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# EVALUATION OF RESISTANCE AND MORPHOLOGICAL PARAMETERS OF DIFFERENT SUNFLOWER CULTIVARS AGAINST CHARCOAL ROT

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**ABSTRACT**

Charcoal rot is one of the major threats to sunflower which causes complete crop loss in epidemic conditions. The genetic resistance of sunflower germplasm could be more economical and durable approach for the management of charcoal rot. In the current experiment, six genotypes obtained from Ayub Agricultural Research Institute, Faisalabad were evaluated for their resistance and morphological parameters against *Macrophomina phaseolina*. Under field conditions, none of the cultivars gave immune or resistant response against the disease. FH-337 was found to be susceptible with 54.87% infection while FH-331, FH-106 and Hycanth-33 appeared moderately susceptible with 25-49% infections. The least affected varieties were FH-259 and DK-40 with moderately resistant status in the range of 10-24%. Yield parameters of resistant varieties were better than susceptible ones when compared under inoculated conditions. Moderately resistant genotypes might have the better genetic makeup and could be selected in breeding programs to find out resistant sunflower germplasm to fulfill the needs of growing population. Moreover, these cultivars could be used for better yield of sunflower.

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## INTRODUCTION

Among oilseed crops, sunflower (*Helianthus annuus* L.) has important position due to short growing time, increased yields, wider growing season, low irrigation requirements, suitability under wide range of soil conditions and higher edible oil contents (Weiss, 2000). The yield of sunflower in Pakistan is lower than that in other sunflower producing countries (Anis et al., 2001). The decreased yield is associated with several biotic and abiotic stresses which include lack of good quality seed and postharvest treatment facilities (Atkinson and Urwin, 2012). In Pakistan, charcoal rot results in huge losses in sunflower (Khan, 2007) which is caused by *Macrophomina phaseolina. M. phaseolina* has necrotrophic mode of nutrition and grows best at higher temperature. The fungus could be isolated both from seed and soil. It reproduces by producing microsclerotia (Khan, 2007). Its transmission from seed to seedling was studied by Kaur (2012). Under epidemic conditions, losses can reach up to 90% or more (Amusa et al., 2007).

This disease was firstly reported from Faisalabad followed by different regions in Sindh, Khyber Pukhtoonkhaw (KPK) and Punjab causing severe yield losses in sunflower (Mirza, 1984). Under favorable conditions, complete destruction of the crop could be observed (Steven et al., 1987). Charcoal rot is of great economic importance and causes decrease in stem height, girth, root and head weight (Ijaz et al., 2012). Disease severity is affected by temperature and moisture conditions. At the time of flowering, low relative humidity and high atmospheric temperature could be the cause of heavy qualitative and quantitative crop losses (Rauf and Sadaqat, 2008).

Apart from sunflower, *M. phaseolina* also destroys wheat, rice, maize, cotton, okra and cucurbitaceous members (Shehzad et al., 1988). The host range of *M. phaseolina* consists of up to more than 500 wild and cultivated plants (Iqbal and Mukhtar, 2014; Purkayastha et al., 2006). Almost 67 host species has been documented from Pakistan (Shehzad et al., 1988). This fungus has varying reports about its ecology i.e. seed, soil and stubble borne (Su et al., 2001). It produces cushion or tuber shaped black sclerotia having 1-8 mm diameter which serve as its survival source (Kaisar and Das, 1988). The plant undergoes many morphological changes after pathogenic attack that include irregular lesions with grey centers and dark brown borders, death of nodes and wilting (Khalili et al., 2016). Fungal sclerotia overwinter on weeds and attack plant roots causing destruction of fibrous roots and blackening of stems (El-Fiki et al., 2004). Pod rot, stem rot and seedling blight are the salient symptoms of charcoal rot (Sinclair, 1982). The fungus interferes with the function of xylem vessels thus causing the plants to wilt and results in premature death (Gupta and Chauhan, 2005). Variation in fungal habitats and excellent sclerotial survival make its chemical control difficult and uneconomical (Iqbal et al., 2014; Khan, 2007). As the most suitable method to avoid charcoal rot is the use of resistant germplasm, the objective of the present studies was to evaluate resistance/susceptibility level and growth parameters of different varieties/lines of sunflower against this disease.

## MATERIALS AND METHODS

**Germplasm collection:** The seeds of six varieties/advance lines (FH-331, FH-259, FH-106, DK-40, Hycanth-33 and FH-37) were obtained from Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan. The experiment was carried out in the Research Area of AARI.

**Design:** All the varieties were sown in augmented design. Recommended agronomic and cultural operations were followed to keep the crop in good conditions. The seeds of all the germplasm were sown on 3 m rows with 25 cm plant to plant and 75 cm row to row distances.

**Sample collection and isolation of pathogen:** Diseased tissues were collected from the infected sunflower cultivars. Infected samples were processed for the isolation of pathogen following the procedure described by Burney et al. (1990). Samples were washed, surface sterilized with 1% sodium hypochlorite and placed on Petri plates enriched with Potato Dextrose Agar medium. The plates were stored in an incubator and observed for fungal growth.

**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 in a drop of methylene blue and covered with the cover slip. This slide was placed under compound microscope and *M. phaseolina* pathogen was identified on the basis of spore shape.

**Maintenance of pure culture:** Once the fungus was identified as pure culture, it was maintained by transferring fungal mycelium into PDA slants, prepared in test tubes. These agar slants were incubated at 27±1ºC for eight days for further multiplication of the fungus.

**Inoculum preparation:** *M. phaseolina* inoculum was prepared with sterile toothpicks of 5 cm size. Toothpicks were loosely packed in glass jars after boiling for one hour in hot water and air drying. The jar was filled with 200 ml of Potato Dextrose Broth (PDB) and autoclaved. After cooling, the autoclaved toothpicks were inoculated with PDA discs of fresh *M. phaseolina* culture. The jar was placed in the incubator until the appearance of grayish mycelium and sclerotia.

**Inoculation:** Sunflower plants were inoculated by prepared toothpicks at 3$^{rd}$ internodes at the time of flowering.

**Data recording:** The data were recorded from 10 randomly selected plants of each variety/line for head diameter, head weight, 100-seed weight and plant height. Percent plant infection of charcoal rot was recorded on the basis of symptoms on stem of the inoculated plants. The disease-rating scale given in Table 1 was used for the evaluation of sunflower germplasm against *M. phaseolina*.

Table 1. Disease rating scale (James, 1971)

| Grade | Disease severity (%) | Status |
|---|---|---|
| 0 | 0 | Immune |
| 1 | 1-9 | Resistant |
| 2 | 10-24 | Moderately resistant |
| 3 | 25-49 | Moderately susceptible |
| 4 | 50-74 | Susceptible |
| 5 | More than 75 | Highly susceptible |

**RESULTS**

The reaction of sunflower germplasm against charcoal rot was evaluated by observing the symptoms on stem of the plants. After toothpick inoculation, data were recorded at weekly intervals. Percent plant infection was increased with the passage of time on all the varieties/lines (Table 2). On an average, 8.86% plant infection was recorded after 20 days of inoculation in all the entries with minimum of 2.62% on FH-259 and maximum (19.29%) on FH-37. After 40 days of inoculation, average plant infection percentage increased to 25.87% while percent plant infection on FH-259 and FH-37 was the minimum (12.27%) and maximum (57.01%) respectively. Percent plant infection increased after 60 days in FH-37 (39.47%) and 88.31% was recorded on FH-37 while average disease infection increased up to 49.61%. Percent plant infection was averaged based on three data (after 20, 40 and 60 days) for all varieties/lines. Minimum (18.12%) infection was recorded on FH-259 and maximum (54.87%) on FH-37.

Symptoms of charcoal rot such as silvery grey lesion, girdling of the stem at soil line, premature plant death and black, spherical micro-sclerotia were observed in the pith of lower stem, just underneath the epidermis and on the exterior of taproot of sunflower plant.

Table 2. Summary of charcoal rot disease on different varieties after varying days.

| Varieties | Percent plant infection after different time interval | | | Average |
|---|---|---|---|---|
| | 20 Days | 40 Days | 60 Days | |
| FH-331 | 9.64 | 18.41 | 48.24 | 25.43 c |
| FH-259 | 2.62 | 12.27 | 39.47 | 18.12 d |
| FH-106 | 9.34 | 23.68 | 55.26 | 29.43 b |
| DK-40 | 3.50 | 17.54 | 23.67 | 14.91 e |
| Hycanth-33 | 8.77 | 26.31 | 42.70 | 25.93 c |
| FH-37 | 19.29 | 57.01 | 88.31 | 54.87 a |
| Average | 8.86 c | 25.87 b | 49.61 a | |

Different letters in a row or column significantly differ at 5 % level of significance

Evaluation of sunflower germplasm resistance in field conditions demonstrated that sunflower cultivars showed different response to *M. phaseolina*. Mean values of selected genotypes showed significant differences in the rate of percent plant infection; hence, the sunflower genotypes were placed into different groups. Response of sunflower under charcoal rot stress showed that all the accessions were variable in their response to disease and none of accessions were found to be completely immune or resistant against *M. phaseolina*. According to disease rating scale, FH-259 and DK-40 were moderately resistant with percent plant infection in the range of 10-24%, FH-331, FH-106 and Hycanth-33 were moderately susceptible by exhibiting 25-49% plant infection and FH-337 was susceptible and falls in the range of 50-74% (Table 3).

**Morphological data of different sunflower varieties:** Data on plant height, 1000 seed weight and head diameter was recorded in inoculated and control plants of all the varieties and lines. All the three parameters were significantly reduced in inoculated plants as compared to control (Table 4). All the cultivars significantly differed from each other with respect to their height. Maximum plant height was 275 cm in case of FH-106 under control conditions which was reduced to 212 cm in inoculated plants. Minimum plant height was observed in Hycanth 33 which was 233.33 cm that reduced to 209 cm in inoculated plants. In control conditions minimum (53.28 g) 1000 seed weight was recorded while maximum was 62.25 g which gradually reduced to 40.93 g and 51.33 g respectively.

Table 3. Sunflower germplasm with different levels of resistance.

| Immune 0% | Resistant 1-9% | MR 10-24% | MS 25-49% | Susceptible 50-74% | Highly susceptible Above 75% |
|---|---|---|---|---|---|
| Nil | Nil | FH-259 | FH-331 | FH-337 | Nil |
| Nil | Nil | DK-40 | FH-106 | | Nil |
| Nil | Nil | | Hycanth-33 | | Nil |

Table 4. Effect of charcoal rot on morphological parameters of sunflower.

| Varieties | Plant Height (cm) | | 1000 Seed weight (g) | | Head Diameter (cm) | |
|---|---|---|---|---|---|---|
| | Control | Inoculated | Control | Inoculated | Control | Inoculated |
| Hycanth-33 | 227.33 f | 209.67 d | 49.28 e | 40.93 e | 21.66 b | 18.50 a |
| DK-40 | 273.33 b | 239.67 a | 55.16 c | 45.39 c | 19.41 c | 14.50 c |
| FH-259 | 241.67 d | 217.67 b | 62.25 a | 51.33 a | 18.35 d | 13.27 d |
| FH-106 | 275.00 a | 212.50 c | 51.86 d | 41.46 d | 16.82 e | 11.43 e |
| FH-37 | 251.00 c | 78.00 f | 55.34 c | 46.18 c | 15.01 f | 10.00 f |
| FH-331 | 234.33 e | 184.33 e | 59.11 b | 48.65 b | 23.75 a | 16.83 b |

Means with similar letters are not significantly different at ($P$<0.05)

## DISCUSSION

The host plant resistance occupies a high value among integrated management techniques because it is easily adopted, requires few inputs and economically advantageous (Iqbal et al., 2010). In the present study, lack of genetic resistance was observed as no variety/line was found immune or resistant when checked according to disease rating scale. Many researchers worked on sunflower cultivars to evaluate their resistance against *M. phaseolina*. Sanaullah et al. (2013) conducted experiment for the evaluation of sunflower cultivars under charcoal rot stress conditions with the findings that all the germplasm showed different levels of resistance against charcoal rot disease and no immune or resistant accession was found. As previously described (Weiss, 2000) variations were found in *M. phaseolina* inoculated plants for resistance. Ullah et al. (2010) demonstrated that no variety was free from charcoal rot in sick field experiment. Ahmad et al. (1991) described that resistance level of four sunflower hybrids was different against charcoal rot disease with no variety free of disease. Kumar and Kaushik (1994) artificially inoculated sunflower germplasm from varied sources and susceptible response was recorded. Sunflower germplasm showed different response against inoculation of *M. phaseolina* (Dalili et al., 2009). In Pakistan, Jalil et al. (2013) concluded his results that out of 24 sunflower accessions, no one was resistant while HBRS-1, A-12 and A-79 showed moderately resistant response. Lodha and Sharma (2002) attributed the higher disease incidence with high sclerotial densities. The fungus manipulates the host machinery to influence the plant hormonal level and disrupting the defense signals (Prins, 2000) for disease progression (Laluk and Mengiste, 2010). The toxins and enzymes released by fungus destroy the cortex of root and gradually form colonies in it (Islam et al., 2012). The cell wall and cell membrane of the plant is disrupted by amylase, cellulase, hemicellulases, lipase and pectinase of *M. phaseolina* (Kaur, 2012).

Biotic stresses pose a negative effect on plant growth and reproduction. There was significant reduction in plant height, 1000 seed weight and head diameter in *M. phaseolina* infected plants as compared to control. All varieties/lines also showed notable differences in the above-mentioned parameters. This difference in plant height might be attributed to varying genetic makeup of two varieties for that trait. Thousand seed weight significantly reduced in *M. phaseolina* infected plants. Abdul and Shabeer (2001) found reduction in grain yield, head diameter, 1000-grain weight, oil content and plant height in susceptible genotypes under charcoal rot stress conditions. Head diameter had positive correlation with 1000 seed weight. It means by reduction in head diameter due to pathogen attack, 1000 seed weight also reduces (Anandhan et al., 2010). Hafeez and Ahmad (2001) described that charcoal rot reduced grain yield, head diameter, 1000-grain weight, oil content and plant height in susceptible genotypes by 14.4%, 20.3%, 15.7%, 15.7% and 16.9%, respectively. Bokor (2007) described that charcoal rot disease resulted in premature death of sunflower, which may be the result of toxins produced by the attacking fungus. The vascular tissues of the sunflower are blocked by the microsclerotia of fungus resulting in premature death of the plants (Ramezani, 2007).

**CONCLUSION**

Two sunflower genotypes, FH-259 and DK-40 were found to be moderately resistant against charcoal rot disease, which need to be checked in future research and advanced breeding experiments in order to release more new lines which are expected to be resistant against *M. phaseolina*.

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