance reaction. Susceptible inbreds and hybrids commonly display numerous necrotic lesions, while moderately resistant inbreds and hybrids often exhibit chlorotic and fleck-type lesions [24]. Carson *et al.* [14] also stated that less aggressive isolates were less efficient in discriminating resistance levels of maize hybrids, indicating the importance of knowing their level of aggressiveness in order to maximize the selection gain when relying on artificial inoculation to evaluate the resistance of maize plants to grey leaf spot.

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It was also observed that results obtained from the studied disease parameters designated highly significant differences among maize varieties, *C. zeae-maydis* isolates and their interactions. Based on different reaction results of maize varieties and *C. zeae-maydis* isolates, high variation in terms of lesion length, initial and final grey leaf spot severity, disease infection rate and AUDPC were observed under greenhouse conditions. Relatively, the disease progress rates were significantly lower on the inoculation of BH-660 and BH-670 maize varieties with all tested isolates of *C. zeae-maydis*. This interaction shows that these maize varieties are resistant to those isolates. Huru-I and Bor-S isolates showed the highest rates of disease development on all tested maize varieties except on BH-660 and BH-670 as compared with the other isolates. This indicates that Huru-I and Bor-S isolates are more aggressive than the other isolates tested on different maize varieties.

On the other hand, the inoculation of the susceptible maize variety, Local-K, with all isolates showed more susceptible reactions with the highest mean AUDPC of 2086.58% - day^{-1}. Based on the interaction of AUDPC value, Huru-I was the most aggressive to all tested maize varieties with the highest mean AUDPC of (1628.08.53%-day^{-1}) followed by Bor-S (1622.16%-day^{-1}). While, the inoculation of susceptible maize variety, Local-K, with all tested *C. zeae-maydis* isolates showed no significant variations on the calculated mean of AUDPC except the isolate of BS-W. Overall, the disease progress rates were significantly lower on the variety BH-660 than all tested maize varieties. Furthermore, significant variety x isolate interaction for the studied disease parameters indicated that the inherent ability of varieties to express resistance reaction against *C. zeae-maydis* was not the same. This investigation is supported by [25], who stated that isolates of *C. zeae-maydis* exhibit a range of aggressiveness when inoculated on maize hybrids. The highest variation in disease reaction among *C. zeae-maydis* isolates tested clearly showed the existence of pathogen variability in Ethiopia. Similar result was also noted by Carson *et al.* [14], who reported on the variation in aggressiveness among isolates of *C. zeae-maydis*. Similarly, Okori *et al.* [26] studied 27 African isolates of *C. zeae-maydis* and also found that significant differences in aggressiveness between isolates.

In terms of dry biomass yield, higher dry biomass yield of maize varieties were measured from plastic pots which relatively obtained lower disease severity, where as the lower dry biomass yield of maize varieties were measured from plastic pots which comparatively having higher disease severity. On the other hand, isolates of *C. zeae-maydis* collected from four zones of south and southwestern Ethiopia showed high variation on all tested maize varieties in affecting their dry biomass yield. Among the isolates, Huru-I was the most aggressive with the highest severity and resulted overall lowest dry biomass yield of the tested maize varieties except on BH-660 as compared with all other tested maize varieties.

The current study pointed out that isolates collected from Illuababora zone (Huru-I) tended to cause more disease than the isolate collected from the other major maize belt areas of south and southwest Ethiopia. Similarly, the highest disease severity was surveyed in Illuababora zone under field conditions as reported in chapter two. Difference in altitude among the assessed sites might contribute for the variation in extent of disease severity. Furthermore, the variations among isolates could be due to variations in the resistance of the host plant and differences in the environment or their interaction. This investigation related with the finding of Nelson [27], who found that an isolates of fungal pathogen is said to be more suitable if it has inherent ability to produce larger lesion and higher disease reaction or efficiency on a specific host genotype relative to other isolate. Bair and Ayer [28] also found that the difference in fitness attributes variation exists among isolates of *C. zeae-maydis*.

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V. Conclusion and Recommendation

In this study, it was recognized that, the latent period affected lesion length, disease severity, and infection and disease development of *Cercospora zeae-maydis* in tested maize varieties, indicating that the latent period becoming longer (delaying in disease development) resulted in reduced disease severity as well as other disease variables of *Cercospora zeae-maydis. Cercospora zeae-maydis* isolate having long latent period are less virulent than thus having short latent period to develop on the host and cause grey leaf spot disease on maize varieties. In the study, isolate Huru-I was identified to be the most aggressive isolate and tended to cause more disease than the isolates collected from the other major maize growing areas of south and southwest Ethiopia. Moreover, the response of both improved and local varieties of maize showed different level of reaction to *Cercospora zeae-maydis.* The result presented in this study demonstrate that variability exists in populations of *Cercospora zeae-maydis* and suggest that varieties developed to reduce disease severity by limiting parameters such as disease severity, lesion length, disease infection rate and AUDPC should be screened against the tested isolates. Therefore, the information provided in this study on the variability of *Cercospora zeae-maydis*is relevant to the question of the stability of resistance to the pathogen. The findings showed the existence of an extensive virulence variation in the country. Hence, it can be concluded that, knowing the virulence variability could play an important role in the development of effective management strategy of this important disease of maize, and it also suggest maize breeding program and developing resistance maize varieties using the aggressive isolate, together with a mix of isolates, in order to test the disease interaction and select for a maize varieties.

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