



Journal of Biological Sciences

ISSN 1727-3048

science
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Screening of Chickpea Germplasm Against *Fusarium* Wilt in Pot Soil, Water Culture and Cultural Filtrate

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Abstract: Out of 31 lines evaluated against chickpea wilt caused by *Fusarium oxysporum* f.sp. *ciceris*, only three lines 99109, 99112 and 99115 were found to be highly resistant, two lines 1040/99 and 99104 were moderate resistant where as 26 lines were susceptible to highly susceptible. A very good correlation was observed among the three screening methods. i.e. screening of the germplasm against the pathogen using pot soil, water culture and spore free culture filtrate of the fungus.

Key words: Screening, wilt, *Fusarium oxysporum* f.sp. *ciceris* chickpea, culture filtrate

Introduction

Chickpea (*Cicer arietinum* L.) is an important legume in the south East and Western Asia, Northern and Eastern Africa and Central and Southern America. Pakistan is the third major producer of chickpea after India and Turkey (Anonymous, 1998). In Pakistan, chickpea is grown over an area of 1118 thousand hectares with production of 607 kg/ha which is lower than that in India producing 813 kg/ha (Anonymous, 1996). This low yield is due to the lack of crop management and diseases affecting the crop. Among the fungal diseases, chickpea will caused by *Fusarium oxysporum* f.sp. *ciceris* is a serious problem in Pakistan (Khan, 1980), especially in thal area the districts of Jhang Lia, Khoshab, Bhukar and Mianwali. Wilt is a serious disease in Pakistan causing annual losses of about 12 million rupees (Sattar *et al.*, 1953). Akhtar (1956) reported about 75 percent damage to the crop in severe cases.

The most ideal and economical way of controlling this disease is the use of host resistance which can be determined by different germplasm screening techniques viz., screening of germplasm in pot soil, water culture and cultural filtrate containing the phytotoxins. Phytotoxins are reported to be helpful not only in understanding the mechanism of pathogens but also in identifying resistant cells in tissue culture or in screening and breeding for disease resistance (Song *et al.*, 1994) reports different levels of resistance/susceptibility in chickpea germplasm.

Materials and Methods

Screening in pot soil: A pure culture of *Fusarium oxysporum* f.sp. *ciceris* was obtained from phytopathological lab of NIAB. A sand maize meal medium was prepared in each of the several 250 ml Erlenmeyer flasks. This medium was autoclaved in the flasks at 15 lb psi for 20 minutes. Each flask was inoculated with a bit of fungus from a pure culture and incubated at 25°C for 15 days. A fungus soil mixture was prepared by hand mixing contents of each flask with 2 kg of non-autoclaved field soil. This soil was taken from a chickpea field where wilt normally occurred. Large (30 cm dia.) earthen pots were filled with inoculated soil approximately 10 kg of soil was required to fill each pot. The pots were watered and kept for 4 days at 25 ± before proceeding to the next step.

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Forty to fifty seeds of highly susceptible cultivar (Aug 424) were sown in pot at 2-3 cm depth. Most plants exhibited wilting after 10 days, which were chopped and incorporated into the soil. This procedure was repeated until over 90% wilt was observed. These pots were than ready for screening. Ten seeds of each of the 31 test lines were planted in a pot. A highly susceptible line (Aug 424) served as control and a resistant line (Paidar 91) were grown as checks. The data on the number of wilted seedling in each pot for each test line were recorded and wilt incidence was calculated for each test line by using the formula.

$$\text{Wilt incidence} = \frac{\text{No. of plants wilted}}{\text{Total No. of plants}} \times 100$$

The data on the mortality due to wilt were taken add the resistance/susceptibility of each line was determined by using a rating scale where 0-10% mortality = highly resistant. 11-20% = resistant 21-30% moderately resistant, 31-50% susceptible and 51-100 % highly susceptible (Iqbal *et al.*, 1993).

Screening in water culture: Fifteen surface sterilized (5 min in 2.5% sodium hypochlorite) seeds of each of the test lines as well as susceptible checkline (Aug-424) were sown in autoclaved riverbed sand placed in 15 cm diameter pot. One pot containing 1 kg sand was used for each test line. Potato dextrose broth was prepared in one 250 ml flask, 100 ml of broth was placed and autoclaved at 15 lb psi for 20 minutes. This broth was then inoculated with a bit of the fungus (*Fusarium oxysporum* f.sp. *ciceris*) growth from a fresh culture tube. The inoculated plates were incubated on a shaker (8 hr each days) at room temperature for 10 days. Entire contents of the flask were diluted with sterilized distilled water to get the final inoculum dilution of 2.5% (about 2.5 liters of water were needed to obtain the desired dilution of the content of one flask). This dilution contained approx. 6.5×10^5 spores/ml. Ten days old (from prior sowing) seedlings were removed from sand. There root system was washed in running water and then rinsed in sterilized distilled water. These plants were shifted to 50 ml conical flask containing 40 ml of the inoculum prediluted. These flasks were placed under florescent

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Table 1: Chickpea varieties/lines showing different levels of resistance/susceptibility against *Fusarium* wilt

Variety/line	Pot experiment %wilting	Through water culture %wilt	Through culture filtrate % wilt	Pedigree
Paidar98/99	50	100	100	-
Ind 98/99	90	100	100	Indian line
290/99	33.3	100	100	992 × 218*
Aug-424	100	100	100	-
588	25	100	100	218 × 620*
881	100	100	100	CM 72 × 542-2*
99102	100	100	100	CM 2954/91
99103	100	100	100	CM 2954/91
99104	37.5	0	0	ICCV 95227
99105	66.6	0	0	PUSA 329
99106	0	50	100	ICCV 95229
99107	0	50	100	ICCV95204
99108	30	100	100	ICCV 95205
99109	0	0	0	CM 738/93
99111	40	75	75	CM 988/93
99112	20	0	0	CM 368/93
99113	66.6	100	100	CM 946/93
99114	62.5	100	100	1966/94
99115	22.2	0	0	CM 1463-2/94
1001/99	22.2	66.66	100	932 × 1130*
1003/99	44.40	100	100	406 × 777*
1004/99	20	33.30	66.60	447 × 222*
1005/99	83.3	100	100	705 × C
1009/99	25	75	75	218 × CM 72*
1014/99	100	100	100	C 27 × 216*
1015/99	50	75	100	620 × 205*
1018/99	33.3	66.6	100	230 × 1012*
1036/99	100	100	100	2-444 × 1159*
1040/99	10	25	25	4112 × 750*
4047/99	20	100	100	4012 × 620*
5007/99	90	100	100	C44 × 750*

*Lines raised by Plant Genetics and Breeding Department, University of Agriculture, Faisalabad

light at $25 \pm 2^\circ\text{C}$ and the flasks were shaken for 8 hrs sowing and lines were rated for their extent of resistance/susceptibility.

Results and Discussion

The green-house screening method is relatively less time consuming cheaper and more reliable as compared to field screening which often has the chances of some escape as the wilt disease is temperature dependent and the level of inoculum may vary at different places. The greening house potscreening of chickpea germplasm revealed that out of 31 testlines evaluated for their level of resistance/susceptibility of *Fusarium* wilt, four lines 99107, 99109, 99106 and 1040/99 were found to be resistant, five lines 1009/99, found to be resistant, five lines 1009/99 588, 1001/99 99108 and 99115 were moderate resistant while all the other lines were susceptible to highly susceptible which could not withstand against wilt. The source of resistance to *Fusarium* wilt in chickpea germplasm are not uncommon and a number of workers have reported the occurrence of high level of resistance to *Fusarium* wilt (Kaushal and Singh, 1990; Yu and Su, 1997).

Chickpea lines that showed upto 20 per cent mortality in pots found resistant to water culture and cultural filtrate of *Fusarium oxysporum* f. sp. *ciceris* but with more than

30% mortality was susceptible (Table 1). However 5 lines 99109, 99112, 99105 and 99104, out of 31 lines having zero percent mortality were found to be highly resistant during water culture and culture filtrate screening. Only one line 1040/99 was moderately resistant, all other lines were highly susceptible. There are several other reports where culture filtrate and water culture were used for the selection of disease resistant plant (Behnke, 1979; Nene *et al.*, 1981; Frane *et al.*, 1991; Alam, 1994). Chickpea varieties/lines showing different levels of resistance/susceptibility against *Fusarium* wilt.

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