COMPARATIVE RESISTANCE OF MAIZE CULTIVARS TO CHARCOAL ROT DISEASE

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ABSTRACT: Present study was aimed to assess the resistance in maize (*Zea mays* L.) cultivars against *Macrophomina phaseolina* which causes charcoal rot in variety of plants. Maize cultivars were sown in artificially inoculated potting soil and the pot experiment was laid in a completely randomized designed for 60 days. None of the maize cultivars displayed complete resistance, whereas most of the cultivars showed susceptibility to the pathogen. Only three cultivars (FH-1228, FH-1025 and FH-1225) were scored in a moderately resistant group. Among the remaining cultivars, two namely FH-1231 and FH-1042 were kept in moderately susceptible and seven (FH1217, FH-1224, FH-1046, FH-1226, FH-929, FH-1232, FH-963) were placed in susceptible group. The plant mortality (62-77%) and growth inhibition index (40%) were significantly greater in susceptible cultivars. Identified moderately resistant cultivars can be used as donors in maize breeding programs for the identification and isolation of resistance genes against charcoal rot disease.

Keywords: Charcoal rot, Disease severity, Growth inhibition index, Macrophomina phaesolins, Susceptible cultivars.

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INTRODUCTION

Maize (*Zea mays* L.) is the most famous and world oldest crop, its grains are enriched with starch (72%), protein (10%), oil (4.8%), sugar (3.0%) and fibers (5.8%), while devoid of cholesterol being planted and exploited in both grain, fodder and industrial raw forms (Ranum *et al*., 2014). Globally, it is an important crop ranked third behind rice and wheat in terms of production (Mboya, 2011). The USA, China and Brazil are the major maize producing countries (Ranum *et al*., 2014). In Pakistan, maize is the 4th largest grown crop and performs a significant role in the development of the national economy by contributing 6.4% of the total food grains yield of the country. Khyber Pakhtunkhwa is the leading province contributing 63% in total maize production followed by Punjab (30%), Sindh (5%) and Baluchistan (3%), respectively (Anonymous, 2013). Despite the availability of optimum soil and climatic conditions for maize cultivation in Pakistan, the grain yield is less than 1.59 times the global average grain yield (Naveed *et al*., 2014).

Amongst the most destructive diseases of maize, the stalk rot or charcoal rot disease incited by well-notorious, sclerotial forming fungus *Macrophomina phaseolina* (Tassi) Goid is responsible for huge loss in crop yield (Ashraf *et al*., 2015). The sclerotia of *M. phaseolina* are heat-resistant, display greater competitive saprophytic ability and can germinate within 48 hours in the vicinity of roots and can survive in soil up to 15 years (Sánchez *et al.*, 2017). Disease symptoms can be seen at any stage of the plant growth because the pathogen can infect plants at all growth stages at the optimum temperature (25–30 °C and prolonged dry weather (soil moisture < 60%) (Purkayastha *et al*., 2006). Infected plants generally displayed wilting symptoms, the disintegration of pith tissue, lodging of plants, premature yellowing of the top leaves and premature leaf drop. When stalk is cut open, microsclerotia are visible as black specks in the shredded vascular bundles (Ashraf *et al*., 2015).

By and large, *M. phaseolina* known as nearly hard pathogen to control using chemical fungicides due to persistent sclerotia and no reliable method is available to combat the pathogen (Khan *et al.,* 2018). Therefore, quest for resistant cultivars certainly appears as substitute for efficient and safer disease management. This essentially necessitates the screening of resistant genotypes of maize against charcoal rot disease. Since around the world few reports are available regarding the resistance of maize cultivar against charcoal rot disease (Gopala *et al*., 2016). Therefore, the present investigation was intended to screen twelve maize cultivars for their resistance against charcoal rot disease.

MATERIALS AND METHODS

Twelve cultivars of maize (FH-1224, FH-1228, FH-929, FH-1042, FH-1232, FH-1226, FH-1046, FH-1231, FH-1225, FH-963, FH-1217 and FH-1025) were procured from Ayub Agriculture Research Institute, Faisalabad, Pakistan.

Two kilograms of pre-fumigated soil (Shoaib *et al*., 2019) was filled in pots (23 cm diameter, 18 cm height) and inoculated with the cultural suspension of the pathogen (30 mL pot^{-1}). The cultural suspension of the *M.*

phaseolina (FCBP 751) was prepared by scratching fungal mass (mycelia + sclerotium) from 7-days old culture prepared at 28 °C on 2% malt extract agar medium. Sclerotial numbers in the suspensions were adjusted (2.0 x 10^5 sclerotia mL^{-1}). After 4^{th} day of pathogen inoculation in soil, the healthy and disinfected seeds of each 12 cultivars were seeded in pre-inoculated soil (3 seeds pot^{-1}). Potted soil for control treatments of each cultivar did not inoculate with the pathogen inoculum. The experiments consisted of 96 pots, 5 pots per treatment, while one plant per pot was maintained till end. The pots were organized in a completely randomized design under natural environmental conditions during April 2016 (temperature 28–30 °C).

Disease evaluation was carried out on 60-days maize plants using 1-4 index scale by splitting open individual plants (Mengistu *et al*., 2007). To get the final disease rating of a cultivar, the mean score of each replication was averaged and the cultivars were categorized (Table-1).

Table-1. Disease rating scale.

Scale	Disease rating	Split stem showing symptoms associated with the scores
1	Resistant	no microsclerotia visible in vascular tissue
>1 and ≤2	Moderately resistant	Very few microsclerotia visible and vascular tissue is not discolored
>2 and <3	Moderately susceptible	Microsclerotia partially covering the vascular tissue and there is minimal discoloration
		Numerous microsclerotia in the tissue and also visible under the outside epidermis, and discolored vascular tissue
3–5	Susceptible	Darkened vascular tissue due to high numbers of microsclerotia both inside and outside of the stem

The disease mortality (%) in maize cultivars was recorded on 60-days maize plants. Growth inhibition index (GII) was measured by taking data on plant growth attributes (length and weight) (Awan *et al*., 2018).

$$Growth\ inhibition\ index = \Sigma \frac{SL + SFW + SDW + RL + RFW + RDW}{Total\ number\ of\ perameters}$$

Where: SL =shoot length; SWF = shoot fresh weight; SDW = shoot dry weight; RL = root length; RFW= root fresh weight; RDW = root dry weight

The data of disease mortality (%) in maize cultivars was analyzed using LSD test. Attributes of the growth in maize cultivars were analyzed through a two-sample t-test. Associations among the investigated attributes were checked by Pearson correlation.

RESULTS AND DISCUSSION

Macrophomina phaseolina, the causal agent of charcoal rot disease has been reported to cause yield losses in maize production regions of Punjab, Pakistan (Ashraf *et al*., 2015) and available disease control methods are not completely effective to get rid of this pathogen (Jordaan *et al*., 2019). Besides, climate change has already created platform for pathogen to play with plants by decreasing rainfall and increasing daily maximum temperatures (Ziervogel *et al*., 2014). Under such circumstances, the *M. phaseolina* could wreak havoc on maize and may cause huge economic losses in maize production. Screening of maize varieties/cultivars for determination of disease response against *M. phaseolina* is essential to identify resistant sources (Ashraf *et al*., 2015) to be used in future for breeding programs. For present study, twelve cultivars of the maize were tested against their response to *M. phaseolina*. The cultivars of maize were classified as resistance, moderately resistance and susceptible using disease scoring scale of Mengistu *et al*. (2007) as presented in Table-2. The categorization of maize cultivars according to the rating scale was consistent with data of plant mortality and growth inhibition index (Fig.-1 and Table-2). None of the maize cultivars exhibited complete resistance to infection caused by *M. phaseolina*. Among twelve cultivars, three namely FH-1228, FH-1225 and FH-1025 exhibited disease rating scores within the range of 1.42–1.58 and plant mortality less than 10% thus kept in moderately resistant group (Table-2). Shoot and root growth attributes of the cultivars in the moderately resistant group were generally non-significantly affected or affected less significantly ($P \leq 0.05$) under the stress of pathogen in comparison to their corresponding un-inoculated plants (Table 3 and 4). Therefore, the growth inhibition index (GII) in this group was low as compared to the other two groups (Fig. 1). Two cultivars viz., FH-1231 and FH-1042 were kept in a moderately susceptible group displayed disease rating score 2.60 and plant mortality of 33% (Table 2). Attributes of the growth in these two cultivars were significantly affected ($P \leq 0.05$, 0.01 or 0.001), whereas, GII was found within a range of 20–23% (Fig. 1). Rests of the seven cultivars (FH-1217, FH-1224, FH-1046, FH-1226 FH-929, FS-0318, FH-1238 and FH-963) were placed in susceptible group on the basis of rating score 4.6–4.8. Susceptible cultivars suffered greatly, which resulted in highest plant mortality (60–80%), significant reduction ($P \leq 0.01$ and 0.001) in the investigated growth attributes along with the maximum GII (Table-3 and 4).

Results regarding correlation coefficients indicated that plant mortality was negatively and significantly ($P \leq 0.05$, 0.01 or 0.001) related with length, fresh dry weight of shoot and root. The correlation between different growth attributes as a reaction of maize cultivars to the pathogen infection was also significant ($P \leq 0.05$ or 0.01) and positively associated (Table-4).

In the present investigation, none of the cultivars displayed complete resistance to the reaction of *M. phaseolina*, which might be attributed to generalist nature of *M. phaseolina*, where genes for specific resistance are still not known in its hosts. So far, resistance is quantitatively controlled, therefore, complete resistance in host is not documented (Coser *et al*., 2017). The difference in the reaction of maize cultivars in response to attempted microbial infection is governed by the underlying resistance genotypic composition of the lines (Manu *et al*., 2017), which has been linked with activation of varying levels of multiple genes, proteins and metabolites (Radwan *et al*., 2013). Pathogen infection resulted in reduction in competitive ability of the cultivars. Therefore, the growth attributes were significantly reduced up to 20%, 30% and 50% in moderately resistant, moderately susceptible and susceptible cultivars, respectively in comparison to their corresponding control as determined through two-sample t-tests. Greater reduction in growth attributes of susceptible cultivars might be linked with the decline in plant fitness caused by an inability of the host protein receptor to identify the pathogen elicitor which may lead to less accumulation of defense-related genes to cope with stress (Khan *et al*., 2018). Therefore, it could be revealed that resistance genes or defense-related genes along their gene product in moderately resistant accessions (FH-1228, FH-1225 and FH-1025), may reduce the risk of infection and/or the replication rate of the pathogen in the host, thus suffered to a lesser extent.

Table-2: Analysis of disease severity and mortality in different maize cultivars screened against *Macrophomina phaseolina* in 60-day maize plant.

Disease rating	Score scale	Maize cultivars	Mortality (%)
Moderately resistance	1.42–1.58	FH-1228	15 **d**
		FH-1025	13 **d**
		FH-1225	17 **d**
Moderately susceptible	2.60	FH-1231	35 **c**
		FH-1042	30 **c**
Susceptible	4.6–4.8	FH1217	62 **b**
		FH-1224	64 **b**
		FH-1046	59 **b**
		FH-1226	67 **b**
		FH-929	80 **a**
		FH-1232	75 **a**
		FH-963	77 **a**

Different letters in a column show a significant difference ($P \leq 0.05$) in replicate mean values as determined by LSD test.

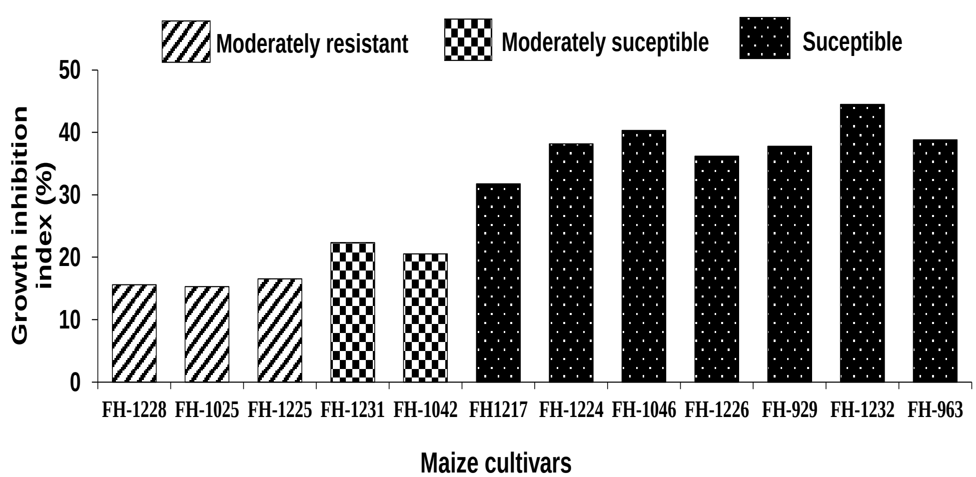

Figure-1: Growth inhibition index(%) of different maize cultivars against the effect of *Macrophomina phaseolina.*

Table-2: Effect of *Macrophomina phaseolina* on growth and biomass of shoot in different maize cultivars.

Maize cultivars	Groups	Shoot length		Shoot fresh weight		Shoot dry weight	
		UN	I	UN	I	UN	I
FH-1228		110±3.06	87±10.16	80±4.61	67±7.02	10.2±0.09	8.6±0.68*
			20.13%		**16.18%**		**15.76%**
FH-1025	**Moderately resistance**	116±1.81	100±9.90	73±4.15	61±3.71	5.3±0.06	4.5±0.30**
			13.49%		**18.17%**		**15.13%**
FH-1225		112±1.99	91±9.20	54±3.79	45±5.53	8.8±0.51	7.3±0.47
			18.85%		**17.38%**		**17.23%**
FH-1231		108±2.85	81±9.69*	79±1.27	63±3.49***	8.9±0.37	7.0±0.54**
			25.93%		**20.08%**		**21.30%**
FH-1042	**Moderately susceptible**	130±5.12	101±11.45*	81±0.69	69±4.54**	10.1±0.34	7.5±0.44***
			23.08%		**15.70%**		**26.04%**
FH1217		111±2.77	83±5.25***	76±2.93	54±4.85***	6.7±0.41	4.3±0.28***
			25.27%		**28.74%**		**36.84%**
FH-1224		114±1.85	81±6.40***	80±4.16	38±3.61***	7.3±0.21	4.3±0.18***
			29.11%		**51.68%**		**41.02%**
FH-1046		114±1.43	81±5.37***	74±2.17	50±4.74***	10.6±0.48	6.2±0.40***
			28.78%		**32.93%**		**41.56%**
FH-1226	**Susceptible**	110±2.63	84±3.50***	55±1.11	34±4.27***	5.8±0.49	3.8±0.50**
			23.36%		**38.25%**		**34.31%**
FH-929		117±1.18	86±2.88***	73±1.63	34±2.26***	7.5±0.59	3.6±0.28***
			26.11%		**53.95%**		**51.96%**
FH-1232		118±1.33	84±4.21***	75±1.48	37±1.72***	8.6±0.34	4.3±0.37***
			28.85%		**51.15%**		**49.84%**
FH-963		105±2.21	75±6.65***	55±1.48	30±1.42***	7.3±0.68	4.8±0.37**
			28.34%		**46.12%**		**34.11%**

ns, *, **, *** non-significant or significant at $P \leq 0.05$, 0.01 and 0.001 using independent two-sample t-test for comparison of inoculated vs. non-inoculated control plants within each genotype. ± show standard errors of means of five replicates. Values in bold letters show percentage inhibition in growth parameters over corresponding control.

Table-3: Effect of *Macrophomina phaseolina* on growth and biomass of root in different maize cultivars.

Maize cultivars	Groups	Root length		Root fresh weight		Root dry weight	
		UN	I	UN	I	UN	I
FH-1228		89±5.22	75±4.31	7.5±0.62	6.6±0.77	2.8±0.15	2.4±0.10
			15.92%		**12.27%**		**13.23%**
FH-1025	**Moderately resistance**	110±4.51	92±4.18*	10.3±0.43	9.4±0.46	1.7±0.49	1.4±0.35
			16.36%		**9.02%**		**19.46%**
FH-1225		109±3.82	90±7.31	8.9±0.57	7.7±0.44	2.5±0.37	2.1±0.33
			11.47%		**13.82%**		**20.34%**
FH-1231		125±2.77	92±7.24**	11.4±0.20	9.5±0.72**	1.8±0.02	1.4±0.13**
			26.20%		**16.55%**		**24.06%**
FH-1042	**Moderately susceptible**	117±2.06	100±3.22**	11.8±0.16	10.1±0.23**	2.9±0.11	2.3±0.12***
			22.90%		**14.18%**		**21.43%**
FH1217		150±1.92	95±5.25***	11.4±0.37	8.0±0.57***	3.3±0.42	2.2±0.19*
			36.47%		**29.80%**		**33.43%**
FH-1224		113±3.15	76±4.68***	9.3±0.19	6.4±0.20***	1.5±0.12	0.9±0.03***
			32.50%		**31.81%**		**42.91%**
FH-1046	**Susceptible**	100±1.18	75±5.38***	9.1±0.33	3.9±0.08***	2.3±0.14	1.0±0.18***
			26.25%		**57.35%**		**55.00%**
FH-1226		113±2.60	79±2.72***	7.7±0.28	3.8±0.21***	1.5±0.19	0.9±0.12**
			30.26%		**50.87%**		**40.17%**
FH-929		103±1.84	77±6.21***	10±0.24	6.8±0.25***	2.0±0.22	1.2±0.04**
			25.30%		**31.06%**		**38.13%**

FH-1232	127±1.48	86±1.47***	11.2±0.3	4.6±0.22***	2.0±0.09	1.1±0.16***
		32.16%		**59.16%**		**45.58%**
FH-963	118±1.18	74±4.60***	9.9±0.31	5.4±0.37***	2.0±0.30	1.2±0.24*
		37.11%		**46.02%**		**41.04%**

ns, *, **, *** non-significant or significant at P ≤ 0.05, 0.01 and 0.001 using independent two-sample T-test for comparison of inoculated vs. non-inoculated control plants within each genotype. ± show standard errors of means of five replicates. Values in bold letters show percentage inhibition in growth parameters over corresponding control.

Table-4: Correlation matrix (Pearson's two-tailed) of phenotypic traits in different cultivars of maize under *Macrophomina phaseolina* stress.

	SFW	SDW	RL	RFW	RDW	MOR
SL	0.58*	0.57*	0.55*	0.63*	0.52*	-0.62**
SFW		0.73**	0.61*	0.69**	0.63*	-0.79***
SDW			0.23^{ns}	0.47^{ns}	0.47^{ns}	-0.71**
RL				0.68**	0.67**	-0.60*
RFW					0.65**	-0.60*
RDW						-0.61*

SL: shoot length, SFW: shoot fresh weight; SDW: shoot dry weight; RL: root length; RFW: root fresh weight; RDW: root dry weight and MOR: Plant mortality.
ns, *, **, *** non-significant or significant at P ≤ 0.05, 0.01 and 0.001, respectively.

Conclusion: Three cultivars namely FH-1228, FH-1225 and FH-1025 were classified as moderately resistant against charcoal rot disease that could be used for future field trial screening in order to pyramidize genes of resistance against *M. phaseolina* in the breeding of new maize cultivars.

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