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ANALYSIS OF RESISTANCE IN SUNFLOWER TO CHARCOAL ROT PATHOGEN *MACROPHOMINA PHASEOLINA*

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Abstract

Four sunflower hybrids viz., NK-212, Cargill-204, SF-100 and Hysun-30 were tested for disease resistance against 26 isolates of *Macrophomina phaseolina* collected from sunflower growing areas in Pakistan. Stem inoculations were made using a tooth-pick and spread of charcoal rot was measured from the point of inoculation. Disease reaction was scored on a 0 to 5 scale; where 0 = no rot and 5 = plant death. Resistance was characterized using analysis of variance. A highly significant main effect for both varieties and isolates was detected. This indicated a highly significant difference in horizontal resistance between varieties and in aggressiveness between the isolates. However, the interaction varieties x isolates was nosignificant, indicatng no evidence of vertical resistance.

Introduction

One of the major problems associated with the production of oilseed sunflower (*Helianthus annus* L.) in Pakistan is the attack of charcoal rot pathogen, *Macrophomina phaseolina* (Ahmad & Burney, 1990). In Pakistan, sunflower is grown in two seasons: spring crop planted in late January or February, while summer crop in late June or early July. The disease occurs in both the spring and summer crops and can completely devastate it, especially in the rainfed (barani) areas. Annual losses due to charcoal rot are reported to be 20-65% (Masirevic *et al.*, 1987).

The control of the disease is difficult since the pathogen is soil borne and has high survival capacity. Therefore, the most effective disease management approach is the use of genetic resistance. The use of a particular genetic control strategy is dependent on the kind of resistance available. The objective of the present study was to provide information on which future genetic control efforts could be based by identifying the type of resistance in sunflower against *M. phaseolina*.

Materials and Methods

Four commercial sunflower hybrids viz., NK-212, Cargill-204, SF-100 and Hysun-32 were tested against 26 isolates of *M. phaseolina* collected from sunflower growing areas in Pakistan.

***M. phaseolina isolates*:** Samples of soil and sunflower tissues infected with *M. phaseolina* were collected from key locations in the sunflower growing areas (Table 1). Collection of soil samples and subsequent isolation of the pathogen was done accord-

Table.1. Source and designation of *Macrophomina phaseolina* isolates obtained from irrigated and rainfed (barani) areas of Pakistan.

Isolate designation	Isolated from	Place of origin	Year
MP-1	Soil(sunflower)	Mangial	1985
MP-2	Soil(sunflower)	Gagan	1985
MP-3	Soil(fallow)	Mianwala	1985
MP-4	Soil(fallow)	Dhulian	1985
MP-5	Soil(millet + peanut)	Kot Sarang	1985
MP-6	Soil(millet)	Biddar	1985
MP-7	Soil(fallow)	Musakhel	1985
MP-8	Soil(millet)	Kundian	1985
MP-9	Soil(millet)	Shahpur	1985
MP-10	Soil(corn)	Gujranwala	1985
MP-11	Soil(millet)	Gujrat	1985
MP-12	Soil(millet)	Dina	1985
MP-13a	Soil(sunflower)	Islamabad	1985
MP-13b	Stem(sunflower)	Islamabad	1985
MP-14	Soil(sunflower)	Punjgran	1985
MP-15	Soil(sunflower)	Tarlai	1985
MP-16	Root(sunflower)	Aroop	1986
MP-17	Root(sunflower)	Kotli Syedian	1986
MP-18	Root(sunflower)	Katorora	1986
MP-19	Root(sunflower)	Manga mandi	1986
MP-20a	Root(sunflower)	Tarlai	1986
MP-20b	Root(sunflower)	Pattoki	1986
MP-21	Root(sunflower)	Faisalabad	1986
MP-22	Root(sunflower)	Rao Bagh	1986
MP-23	Root(sunflower)	D.I.Khan	1986
MP-24	Seed(sunflower)	Islamabad	1986

ing to Ahmad *et al.,* (1988). Five soil samples taken from each location with the help of a soil sampler were pooled together air dried and passed through a 10 mesh screen. A 5 g soil sample suspended for 10 minutes in 1% sodium hypochlorite (NaOCl) in a blender in three comminutions, each of 10 sec duration at intervals of 5 min., was poured on to a 45 µm (325 mesh) sieve and washed with distilled water for 2-3 minutes. The washed residue on the sieve was transferred into molten chloronebmercuric chloride-Rosebengal agar (CMRA) at 45^{O}C, agitated and immediately poured into Petri dishes, the dishes were incubated in dark for one week at 33^{O}C and examined for colonies of *M. phaseolina*.

Table.2. Reaction of four sunflower hybrids to twenty six isolates of *Macrophomina phaseolina*.

Isolate	Mean disease severity rating*			
	NK-212	Cargill-20	4SF-100	Hysun-30
MP-1	3.87	3.53	1.97	4.63
MP-2	3.67	3.37	2.13	4.10
MP-3	3.77	3.43	2.37	4.30
MP-4	3.40	2.93	2.10	3.90
MP-5	3.63	3.33	3.03	4.30
MP-6	2.60	3.00	2.00	3.73
MP-7	3.53	3.27	2.23	3.10
MP-8	3.60	2.70	1.77	3.53
MP-9	3.33	3.30	2.53	4.73
MP-10	3.23	2.77	1.83	3.93
MP-11	3.57	3.17	1.87	4.73
MP-12	3.37	3.40	1.50	3.80
MP-13a	3.43	3.00	2.63	4.53
MP-13b	4.57	3.73	2.63	4.60
MP-14	3.83	2.87	1.53	2.47
MP-15	3.93	2.50	2.53	4.43
MP-16	3.67	2.83	2.53	4.03
MP-17	3.67	3.37	2.73	4.77
MP-18	3.93	2.77	1.77	3.53
MP-19	4.07	3.03	2.43	4.07
MP-20a	3.70	3.00	1.77	4.73
MP-20b	3.13	3.53	1.60	3.47
MP-21	3.47	2.10	1.37	4.07
MP-22	1.10	1.90	1.37	3.17
MP-23	3.13	1.67	1.13	3.93
MP-24	3.57	3.13	2.83	4.40

* On A 0-5 scale, 0 = Immune, 5 = Highly susceptible.

Infected sunflower tissues (roots, stem and seeds) were processed for isolation as described by Burney *et al.*, (1988). The stem and seed samples were washed in running tap water for 5 min. and the root samples for half an hour, surface sterilized in 1% sodium hypochlrite and plated on CMRA. The plates were incubated in dark at 33^{o}C and observed for *M. phaseolina* colonies. The isolates were designated (Table 1) and preserved in paraffin oil at 4^{o}C.

***Inoculum preparation*:** Inoculum of *M. phaseolina* isolates was produced on sterile toothpicks. Toothpicks were boiled in water for an hour, air dried and loosely packed in glass jars containing 200 ml of potato dextrose broth covering lower one third of the toothpicks. Jars were autoclaved for 20 minutes at 15 psi with their lids loosely

Table.3. Analysis of variance of reaction of four sunflower hybrids against 26 isolates of *Macrphomina phaseolina*.

Source	DF	SS	MS	F. ratio
Hybrids	3	175.2	58.4	92.4**
Isolates	25	50.8	2.0	3.2**
Hybrids x Isolates	75	33.7	0.4	0.70N.S.
Error	208	131.5	0.6	
Total	311	391.2		

** = Significant at 0.001. N.S. = non-significant.

screwed. When cooled, toothpicks were inoculated with PDA discs of fresh cultures of *M. phaseolina* isolates. The jars were incubated at 33°C in the dark till the toothpicks were covered with greyish mycelium and dark coloured sclerotia.

***Plant culture, inoculation and Characterization of Resistance*:** Sunflower hybrids viz., NK-212, Cargill-204, SF-100 and Hysun-30 were sown in 4 rows in 4.0x3.5 m plots in a randomized complete block design with four replicates. Row and plant spacing was 0.8 m and 23.0 cm respectively. Plants were inoculated at the time of flowering inserting toothpick inoculum into the 3rd internode of each plant through a hole made with an electric drill. The bit of the drill was surface sterilized with alcohol each time before making the hole. Disease reaction was scored on a 0 to 5 scale; where 0 = no rot and 5 = plant death. Resistance was characterized using an analysis of variance (Vanderplank, 1984) on a BBC computer where resistance is defined biometrically in terms of only two variables: host and pathogen.

Results and Discussion

Mean disease severity ratings of sunflower hybrids tested against 26 isolates of *M. phaseolina* are given in Table 2. The analysis of variance showed a highly significant main effect for both varieties and isolates (Table 3). This indicated a highly significant difference in horizontal resistance between sunflower varieties and in aggressiveness between the isolates of *M. phaseolina* tested. However, the interaction varieties x isolates, was insignificant. This indicated that with the particular sunflower hybrids and the *M. phaseolina* isolates and the parameter of charcoal rot incidence used in the present study, there was no evidence of vertical resistance.

Overall SF-100 could be ranked as most resistant followed by Cargill-204, NK-212 and Hysun-30. In the present study (Table 2) the disease severity ratings of hybrid SF-100 and Cargill-204 are low for all isolates in SF-100 than Cargill-204 except for MP-5 and MP-15 where ratings are at par. Thus in the present case SF-100 is likely to be more resistant in most field situations than Cargill-204.

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