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Elucidation of host resistance for chickpea wilt (*Fusarium oxysporum* f. sp. *ciceris*)

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ABSTRACT

A total of 1890 elite chickpea breeding lines were phenotyped against wilt disease of chickpea incited by *Fusarium oxysporum* f.sp. *ciceris* (race 2) during 2004-2011 and were also evaluated for yield potential. Finally, 67 best performing lines were further evaluated for wilt resistance during 2012-15. Pooled data of three years indicated that the wilt incidence in elite breeding lines of chickpea varied from 6.7 to 19.8%. Significant (P< 0.001) difference in wilt incidence was noticed among the elite breeding lines due to the presence of unique genetic diversity. In the present study, ten lines *viz.* IPC2010-121 (3249 kg/ha), IPC2005-45 (2628 kg/ha), IPC2007-04 (2758 kg/ha), IPC2007-36 (2568 kg/ha), IPC2010-03 (3042 kg/ha), IPC2010-05 (2767 kg/ha), IPC2010-78 (3042 kg/ha), IPC2010-173 (2975 kg/ha), IPC2011-94 (3418 kg/ha) and IPC2012-03 (3030 kg/ha) were showed resistance against Fusarium wilt with higher yield. The resistant lines identified in the present study can be utilized in breeding programmes for developing wilt resistant cultivars in chickpea. Some of the high yielding resistant lines identified can be used for cultivation after fulfilling required criteria for releasing variety for cultivation.

Key words: Elite breeding lines, Race 2, Resistance, Screening, Wilt

Chickpea (*Cicer arietinum* L.) is an important pulse crop after peas and common bean grown over 50 countries (Gaur *et al.* 2014). It is used as an important source of dietary protein and plays pivotal role in human nutrition and animal feed. India is the major producer of chickpea with an area of 8.25 million ha with a production of 7.33 million tons (Anonymous, 2016). The productivity of chickpea is less than the potential productivity due to its vulnerability to various biotic and abiotic stresses throughout the growing season. Among biotic stresses, wilt caused by *Fusarium oxysporum* f. sp. *ciceris* (Padwick) Matuo and K. Sato (*Foc*), is a highly destructive pathogen of chickpea worldwide and is estimated to cause 10-90% annual yield loss across the world (Jimenez-Diaz *et al.* 1989). In India, wilt causes 10-15% annual yield losses (Singh and Dahiya, 1973). However, losses depends on the stage of crop infection, early and late wilting causes 77- 94% and 24-65%, respectively (Haware and Nene, 1980). Efforts of several national as well as international chickpea breeding programmes yielded several resistant cultivars against wilt disease but, resistance is not durable due to high variability in the pathogen. The variability in the pathogen has been distinguished into eight races across the world (Haware and Nene, 1982 and Jimenez-Gasco *et al.* 2001). However, recent study indicated that, existence of all eight races on a new set of chickpea differentials in India (Dubey *et al.* 2012). Management of chickpea wilt is difficult by cultural and chemical methods because the pathogen survives in the soil for many years in absence of host (Haware *et al.* 1996). Currently, deployment of diverse resistance sources is more effective, economic and eco-friendly strategy for management of chickpea wilt (Govil and Rana, 1994). Consequently, considerable efforts have been made to identify resistance sources against wilt across the world (Halila and Strange, 1997, Pande *et al.* 2006, Sharma *et al.* 2012 and Saabale *et al.* 2017) and several are being utilized in breeding programs. But, host resistance in disease management is seriously curtailed as a result of genetic breakdown or change in the virulence of the pathogen making imperative to continuously search for resistant sources against wilt pathogen. In present study, evaluation of elite breeding lines of chickpea was undertaken to identify stable wilt resistant sources against Fusarium wilt (race 2) in artificially developed sick plot.

MATERIALS AND METHODS

Plant material: A total of 1890 'desi' and 'kabuli' elite breeding lines were developed under chickpea breeding program of Division of Crop Improvement, ICAR-IIPR, Kanpur, India. These elite breeding lines were evaluated against wilt disease caused by *F. oxysporum* f. sp. *ciceris* (race 2) in sick plot and simultaneously, these lines were evaluated for their yield in normal field during 2004-05 to 2010-11. Seeds of checks *viz*., JG 62 (Susceptible check) and JG 315 (Resistant check) were obtained from Division of Crop Protection, IIPR, Kanpur, India.

Wilt sick plot: Fusarium wilt sick plot is located at 26.49^0 north latitude and longitude of 80.27^0east. The soil is alluvial with a pH 7.9 and area receives an annual average rainfall of 885mm. The Fusarium wilt sick plot of size 3000 m^2 developed at Kanpur during late 1980s against *F. oxysporum* f. sp. *ciceris* race 2, a predominant race of Northern India. Sick plot used in the present study is located in North East Plain Zone (NEPZ) of India and is designated for screening of All India Coordinated Research Project (AICRP) and International Crop Research Institute for Semi-Arid Tropics (ICRISAT) entries. Acceptable threshold inoculum level (~7460 CFU/g of soil) of sick plot was maintained by incorporating wilted plants along with wilt inoculum prepared in laboratory on sorghum grains using dynamic culture of *Foc* race 2 maintained in the laboratory.

Field screening of genotypes and Analysis: A total of 67 promising chickpea elite breeding lines identified over the years were revalidated in the wilt sick plot during 2012-13, 2013-14 and 2014-15. The pedigrees and yield of 67 selected breeding lines used in this study are presented in Table 1. The experiment was laid in a randomized complete block design (RCBD) with two replications. Each genotype was planted in 2 rows of 4m length with row to row spacing of 30 cm and plant to plant distance of 10cm. The chickpea genotype JG 62 was used as susceptible check and WR-315 genotype was used as a resistant check after every 10 test rows to serve as indicators.

Data on periodical wilt incidence was recorded during seedling, flowering/podding and at near maturity stages of the crop. Cumulative percent wilt incidence of each test genotype was calculated using formula as Disease incidence (%) = (Number of infected plants/ Total number of plants) x 100. The reaction of test genotypes was determined by following disease rating scale of Sharma *et al.* (2012). 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). Data on disease incidence was subjected to log transformation (Gomez and Gomez, 1984) to make error variance homogeneous. Analysis of variance (ANOVA) was calculated to study the effect of genotype (G), year (Y) and their interaction (G×Y) assuming year and replications are fixed and genotype as random using SAS software version 9.1. Cary, NC: SAS Institute Inc).

RESULTS AND DISCUSSION

Incorporation of disease resistance involves pairwise crossing of compatible parents, usually one parent is chosen for disease resistance while other chosen parent has suitable agronomic features for higher yield. Bhardwaj *et al.* (2012) used similar crossing strategy for incorporating the resistance in the progenies. In the present study, evaluation of elite breeding lines developed at IIPR Kanpur were undertaken in a Fusarium wilt sick plot having the threshold inoculum level of ~7460 Colony Forming Units (CFU) per gram of soil. Hence, it was unlikely that test lines would have chance to escape from infection. However, previous studies showed that, Foc population 1795±253 CFU/g of soil is adequate for identifying resistance sources (Halila and Strange, 1997). Similar wilt sick plots were developed in ICRISAT, Hyderabad, India (Nene *et al.* 1981) and Santaella, Cordoba, Spain (Jimenez-Diaz *et al.* 1991) for large scale screening. In the preliminary trial, 1890 breeding lines showed wide variation for Fusarium wilt incidence and ranged from 0 to 100 per cent (Data not shown). Among which, 67 promising breeding lines of chickpea were subjected for reconfirmation. Based on the mean percent wilt incidence of all the three years (2012-13, 2013-14 and 2014-15) of evaluation, 24 breeding lines were found resistant (d" 10% incidence) and 36 were moderately resistant (10-20% incidence) among *desi* type whereas, among *kabuli* genotypes two were resistant and five were moderately resistant (Table 1).

Table 1. Percent Fusarium wilt incidence, yield and pedigree of elite breeding lines of chickpea

Sl. No.	Genotype	Disease incidence (%)				Yield (kg^{-1} ha)	Pedigree
		2012-13	2013-14	2014-15	Pooled data		
1	IPC2005-45	8.45	8.00	3.55	6.67	2628	DCP92-3 × IPC 71
2	IPC2005-19	8.10	4.70	8.60	7.13	1844	DCP92-3 × IPC 92-1
3	IPC2007-04	5.10	11.40	5.05	7.18	2758	DCP92-3 × IPC94-19
4	IPC2010-78	6.90	7.30	8.20	7.47	3042	CSG962×ICCV96029
5	IPC2007-36	2.85	12.15	7.95	7.65	2568	DCP92-3×IPC92-3
6	IPC2011-94	5.65	10.30	7.60	7.85	3418	JG16×IPC99-18
7	IPC2010-03	7.85	12.45	3.40	7.90	3042	IPCK96-3 ×IPCK2004-1
8	IPC2007-50	6.70	8.65	8.50	7.95	2440	GNG496×KWR108
9	IPC2007-28	8.90	11.55	3.55	8.00	1422	DCP92-3× SAKI9516
10	IPC2011-76	5.95	10.45	7.95	8.12	1958	CSG-89-26×FG711
11	IPC2009-66	12.00	10.90	2.05	8.32	1453	14222767
12	IPC2011-31	11.60	10.00	3.45	8.35	2185	BG256×PHLEG5
13	IPC2011-28	10.00	7.90	8.05	8.65	2136	HC-5×GL23138
14	IPC2010-121	11.55	9.40	6.35	9.10	3249	IPC1997-7×IPC1995-1
15	IPC2005-41a	6.25	10.10	11.40	9.25	1097	KPG143-2×T39-1
16	IPC2010-128	9.10	10.30	8.85	9.42	1775	ICC30163
17	IPC2010-173	10.65	7.45	10.15	9.42	2975	IPC94-132×BGD1122
18	IPC2005-62	9.65	4.80	14.00	9.48	1911	DCP92-3×T39-1
19	IPC2005-24	11.55	3.40	13.70	9.55	1883	DCP92-3×KPT-1
20	IPC2012-03	7.45	8.75	12.95	9.72	3030	IPC98-12×B1025
21	IPC2005-18	8.00	5.00	16.45	9.82	1889	(DCP92-3×IPC71)×ICC4958
22	IPC2005-26	9.50	4.70	15.40	9.87	1097	KPG143-2×T39-1
23	IPC2010-05	12.75	7.30	9.75	9.93	2767	ANNIGERI ×TYSON

24	IPC2012-198	12.20	11.30	6.40	9.97	1916	C214×K850
25	IPC2009-43	12.70	8.00	11.25	10.65	2886	PGD84-16 × 86-18
26	IPC2005-52	8.50	11.20	12.30	10.67	1300	BG362×BG256
27	IPC2012-99	9.25	11.65	12.25	11.05	1419	GC98×ICC14203
28	IPC2007-51	8.85	13.25	11.80	11.30	2886	PGD84-16×H86-18
29	IPC2010-152	15.05	8.85	11.35	11.75	2843	BG256×JG16
30	IPC2010-71	14.00	13.80	7.80	11.87	2158	DCP92-3×ICCV96029
31	IPC2005-46	9.30	7.75	18.60	11.88	1825	(H82-94×H95-67)×HK89139
32	IPC2005-37	15.45	8.75	11.60	11.93	2072	L149×H82-2
33	IPC2010-123	13.75	11.90	10.25	11.97	3042	CSG8962×K850
34	IPC2005-44	13.85	8.85	13.85	12.18	1830	KPG143-2×T39-1
35	IPC2005-41B	10.80	9.05	16.75	12.20	-	KPG143-2×T39-1
36	IPC2004-3	16.25	5.20	15.55	12.33	1610	PA079×9301
37	IPC2012-48	10.40	13.10	15.05	12.85	2111	DCP92-3×BPM
38	IPC2008-10	16.25	9.60	12.75	12.87	2218	PGD84-16×ICC87322
39	IPC2005-15	12.75	11.25	14.70	12.90	1994	H82-2×ICCV10
40	IPC2005-35	15.00	7.55	16.35	12.97	3014	KATILA×BG362
41	IPC2011-99	6.20	18.45	14.70	13.12	3518	BG256×ICC269082
42	IPC2010-185	18.15	9.75	11.55	13.15	2560	CP116-15-22
43	IPC2009-153	18.90	14.00	6.85	13.25	2483	DOLLAR×IPCK96-3
44	IPC2010-61	16.95	13.65	11.10	13.90	2069	ICCV96030×*CicerPinnatifidum*
45	IPC2007-48	15.40	14.95	13.20	14.52	2600	BG372×ICCV96029
46	IPC2005-59	17.65	11.90	14.30	14.62	2628	DCP92-3×IPC71
47	IPC2009-187	12.35	11.75	20.25	14.78	2667	IPC97-7×IPC95-1
48	IPC2004-8	16.35	11.15	17.40	14.97	1610	PA079×9301
49	IPC2005-54	15.15	11.50	18.30	14.98	2511	PG5×GNG469
50	IPC2011-65	11.05	13.05	22.00	15.37	3313	IPC94-94×IPC2000-4
51	IPC2010-28	12.50	19.25	14.85	15.53	2684	JG315×ICCV92944
52	IPC2010-120	19.00	16.30	12.30	15.87	2125	KWR108×JG62
53	IPC2007-98	15.60	19.60	13.25	16.15	1618	GNG479×ICCV96029
54	IPC2005-34	18.60	13.25	16.85	16.23	2314	ICCV4953×H82-2
55	IPC2012-192	14.95	15.10	19.50	16.52	2222	JG315×RSG865
56	IPC2005-30	21.85	9.55	19.60	17.00	1722	GNG469×KWR108
57	IPC2010-41	17.70	22.00	16.45	18.72	622	C-8
58	IPC2010-207	12.70	21.90	22.40	19.00	2239	CP211-2-3
59	IPC2012-197	19.70	19.35	18.85	19.30	2527	IPC2000-1×ICC14880
60	IPC2010-146	20.00	23.15	16.35	19.83	2357	IIPC94-132×ICCCV96029
61	IPCK2012-306	6.95	9.40	5.55	7.30	1622	ICARDA17122
62	IPCK2012-258	6.40	9.00	10.40	8.60	1533	IPCK96-3×DOLLAR
63	IPCK2012-154	15.90	12.75	7.55	12.07	1055	ICARDA16116
64	IPCK2012-310	12.10	7.65	19.15	12.97	1433	JGK1×ICC16144
65	IPCK2012-293	11.95	13.10	18.60	14.55	1211	ICARDA17103
66	IPCK2012-269	12.60	18.40	16.60	15.87	1333	ICARDA17103
67	IPCK2012-278	14.25	23.80	15.45	17.83	1255	ICARDA17107

Analysis of variance (ANOVA) showed significant variation among the 67 elite breeding lines at 1% level of significance suggesting that genotypes have considerable variation for the wilt reactions and non significant difference was observed across the years indicated that the disease reactions were stable. Description of the statistics of wilt incidence of elite breeding lines is given in Table 2. This signifies that the differences noticed in wilt disease incidence were mainly due to the genotypes. Pooled data analysis showed that ($P < 0.05$), incidence of Fusarium wilt in genotypes had almost no interaction effect of environment over the years of screening under sick plot.

Based on mean incidence of 3 years data, the breeding lines which showed consistent resistant reaction in all the years of testing with higher yields *viz*. IPC 2010-121 (3249kg/ha), IPC 2005-45 (2628 kg/ha), IPC 2007-04 (2758 kg/ha), IPC 2007-36 (2568 kg/ha), IPC 2010-03 (3042 kg/ha), IPC 2010-05 (2767 kg/ha), IPC 2010-78 (3042 kg/ha), IPC 2010-173 (2975 kg/ha), IPC 2011-94 (3418 kg/ha) and IPC 2012-03 (3030 kg/ha) were presented in Table 1. The variations occurred in per cent wilt incidence and time of wilt development may be attributed to genetic makeup of the host plant (Upadhyaya *et al.* 1983). Huisman (1982) reported that,

Table 2. Analysis of variance for Fusarium wilt incidence in elite breeding lines of chickpea

	Source	Anova SS	Mean Square	F Value	Pr > F	R-Square
Pooled data (three years)	Genotype	113.36	1.69	4.87	<.0001	0.74
	Replication	0.02	0.02	0.05	0.8222	
	Year	0.76	0.38	1.09	0.3391	
	Genotype*Year	91.64	0.68	1.97	<.0001	
2012-13	Genotype	55.96	0.84	5.63	<.0001	0.85
	Replication	0.68	0.68	4.58	0.036	
2013-14	Genotype	60.95	0.91	4.22	<.0001	0.81
	Replication	0.39	0.39	1.81	0.1828	
2014-15	Genotype	88.09	1.31	1.96	0.0033	0.66
	Replication	0.18	0.18	0.27	0.6022	

differences among genotypes against Fusarium wilt may be also due to variations in roots of chickpea genotypes, which compensate by production of new roots. The data on overall percentage wilt incidence indicated that '*kabuli*' breeding lines are more susceptible to wilt compared to '*desi*' types. The results are in conformity with the results of Nene and Haware (1980) and Jimenez-Diaz *et al.* (1991). The data in our study also showed that, only small percentage of genetic resistance was observed in elite breeding lines of chickpea. Haware *et al.* (1992) identified only1.2% of accessions collected across the globe were resistant to *race1*. Pande *et al.* (2006) reported 12% of mini-core accessions (211) were highly resistant against Fusarium wilt.

The study concludes that immunity in chickpea against Fusarium wilt is rather scarce. However, desi types of chickpea are more tolerant than kabuli types. The difference in per cent wilt incidence may be because of different genes involved in imparting resistance. The information generated on these test lines could be a great value for plant breeders in their efforts in development of race specific (Race-2) resistant chickpea cultivars.

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