

Diversity of chickpea germplasm against wilt disease caused by *Fusarium oxysporum* f. sp. *ciceris*, Race 2

PR SAABALE*¹, MANJUNATHA L², RK MISHRA², NAIMUDDIN² and DN GAWANDE

ABSTRACT

A total of 123 'desi' chickpea germplasm were evaluated against *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *ciceris* (Padwick) Matuo & K. Sato (race 2) under artificial epiphytotic conditions during 2015-16 and 2016-17. During 2015-16, 22 genotypes were found resistant, 11 moderately resistant, 17 susceptible and 73 highly susceptible. The percent incidence in susceptible check was recorded 100 percent and in resistant check it was less than 10 percent. The local severity index (LSI) was 55.2 percent at physiological maturity stage of the crop. In a total, 17.8 percent germplasm accessions revealed resistance reaction (<10% disease incidence) and 8.9 percent revealed moderately resistant reaction (10.1 to 20%). Based on previous year observations, 22 resistant and 11 moderately resistant lines were further evaluated in 2016-17. Based on pooled data of two years, 12 lines viz., EC-267154, EC-267308, EC-489845, EC-489882, EC-489919, EC-489991, EC-498818, IC-83523, IC-83538, IC-83539, IC-83748 and IC-83757 were found resistant to the disease. These identified resistant lines can be exploited for breeding programs to develop resistant varieties.

Key words: Diversity, *Fusarium*, Germplasm, Race 2, Resistance, Wilt

¹Indian Institute of Pulses Research, Regional Centre, Dharwad-580005, Karnataka; ²Indian Institute of Pulses Research, Kanpur-208024, Uttar Pradesh;

*E-mail: sparashu@gmail.com

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Dr. P. Jayamani, Tamil Nadu Agricultural University, Coimbatore

Chickpea (*Cicer arietinum* L.) is the world's third most important pulse crop known for its nutritive value in all tropical and subtropical countries. It plays vital role in human and animal nutrition (20-26% dietary proteins) and increases soil fertility by fixing atmospheric nitrogen. India share is approximately 70% of world chickpea production with an area of 9.63 million ha with 9.38 million tons production and an average productivity 974 kg/ha (Anonymous, 2018). The production of chickpea is largely affected by many biotic and abiotic factors. Amongst biotic stresses, *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *ciceris* (Padwick) Matuo & K. Sato, is one of the major causes for low productivity in chickpea (Haware and Nene, 1982a). The disease occurs in all chickpea growing states and is estimated to cause an annual yield loss of 10-15% (Singh and Dahiya, 1973), but under favorable conditions, yield loss up to 100 per cent has been recorded (Halila and Strange, 1996). Moreover, losses caused by *Fusarium* wilt depend upon the stage of the crop infected; early wilting causes 77-94% losses while late wilting causes 24-65% (Haware and Nene, 1980). The disease can occur at all stages of plant growth but high incidence occurs at flowering and podding stages. The pathogenicity test indicates that *Fusarium* wilt of chickpea exhibits two pathotypes viz., yellowing and wilting pathotype (Trapero-Casas and Jimenez-Diaz, 1985). Based on a

set of differential chickpea cultivars eight races (races 0, 1A, 1B/C, 2, 3, 4, 5, and 6) of pathogen have been reported worldwide. Among them, 0 and 1B/C cause yellowing syndrome and 1A, 2, 3, 4, 5, and 6 cause wilting syndrome (Jimenez - Diaz *et al.*, 1993). Races 1A, 2, 3 and 4 have been reported from India (Haware and Nene, 1982b). Recently, a change in race scenario in Indian populations of *Foc* has been reported by Dubey *et al.* (2012) on a new set of chickpea differentials wherein he reported existence of all 8 races. Currently, though chickpea wilt can be managed to some extent by cultural practices and chemical seed treatment methods, host resistance can offer complete protection and is an economical and eco-friendly strategy for management of chickpea wilt (Govil and Rana, 1994). Consequently, considerable efforts have been made across the globe to identify resistance sources against wilt (Pande *et al.* 2006, Sharma *et al.* 2012, Saabale *et al.* 2017 and Saabale *et al.* 2019) and several are being utilized in breeding programs. But, periodical change in the virulence of pathogen seriously curtailed host plant resistance longevity due to genetic breakdown thus making it imperative to continuously search for resistant sources. In present study, evaluation of germplasm lines of chickpea was undertaken to identify stable wilt resistant sources against *Fusarium* wilt (race 2) in an artificially developed sick plot.

MATERIAL AND METHODS

Seed Source: A total of 123 'desi' chickpea germplasm lines collected from NBPGR, New Delhi, India were evaluated against wilt disease caused by *F. oxysporum* f. sp. *ciceris* (race 2) in sick plot under artificially inoculated conditions during 2015-16 and 2016-17. JG 62 (Susceptible check) and JG 315 (Resistant check) were obtained from Division of Crop Protection, ICAR-IIPR, Kanpur, India.

Wilt sick plot: The Sick plot is located at 26.49° north latitude and 80.27° east longitude. The soil type is alluvial with a pH 8.0 and area receives an annual average rainfall of 880 mm. The *Fusarium* wilt sick plot of size 3000 m² is located in North East Plane Zone (NEPZ) of India against *F. oxysporum* f. sp. *ciceris* race 2. It contains threshold inoculum level (~7000 CFU/g of soil) and is designated to evaluate entries of All India Coordinated Research Project (AICRP) for NEPZ region. Threshold of inoculum was maintained by adding inoculum prepared in laboratory on sorghum grains using dynamic culture of *Foc* race 2.

Sickplot evaluation of chickpea germplasm for wilt resistance: A total of 123 chickpea germplasm lines were evaluated under artificial epiphytotic conditions during 2015-16. The promising lines identified were revalidated during 2016-17 for confirmation. The experiment was laid in a randomized complete block design (RCBD) with two replications. Each genotype was planted in 2 rows of 4 m length with row to row spacing of 30 cm and plant to plant distance of 10 cm. The chickpea genotype JG 62 and JG 315 were used as susceptible and resistant check respectively at every 10 test rows as indicators.

The observations were recorded on wilt starting from seedling to maturity of the crop. Cumulative percent wilt incidence of each test genotype was calculated using the formula as: Disease incidence (%) = (Number of infected plants/ Total number of plants) x 100.

The test genotypes were grouped as resistant (0-10% incidence), moderately resistant (10.1-20% incidence), susceptible (20.1-50%) and highly susceptible (>50.0 % mortality) based on disease rating scale given by Sharma *et al.* (2012). Local Severity Index (LSI) indicates severity of a disease at a location and was calculated by using formula LSI (%) = (Total wilt incidence in a given location/total number of test entries) x 100.

RESULTS AND DISCUSSION

Fusarium wilt is an important disease in all chickpea growing agro-climatic zones of India.

Cultivation of resistant varieties is an only effective and economically viable option for management of wilt disease (Jimenez-Diaz *et al.* 1993). In the present study, phenotyping of 123 germplasm lines against *Fusarium* wilt was undertaken in a sick plot having the threshold inoculum level of ~7000 Colony Forming Units (CFU) per gram of soil. In previous study, Foc population 1795±253 CFU/g of soil was reported to be adequate for efficient screening (Halila and Strange, 1997). As a result, many international and national institutes developed sick plots (Nene *et al.* 1981; Jimenez-Diaz *et al.* 1991) for large scale screening of chickpea germplasm lines.

In the present study, 123 lines exhibited wide variation for *Fusarium* resistance and disease incidence varied from 0 to 100 percent. Among 123 lines, 22 genotypes showed resistant, 11 were moderately resistant, 17 were susceptible and 73 were showed highly susceptible reaction. These genotypes may be divided into early wilting and late wilting genotypes. Percentage of early wilting ranged between 1.1 to 90.4 percent and late wilting ranged between 1.4 to 100 percent (Table 1). At seedling stage, 30 genotypes were sensitive, however, at flowering and podding stage 56 genotypes appeared to be sensitive. Similarly, previous workers also reported variation in wilt resistance at seedling and reproductive stage (Haware *et al.* 1996). High temperature coupled with moisture stress might play an important role in the development of the disease during reproductive stage. Consequently, most of the genotypes were resistant during seedling stage. The susceptible check exhibited 100 percent wilt incidence within 40 days after sowing and a local severity index (LSI) of 55.2 percent indicating that screening was reliable. In the current study, 17.8 per cent germplasm accessions revealed a resistance reaction (<10% disease incidence) at physiological maturity stage against race 2. Similarly, in earlier studies, a small fraction of land race (ILCs) accessions (5.0 %) collected across the globe were found resistant to *Fusarium* wilt race II (Saable *et al.* 2017). Pande *et al.* (2006) and Haware *et al.* (1992) had reported that only 12 percent of minicore collections and 1.1 percent global collections were resistant to *Fusarium* wilt race I another race.

Based on previous year observations, 22 resistant and 11 moderately resistant lines were selected and were subjected to revalidation for confirmation of resistance. Similar results were obtained during revalidation except for a few lines, which, signifies the differences in response due to genotype. In pooled data, wilt incidence of chickpea varied from 0.7 to 37.6%. Based on data of two years, 12 lines *viz.*,

Table 1. List of germplasm lines evaluated against Fusarium wilt during 2015-16

Genotype	Early wilt (%)	Late wilt (%)	Total wilt (%)	Genotype	Early wilt (%)	Late wilt (%)	Total wilt (%)	Genotype	Early wilt (%)	Late wilt (%)	Total wilt (%)
EC-223519	0.0	8.2	8.2	EC-489913	26.5	57.8	84.3	IC-83529	5.9	11.7	17.7
EC-261136	0.0	72.3	72.3	EC-489919	0.0	5.2	5.2	IC-83530	0.0	8.9	8.9
EC-267154	0.0	4.9	4.9	EC-489928	5.2	46.5	51.8	IC-83534	2.6	14.9	17.5
EC-267240	0.0	47.6	47.6	EC-489930	31.2	48.5	79.7	IC-83535	0.0	18.3	18.3
EC-267261	17.1	50.1	67.2	EC-489950	54.9	45.1	100.0	IC-83536	2.1	97.9	100
EC-267265	9.5	48.4	58.0	EC-489974	0.0	21.5	21.5	IC-83537	2.8	21.6	24.4
EC-267272	16.4	47.7	64.1	EC-489975	0.0	22.7	22.7	IC-83538	0.0	6.5	6.5
EC-267293	1.7	12.9	14.6	EC-489989	19.0	79.6	98.6	IC-83539	1.1	8.8	9.9
EC-267308	0.0	1.5	1.5	EC-489991	0.0	3.1	3.1	IC-83541	0.0	13.2	13.2
EC-267316	1.3	79.2	80.5	EC-489992	45.3	54.7	100.0	IC-83542	0.0	5.1	5.1
EC-267437	0.0	3.0	3.0	EC-490005	30.1	20.0	50.2	IC-83543	10.7	52.5	63.2
EC-267484	47.6	52.4	100	EC-490010	52.7	47.3	100.0	IC-83545	8.7	42.1	50.9
EC-267504	1.8	35.7	37.5	EC-490013	63.6	36.4	100.0	IC-83546	13.3	68.4	81.8
EC-267511	0.0	15.5	15.5	EC-490014	58.1	41.9	100.0	IC-83549	9.7	48.2	57.9
EC-440552	3.8	4.9	8.7	EC-490024	73.5	26.5	100.0	IC-83551	0.0	12.8	12.8
EC-441725	3.2	13.1	16.3	EC-490027	4.1	32.1	36.2	IC-83554	0.0	67.8	67.8
EC-441805	48.6	51.4	100	EC-490028	50.0	45.1	95.1	IC-83555	0.0	71.7	71.7
EC-442202	73.4	26.6	100	EC-490030	5.2	28.8	34.0	IC-83556	0.0	5.4	5.4
EC-442226	74.5	25.5	100	EC-490034	52.6	41.8	94.4	IC-83558	0.0	6.6	6.6
EC-442228	0.0	32.6	32.6	EC-490038	42.1	57.9	100.0	IC-83568	84.5	15.5	100
EC-44229	19.4	77.2	96.6	EC-490039	24.5	75.5	100.0	IC-83569	7.6	12.4	20.0
EC-442300	5.4	34.9	40.4	EC-490040	23.4	76.6	100.0	IC-83570	0.0	4.0	4.0
EC-442348	56.2	43.8	100	EC-490042	17.8	82.2	100.0	IC-83571	6.7	68.1	74.8
EC-442380	90.4	9.6	100	EC-490045	25.2	69.5	94.7	IC-83572	32.1	67.9	100
EC-442407	43.8	56.2	100	EC-498765	33.9	59.5	93.5	IC-83573	17.1	82.9	100
EC-442409	50.0	50.0	100	EC-498767	11.9	73.4	85.3	IC-83747	16.1	83.9	100
EC-442519	2.9	74.4	77.3	EC-498773	12.4	67.8	80.2	IC-83748	0.0	3.8	3.8
EC-449853	3.1	54.4	57.5	EC-498779	4.5	57.7	62.2	IC-83750	0.0	8.7	8.7
EC-489845	0.0	10.0	10.0	EC-498780	0.0	71.1	71.1	IC-83751	3.8	3.1	6.9
EC-489861	8.1	66.0	74.2	EC-498812	0.0	21.6	21.6	IC-83753	18.6	70.1	88.7
EC-489865	9.2	41.0	50.2	EC-498818	0.0	1.4	1.4	IC-83757	0.0	7.6	7.6
EC-489867	0.0	75.4	75.4	EC-498823	0.0	53.4	53.4	IC-83758	63.4	36.6	100
EC-489869	5.2	62.6	67.8	EC-498827	10.2	64.7	75.0	IC-83760	4.6	46.2	50.8
EC-489870	1.7	50.4	52.1	EC-267308	0.0	27.8	27.8	IC-83761	3.1	56.7	59.8
EC-489881	7.2	59.3	66.5	IC-83518	0.0	50.2	50.2	IC-83763	54.0	46.0	100
EC-489882	0.0	8.7	8.7	IC-83519	0.0	14.8	14.8	IC-83764	3.0	97.0	100
EC-489883	15.8	62.1	77.9	IC-83522	1.9	24.7	26.6	IC-83765	0.0	100	100
EC-489886	12.6	37.4	50.0	IC-83523	0.0	4.6	4.6	IC-83765	2.0	98.0	100
EC-489887	0.0	35.0	35.0	IC-83524	13.7	57.4	71.1	IC-83766	0.0	100	100
EC-489905	0.0	19.0	19.0	IC-83525	0.0	23.3	23.3	JG62 (S)	66.6	33.4	100
EC-489907	0.0	28.7	28.7	IC-83526	2.5	14.7	17.2	JG315 (R)	0	4.0	4.0
EC-489910	23.3	28.1	51.4	IC-83527	0.0	11.5	11.5				
LSI			55.2								

EC-267154, EC-267308, EC-489845, EC-489882, EC-489919, EC-489991, EC-498818, IC-83523, IC-83538, IC-83539, IC-83748 and IC-83757 were found resistant and 15 accessions moderately resistant (Table 2) to Fusarium wilt. The variations observed in per cent wilt incidence and time of wilt development may be due to genotype (Upadhyaya *et al.* 1983) and variations in genotype sensitivity to toxins of pathogen (Sutherland and Pegg, 1992). Variations in genotypes

may also be due to ability of chickpea genotypes to compensate damage caused by pathogen with production of new roots (Huisman, 1982).

The study concludes that immunity in chickpea against Fusarium wilt is rather scarce. Though many resistance sources are being identified across the globe, a continuous change in the genetic makeup of the pathogen warrants search for new resistant sources. The identified resistant germplasm in the present study

Table 2. Percent incidence in chickpea genotypes during 2015-16 and 2016-17

Genotype name	2015-16	2016-17	Pooled data	Reaction
EC-223519	8.2	43.8	26.0	S
EC-267154	4.9	15.2	10.0	R
EC-267293	14.6	60.6	37.6	S
EC-267308	1.5	9.5	5.5	R
EC-267437	3	17.9	10.4	MR
EC-440552	8.7	12.2	10.5	MR
EC-441725	16.3	12.3	14.3	MR
EC-489845	9.9	2.1	6.0	R
EC-489882	8.7	6.0	7.4	R
EC-489905	18.9	11.4	15.1	MR
EC-489919	5.2	8.7	6.9	R
EC-489991	3.1	11.7	7.4	R
EC-498818	1.4	0.0	0.7	R
IC-83519	14.7	32.4	23.5	S
IC-83523	4.6	4.3	4.5	R
IC-83526	17.2	15.0	16.1	MR
IC-83527	11.4	19.7	15.5	MR
IC-83529	17.6	16.0	16.8	MR
IC-83534	17.5	25.6	21.5	S
IC-83535	18.3	12.3	15.3	MR
IC-83538	6.4	9.8	8.1	R
IC-83539	9.8	4.4	7.1	R
IC-83541	13.1	19.5	16.3	MR
IC-83542	5.1	52.0	28.6	S
IC-83551	12.8	11.2	12.0	MR
IC-83556	5.4	22.1	13.8	MR
IC-83558	6.5	26.8	16.6	MR
IC-83569	20	13.6	16.8	MR
IC-83570	3.9	45.8	24.9	S
IC-83748	3.7	16.2	10.0	R
IC-83750	8.6	18.7	13.7	MR
IC-83751	6.9	27.3	17.1	MR
IC-83757	7.6	8.8	8.2	R
Resistant check	4.0	5.2	4.6	R
Susceptible check	100	100	100	S

can be of great value to plant breeders in their efforts to develop race specific (Race II) resistant chickpea cultivars.

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