



Host status of sunflower (*Helianthus annuus* L.) against Charcoal rot caused by *Macrophomina phaseolina* in arid region of Bahawalpur, Pakistan

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Abstract

The present study was aimed to identify resistance in elite sunflower genotypes against charcoal rot disease for variety improvement program. Sixteen sunflower genotypes were selected to check the disease severity of charcoal rot. It was revealed that all the genotypes were susceptible to charcoal rot disease. The genotype 14068 was highly susceptible against the disease with high reduction in yield; however, genotypes 14013, 14052, 14082 and 14095 were resistant against the disease based on rating scale and resulted in high yield in arid region of Bahawalpur. Resistant genotypes need to be selected in breeding program to release new sunflower lines highly resistant against charcoal rot to feed ever increasing population of the world.

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Introduction

Sunflower (*Helianthus annuus* L.) is the fourth major crop in the world which belongs to the family "Asteraceae" (Rodriguez *et al.*, 2002). Sunflower production decreased due to the presence of numerous diseases but charcoal rot was reported a huge threat for its yield production (Ijaz *et al.*, 2013; Iqbal and Mukhtar, 2014; Iqbal *et al.*, 2014). It is a fungal disease, which leads to the death of plant. *Macrophomina phaseolina* was 1st time reported from Sri Lanka in 1927 and first time in Pakistan Charcoal rot was stated in Faisalabad in 1982 and far ahead it spread to across the regions of Khyber Pakhtunkhwa, Sindh, and Punjab. Nearly 60% yield loss in sunflower production has been reported due to charcoal rot (Steven *et al.*, 1987; Khan, 2007). Symptoms showed that *M. phaseolina* are root preventing and tubers producing fungi which is also narrated as the seed, soil and stubble borne disease (Smith, 1969; Mirza, 1984; Kaiser and Das, 1988; Liddell, 1999; Gupta *et al.*, 2012). Lacerations of silver gray color produce proximate to the base which leads to the decline of root, stem and ripening.

Affected plants show less concentration of oil, reduced seed size and poor seed filling (Bajwa *et al.*, 2007). This fungus was also reported in some grass species, legumes and many other species (Ali and Dennis, 1992; Su *et al.*, 2001).

Round about 500 species in world and 67 in Pakistan were reported as the host of *M. phaseolina*. In circumstances of waterless soils it can also live on for 10 months. Population of active sclerotia is directly related to the cruelty of the disease (Khan, 2007). In mostly fungus diseases soil is the main source of inoculum but in case of *M. phaseolina* it is not compulsory (Norton, 1953; Anis *et al.*, 2010). The disease decreases mean seed weight seedling vigor, germination index and oil contents, while iodine and protein contents increases (Ijaz *et al.*, 2013). Disease harshness and inoculum level in seed bed showed the positive association for *M. phaseolina* (Khan, 2007).

Infection of sunflower seed by *M. Phaseolina* has been found to reduce the seed size and germination rate, instead of generating charcoal rot and seedling blight disease (Fakhir *et al.*, 1976).

Its chemical control is difficult and inefficient due to variation in its behavior but some extracted composites of plants can be used to resist this disease. These derived compounds could be used in future as the pathway to produce the pesticides for *M. phaseolina* (Duke *et al.*, 2000). Still resistant cultivars are the only source to combat the pathogen but no resistant cultivar in contradiction to this fungus is available (Ahmad and Burney, 1990; Khan, 2007). Resistance against seed-borne fungi is necessary which remove its harmful effects through integrated approaches (Vaidehi *et al.*, 2002). The recent work reports on the susceptibility/resistance of sixteen sunflower varieties to charcoal rot caused by *M. phaseolina* aiming to find out the resistant genotypes to be selected in breeding program to release new sunflower lines highly resistant against charcoal rot to feed ever increasing population of the world.

Materials and methods

Experiment consisted of sixteen sunflower genotypes detailed in Table 1, developed and maintained by the National Agriculture Research Center (NARC), Islamabad. The trial was conducted in Plant Pathology section research area of Regional Agriculture Research Institute (RARI) Bahawalpur during May to August 2015. The experiment was conducted to screen out the sunflower varieties/lines against charcoal rot of sunflower caused by *M. phaseolina* under natural conditions in sick field and to find out the yield loss data of each entry in response to the disease.

Collection of diseased samples (Stem)

For the identification of *M. phaseolina*, stalk samples of sunflower showing charcoal rot disease symptoms were collected from experimental trial of oil seed section of RARI and farmers' fields. These diseased samples were labeled properly,

cut into small pieces and packed in sterilized polythene bags and were brought to the Plant pathology laboratory of RARI, Bahawalpur for isolation and identification of the Pathogen.

The pathogen was isolated by agar plate method isolation technique used by (Ricker and Ricker, 1936). In this technique diseased portions of sunflower stem were cut into small pieces i.e. 1 to 4 mm in size. These pieces were disinfested with 1 % Clorox solution for 1 minute and washed by dipping three times in distilled water. The pieces were then placed on sterilized blotter paper to dry.

The Potato Dextrose Agar medium containing (Dextrose 20 g, Agar 15 g, Potato starch 4 g and 1000 ml water), a standard medium for isolation of fungi was prepared, autoclaved at a temperature of 121 °C with 15psi pressure for 20 minutes for sterilization. After cooling of medium about 50 °C, 50ml of sterilized medium was poured in petridishes (9cm) under aseptic conditions in laminar flow chamber.

The blotter dry pieces of samples were placed on PDA plates at equal distance by using sterilized forceps. Five disease sample small pieces were placed in each plate for proper growth on PDA. The plates were then kept in an incubator with set temperature of 32±1°C which favors the growth of the fungus *M. phaseolina*. White cottony colonial growth of *M. phaseolina* appeared on the medium plates after 3-4 days.

Identification of pathogen

At the appearance of cottony growth on PDA the mycelial growth of pathogen was taken out with the help of sterilized needle, placed on the surface of glass slide having a drop of methylene blue and then covered with the cover slip. This slide was placed under compound microscope and *M. phaseolina* pathogen was identified.

Maintenance of pure culture

Once the fungus was identified as pure culture, it was maintained by transferring fungal mycelium into Potato Dextrose Agar slants, prepared in test tubes. These agar slants were incubated at 27±1°C for eight days for further multiplication of the fungus.

Disease incidence

On identification of fungus (*M. phaseolina*) disease incidence data of each entry was recorded by using the following formula.

$$\text{Disease incidence} = \frac{\text{Disease infected plants}}{\text{Total no. of plants}} \times 100$$

The entries were evaluated on the basis of percent plant infection and arranged according to the following six point 0-5 (0= no disease symptoms on the external stem to 5= premature death of plant) disease severity rating scale for charcoal rot of sunflower caused by *M. phaseolina* (James, 1971) (Table 2).

Statistical analysis

All sixteen sunflower genotypes having selected treatments along with three replications in the field were planted to ensure homogeneous condition within a block. Row to row and plant to plant distance was maintained as 75cm and 30 cm, respectively. Plant morphological, yield and disease intensity data was analyzed under Randomized Complete Block Design (RCBD) and mean values were compared by measuring standard error of means.

Results and discussion

As the experiment was conducted on infested soils with *M. phaseolina* to find out the resistant sunflower genotypes (*H. annuus* L.). Symptoms of charcoal rot were observed on sunflower plant (Gulya *et al.*, 2002). Symptoms, first observed on plant completing its physiological maturity, consisted of silvery grey lesion girdling the stem at soil line, premature plant death, and reduced head diameter, the pith in the lower stem was either completely absent or was compressed into horizontal layers. Black, spherical micro-sclerotia were observed in the pith of lower stem, just underneath the epidermis and on the exterior of tap root.

The fungus associated with infected sunflower genotypes stem was isolated and identified as *M. phaseolina* with the help of available literature (Cloud and Rupe, 1991) and

already isolated and identified pure culture. Evaluation of sunflower germplasm resistance in field conditions demonstrated that sunflower cultivars showed different response to *M. phaseolina*. The sunflower genotypes were significantly different on

reaction to *M. phaseolina* as shown in Table 3. Mean values of selected genotypes showed significant difference in the rate of disease incidence; hence the sunflower genotypes were placed into different groups.

Table 1. Sunflower germplasm collected from National Agricultural Research Center, Islamabad.

S. No	Varieties/lines	S. No	Varieties/lines
1	14001	9	1452
2	14005	10	14068
3	14009	11	14071
4	14013	12	14082
5	14021	13	14092
6	14035	14	14095
7	14041	15	Check-1
8	14048	16	Check-2

The impact of *M. phaseolina* for charcoal rot disease reaction was recorded carefully by tentative external disease symptoms on the stem of plant at maturity stage of crop.

Development of resistant varieties is the cheapest source for the management of sunflower charcoal root. The use of resistant cultivar is considered as one of the most important method.

Table 2. Disease rating scale to measure disease incidence.

Scale	Disease Incidence %	Reaction
0	0%	Immune
1	1-9%	Resistant
2	10-24%	Moderately resistant
3	25-49%	Moderately susceptible
4	50-74%	susceptible
5	75% and above	Highly susceptible

There are different studies about reaction of sunflower to *M. phaseolina*. Some sunflower varieties or lines were studied about reaction of sunflower to *M. phaseolina*. Sixteen sunflower varieties or lines were studied in the field condition against *M. phaseolina*. Disease incidence was estimated at flowering stage.

Response of sunflower under charcoal rot stress showed that all the accessions were variable in response to disease and none of accessions were found to be completely immune against *M. phaseolina*. Study on pathogenicity of *M. phaseolina* indicated high level of variations in

pathogenicity of fungus (Su *et al.*, 2001). Investigation of *M. phaseolina* isolates showed great variability in pathogenicity among isolates from different host species (Ullah *et al.*, 2010).

Therefore, 14013, 14052, 14082 and 14095 were found resistance to charcoal rot disease because they had the disease incidence among the 1-9% according to ranking scale. The varieties 14009, 14021, 14035, 14041, 14048 and Check-1 had 10-24% disease incidence were moderately resistant. Three varieties namely 14005, 14092 and check-2 showed moderately susceptible reaction due to 25-49% disease incidence and

two varieties 14001 and 14071 showing 50 to 74% disease incidence were susceptible to disease infection and caused reduction in yield of sunflower. Only one entry 14068 had disease

incidence more than 75% so these accessions were categorized as highly susceptible and strongly un-recommended for sowing as shown in table 3.

Table 3. Varietal response to charcoal rot disease incidence (*Macrophomina phaseolina*).

Scale	Disease incidence	Reaction	Name of entries
0	0%	I(Immune)	Nil
1	1-9%	R(Resistant)	14013, 14052, 14082, 14095
2	10-24%	MR(Moderately resistant)	14009,14021,14035,14041,14048 & check-1
3	25-49%	MS(Moderately susceptible)	14005,14092,check-2
4	50-74%	S(Susceptible)	1,400,114,071
5	75% & above	HS(Highly susceptible)	14068

Comparison of yield loss with percent disease incidence

Mean values expressed in (Fig. 1) showed 1000 seed weight loss in percentage taken from five healthy and disease plants of each replication of sunflower.

Weight of 1000 seeds was positively and significantly correlated with the disease reaction. Disease severity data of charcoal rot indicated significant effect on germplasm disease rating (Fig 2). Interaction of disease rating and seed weight loss was significant.

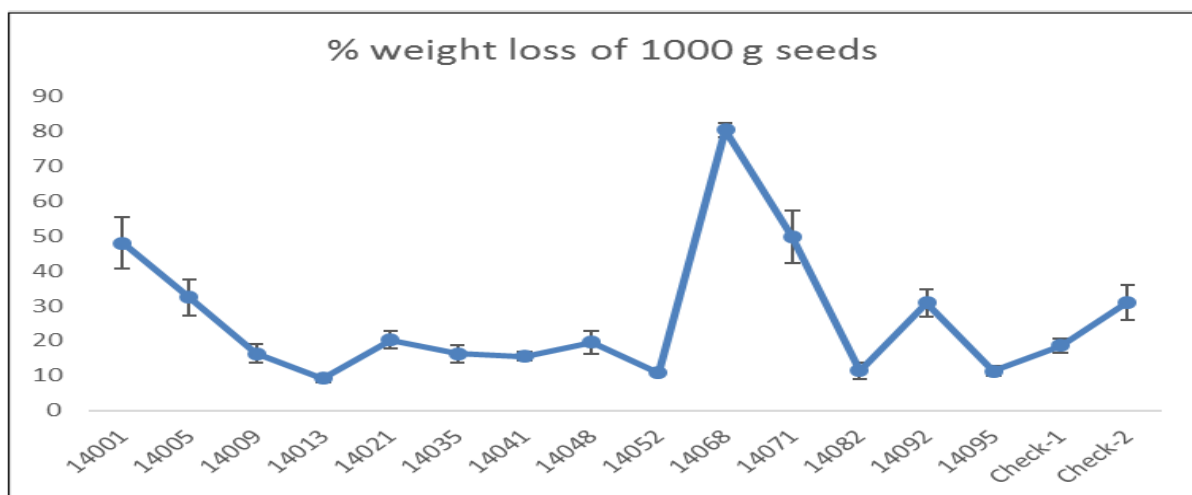


Fig. 1. % weight loss of 1000 g seeds.

This indicates that charcoal rot disease severity appearing in different germplasm vary from variety to variety. *M. phaseolina* has marked effect on growth of different genotypes and seed weight loss of sunflower crops. Charcoal rot caused reduction in seed weight by 8.79%, 4.70%, 6.63% and 7.39%, infected seeds from infected plants weight 9.28%, 10.81%, 11.31% and 11.42% less than healthy plants of genotypes 14013, 14052, 14095 and 14082 showing resistant reactions (R) respectively. Genotypes 14009, 14021, 14035, 14041, 14048, and check-1 having disease

incidence 14.27%, 14.00%, 22.64%, 10.68% 12.06% and 19.70% were categorized as moderately resistant (MR) and showing infected plants' seed weight loss 16.26%, 20.08%, 17.51%, 15.56%, 19.54% as compared to 18.07% from healthy plants.

The varieties 14005, 14092 and check-2 responded by showing moderately susceptible reaction (MS) having disease incidence 29.71%, 35.28% and 39.00% indicated seed weight loss of infected plants 32.4% 30.81% and 30.96% of healthy plants respectively.

Two germplasm 14001 and 14071 represented susceptible reaction (S) with disease incidence 53.20% and 61.97% and infected plant seed weight loss 47.9% and 49.8% from healthy plants.

14068 exhibited highly susceptible (HS) reaction with disease incidence 82.51% and seed weight loss of 80.24% from infected plants to healthy plants.

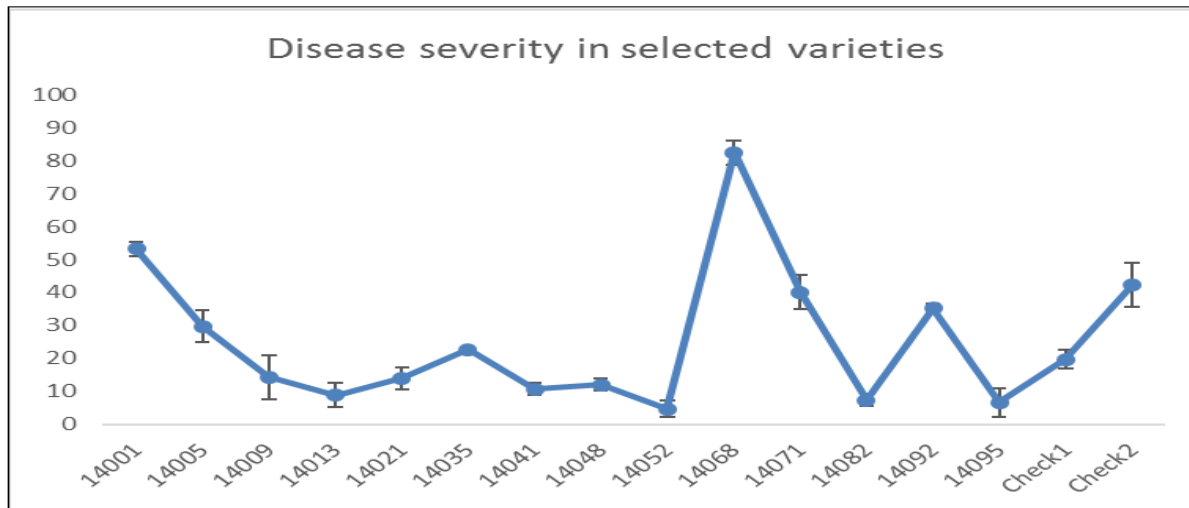


Fig. 2. Disease severity in selected varieties.

The accessions 14013, 14052, 14082, and 14095 showing resistant reactions, have 4- 9% disease incidence and caused 9-12% yield loss. Six genotypes i.e.14009, 14021, 14035, 14041, 14048 and check-1 showed moderately resistant (MR) response with disease incidence 10-23% and yield loss 15-21%. Next three varieties 14005, 14092, and check-2 indicated moderately susceptible (MS) reaction with disease incidence 29-39% and yield loss from 30-33%. Two accessions 14001 and 14071 were categorized as susceptible(S) showing the disease incidence 53-62% and yield 47-50 % and highly un- recommended for sowing.

The last one 14068 which shows the highest disease incidence 82.51% and yield loss 80.24% was strictly restricted from sowing.

The four accessions 14013, 14052, 14082, and 14095 showing resistant reactions and minimum yield loss are considered as good accessions and recommended for sowing. Six moderately resistant entries i.e.14009, 14021, 14035, 14041,14048 and check that showed deviation in response from four resistant varieties in disease incidence and yield loss but also considered good and recommended for sowing.

Conclusion

Four sunflower genotypes, 14013, 14052, 14082 and 14095 were proved as resistant against Charcoal rot disease which may be released for commercial sunflower production in arid zones of Pakistan. Moreover, QTL analysis of these elite genotypes need to check in future research and advanced breeding of these genotypes with diverse sunflower germplasm will help to release more new lines which are expected to be resistant in various edaphic and biotic stresses.

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