



Full Length Article

Adaptability Trials of Sesame Germplasm Against *Macrophomina phaseolina* (Tassi) Goid by using AMMI Biplot Analysis in Pakistan

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Abstract

The current research was conducted to exploit the adaptability/resistance status of different sesame varieties/advanced lines of sesame germplasm against charcoal rot under different environmental conditions through AMMI Biplot analysis. Ten varieties/ advanced lines of sesame viz. TS-5, 10003, 40009, 50009, 50011, 50022, 16001, 40004, Black Till and TH-6 were collected from Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. The seeds of sesame varieties/ advanced lines were sown at six different locations namely Faisalabad, Toba Tek Singh, Piplan, Bhakhar, Mandi Baha Uddin and Bahawalpur with three replications under Randomized Complete Block Design. Statistical analysis was performed through R-statistical software, version 3.5.1. Combined analysis of variance was used to assess the level of significance whereas Genotype × Environment Interaction Table was used to separate means of data regarding disease incidence. Further, Additive Main Effects and Multiplicative Interaction (AMMI) Biplot was performed for stability analysis. Five sesame genotypes namely 40004, 40009, 50011, 50022, Black Till and TS-5, respectively, expressed resistant response at various locations with varying disease incidences whereas 50009 exhibited moderately susceptible response at Bhakhar, M.B. Din, Piplan and T.T. Singh with (36.76, 39.71, 40.95 and 46.98) percent disease incidences respectively. Likewise, two sesame advanced lines 10003 and 16001 expressed susceptible response at all locations with disease incidences of 64.547, 67.050, 66.75, 72.54, 77.38, 71.56 percent and 74.42, 72.03, 77.20, 77.27, 65.55, 69.06 percent correspondingly. It was concluded that resistant varieties are the most economical management strategy for farmers against charcoal rot of sesame. © 2020 Friends Science Publishers

Keywords: *Sesamum indicum*; Charcoal rot; Adaptability/resistance status; AMMI Biplot analysis

Introduction

Sesame (*Sesamum indicum* L.) is one of the most imperative oilseed crops in Pakistan that belongs to family Pedaliaceae (Ahmad *et al.*, 2018). Sesamum seeds are considered as the much primitive oilseed crop dating back to 1600 BC. Now, it is widely grown in the tropical areas of Asia and Africa (Saydut *et al.*, 2008). It has high nutrition value as it contains sesamin, sesamol, sesaminol and sesamolol that maintains the low-density lipoprotein fats (Banerjee and Kole, 2009). The consumption of 100 g sesame seed provides carbohydrates (24.05 g), proteins (18.08 g), fats (50.87 g), total dietary fibres (5.5 g), calcium (960 mg), iron (19.2 mg), magnesium (362 mg), phosphorus (659 mg), sodium (12 mg), potassium (582 mg), thiamine (0.240 mg), riboflavin (0.200 mg), pantothenic acid (0.052 mg), vitamin B6 (0.816 mg), vitamin A (3 µg), saturated fatty acid (1.252 g), monosaturated fatty acids (3.377 g) and polyunsaturated fatty acids (3.919 g) (Nagendra *et al.*, 2012). In the world, it is cultivated on area of 1.05 million ha while Pakistan contributes only 8.01 thousand

ha in total cultivated area. Similarly, in the world, the average yield and production is 5778 kg/ha and 611.15 thousand tonnes, respectively, whereas in Pakistan, the sesame yield is 4103 kg/ha as well as production is 3.29 thousand tonnes (FAOSTAT, 2016-17).

There are numerous biotic constraints in successful cultivation and harvesting of sesame. Among those production inhibiting biotic restrains, charcoal rot of sesame caused by *Macrophomina phaseolina* (Tassi) Goid is the most destructive disease (Ibrahim and Abdel-Azeem, 2015). The losses in yield under field conditions owing to *M. phaseolina* are ranging from 22-53% with 37 percent disease incidence (Gupta *et al.*, 2018) whereas yield losses of 5-100 percent in Egypt has also been observed under favourable environmental conditions (EL-Bramawy and Wahid, 2006). The pathogen has anxiously diminished the yield of sesame approximately 27 million bushels per annum in USA (Chattopadhyay and Sastry, 2002) with the predicted value of \$US 146 million (Bashir *et al.*, 2017).

Macrophomina phaseolina is a necrotrophic thermophilic soil-borne plant pathogen having two asexual subphases i.e. saprophytic phases and pathogenic phase. It produces microsclerotia and mycelia in the saprophytic phase whereas microsclerotia, mycelia and pycnidia are produced during pathogenic phase (Kaur *et al.*, 2012). The genus *Macrophomina* is a monotypic genus because it comprises only one species namely “phaseolina” (Sutton, 1980). The characteristic symptoms owing to *M. phaseolina* on sesame are spindle shaped lesions with brown margins and light grey centres having dispersed pycnidial bodies. Infected plants depict wilting, drying, shredding of leaves ultimately the decaying of host plant after severe infection (Beas *et al.*, 2006). The visual appearance of *M. phaseolina* in petri-plates varies from black to brown or grey but it becomes dark with the passage of time. Numerous aerial mycelia are produced with sclerotia imbedded within the hyphae. The hyphae are septate and black in appearance. Microsclerotia are also in black colour with different sizes, ranging from 50-150 µm whereas pycnidia are dark to greyish in colour with globose or flattened structure having diameter of 100 to 200 µm (Yang and Navi, 2005; Kaur *et al.*, 2012).

Environmental factors imperatively influence the genotypic characters of host plant by promoting virulence factors of pathogens and subsequently reduce the maximum cultivation of sesame in a certain geographic location. Genotype by environment interaction (GGE) biplot analysis is an eminent method to determine yield stability and suitable variety selection consider genotypes as main effect under multi-geographic locations (Shim *et al.*, 2015). Similarly, additive main effects and multiplicative interaction (AMMI) biplot analysis is an effective tool which is used to separate multiplicative components and additive main effects through principal component analysis. The comparison of multilocation trials with genotypes as well as main effects and multiplicative interaction is also helpful to enhance the yield by understanding all yield limiting factors (Yan and Hunt, 1998). The GGE comprises numerous interaction methods of biplot analysis by which genotype by environment (G×E) interactions can visually be addressed (Yan and Kang, 2003; Yan and Tinker, 2006). The GGE and AMMI biplots analysis are extensively used for diversity, stability, adoptability trials, for the source of resistance and better yield performing cultivars in a set of environmental interactions (Pourdad and Moghaddam, 2013).

Various conventional (application of plant extracts, use of antagonistic organisms, plant growth regulators, management through fertilizers, disease management through organic manures, soil solarization, application of fungicides etc) and molecular approaches (systemic induce resistance, systemic acquired resistance, DNA markers, proteomics, transcriptomics, comparative genomics, computational biology etc.) are available for the management of sesame charcoal rot disease. Among all methods, few are not practicable and attainable for poor farmers due to higher cost benefit ratio, less efficiency under

diverse climatic conditions whereas others impose direct or indirect impact on human health such as indiscriminate use of fungicides for diminishing disease incidence. Thus, it is dire need to explore such disease inhibiting or management approaches which are economically adoptable, environmentally friendly and easily available for farmers (Ahmed *et al.*, 2013). Therefore, the ideal and most apposite way to combat the disease and hinder the severe yield losses is the use of climate resilient and genetically resistant sesame advanced genotypes. The source of resistance through screening of existing germplasm against *M. phaseolina* under natural field conditions is pre-requisite to accomplish the purpose (Meena *et al.*, 2018). Thus, indispensable efforts were carried out to assess the genotypes of sesame under natural field conditions. The research was conducted to evaluate the existing sesame germplasm/ advanced lines under natural field conditions against charcoal rot disease caused by *Macrophomina phaseolina* (Tassi) Goid for the source of resistance. Genotypes possessing resistant genes against virulent pathogen will be submitted for varietal approval based on data of continuous multilocal research trials to find the resistant source.

Materials and Methods

Identification, Isolation and Purification of *Macrophomina phaseolina*

Sesame plants with distinctive symptoms of Charcoal rot disease were collected from experimental research field and brought in the Oilseeds Plant Pathology Lab. for isolation of virulent pathogen. Infected roots were washed carefully with running tap water, cut into pieces of 3-5 mm and surface sterilized with 1% sodium hypochlorite (NaOCl) solution. The roots were air dried for 2-3 min by keeping on sterilized filter paper in 9 cm petri plate. The infected roots (3-4 pieces) were placed in petri plate containing 20 mL Potato Dextrose Agar (PDA) medium. Petri plates were incubated at 25°C ± 2 for 48-72 h (Sarwar *et al.*, 2005). Maximum fungal growth was observed in plates, a temporary slide of pathogen was prepared for identification. After identification and confirmation through literature as well as comparison with the isolated *Macrophomina phaseolina* under stereomicroscope, the purified fungal colonies were multiplied for further use in the research (Soesanto *et al.*, 2011).

Preparation of Sick Field

Highly susceptible single stem variety of Sesame i.e. TH-6 was sown in experimental research area of Plant Pathology at Oilseeds Research Institute, Faisalabad, Pakistan. The suspension of *M. phaseolina* pure culture possessing 1×10⁶ sclerotia and pycnidia/ mL of H₂O was prepared, and concentration was confirmed through haemocytometer. TH-6 was inoculated through drenching with two successive

irrigation of 7 days interval by placing the bottle having inoculum near inlet of water. Similarly, 4 mL of sterilized water was poured on 5-8 days old pure culture of *M. phaseolina* in petri plates, shake them gently and 4 plates were transferred in 250 mL water in beaker. 5 mL pure culture from beaker was also drenched near root zone of each plant before irrigation to enhance the inoculum density. Disease symptoms appeared on 30-45 days old plants, the diseased plants were homogeneously mixed in the soil and the field was irrigated to increase decomposition of plant debris as well as to enhance pathogen infection density on plants during research.

Establishment of Disease Screening Nursery

Ten varieties/ advanced lines of sesame namely TS-5, 10003, 40009, 50009, 50011, 50022, 16001, 40004, Black Till and TH-6 were collected from Oilseeds Research Institute (ORI), Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. The experiment was sown at six different locations i.e. Faisalabad, Toba Tek Singh, Piplan, Bhakkar, Mandi Baha Uddin and Bahawalpur. Each row of (3.5 m) 350 cm was prepared and seed was sown through hand drill in three replications at Experimental Research Area of Plant Pathology, ORI, AARI, Faisalabad. After 7-10 days, seedlings were observed, and the experiment was visited daily to visualize the germination rate in the field. After 15 days of seedling emergence, 30-40 healthy sesame plants were sustained in each row by maintaining the P×P and R×R distance of 8-10 cm and 45 cm under Randomized Complete Block Design (RCBD). All weak and unwanted plants were pulled off to diminish the nutritional competition. All the horticultural practices including hoeing, earthing up, weed eradication and irrigation were followed to keep the plants in good health. Data of disease incidence was recorded after 10-15 days interval from all experimental location at different regions.

Statistical Analysis

Experiment was conducted under randomized complete block design (RCBD). Statistical analysis was performed using R. Combined analysis of variance was used to assess the level of significance whereas Genotype × Environment Interaction table was used to separate means of data regarding disease incidence. Further, Additive Main Effects and Multiplicative Interaction (AMMI) Biplot was performed for stability analysis.

Results

Genotypes Adaptability/resistance Status through Additive Main Effects and Multiplicative Interaction (AMMI) Analysis

Separate Analysis of Variance for each environment

revealed that the genotypes performed statistically significant response to control the Disease Incidence (%) of Sesame charcoal rot. The performance of ten genotypes was different at various locations (Table 1). Combined analysis of variance not only showed that the performance of the genotypes was not the same ($F = 440.053$, $p < 0.001$) but also revealed that their performance against charcoal rot of sesame varies across the environments ($F = 4.248$, $p < 0.001$) (Table 2). The significant interaction between genotypes and environments implied that the performance of genotypes was not stable across environments and all the genotypes performed differently across environments. The same phenomena were depicted by genotypes by environment means and ranks of genotypes means within environments (Table 3 and 4). The genotypes 16001, 10003 and 50009 depicted the highest disease incidence on sesame at six different locations as compared to remaining genotypes and showed susceptibility (Disease rating 5). The genotype 16001 expressed the highest disease incidence in four (Bahawalpur, Bhakkar, Faisalabad and M.B. Din) out of the six locations and the second highest disease incidence in the other two locations (Piplan and T.T. Singh) (rating 5). Similarly, the genotype 10003 showed the highest disease incidence in two (Piplan and T.T. Singh) out of the six locations and the second highest disease incidence in three locations (Bhakkar, Faisalabad and M.B. Din) and the third highest disease incidence in Bahawalpur (rating 5). Genotype 50009 was the third most sensitive among ten genotypes. It remained third most diseased in four (Bhakkar, Faisalabad, Piplan and T.T. Singh) out of six localities by exhibiting moderately susceptible response (rating 4) while at Bahawalpur it was found second most diseased genotype after showing susceptible response (rating 5). The genotypes 40004, TS-5 and 50011 were found to be the resistant genotypes. The genotype 40004 expressed resistance response at two locations (Piplan, Bhakkar) out of six localities and ninth most resistant genotype at Faisalabad and T.T. Singh (rating 2) as well as it was found seventh and sixth resistant genotype at M. B. Din and Bahawalpur respectively (rating 3). Genotype TS-5 was found to be resistant for Bahawalpur, Faisalabad and Piplan environments while ninth most resistant in two locations (Bhakkar, M.B. Din) and the most appropriate for T.T. Singh owing to its highest resistance against charcoal rot disease of sesame (rating 2). Likewise, genotype 50011 remained highly resistant at Faisalabad and Bahawalpur (rating 2) whereas the eighth at two localities (Bhakkar, T.T. Singh) and the sixth moderately resistant response at M.B. Din and Piplan (rating 3). The genotype 50022 expressed moderately resistant response at two locations (Bahawalpur, Bhakkar) (rating 3) while showed resistant response at Faisalabad and T.T. Singh, (rating 3). It remained ninth resistant genotype at Piplan and tenth most resistant in the environment of M. B. Din (Disease rating 2). Likewise, the genotype 40009 expressed moderately resistant response at three (Faisalabad, Piplan, T.T. Singh) out of six locations while

Table 1: Individual Analysis of Variance for each Environment

SOV	df	Sum of Squares (SS)					
		Bahawalpur	Bhakkar	Faisalabad	M.B. Din	Piplan	T.T. Singh
Genotypes	9	21017***	14125***	19935***	17939***	17940***	17784***
Residuals	20	670	263	482	326	293	920

Table 2: Combined Analysis of Variance for the assessment of resistant source

SOV	Df	SS	MS	F	P
Environments	5	336.480	67.296	6.508	0.004
Replication (Env.)	12	124.080	10.340		
Genotypes	9	103734.959	11526.107	440.053	<0.001
Gen: Env	45	5006.836	111.263	4.248	<0.001
PC1	13	3246.591	249.738	9.535	<0.001
PC2	11	946.380	86.035	3.285	<0.001
Pooled Deviation	31	813.865	26.254	1.002	0.475
Residuals	108	2828.792	26.193		

Table 3: Genotypes by Environment Interaction Means

Genotypes	Locations					
	Bahawalpur	Bhakkar	Faisalabad	M.B. Din	Piplan	T.T. Singh
10003	64.547	67.050	66.750	72.547	77.383	71.560
16001	74.423	72.037	77.200	77.277	65.550	69.063
40004	16.920	7.777	6.983	15.883	6.487	7.133
40009	7.270	17.470	16.800	13.893	16.717	19.653
50009	71.407	34.760	53.247	39.713	40.953	46.983
50011	7.293	15.570	6.533	16.530	16.380	9.033
50022	16.217	16.660	8.057	7.410	7.710	9.730
Black Till	17.093	27.627	16.700	19.397	9.113	14.357
TH-6	43.550	33.103	40.977	42.747	36.500	41.290
TS-5	8.653	7.780	7.800	9.123	7.997	6.853

Table 4: Ranks of *Brassica napus* Genotypes within different Environments

Ranks	Locations					
	Bahawalpur	Bhakkar	Faisalabad	M.B. Din	Piplan	T.T. Singh
1	16001	16001	16001	16001	10003	10003
2	50009	10003	10003	10003	16001	16001
3	10003	50009	50009	TH-6	50009	50009
4	TH-6	TH-6	TH-6	50009	TH-6	TH-6
5	Black Till	Black Till	40009	Black Till	40009	40009
6	40004	40009	Black Till	50011	50011	Black Till
7	50022	50022	50022	40004	Black Till	50022
8	TS-5	50011	TS-5	40009	TS-5	50011
9	50011	TS-5	40004	TS-5	50022	40004
10	40009	40004	50011	50022	40004	TS-5

sixth at Bhakkar and eighth at M.B. Din localities (Disease rating 3). The same genotype depicted resistant response at Bahawalpur location under disease rating 2. The genotype TH-6 showed moderately susceptible response at five locations viz. Bahawalpur, Bhakkar, Faisalabad, Piplan and T. T. Singh whereas it also expressed moderately susceptible response at third of M.B. Din (Disease rating 4). Black Till genotype depicted resistant response at Piplan (rating 2) and remained at sixth after showing moderately resistant response (rating 3) in two locations (Faisalabad, T.T. Singh). It showed moderately susceptible response at fifth in Bhakkar (rating 4) as compared to Bahawalpur and M.B. Din localities where it remained at fifth but exhibited moderately resistant response (rating 3) (Table 5 and Fig. 1). The Additive Main Effects and Multiplicative Interaction

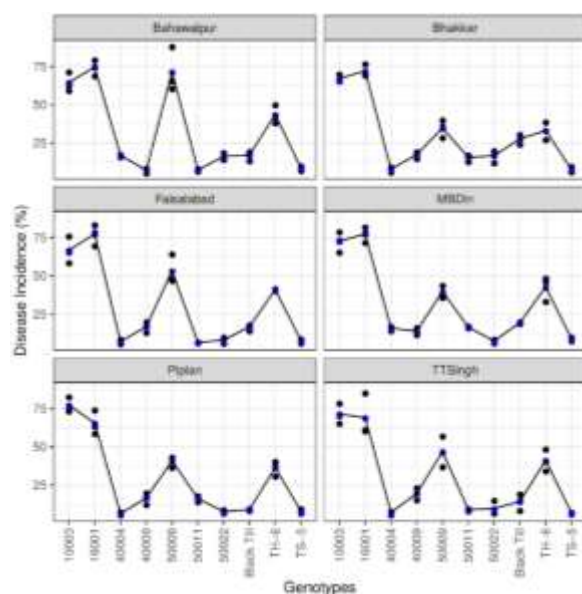
(AAMI) analysis indicated almost 84% variability or adaptability in all genotypes at six diverse environments. The main effects i.e. genotypes, and the environmental interaction expressed the suitability/ adaptability of genotypes in different environments (Fig. 2).

Discussion

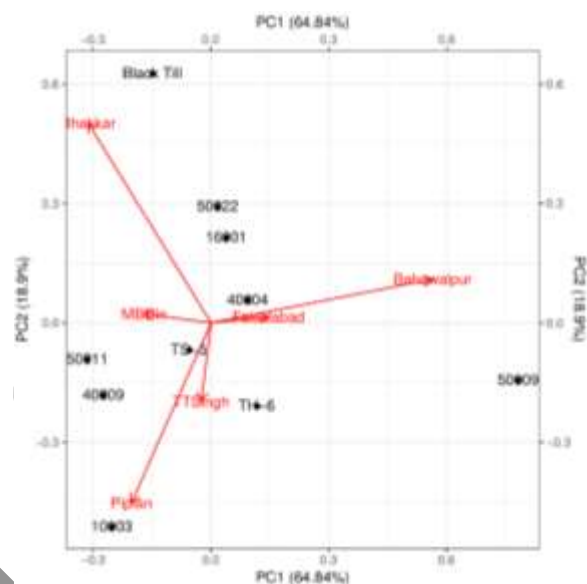
Charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid is a potential threat in sesame growing areas of Pakistan, causing huge losses with disease incidence ranging from 30-37% in the field under conducive environmental conditions (EL-Bramawy and Wahid, 2006; Bashir et al., 2017). Shredding of leaves, wilting, drying and decaying are the characteristic symptoms this disease

Table 5: Modified disease rating scale for charcoal rot of sesame

Disease Rating	Description	Response	Symbol
0	No symptoms on plants	Immune	I
1	1 % or less plants infected	Highly Resistant	HR
2	0.01-10 % plants infected	Resistant	R
3	10.01-20 % plants infected	Moderately resistant	MR
4	20.01-50 % plants infected	Moderately Susceptible	MS
5	> 50.01 % plants infected	Susceptible	S


Fig. 1: Charcoal rot disease incidence on sesame advance genotypes at six different locations

(Beas *et al.*, 2006). High temperature, moisture, soil physical and chemical characteristics, virulent pathogen races, inoculum density and time of infection influence the development of symptoms (Akhtar *et al.*, 2011). Moreover, resistant genotypes not only reduce the disease incidence but also tolerate fungicide toxicity (Banerjee and Kole, 2009). The use of molecular techniques for identification, selection and insertion of resistant genes is costly and non-durable due to higher genomic evolutionary processes but screening for the source of resistance through conventional breeding under diverse environmental conditions is an easily accessible and durable approach to farmers (Meena *et al.*, 2018). That is why, ten sesame varieties/advanced lines were assessed against Charcoal rot disease in six different environmental regions and it was observed that resistance is prevailing in some genotypes (40004, 50011, 50022 and TS-5) but, the resistance status of genotypes may be decreased if specific genotypes are continuously used in a geographically specific area as well as due to more virulent aggressive strains of pathogens. Durable, field or polygenic resistance diminishes the severity of disease owing to the presence of many genes in host plant as compared to non-durable or monogenic resistance. However, the multilocal conventional hybridization breeding


Fig. 2: Stability analysis for additive main effects and multiplicative interaction

program for the source of resistance in host plants is also an imperative possible solution to reduce the disease (Meena *et al.*, 2018). Resistant source should be incorporated directly or indirectly through conventional breeding to restore the genotypes at desirable level. During selection of resistant source, two segregating generations (F_3 and F_4) were exposed to sesame charcoal rot pathogen (*Macrophomina phaseolina*) under natural field conditions during two successive seasons (2004 and 2005). The highly significant variability regarding disease incidence was observed in five crosses i.e. $P_1 \times P_2$, $P_1 \times P_4$, $P_1 \times P_5$, $P_2 \times P_6$ and $P_3 \times P_4$. Such crosses in conventional breeding might be helpful for the selection of stable source of resistance and will aid breeders to achieve better sesame cultivars with charcoal rot resistance (El-Bramawy and Wahid, 2006). Similarly, thirty-six (36) F_1 genotypes were evaluated under artificial screening as well as sick plot conditions against root rot of sesame and three genotypes namely ORM-7, ORM-14 and ORM-17 were found resistance against disease (Thiyagu *et al.*, 2007).

Statistical models like AMMI and GGE (Gauch *et al.*, 2008) are helpful for stability analysis of genotypes against aggressive virulent pathogens (Belay *et al.*, 2018). The GGE and AMMI Bi Plot analysis also play a

pivotal role for selection of resistant source in multilocation trails (Yaseen et al., 2014) as well as to check the variability and stability in sesame genotypes against charcoal rot disease (Kaur et al., 2012).

Conclusion

It is concluded that the genotypes 40004, TS-5, 50011 and 50022 exhibited resistant response against Charcoal rot of sesame caused by *Macrophomina phaseolina* at different localities. The genotype 40004 is much suitable for Bhakkar, Faisalabad, Piplan and T.T. Singh whereas genotype TS-5 is better for Bhakkar, Faisalabad, MB Din and T.T. Singh. Similarly, the genotype 50011 exhibited resistant response against disease at three location (Bahawalpur, Faisalabad and T.T. Singh) while genotype 50022 is also good for three localities including MB Din, Piplan and T.T. Singh.

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