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Cultural and morphological variability of *Macrophomina phaseolina* (Tassi) Goid causing charcoal rot of sunflower in Sargodha Pakistan

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Abstract

This study was conducted to determine the morphological identification of charcoal rot (*Macrophomina phaseolina*) of sunflower. The cultural characterises and morphological tools were used for the identification of *M. phaseolina* isolates. The wilting, gray discoloration at the base of plants and sclerotia were recorded from sunflower fields growing at College of Agriculture (COA), Sargodha, Pakistan. A total seventeen isolates were isolated. The isolation was conducted on potato dextrose agar (PDA) and sterile soil method was used for long-term preservation. The colony color of four isolates was grey while seven isolates were exhibitingblackish grey and remaining six isolates were grayish white color. The mycelial growth pattern of *M. phaseolina* was blackish grey, lesser cottony, straight and grayish white. The colony appearance was varied from, very less feathery, less feathery, more feathery and maximum feathery. The oblong and round shape sclerotia were recorded in all isolates. The range of the average radial growth of individual isolates was 79.4 to 91.2 mm. The maximum average sclerotial population/microscopic field was record in MP_{11} while 16.4 was minimum in MP_1 143.2 were the highest number of Sclerotia/9 mm disc in isolate MP_{12}.The production of maximum sclorita plays a vital role on the virulence of *M. phaseolina* and it is supposed to be highly virulent. This isolates will be further used for molecular identification, screening of available sunflower germplasm and developing integrated management strategies of charcoal rot.

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Introduction

Sunflower (*Helianthus annuus* L.) plays a significant role in oilseed production and it is becoming an important oil seed corps in many countries of the world. In edible oil production, sunflower crop is ranked at third position after soybean and groundnut (Meric, 2003). Sunflower is short duration crop and it was first introduced in Pakistan in the sixties to minimize the oil gap (Mirza and Beg, 1984; Bhatti and Soomro, 1996). It can grow autumn as well as spring crop in different agro-ecological zones of barani and irrigated areas of Pakistan (Samiullah, 2000).

Charcoal rot (*Macrophomina phaseolina),* collar rot *(Sclerotinia rolfsii)*, head rot (*Rhizopus* sp. and *Sclerotinia sclerotiorum*) leaf spots (*Alternaria helianthi* and *Sepotoria helianthi*) and rust *(Puccinia helianthi)* has been reported in sunflower crop of Pakistan (Ahmed *et al.,* 1991). Among all these charcoal rot is getting an alarming threat in main sunflower growing areas of Pakistan. *Macrophomina phaseolina* (Tassi) Goid is an important soil borne pathogens which has a wide hosts range including agricultural crops, several legumes and cereals (Dhingra *et al.*, 1981; Sinclair *et al.*, 1982).

This pathogen has caused 90% yield losses under favourable conditions and it attacks on root, stem and fruit of more than 500 plant species worldwide (Viana and Souza, 2002; Khan, 2007; Gupta *et al.*, 2012). The appearance of dark lesions on the epicotyls and hypocotyls, wilting and death of entire plant was recorded due to obstruction of xylem vessels. Black microsclerotia and dark mycelia can be observed on infected sunflower plants. The cultural and morphological characteristics are differing among the isolates of *M. phaseolina* isolates. The isolates are differing in various morphological (Mayek *et al.*, 1997), physiological (Mihail 1995), pathogenic (Mayek-Perez *et al.*, 2001; Aboshosha *et al.,* 2007) and genetic variations (Chase *et al.,* 1994; Jana *et al.*, 2005; Reyes-Franco *et al.,* 2006; Farhana *et al.,* 2013). The present study aims at isolation, preservation and identification (cultural and morphological) of *M. phaseolina* causing charcoal rot of sunflower in Sargodha Pakistan.

Material and methods

Sample collection

The infected stems of sunflower with microsclerotia and characteristic symptoms of charcoal rot were collected form the infected field of sunflower located in University College of Agriculture (UCA) Sargodha Pakistan. The samples were brought to Plant Pathology Laboratory and stored at 4°C until processed for isolation and morphological identification.

Isolation and purification

The infected segment of stem bark tissues were cut into 5mm small pieces and surface sterilization was performed with 1% NaOCl. The sterile segments were rinsed thrice in sterile distilled water for 3 min and shifted to double layer of sterile filter papers to remove the moisture.

The sterile segments were further transferred to Potato Dextrose Agar (PDA) medium and incubated at 25±2°C. For the purpose of purification, the agar plug with fungal growth was shifted in the center of 9mm sterile PDA Petri dish and incubated at 25±2°C. Sterile soil method was used for long term preservation of *M. phaseolina*. About one-third loam soil was filled in McCartney vials and double sterilization of vials was conducted. One third sterile distilled water was added in the pure culture Petri dish of *M. phaseolina* and sterile needle was used to harvest the fungal culture for mycelial and spore suspension. 1mL of the suspension was shifted into sterile vials and vials were incubated for 10 days at 25±2°C (Atkinson, 1954). For long term preservation, the vials were further stored at 4°C.

Morphological identification

The cultural and morphological tools viz colony color, texture, radial growth, branching pattern, size, shape and number of sclerotia were used for morphological identification. The radial growth and sclerotial

morphology was recorded on seventh day (Gavali *et al.*, 2017).

Result

The initial symptoms of charcoal rot of sunflower were observed after flowering. The plants were exhibiting general wilting during the midday heat and recovery was recorded in the evening as temperature decline (Fig 1A). Gray discoloration at the base of infected plant was observed and small, black flecks or sclerotia were more dominant (Fig 1A).

Three coloar in colony characteristics viz grey (MP_1, MP_6 MP_7 and MP_8) blackish grey (MP_2, MP_5, MP_9, MP_{10}, MP_{12}, MP_{15}, and MP_{17}) and grayish white (MP_8, MP_4, MP_{11}, and MP_{14}) were recorded in all isolates (Table 1 & Fig 1B).

Isolates were also assigned into groups on the basis of mycelial growth and colony texture. MP_2, MP_5, MP_9, MP_{10}, MP_{12}, MP_{15} and MP_{17} isolates were exhibiting blackish grey mycelial growth while lesser cottony growth was appeared in MP_8, MP_4, MP_{11}, and MP_{14} isolates respectively. MP_1, MP_6 MP_7 and MP_8 showed straight and grayish white mycelial growth. Only four isolates (MP_4, MP_8, MP_{13} and MP_{17}) were exhibiting the maximum feathery colony (Table 1).

Table 1. Cultural characteristics and morphological identification of *M. phaseolina* causing charcoal rot of sunflower in Sargodha, Pakistan.

Isolate	Colony color		Colony appearance	Branching pattern	CPO	FSB	SS	RG mm	*SP/ 9mm disc	*SP/MF	*SD µm
	Reverse	In front									SD
MP_1	Black	Grey	Very less feathery	Right angle	Presence	Presence	Oblong	79.4	58.4	16.4	22.1-26.5
MP_2	Black	Blackish grey	Less feathery	Acute angle	Presence	Presence	Round	80.23	108.6	27.4	22.23-28.4
MP_3	Black	Grayish white	More feathery	Acute angle	Presence	Presence	Oblong	81.3	102.4	25.2	20.4-27.92
MP_4	Black	Grayish white	Maximum feathery	Right angle	Presence	Presence	Oblong	88.4	83.4	35.1	18.34-28.34
MP_5	Black	Blackish grey	Less feathery	Acute angle	Presence	Presence	Oblong	84.5	91.4	25.2	19.23-26.76
MP_6	Black	Grey	Very less feathery	Right angle	Presence	Presence	Round	87.6	99.2	29.2	22.32-41.2
MP_7	Black	Grey	More feathery	Right angle	Presence	Presence	Oblong	91.20	126.4	25.2	30.6-38.72
MP_8	Black	Grey	Maximum feathery	Acute angle	Presence	Presence	Round	82.34	90.2	26.2	23.9-36.8
MP_9	Black	Blackish grey	Less feathery	Acute angle	Presence	Presence	Round	88.2	67.8	32.2	21.5-31.4
MP_{10}	Black	Blackish grey	Less feathery	Right angle	Presence	Presence	Oblong	83.43	85.4	32.4	20.4-24.9
MP_{11}	Black	Grayish white	More feathery	Acute angle	Presence	Presence	Round	85.12	77.2	24.4	15.2-16.6
MP_{12}	Black	Blackish grey	Very less feathery	Right angle	Presence	Presence	Oblong	87.32	143.2	40.5	18.3-20.7
MP_{13}	Black	Grayish white	Maximum feathery	Acute angle	Presence	Presence	Oblong	88.32	72.4	31.4	28.23-39.7
MP_{14}	Black	Grayish white	Less feathery	Acute angle	Presence	Presence	Round	80.32	80.6	33.0	23.9-27.34
MP_{15}	Black	Blackish grey	More feathery	Acute angle	Presence	Presence	Oblong	82.54	84.4	33.4	23.2-28.23
MP_{16}	Black	Grayish white	Very less feathery	Right angle	Presence	Presence	Round	85.32	79	33.4	23.5-29.4
MP_{17}	Black	Blackish grey	Maximum feathery	Right angle	Presence	Presence	Round	84.12	67.4	32.6	22.2-38.5

CPO=Construction at the point of origin, FSB: Formation of Septum in the branch near the origin, SS= Sclerotial shape, RG= Radial Growth, SP= Sclerotial population, SP/MF= Sclerotial population/ microscopic field, SD = Sclerotial Diameter, * = Mean for five observation.

The oblong shape and irregular edges of sclerotia were recorded in MP_1, MP_3, MP_4, MP_5, MP_7, MP_{10}, MP_{12}, MP_{13} and MP_{15} and round shape with regular edges sclerotia were observed in remaining isolates (Table 1). Right (8 isolates) and acute angle (9 isolates) types were recorded in branching pattern. After 7 days, the average radial growth of individual isolates ranged from 79.40 to 91.20 mm. The maximum radial growth was recorded in MP_7 while MP_1 was exhibiting the minimum. Sclerotial

population / microscopic field was varied from 15.20 to 46 and the maximum average sclerotial population / microscopic field was record as 40.5 in MP_{11} while 16.4 was minimum in MP_1. 143.2 were the highest number of Sclerotia/9 mm disc in isolate MP_{12} while 58.4 was minimum in MP_1 (Table 1 & Fig IC).

Discussion

Macrophomina phaseolina is seed-borne as well as soil born fungus. This pathogen creates a hindrance in the flow of nutrients and water to the head and cause wilting. The wilt became permanent and ultimately death of entire plant was recorded (Fig 1A). Symptomology plays a vital role in diagnosis of *M. phaseolina* but it is not a reliable method for the confirmation as wilting may developed due to different biotic and abiotic factors. The cultural characteristic and morphological tools were further used for the reliable confirmation of charcoal rot infecting sunflower in Sargodha, Pakistan. *M. phaseolina* has a wide host range study and morphological variability in terms of growth, color, pycnidium production and chlorate sensitivity and pathogenicity was recorded in different hosts (Riaz *et al.*, 2007). Seventeen isolates were isolated from infected plant samples of sunflower collected from UCA Sargodha.

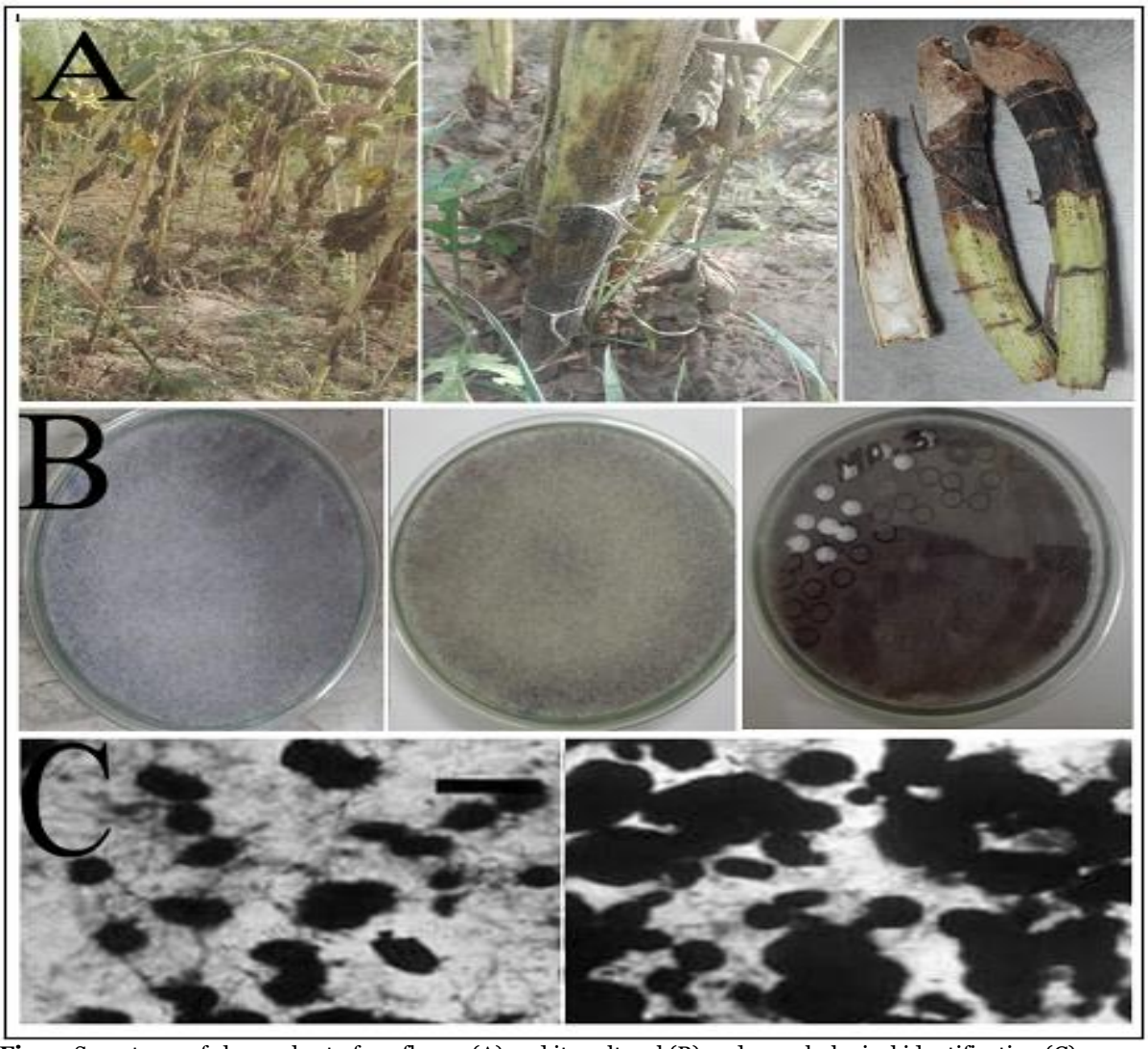

Fig. 1. Symptoms of charcoal rot of sunflower (A) and its cultural (B) and morphological identification (C).

The cultural and morphological variation among *M. phaseolina* isolates were recorded in this study. Previously variation among cultural and morphological characters of *M. phaseolina* isolates were reported by Karunanithi *et al.* (1999); Atiq *et al.* (2001); Jana *et al.*, 2005; Saleh *et al.* (2010) and

Linhai *et al*., (2011). Elven isolates of *M. phaseolina* were morphologically identified from Akkalkot, Barshi, Karmala, Madha, Malshrius, Mohol, North Solapur, Pandharpur, Sangola, South Solapur and located in India and all the isolates were exhibiting morphological variability (Gavali *et al*., 2017). A total number of 65 isolates of *M. phaseolina* were isolated from Banu, Bhakkar, Chakwal, Dera Ghazi Khan, Faisalabad, Islamabad, Kohat, Layyah, Mianwali, Muzaffargarh, Narowal, Rawalpindi and Sialkot located in different agro ecological regions of Khyber Pakhtunkhwa and Punjab (Iqbal *et al*., 2014). All the isolates were exhibiting variation in their cultural and morphological characters characterises. A comprehensive survey of sunflower was conducted in Sindh and 32 isolates of *M. phaseolina* were collected from Badin, Dadu, Hyderabad, Khairpur, Mirpurkhas, Sanghar, Shaheed Benazirabad, Sukkur, Tando Muhammad Khan and Thatta (Wagan *et al*., 2018). The variation in colony color (gray and blackish-gray to grayish-black), pattern (dense, feathery and restricted) and sclerotia size were reported (Wagan *et al*., 2018). It was previously concluded that the degree of production of sclerotia is positively correlated with the virulence of charcoal rot of maize caused by *M. phaseolina* (Shekhar *et al.,* 2012).

Conclusion

It is concluded that the degree of variability in cultural characteristics and morphological identification of seventeen isolates of *M. phaseolina* were observed from Sargodha causing charcoal rot of sunflower. The production of maximum sclorita has positively impact on the virulence of *M. phaseolina*. The maximum numbers of sclorita were observed in MP_{12} isolates and it is supposed to be highly virulent among all isolates and this isolates will be further used for molecular identification, screening of available sunflower germplasm and developing integrated management strategies of charcoal rot.

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