Genetic Resistance in Desi and Kabuli Chickpea Lines to Fusarium Wilt Caused by *Fusarium oxysporum* f. sp. *ciceris*

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Abstract

Twenty five lines each of desi and of kabuli chickpea (Cicer arietinum L.) were evaluated for Fusarium wilt resistance during 2008-09 season in the field (wilt sick plot) and greenhouse at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. Fifteen desi and nine kabuli lines were found resistant (d"10% mortality) to Fusarium wilt. Significant positive correlation was found between greenhouse and field screening techniques (r = > 0.84, P < 0.0001). Additionally, phenological traits and yield were also recorded for all the lines in the disease free field at ICRISAT, Patancheru. Six wilt resistant desi lines (ICCV 09118, ICCV 09113, ICCV 09115, ICCX-030042-F4-P12-BP-BP, ICCX-030037-F4-P9-BP-BP, ICCX-030042-F4-P1-BP-BP) and two kabuli lines (ICCV 09308, ICCV 09314) matured early between 99-107 days and yielded more than the control cultivars JG 11 for desi (2208 kg/ha yield) and JGK 1 for kabuli (2243 kg/ha). These early maturing, high maturng, high yielding and wilt resistant desi and kabuli chickpea lines can be useful sources for breeding wilt resistant varieties.

Keywords: Chickpea, *Fusarium oxysporum* f. sp. *ciceris,* resistance

Introduction

Chickpea (*Cicer arietinum* L.) is the third most important grain legume cultivated in over 50 countries in Asia, Africa, Oceania, America and Europe. India accounts for 64% of the global chickpea production (FAO 2007). Average global productivity of chickpea (800 kg ha^{-1}) is far below the actual yield potential because the crop is attacked by a number of diseases throughout the growing season (Pande *et al.,* 2006). Among the diseases, Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceri*s (Padwick) Matuo & Sauto (FOC) is highly destructive and worldwide in occurrence (Nene *et al*., 1989, Halila and Strange 1996, Kraft *et al*., 2004). The pathogen penetrates the vascular bundles of the roots of chickpea plants and stops or reduces the water uptake to the foliage. The infected plants ultimately wilt and die. The disease can occur at all stages of plant growth right from seedling to maturity and causes annual yield losses of 10-90 % annually (Jalali and Chand 1992, Jimmez-Diaz *et al*., 1989). In susceptible genotypes, under favourable environmental conditions, wilt causes 100% yield losses (Haware, 1990).

Due to difficulty in application of cultural and chemical control for the management of the disease, wilt resistant cultivars provide effective and economical control of this disease. At International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), wilt sick plot has been developed for field screening and wilt-resistant sources identified (Nene *et al*., 1981; Nene and Haware, 1980; Haware *et al*., 1981, 1992; Pande *et al*., 2006, 2007; Gaur *et al*., 2006). Ffield screening in wilt sick plots is effectively used to cull out the ultra susceptible germplasm and breeding lines. However, the probability of presence of other soil-borne pathogens that interfere with the FOC in the sick plot cannot be overlooked and hence, it would be rather necessary to confirm field resistance to wilt following the precise greenhouse screening technique. The present investigations were undertaken to identify additional sources of resistance to Fusarium wilt in the newly developed desi and kabuli chickpea lines using both the field and greenhouse screening techniques. Attempts were made to evaluate these lines for agronomic yield and traits.

Materials and methods

Seed source

Seeds of 50 advanced breeding lines of chickpea, 25 each of desi and kabuli and of released cultivars of desi and kabuli types used as a control cultivar checks for comparison of agronomic traits were obtained from the chickpea breeding program at ICRISAT, Patancheru, India. Seeds of all the

susceptible and resistant lines used as a control for Fusarium wilt were obtained from the Department of Legumes Pathology, ICRISAT.

Field screening

Fusarium wilt resistance screening of 50 desi and kabuli breeding lines was conducted during the 2008-09 post-rainy season under artificial epiphytotic conditions in the wilt sick plot at ICRISAT, Patancheru. The experiment was conducted in randomized block design in two replications. For each line, 40 seeds were sown in a 4-m row with seed - seed spacing of 10cm and row - row spacing of 40cm. As wilt manifests from seedling to maturity, the resistance in 50 newly developed chickpea lines was compared with JG 62 and K 850 representing wilt susceptible checks for seedling (early wilter) and vegetative-flowering (late wilter) growth stages respectively. Additionally, a wilt resistant cultivar WR 315 was also sown for comparing the level of resistance in these test lines. The sowing arrangement of test, susceptible and resistant lines included four test rows followed by susceptible checks and resistant check throughout the field. Sowing was done in mid-October and harvesting in February. Data on disease incidence were recorded periodically at 30, 60 and 90 days after sowing.

Greenhouse screening

All the newly developed breeding lines were evaluated for wilt resistance in the greenhouse following root dip inoculation technique (Pande *et al.,* 2006). Chickpea lines along with wilt susceptible [JG 62 (early wilter) and K 850 (late wilter)] and resistant cultivar (WR 315) were raised in polythene bags filled with sterilised river sand in a greenhouse maintained at 25±1°C for eight days. Inoculum was prepared from a single conidial culture of *F. oxysporum* f. sp. *ciceris* isolated from wilt infected plants collected from ICRISAT wilt sick plot. For mass inoculum preparation, a 7-mm disc of actively growing *F. oxysporum* f. sp. *ciceris* culture was put into a 250ml conical flask containing 100ml of sterilized potato dextrose broth and incubated for seven days in incubator shaker at 25±1°C and 125 rpm. The culture was then homogenized in sterilized distilled water and adjusted to 6 x 10^5 conidia ml^{-1} using a haemocytometer for use as an inoculum. Eight-day-old seedlings of each test line as well as susceptible and resistant control cultivars grown in sterilized river sand were uprooted, cleaned with tap water and root inoculated by dipping in inoculum suspension for 1-2 minutes to enable conidia to adhere to the roots. Inoculated seedlings were transplanted in pre-irrigated sterile vertisol and sand (3:1) in pots and incubated in a greenhouse at 25±3°C. Thirty seedlings of each line were tested in three replications in a randomized complete block design (RCBD). Inoculated seedlings were observed for wilt symptoms from 15 to 60 days after inoculation at a 5-day interval and the experiment was repeated once.

Agronomic traits

One set of these lines was evaluated for agronomic traits and yield in the disease free field at ICRISAT. The experiment was conducted in vertisols under rainfed conditions in a RCBD with three replications. Each test line was sown in 4 rows of 4 m length with row-to-row spacing of 40 cm and plant-to-plant spacing of 10cm. Chickpea cultivars JG 11 and JAKI 9218 in case of desi and KAK 2 and JGK 1 in kabuli were used as a control cultivar checks as they are farmer preferred cultivars released in India particularly for central and southern India where the chickpea is grown under residual soil moisture and crop season is short. Standard package of practices were followed to ensure a healthy crop. Seeds were treated with fungicide (2g thiram + 1g carbendazim kg^{-1} seed) before sowing for reducing seed and soil borne diseases. Fertilizer application included 20-30kg nitrogen (N) and 40-60kg phosphorus (P) ha^{-1}. Data were recorded on days to 50% flowering, days to maturity, 100-seed mass (g) and seed yield (g/plot).

Disease assessment and analysis

Data on disease incidence (per cent plant mortality) was recorded using the following formula

$$\text{Disease incidence (\%)} = \frac{\text{Total number of wilted plants}}{\text{Total number of plants}} \times 100$$

Depending on the experimental design, data on all the parameters including wilt incidence in field, greenhouse and agronomic traits were subjected to statistical analysis using GENSTAT statistical package. Correlation coefficient between field and greenhouse screening techniques was also calculated using GENSTAT statistical package.

Results and discussion

Resistance in desi chickpea

In the field screening in wilt sick plot, 15 lines were found resistant (d"10% incidence), seven moderately resistant (10-20% incidence), two susceptible (20-40%) and one highly susceptible (>40%) to Fusarium wilt. The early wilt susceptible check (JG 62) showed 100% plant mortality with in 30 days of sowing throughout the field. Late wilt susceptible check (K 850) showed >80% plant mortality after 60 days of sowing and resistant check (WR 315) had 0% plant mortality till harvesting. In the greenhouse, of the 25 lines, 24 lines were resistant (d" 10 % incidence) and one highly susceptible to Fusarium wilt. Fifteen lines found resistant in field screening showed resistant reaction in greenhouse (Table 1).

Table 1. Evaluation of advanced breeding lines of desi chickpea for phenology, yield and resistance to Fusarium wilt in field and greenhouse conditions

Advanced breeding desi lines	Agronomic traits[a]				Percent wilt incidence	
	Days to 50% flowering	Days to maturity	100-seed mass (g)	Seed yield (kg/ha)	Field[b]	Greenhouse[c]
ICCV 09101	49	110	18.3	2173	2.7	2.0
ICCV 09102	44	110	19.6	2112	10	0.0
ICCX-030034-F4-P8-BP-BP	47	110	20.8	2141	2.4	0.0
ICCV 09107	44	108	20.9	2239	6.3	0.0
ICCX-030038-F4-P4-BP-BP	45	106	22.8	1543	2.2	6.0
ICCX-030038-F4-P6-BP-BP	43	102	21.3	2104	10	0.0
ICCX-030042-F4-P1-BP-BP	46	107	21.6	2346	10.0	4.0
ICCV 09110	46	106	17.3	2200	4.9	0.0
ICCV 09111	45	107	22.0	2145	10.0	0.0
ICCV 09112	45	113	21.4	2517	8.0	0.0
ICCV 09113	48	102	21.2	2446	7.7	0.0
ICCV 09115	50	103	19.2	2592	9.8	0.0
ICCX-030037-F4-P9-BP-BP	51	103	22.6	2618	8.0	0.0
ICCX-030042-F4-P12-BP-BP	53	103	22.8	2303	9.0	2.0
ICCV 09118	47	101	20.2	2498	10.0	0.0
JG 11 (check)[d]	45	108	22.3	2208	14.0	10.0
JAKI 9218 (check)[d]	46	107	24.2	1918	16.5	12.0
JG 62 (early sus. check)[e]	-	-	-	-	100	100
K 850 (late sus. check)[f]	-	-	-	-	82	85
WR 315 (resistant check)[g]	-	-	-	-	0.0	0.0
SEM	1.22	1.61	0.44	102.2	2.11	1.76
SED	1.73	2.28	0.62	144.47	1.59	2.49
CV (%)	4.56	2.62	3.45	16.34	9.3	4.6
LSD (5%)	3.47	4.57	1.25	289.89	3.2	5.0

[a]Mean of two replications evaluated in disease free field; [b]Mean of two replications evaluated in wilt sick plot; [c]Mean of three replications evaluated in greenhouse; [d]Control cultivar checks for comparison of; phenological data; [e]Early susceptible check for Fusarium wilt; [f]Late susceptible check for Fusarium wilt; [g]Resistant check for Fusarium wilt

Days to 50% flowering varied between 43-53 days in the resistant/moderately resistant desi lines while maturity ranged between 101-113 days (Table 1). Majority of the lines matured earlier or were similar in maturity with the control cultivar checks JG 11 and JAKI 9218. Seven lines ICCV (9108, ICCX-030038-F4-P6-BP-BP, ICCV 9113, ICCV 09115, ICCX-030037-F4-P9-BP-BP, ICCX-030042-F4-P12-BP-BP and ICCV 09118 showed significant difference in maturity days 9101-103 days) as compared to the control cultivar JAKI 9218 (107 days). The 100-seed mass of the desi lines varied from 17.3 to 24.2 g. Significantly higher yield (2498-2741 kg ha^{-1}) was recorded in four lines ICCV 09112, ICCV 09115, ICCV 09118 and ICCX-030037-F4-P9-BP-BP than the best control cultivar check JG 11 (2208 kg ha^{-1}). Three lines ICCV 09118, ICCX-030037-F4-P9-BP-BP and ICCV 09115 had the best combination of earliness (maturity – 101-103 days), yield (2498-2618 kg ha^{-1}) and reaction to Fusarium wilt (d"10% incidence) both in the field and greenhouse (Table 1).

Resistance in kabuli chickpea

Among kabuli types, seven lines were found resistant (d"10% incidence), five moderately resistant (10.1-20.0 % incidence), eight susceptible (20-40%) and five highly susceptible (>40%) to Fusarium wilt. All the seven resistant lines found in the field showed resistant reaction in the greenhouse. It was found that two lines that showed moderately resistant reaction in the field showed resistant reaction in the greenhouse. However, of the five moderately resistant lines in the field, one had resistant reaction in the greenhouse. All the susceptible lines showed late wilting reaction.

Table 2. Evaluation of advanced breeding lines of kabuli chickpea for phenology, yield and resistance to Fusarium wilt in field and greenhouse conditions

Advanced breeding Kabuli lines	Phenological traits[a]				Per cent wilt incidence	
	Days to 50% flowering	Days to maturity	100-seed mass (g)	Seed yield (kg/ha)	Field[b]	Greenhouse[c]
ICCV 09301	38	105	40.7	2315	10.0	8.0
ICCV 09303	40	106	42.0	2352	10.0	2.0
ICCV 09308	35	99	44.2	2091	10.0	2.5
ICCV 09311	39	104	44.4	2243	3.6	0.0
ICCV 09314	39	100	38.5	1959	10.0	10.0
ICCV 09315	42	103	45.6	1944	9.09	4.0
ICCX-030177-F4-P23-BP-BP	37	103	38.3	2040	0.0	0.0
KAK 2 (check)[d]	40	108	35.2	1875	10.0	12.0
JGK 1 (check)[d]	38	103	35.5	2243	15.5	14.0
JG 62 (early sus. check)[e]	-	-	-	-	100	100
K 850 (late sus. check)[f]	-	-	-	-	85	80
WR 315 (resistant check)[g]	-	-	-	-	0.0	0.0
SEM	0.63	1.20	1.13	77.70	1.32	1.16
SED	0.89	1.70	1.60	109.9	1.36	1.64
CV (%)	2.82	2.02	4.29	12.87	6.6	8.3
LSD (5%)	1.78	3.41	3.22	220.55	2.8	3.3

[a]Mean of two replications evaluated in disease free field; [b]Mean of two replications evaluated in wilt sick plot; [c]Mean of three replications evaluated in greenhouse; [d]Control cultivar checks for comparison of phenological data; [e]Early susceptible check for Fusarium wilt; [f]Late susceptible check for Fusarium wilt; [g]Resistant check for Fusarium wilt

Among the resistant/moderately resistant lines, all the lines were earlier in maturity (99-106 days) than the control cultivars KAK 2 (maturity days-108 days) and five lines were equal or earlier in maturity than the control cultivar check JGK 1 (maturity days-103 days) (Table 2). Seed size (38.5 to 45.6 g) was significantly more in all the lines than both the control cultivar checks KAK 2 (35.2 g) and JGK 1 (35.5 g). Significantly higher yield was recorded in seven lines as compared to control cultivar KAK 2 (1875kg/ha) and most of the lines had yield statistically at par with the control cultivar JGK 1 (2243kg/ha).

Breeding for Fusarium wilt is an important goal in chickpea across the world. Considerable progress has been made in the identification of wilt resistant sources and development of wilt resistant and high yielding cultivars. During 1976 to 1985, more than 13,500 germplasm accessions available at the ICRISAT gene bank were screened in the wilt sick plot against race 1 of *F. oxysporum* f.sp. *ciceris* (Haware *et al*., 1992). They reported 160 accessions resistant to Fusarium wilt through field and greenhouse screening and majority of these lines (150) were of desi type and only 10 were of kabuli types. Since then there has been a significant change in the scenario of chickpea cultivation in India. The expansion of irrigated agriculture in northern India has led to displacement of chickpea with wheat in larger area. As a result, the chickpea area got reduced from 5.1 m ha to 0.8 m ha in northern states, while it increased from 2.1 m h to 5.3 m ha in central and southern India. Therefore because of the increasing importance of the chickpea crop and its expansion in drier areas, it is important to identify additional sources of resistance to wilt both in desi and kabuli types.

Comparison of field and greenhouse screening techniques

Results of field and greenhouse screening were comparable for Fusaium wilt evaluation (Figures 1 and 2). The correlation coefficient for desi chickpea lines evaluated for Fusarium wilt in field and greenhouse was highly significant (r=0.84, $P<0.0001$). The susceptible control line JG 62 showed 100% wilt incidence and wilt resistant cultivar 0% incidence in both the techniques. Similarly, the disease incidence of ten kabuli chickpea lines was compared both in the field and greenhouse (Figure 2). The correlation coefficient for kabuli chickpea lines evaluated for Fusarium wilt in field and greenhouse was highly significant (r=0.88, $P<0.0001$).

Present study showed significant correlation between greenhouse and field screening technique. However, confirmation of resistance in greenhouse is an important tool in breeding programs focused on Fusarium wilt resistance. Screening in a controlled environment allows breeding material to be challenged with well-characterized isolates without interaction with other phytopathogenic

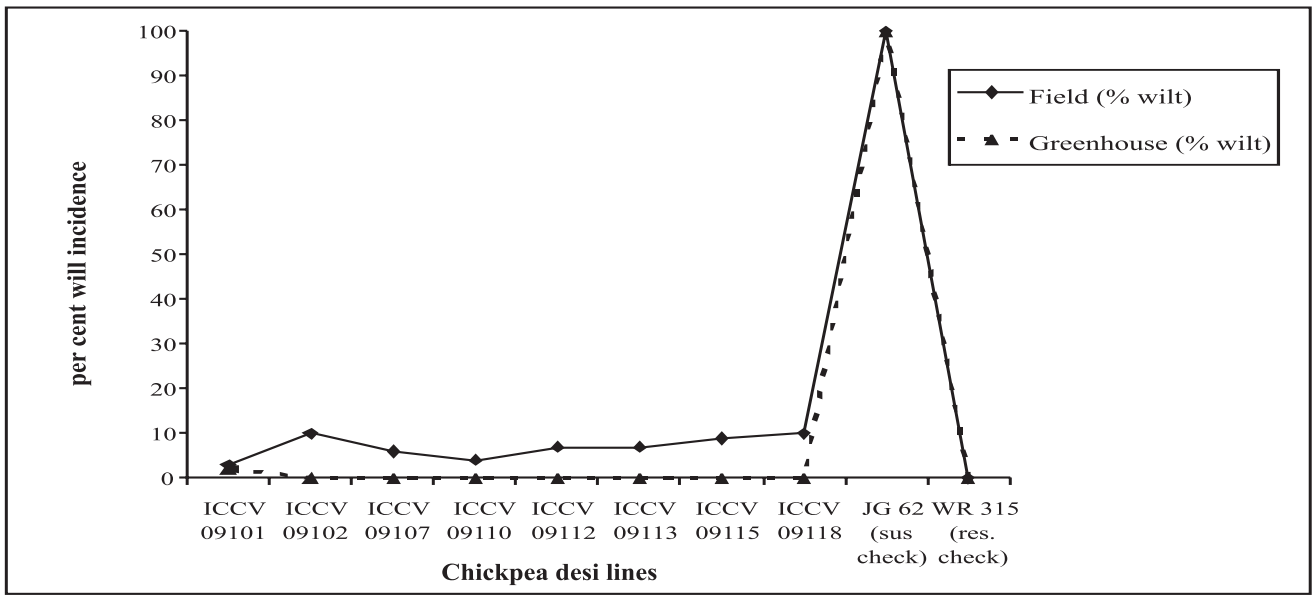

Figure 1. Comparison of Fusarium wilt reaction in desi chickpea cultivars under field and greenhouse conditions

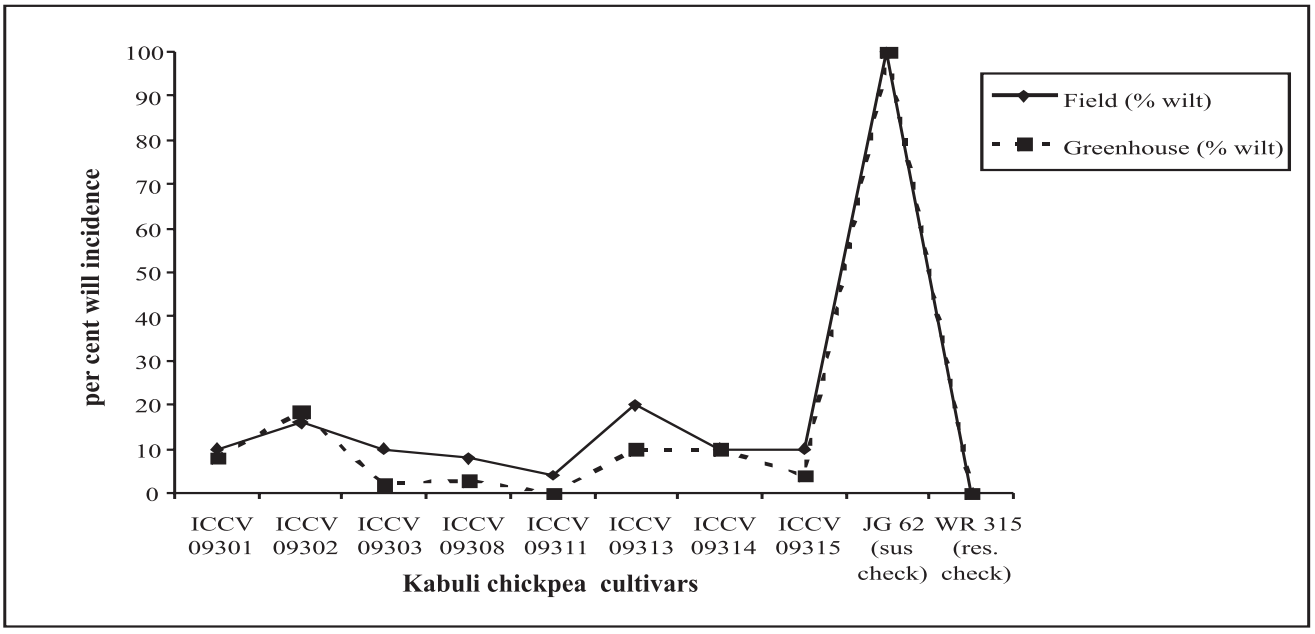

Figure 2. Comparison of Fusarium wilt reaction in kabuli breeding lines under field and greenhouse conditions

organisms. Environmental factors such as temperature and photoperiod can be easily managed to establish optimum conditions for Fusarium wilt development. Further, advantages of greenhouse screening include repeatability, uniformity, season independence and reduced risk of the disease spreading to other chickpea crops. Effective screening for disease resistance requires accurate simulation of natural environmental conditions where plants are exposed to the optimum level of inoculum (Porta-Pugilia and Aragona, 1997). Further, the scenario of *F. oxysporum* f.sp. *ciceris* has also changed and there are reports of more than one races from one location (Sharma and Muehlbauer, 2007 and Sharma *et al*., 2009). Also in the field confounding effect of other soil borne pathogens particularly *Rhizoctonia bataticola* (Taub.) Butler causing dry root rot and *Scleriotium rolfsii* Sacc. causing collar rot can not be detected. These pathogens cohabitate with FOC and interfere with its reaction in wilt sick plot. Therefore, there is need for confirmation of resistance identified in the field to wilt in the greenhouse.

In chickpea, short-duration varieties are very much needed as the crop is generally grown under rainfed conditions on residual soil moisture. Early maturity coupled with wilt resistance is important for its adaptation to short-season environments and for escape from terminal stresses. The development of wilt resistant medium to large seeded, early maturing desi and kabuli varieties has helped in expansion of chickpea area to southern and central India and similar environments in Africa and elsewhere, which has typically short-season tropical environment (Gowda and Gaur 2004). Gaur *et al*. (2006) suggested that it is possible to breed extra large seeded kabuli varieties with high resistance to wilt. The new early maturing, high yielding desi and kabuli chickpea lines with high level of resistance to wilt identified in this work could be utilized as valuable breeding sources for chickpea improvement program in India.

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