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Screening of Chickpea Genotypes for Resistance against *Fusarium* Wilt

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Authors' contributions

This work was carried out in collaboration among all authors. Author SS designed the study. Authors SK and SS performed the work, statistical analysis, wrote the protocol and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Chickpea (*Cicer arietinum*) is one of the world's major legume crops and suffers substantial damage from wilt disease incited by *Fusarium oxysporum* f. sp. *ciceri* (Padwick) with yield loss over 60 per cent. The screening for new resistance chickpea genotypes against this disease is an alternative approach to avoid indiscriminate use of chemical pesticides. In this study 55 chickpea genotypes were screened against *Fusarium* wilt. Out of 55 chickpea genotypes studied, only one genotype was found to be resistant and 12 were found to be moderately resistance. Nineteen genotypes showed moderately susceptible. However, nineteen and four genotypes showed susceptible and highly susceptible reaction for wilt disease, respectively.

Keywords: Chickpea (Cicer arietinum); Fusarium oxysporum f. sp. ciceri (Padwick); disease resistance; wilt incidence.

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1. INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the important legume crops grown in the Mediterranean basin and world-wide [1]. It is third pulse crop in the world after dry bean (*Phaseolous vulgaris* L.) and dry pea (*Pisum sativum* L.) [2]. Chickpea is member of sub-family *Papilionaceae*, family *leguminaceae* and originated in the Middle East and subsequently spread over 45 countries with arid, semi-arid and sub-tropical environments. Chickpea is valued for its nutritive seed composition which is high in protein content and used increasingly as a substitute for animal protein [3]. It has ability to fix nitrogen and enrich the soil [4]. Chickpea is also a good source of minerals such as Ca, P, Mg, Fe, K and β-carotene.

India accounts for approximately 75 percent of global chickpea production. Chickpea contributes about 67 per cent to *Rabi* pulse production and 46 per cent of total production of India. It occupies an area of about 8.35 million hectare with annual production of 7.17 million tons with productivity of 859 Kg per hectare (Directorate of economics and statistics, 2015-16). Many factors contribute towards chickpea's low yield but the pathological constraints are the most important. Chickpea wilt caused by *Fusarium oxysporum* Schlechtend Fr. f. sp. *ciceris* (Padwick) is the most important soil-borne disease of chickpea throughout the world and particularly in the Indian Sub-continent, the Mediterranean Basin and California [5,6,7]. *F. oxysporum* f. sp. *ciceri* may survive in soil and on crop residues as chlamydospores for up to six years in the absence of susceptible host and spread by means of both soil and infected seed. Attacks of the *Fusarium* wilt pathogen can destroy the crop completely or cause a significant annual yield loss, especially in low rainfall regions which is a permanent threat to the chickpea causing wilt syndrome. *F. oxysporum* f. sp. *ciceri* produce mycotoxins. *Fusarium* wilt of chickpea is prevalent in almost all chickpea-growing areas of the world and its incidence varies from 14 to 32 % in the different states of India [8]. This disease causes yield losses up to 100 % under favorable conditions [9,10].

Management of this pathogen is currently being carried out by use of chemical fungicides. Although fungicides have shown promising results in controlling the pathogen; phytotoxicity and fungicidal residues along with environmental contamination and human health hazards prevents their large-scale use. Therefore, identification of chickpea genotypes against *Fusarium* wilt through screening in natural environmental condition is an attractive way to control this disease in eco-friendly manner.

2. MATERIALS AND METHODS

2.1 Identification of Chickpea Genotypes for Resistance against *Fusarium* Wilt

Studies were undertaken to identify the resistance of chickpea germplasms against *Fusarium* wilt. Field experiments were conducted during *Rabi,* 2017-18 in the chickpea wilt sick plot of Tirhut College of Agriculture, Dholi, Muzaffarpur (Dr. Rajendra Prasad Central Agricultural University, Bihar, Pusa, Samastipur) during the year 2017-18. A total of 55 Chickpea genotypes, collected from All India Coordinated Research Project on Chickpea, T.C.A., Dholi were assessed for their reaction against Chickpea wilt in the wilt sick plot by infector row technique in an augmented design having two replications by planting 2 rows of two test entries each, alternated with one row of JG 62 as susceptible check and spreader. Each test entry was planted in a row 4 meter in length with row to row distance 30 cm and plant to plant distance 10 cm. General cultural practices were adopted to maintain the experiment except that fungicide sprays were not applied in order to encourage the pathogen. Disease observations on wilt incidence were recorded from seedling stage to maturity at 15 days interval. The plots were periodically observed for number of wilted plants and at reproductive stage, data on wilted plants of test entries were computed at 100% killing of the susceptible check. The percent wilt incidence of each test entry was calculated by the following formula:

Wilt Incidence = Number of plants wilted/ Total number of plants* 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).

2.2 Isolation and Purification of Causal Organism of Chickpea Wilt

The diseased samples of chickpea showing typical wilt symptoms were collected in *Rabi* season of 2016 from a chickpea wilt sick plot, TCA., Dholi. The diseased samples were carefully placed in polythene bags, properly tagged and brought to the laboratory and subjected to microscopic examination and tissue isolation. Infected chickpea plant showing typical wilt symptoms were used to isolate the pathogen. These infected aerials parts were thoroughly washed in running tap water to remove the adhering soil. These were then cut into small pieces with the help of a sterilized scalpel, washed in sterilized water, surface sterilized by dipping in 0.1 per cent mercuric chloride ($HgCl_2$) for 30 seconds rinsed thrice in sterilized distilled water and transferred onto Potato Dextrose Agar (PDA) medium in Petri plates. The plates were incubated at 25±1ºC for growth. 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. Koch's postulate was demonstrated for the isolated pathogen. The pathogen was sub-cultured at monthly intervals and maintained at 4ºC in a refrigerator.

Table 1. Grades of resistance

Category	Per cent disease incidence
Resistance	0 to 10
Moderately resistant	10.1-20
Moderately susceptible	20.1-30
Susceptible	30.1-50
Highly susceptible	Above 50

3. RESULTS

3.1 Screening of Chickpea Genotypes for Resistance against *Fusarium* Wilt

To locate sources of host resistance against the *Fusarium* wilt pathogen, a total fifty five chickpea genotypes, collected from AICRP on Chickpea, T.C.A., Dholi were assessed in a wilt sick plot during *Rabi,* 2017-18. The mortality of the susceptible check (JG 62) in this season was 100%, indicating the uniformity of inoculum in the wilt sick plot. Isolations of *Fusarium* wilt pathogen from dead plants indicated that mortality was due to *Fusarium* wilt. The results revealed that there was huge variation exist among genotypes studied. All the studied genotypes were grouped into different categories of resistance and susceptibility based on grading scale used in AICRP on Chickpea [11] (Table 1). Results of disease reaction of genotypes during *Rabi,* 2017-18 are presented in Table 2 and Table 3. In the present study, the wilt disease incidence varied from 9.13 to 100 per cent. The minimum disease incidence was observed in KWR 108 whereas, JG 62 showed maximum disease incidence. Based on this study, only one entry KWR 108 was classified as resistant as it exhibited 9.13% wilt incidence. Twelve genotypes i.e BG 3075, NBeG 776, PG 170, BG 3076, GJG 1403, AKG 1303, CSJ 866, H 12-1, Phule G 0818, H 13-36, H 13-03 & Pusa 256 were found to be moderately resistant with wilt disease incidence ranging from 11.20% (BG 3076) to 19.79 % (BG 3075). Nineteen genotypes i.e. GNG 2300, DCP 92-3, H 12-26, RKG 13-380, PG 177, CSJ 907, IPC 2013-21, GNG 2300, BDNG 2015-1, PG 214, RKG 13-75, PG 172, RG 2011-02, IPC 2012-108, GJG 1416, WR315, PG 158, JG315, & BG 372, were found to be moderately susceptible against wilt disease. The disease incidence of moderately susceptible genotypes ranges from 20.81% (RKG 13-75 and BG 372) to 29.30% (BDNG 2015-1). Nineteen genotypes like GJG 1318, GCP 101, AKG 1109, NDG 15-6, GL 13042, GNG 2325, H 12-63, BRC 3, Phule G 0405, Phule G 0819, NBeG 738, JG 2016-44, GJG 1320, GNG 2264, NBeG 807, RVSSG 42 and BRC-1 were found to be susceptible for wilt disease and showed more than 30% wilt incidence.

4. DISCUSSION

Chickpea (*Cicer arientinum* L.) is a premier *rabi* season pulse crop of the Indian subcontinent. It is grown in semi-arid and tropical climate. It originated from middle east and now grown in 45 countries across the world. The major states producing chickpea are Madhya Pradesh, Uttar Pradesh, Bihar and Maharashtra. Vascular wilt caused by *F. oxysporum* f.sp. *ciceri* is one of the most important disease of chickpea worldwide and considered as most devastating for the production of chickpea [12].

The present investigation was undertaken to identify chickpea genotypes for resistance against wilt induced by *F. oxysporum* f.sp. *ciceri* (Padwick) which is widely prevalent in moderate to high severity in different parts of Bihar. A plethora of reports showed the existence of resistant chichpea genotypes against wilt disease [13,14,15]. However the pathogen also

evolves to overcome host resistance over time [16] and can vary by location, which makes re-evaluation important. On the basis of per cent wilt incidence, the genotypes were categorized

Table 2. Screening of chickpea genotypes against *Fusarium* wilt during *Rabi*, 2017-18

Sl. No.	Entries	*Rabi*, 2017-18			
		Wilt incidence (%) ‡			Disease reaction
		R_1	R_2	Average	
1	GJG 1318	45.00	16.67	30.83	S
2	GNG 2300	25.93	23.91	24.92	MS
3	GCP 101	64.00	15.00	39.50	S
4	DCP 92-3	37.50	13.04	25.27	MS
5	AKG 1109	45.45	22.86	34.16	S
6	BG 3075	33.33	6.25	19.79	MR
7	NBeG 776	13.64	20.00	16.82	MR
8	H 12-26	36.36	13.89	25.13	MS
9	PG 170	12.90	17.39	15.15	MR
10	NDG 15-6	47.46	48.00	47.73	S
11	GL 13042	30.00	50.00	40.00	S
12	RKG 13-380	32.00	19.35	25.68	MS
13	GNG 2325	34.62	40.54	37.58	S
14	PG 177	36.36	20.00	28.18	MS
15	BG 3076	17.14	5.26	11.20	MR
16	H 12-63	55.17	31.58	43.38	S
17	BRC 3	35.71	28.13	31.92	S
18	Phule G 0405	32.00	56.25	44.13	S
19	CSJ 907	36.36	20.00	28.18	MS
20	Phule G 0819	30.00	50.00	40.00	S
21	JG 2016-43	44.44	65.71	55.08	HS
22	IPC 2013-21	25.81	30.30	28.05	MS
23	NBeG 738	40.00	41.18	40.59	S
24	GJG 1403	20.69	13.89	17.29	MR
25	JG 2016-44	51.52	38.71	45.11	S
26	GNG 2300	30.43	26.09	28.26	MS
27	GCP 105	59.52	43.75	51.64	HS
28	BDNG 2015-1	34.78	23.81	29.30	MS
29	GJG 1320	61.11	20.83	40.97	S
30	PG 214	23.08	19.35	21.22	MS
31	AKG 1303	12.50	10.00	11.25	MR
32	CSJ 866	0.00	30.43	15.22	MR
33	RKG 13-75	13.04	28.57	20.81	MS
34	GNG 2264	47.83	34.78	41.30	S
35	H 12-1	10.26	20.00	15.13	MR
36	PG 172	25.00	22.22	23.61	MS
37	NBeG 807	18.75	43.48	31.11	S
38	RG 2011-02	24.24	27.27	25.76	MS
39	RVSSG 42	26.67	37.50	32.08	S
40	IPC 2012-108	33.33	25.00	29.17	MS
41	BRC-1	32.00	30.00	31.00	S
42	GJG 1416	32.43	22.50	27.47	MS
43	Phule G 0818	16.67	21.43	19.05	MR
37	GL 13001	54.29	57.50	55.89	HS
45	H 13-36	17.50	17.50	17.50	MR
46	BGD 138	45.00	36.36	40.68	S
47	H 13-03	22.22	12.00	17.11	MR
48	C 235	46.43	43.59	45.01	S
49	WR315	29.03	22.58	25.81	MS
50	PG 158	17.39	37.93	27.66	MS
51	JG315	15.38	34.38	24.88	MS
52	Pusa 256	16.00	14.29	15.14	MR
53	BG 372	13.04	28.57	20.81	MS
54	KWR 108	7.14	11.11	9.13	R
55	JG-62 (S-check)	100.00	100.00	100.00	HS

‡: Means, R: Resistance, MR: Moderately resistance, MS: Moderately Susceptible, S: Susceptible, HS: Highly susceptible

Table 3. Disease reaction of chickpea genotypes against *Fusarium* wilt during *Rabi*, 2017-18

Genotypes	Total no. of genotypes	Disease reaction
KWR 108	1	R
BG 3075, NBeG 776, PG 170, BG 3076, GJG 1403, AKG 1303, CSJ 866, H 12-1, Phule G 0818, H 13-36, H 13-03, & Pusa 256	12	MR
GNG 2300, DCP 92-3, H 12-26, RKG 13-380, PG 177, CSJ 907, IPC 2013-21, GNG 2300, BDNG 2015-1, PG 214, RKG 13-75, PG 172, RG 2011-02, IPC 2012-108, GJG 1416, WR315, PG 158, JG315, & BG 372	19	MS
GJG 1318, GCP 101, AKG 1109, NDG 15-6, GL 13042, GNG 2325, H 12-63, BRC 3, Phule G 0405, Phule G 0819, NBeG 738, JG 2016-44, GJG 1320, GNG 2264, NBeG 807, RVSSG 42, BRC-1, BGD 138, & C 235	19	S
JG 2016-43, GCP 105, GL 13001, & JG-62	4	HS

R: Resistance, MR: Moderately resistance, MS: Moderately Susceptible, S: Susceptible, HS: Highly susceptible BGD 138, & C 235. However, four genotypes, JG 2016-43, GCP 105, GL 13001, & JG-62 showed highly susceptible reaction exhibited more than 50% wilt incidence

as resistant, moderately resistant, susceptible and highly susceptible. Similar studies were made by Zote, et al. [17] who studied sources of resistance to chickpea wilt and reported that none of the 42 lines of *Cicer arietinum* tested in a wilt sick plot infested with *F. oxysporum* f. sp. *ciceri* were highly resistant, 4 developed less than 10% and 6 others less than 29% disease. While, Kumar, et. al. [18] who screened one hundred one genotypes of chickpea for resistance to *Fusarium* wilt disease caused by *Fusarium oxysporum* f. sp. *ciceri* during *Rabi,* 2014-15. It was observed that 57 lines were resistant, 28 were tolerant while 16 were susceptible to the wilt disease at seedling stage. Whereas, 31 genotypes were resistant, 26 were tolerant and 44 were susceptible at reproductive stage. Cultivation of resistant cultivars is the most effective and economical way of controlling the disease [19]. The current study was conducted to identify resistant cultivars against the prevalent isolate of wilt existing in this area. The genotypes that showed resistance or moderately resistance are most suitable for exploitation in breeding programs for the development of resistant cultivars against wilt or for direct sowing in wilt prone areas. This study provides us valuable information about the resistance sources, which exist in the country collection of chickpea germplasm against a virulent isolate of *F. oxysporum* f. sp *ciceri* in India.

5. CONCLUSION

In present investigation an attempt was made to screen 55 chickpea genotypes against *Fusarium* wilt in chickpea wilt sick plot. Out of fifty five chickpea genotypes, only one entry KWR 108 showed resistance against *F. oxysporum* f. sp. *ciceri*. Our investigation also identified 12 moderately resistance genotypes. The identified resistance genotypes may be used in further chickpea improvement programme.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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