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Screening for resistance of chickpea genotypes against Fusarium wilt (*Fusarium oxysporum* f. sp. *ciceris*)

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Abstract
Chickpea (*Cicer arietinum* L.) is a major pulse crop of India, grown in diverse agro-climatic conditions. *Fusarium* wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceris* is one of the highly destructive disease causes up to 90% yield losses depending on unfavorable environmental condition. In the present study, 40 chickpea genotypes were Forty genotypes of Desi and Kabuli procured from ICAR-IIPR, Kanpur, India and screened by artificial inoculation, at Department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, U.P. Study suggested that a considerable variation among the genotypes for disease reaction. Under pot culture, reaction of genotypes against the *Fusarium* wilt varied from resistant Based on result obtained in pot culture four genotypes DCP 92-3, IPC 14-28, IPC 13-70 and IPC 05-28 were showed resistant reaction with 0-10 per cent disease incidence of Fusarium whereas, whereas five genotypes *viz.*, IPC 10-72, IPC 10-217, IPC 11-30, IPC 12-108 and IPC 11-12 were showed moderately resistant reaction with 11-20 per cent disease incidence under sick pot condition. Rest all the tested genotypes showed tolerant to highly susceptible with 21-100 disease incidence. This screening of genotypes will further help to utilize in the crop improvement programmer.

Keywords: *Fusarium oxysporum* f. sp. *ciceri* (Padwick), Disease resistance, Wilt incidence

1. Introduction
Chickpea (*Cicer arietinum* L.) is a major pulse crop of India, grown in diverse agro-climatic conditions. It is also known as Gram, Bengal gram, Chana and Garbanzo bean. India is largest producer of chickpea in world, sharing 65.25 per cent in area and 65.49 per cent in production. In India, chickpea is grown on 9.85 million ha area with production 11.99 million tonnes and productivity 1217 kg/ha. In India, more than 90 per cent of gram is produced by the states of Madhya Pradesh, Maharastra, Rajasthan, Karnataka, Uttar Pradesh, Andhra Pradesh, Gujarat, Jharkhand, Chhattisgarh and Telangana contributes about 93 per cent towards the total acreage. The production of chickpea in Uttar Pradesh is 0.84 million tonnes with productivity of 1376 kg/ha, which covered nearly 0.61 million ha of area. Uttar Pradesh contributes about 7.01 per cent share in total production and 6.20 per cent of total acreage of country.
Several biotic and abiotic factors are responsible for low productivity of chickpea. According to a survey conducted in 55 countries of the world in the year 1995, the number of pathogens causing various diseases in chickpea had been reported to be 172, which include 67 fungi, three bacteria, 22 viruses and phytoplasma and 80 nematodes (Nene *et al.*, 1996) [12]. Among, *F. oxysporum* f. sp. *ciceri* causing wilt of chickpea is major concern to legume pathologists and breeders due to its impact on economic chickpea production. *Fusarium* wilt caused by *Fusarium oxysporum* Schlecht emend Snyd and Hans. f. sp. *ciceri* (Padwick) was first described by Padwick in 1940 [13]. It is an economically important disease having potential of causing tremendous losses in chickpea. Wilt of chickpea was first reported by Butler (1918) [3] from North-west province of undivided India in 1906-07. The wilt disease of chickpea was reported in Uttar Pradesh, Bihar and Burma in 1922 by Perl and Mckerral (1923) [10]. The fungus is both seed and soil-borne and may survive in soil for up to six years even in the absence of the host (Haware *et al* 1986) [9]. The primary infection is through chlamydospores or mycelia. The pathogen survives well in roots and stem; even in apparently healthy looking plants growing among diseased ones harbouring enough fungus.

The continuous application of systemic fungicides is not a permanent solution to complete eradication of disease from infected site despite often development of wilt resistance pathotypes (Haseeb 2014) [7]. To overcome this major limitation development of resistant varieties of chickpea is one of the sustainable alternative approaches for the management of this disease. Therefore, current development of wilt resistant cultivar, conservation and screening of genotypes against specific pathotype are most important steps for sustainable farming. Furthermore, many agricultural management practices are inadequate which are mainly depending on intensive use of fungicide and finally not able to reduce severity of soil borne disease. In the past, rigorous work was initiated on host plant resistance for economic management of this disease (Haware, 1990 [8]). However, deployment of resistant varieties was not extensive due to undesirable agronomical character associated with wild donor parent of chickpea as well as high degree of pathogenic variability among the population of *F. oxysporum* f. sp. *ciceri* (Dubey *et al*., 2012) [6]. These condition possess difficulties among farmers as well as research community for getting maximum yield of this crop. Therefore, present study was undertaken with the hypothesis to identify resistance cultivar of chickpea against wilt disease collected from diverse genetic resource.

2. Materials and Methods

2.1 Isolation, purification and identification of *Fusarium oxysporum* f. sp. *ciceris*

Infected chickpea plants showing characteristics symptom were collected from CRC of Sardar Vallabhbhai Patel University of agriculture and technology, Meerut, U.P. The diseased samples were carefully placed in polythene bags, properly tagged and brought to the laboratory and subjected to microscopic examination and tissue isolation. The infected samples were washed with running tap water to remove soil particles and then cut into small bits with the help of a sterilized scalpel, about 5mm size involving healthy as well as diseased portion from root portions showing characteristic diseased symptoms like browning of vascular tissue. The tissue bits were surface sterilized with 1 per cent sodium hypochlorite solution for 40-60 seconds followed by rinsing twice in sterilized distilled water to remove traces of sodium hypochlorite. These surface sterilized pieces were transferred on to sterilized tissue paper and allowed to air dry for two minutes. Later on, four tissue bits were transferred on Potato Dextrose Agar (PDA) in Petri plates under aseptic conditions. Plates were incubated at 26±2 ˚C in BOD incubator for 3 to 4 days. Early growing fungal mycelia was transferred to another PDA plats and allowed to grow for next seven days at 26±2 ˚C.

The culture was further purified by growing hyphal tips produced on such plates and maintained on PDA slants for further use. The pathogen was identified as *F. oxysporum* f. sp. *ciceri* based on morphological characteristics. Pathogensity was demonstrated for the isolated pathogen. The pathogen was sub-cultured at monthly intervals and maintained at 4 ˚C in a refrigerator.

2.2 Evaluation of chickpea genotypes for resistance against *Fusarium* wilt

Forty genotypes of Desi and Kabuli procured from ICAR-IIPR, Kanpur were screened separately for host plant resistance against *Fusarium* wilt in pot at Department of plant pathology, Sardar Vallabhbhai Patel University of agriculture and technology, Meerut. The inoculums of *F. oxysporum* f. sp. *ciceri* at 25 g kg^{-1} were thoroughly mixed with autoclaved soil and filled in pot previously surface sterilized by five per cent sodium hypochlorite solution. These pots were washed and incubated for ten days. On seventh day, 10 seeds of susceptible each genotypes of chickpea which were sterilized with one per cent sodium hypochlorite solution for two minutes and washed with distilled water were sown in each pot with three replications. In case of control, chickpea seeds were sown in pots containing uninoculated soil.

Observations on per cent wilt incidence were recorded from seedling stage up to pod initiation stage in field condition and up to 40 DAS in pot condition.

The following formula used to calculate wilt disease incidence

$$\text{Per cent disease incidence} = \frac{\text{Number of plants wilted}}{\text{Total number of plants observed}} \times 100$$

The chickpea genotypes were later grouped into different categories of resistance and susceptibility based on grading scale used in All India Coordinated Research Project on Chickpea [11] from highly susceptible to Resistant. Data regarding wilt incidence was computed according to grades of resistance (Table 1).

Reaction	Per cent wilt incidence
Resistant	0 - 10
Moderately resistant	11 - 20
Tolerant	21 - 30
Susceptible	31 - 50
Highly susceptible	>50

3. Result and Discussion

To locate sources of host resistance against the *Fusarium* wilt pathogen, a set of 40 chickpea genotypes procured from ICAR-IIPR, Kanpur were screened against *Fusarium* wilt disease in wilt sick pot tray. The genotypes were screened at starting from 7 days to 40 days after sowing. On the basis of per cent diseases incidence, disease reaction categories were assigned to each genotypes (Table 2). All 40 genotypes were showed a range of 6.67-100 per cent wilt incidence (Table 1). Among four genotypes *viz.*, DCP 92-3, IPC 14-28, IPC 13-70 and IPC 05-28 were showed resistant reaction with 0-10 per cent disease incidence whereas five genotypes *viz.*, IPC 10-72, IPC 10-217, IPC 11-30, IPC 12-108 and IPC 11-12 were showed moderately resistant reaction with 11-20 per cent disease incidence. Six genotypes *viz.*, IPC 15-165, IPC 15-267, IPC 16-107, IPC 10-142, IPC 11-28 and IPC 97-29 were showed tolerant reaction with 21-30 per cent disease incidence and eleven genotypes viz., IPC 06-77, IPC 14-10, IPC 10-134, IPC 10-62, IPC 11-247, IPC 15-133, IPC 18-52, IPC 07-28, IPC 04-98, IPC 11-112 and IPC 04-52 showed susceptible reaction with 31-50 percent diseases incidence whereas fourteen genotypes *viz.*, ICC 244263, IPC 15-12,

IPCK 15-17, IPC 15-127, IPC 05-62, IPC 04-01, IPC 13-74, IPC 14-120, IPC 07-100, IPC 12-49, SA 1, IPC 13-33, ICC 5434 and IPC 14-51 were showed highly susceptible reaction with more than 50 percent disease incidence. The results concord with the study of Nathawat *et al.* (2018) [18] evaluated 60 lines of chickpea revealed that none of the entries was found completely free from the wilt disease. However, 9 entries viz., CSJK-54, CSJ-515, GNG-1581, GNG-2207, GNG-2226, H-11-41, PG-0109, IPC-2010 72, IPc-2010-112 were categorized as resistant showed disease incidence of 1–10 per cent. While, 13 entries showed moderately resistant reaction (10.1–20 per cent) rest of the genotypes exhibited susceptible (30.1–50 per cent) to highly susceptible (above 50 per cent) reactions to the pathogen. Yadav and Kumar (2019) [14] also screened chickpea genotypes against *Fusarium* wilt and classified genotypes into different disease reaction based on wilt incidence. Earlier workers Bajwa *et al.* 2000 [2]; Zope *et al.* 2002 [17]; Chaudhary *et al.* 2007 [4]; Tripathi *et al.* 2007 [15]; Dubey and Birendra Singh, 2008 [5]; Ved Ratan and Biswas, 2010 [16]. The present findings are agreeable with these works in categorizing genotypes into different disease reaction.

Table 1: Screening of chickpea genotypes against *Fusarium* wilt in sick pot condition

S. No.	Genotypes	Total plant	Wilted plant	Percent wilt incidence	Reaction
1.	IPC 15-165	30	08	26.67	Tolerant
2.	IPC 15-267	30	09	30.00	Tolerant
3.	IPC 06-77	30	12	40.00	Susceptible
4.	ICC 244263	30	19	63.33	Highly susceptible
5.	IPC 14-10	30	15	50.00	Susceptible
6.	IPC 15-12	30	22	73.33	Highly susceptible
7.	IPCK 15-17	30	24	80.00	Highly susceptible
8.	IPC 10-134	30	13	43.33	Susceptible
9.	IPC 15-127	30	21	70.00	Highly susceptible
10.	IPC 10-72	30	6	20.00	Moderately resistant
11.	IPC 16-107	30	10	30.00	Tolerant
12.	IPC 10-62	30	12	40.00	Susceptible
13.	IPC 05-62	30	17	56.66	Highly susceptible
14.	IPC 10-142	30	7	23.33	Tolerant
15.	IPC 11-247	30	15	50.00	Susceptible
16.	IPC 10-217	30	5	16.67	Moderately resistant
17.	DCP 92-3	30	2	6.67	Resistant
18.	IPC 15-133	30	11	36.67	Susceptible
19.	IPC 11-28	30	09	30.00	Tolerant
20.	IPC 11-30	30	5	16.67	Moderately resistant
21.	IPC 14-28	30	2	6.67	Resistant
22.	IPC 13-70	30	3	10.00	Resistant
23.	IPC 12-108	30	6	20.00	Moderately resistant
24.	IPC 18-52	30	14	46.66	Susceptible
25.	IPC 04-01	30	30	100.00	Highly susceptible
26.	IPC 13-74	30	28	93.33	Highly susceptible
27.	IPC 14-120	30	24	80.00	Highly susceptible
28.	IPC 07-100	30	30	100.00	Highly susceptible
29.	IPC 12-49	30	25	83.33	Highly susceptible
30.	SA 1	30	28	93.33	Highly susceptible
31.	IPC 13-33	30	23	76.67	Highly susceptible
32.	ICC 5434	30	19	63.33	Highly susceptible
33.	IPC 07-28	30	14	46.67	Susceptible
34.	IPC 14-51	30	22	73.33	Highly susceptible
35.	IPC 04-98	30	15	50.00	Susceptible
36.	IPC 11-112	30	11	36.67	Susceptible
37.	IPC 97-29	30	8	26.67	Tolerant
38.	IPC 05-28	30	2	6.67	Resistant
39.	IPC 11-12	30	5	16.67	Moderately resistant
40.	IPC 04-52	30	12	40.00	Susceptible
41.	JG-62 (check)	30	30	100	Highly susceptible

Table 2: Disease reaction of chickpea genotypes against *Fusarium* wilt

S. No.	Disease Reaction	% Disease Incidence	Total no. of Genotypes	Chickpea genotypes
1.	Resistant	0-10	04	DCP 92-3, IPC 14-28, IPC 13-70, IPC 05-28
2.	Moderately resistant	11-20	05	IPC 10-72, IPC 10-217, IPC 11-30, IPC 12-108, IPC 11-12
3.	Tolerant	21-30	06	IPC 15-165, IPC 15-267, IPC 16-107, IPC 10-142, IPC 11-28, IPC 97-29
4.	Susceptible	31-50	11	IPC 06-77, IPC 14-10, IPC 10-134, IPC 10-62, IPC 11-247, IPC 15-133, IPC 18-52, IPC 07-28, IPC 04-98, IPC 11-112, IPC 04-52
6.	Highly susceptible	>50	14	ICC 244263, IPC 15-12, IPCK 15-17, IPC 15-127, IPC 05-62, IPC 04-01, IPC 13-74, IPC 14-120, IPC 07-100, IPC 12-49, SA 1, IPC 13-33, ICC 5434, IPC 14-51

4. Conclusion

In the current study, an effort was made to screen 40 different genotypes of chickpea against *Fusarium* wilt in a sick pot. Four of the forty genotypes of chickpeas showed resistance to *F. oxysporum* f. sp *ciceri*. Five moderately resistant genotypes were also found by our investigation. The identified resistant genotypes may be utilized in future chickpea improvement programmers.

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6. Conflicts of interest

We solemnly declare that there is no conflict of interest to publish this manuscript.

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