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GENETIC POTENTIAL OF CHICKPEA GERMPLASM AGAINST Fusarium WILT (*Fusarium oxysporum.* f.sp. *ciceris*)

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A B S T R A C T

Sixty four advance lines of chickpea were evaluated against *Fusarium* wilt by sowing them in sick plot in research area of Plant Pathology Research Institute Faisalabad following augmented design to find out the resistance source. Three susceptible varieties CM-44, CM 72 and CM 2006 were used as check. Out of these 64 advance lines,7 lines TGxKo1, TGx1110, TGx1219, TG1307, TGx1232, TGx1218 and TGx1113 were found highly resistant ranging 0-10% plant mortality, 11 advance lines were resistant showing 11-20% plants mortality and 10 moderately resistant ranging 21-30% plant mortality. Remaining advance lines showed in susceptible and highly susceptible range showing 31-50% and more than 50% plant mortality, respectively. Highly resistant and resistant genotypes may be exploited for the development of resistant cultivars against wilt.

Keywords: Chickpea, Screening, *Fusarium oxysporum*

INTRODUCTION

Chickpea *(Cicer arietinum* L*)* is an important pulse crop which belongs to family *Fabaceae* ranking third after dry beans (*Phaseolus vulgaris* L.) and dry peas (*Pisum sativum* L.) (Dhar and Gurha, 1998).The Kabuli and Desi chickpea is grown throughout the world with different names i.e. Chickpea (UK), Garbanzo (Latin America), Bengal gram (Indian), Hommes, Hamaz (Arab world), Shimbra (Ethiopia), Nohud and Loblebi (Turkey). Chickpea is self-pollinated rabi crop, upto 1% cross-pollinated (Smithson *et al*., 1985; Singh, 1987). Chickpea is a rich source of protein, carbohydrates, vitamins, minerals and fibers, not only consumed as a pulse but also used in preparing a variety of snack foods, sweets and condiments and green fresh chickpeas are commonly consumed as a vegetable. According to FAO, 2014 chickpea is cultivated on area of 14.81 million hectares with 14.24 million tonns production around the world .In Pakistan it was grown on area of 0.99 million hectares with the production 0.75 million tonnes. The Thal area which comprised on districts Khushab, Mianwali, Chakwal, Bhakkar, Layyah, Faisalabad and Jhang (Punjab Province), Dera Ismail Khan, Karak and Bannu (North-West Frontier Province), Jafar abad and Dera Allah Yar (Balochistan Province) contributes about 80% of its production (Khan *et al*., 1991).Optimum temperature and crop water requirement for the growth of chickpea ranges from 18°C to 30°Cand 340 mm to 346mm respectively. (Devasirvatham *et al*., 2012; Lemma *et al*., 2016).

During last decade due to different biotic and biotic factors, the production of chickpea remained static or declined.(Ahmad., 2010). According to Nene *et al*., 1996 from all over the world more than 50 pathogens of chickpea has been identified but only a few of them have a potential to devastate the crop. Among the other diseases whish diminished the cultivation of crop *Fusarium* wilt is an acute disease of chickpea in Pakistan caused by *Fusarium oxysporum* f.sp.*ciceris* which is both seed as well assoil borne pathogen. (Pande *et al*.,2007). This pathogen has two pathotypes and eight pathogenic races on chickpea (Jorge *et al*., 2005). In case of yellowing pathotype vascular discoloration with plant death took place between 40 days Whereas in wilting pathotype plant death followed by chlorosis, flaccidity

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and vascular discoloration appear within 20 days after inoculation (Jimenez-Diaz *et al*., 1993; Haware and Nene, 1982).Fungus can survive on crop residues upto 6 years(Haware *et al*., 1986).The importance of disease is evident by the fact that in Punjab during 2008-09,its prevalence incidence and severity ranged from 92.58 ±3.69, 82.52±5.14 and 7.00±0.48,respectively (Ahmad., 2010).When the pathogen attacks, the plants show pale appearance with falling of leaves. The roots on examination showed brown discoloration of internal tissues which cause flagging and wilting resulting in severe yield losses (Haqqani *et al*., 2000). Epidemiological factors play key role in disease occurrence and maximum damage occurs at 25-30^{0}C.The temperatures below 15^{0}C and above 35^{0}C adversely affect the growth of fungus (Farooq *et al*., 2005). In Pakistan this disease can cause yield losses upto 10-50% every year (Ikramul and Farhat, 1992).Detection of new varieties is important, because prevailing resistant variety may become susceptible to new physiological races of the pathogen. Present study was conducted to evaluate newly developed genotypes of chickpea for resistance against wilt in order to identify the source of resistance. The results of this study will be useful for breeders to design effective resistance breeding program in chickpea.

METERIALS AND METHODS

Sick Plot Preparation: Sick plot was prepared and maintained by incorporating diseased plant debris followed by repeated cultivations of susceptible variety until the wilt incidence became more than 90%. The pathogen *Fusarium oxysporum* was isolated from diseased plants and was maintained on potato dextrose agar (PDA) medium at 25± 2˚C. For mass multiplication of pathogen, the Sorghum seeds were first were soaked in tap water overnight and then surface dried by spreading on paper towels. Surface dried seeds were put into conical flasks @ 250g/flask and the flasks were closed by inserting cotton plugs. These flasks were autoclaved at 15 psi for 20minutes. The sterilized flasks after cooling were inoculated with 7 days old actively growing culture of *F. oxysporum* f. sp. *ciceri* by adding 4 mm agar plugs with sterile cork borer. These flasks were incubated at 25^{o}C for 7 days. The cultured inoculum was incorporated in wilt sick plot (Nikam *et al.,* 2007).

Screening of germplasm: During November 2013, 64advance lines of chickpea were sown in research area of Plant Pathology Research Institute, Faisalabad (Pakistan). These varieties/lines were obtained from Arid Zone Research Institute, Bhakkar (Pakistan). Each line was planted in a single row of 3m length, with plant to plant distance 15cm and row to row 30cm. The experiment was conducted in augmented design. Germplasm was divided into six sets, four sets consisted of eleven advance lines and two sets consisted of ten advance lines excluding susceptible varieties CM 44, CM 72 and CM 2006 which were used as check in each set. All agronomic practices were same for all varieties/ lines.

Data collection: According to Ahmad *et al*., 2010 disease incidence is always higher at reproductive stage (0-57%) as compared to seedling stage (0-29.3%). So data for disease incidence was recorded in mid of March 2014 at reproductive stage and calculated by formula given below.

$$\text{Wiltincidence (\%)} = \frac{\text{Number of wilted plant}}{\text{Total number of plant}} = \times 100$$

The level of susceptibility and resistance of each test line/variety was determined by using 1-9 rating scale suggested by Iqbal *et al.,* 2005.

Table 1. Disease rating scale for evaluation of chick pea germplasm against Fusarium wilt disease

Disease Rating	% Infection	Disease response
1	0-10% plant wilted	Highly Resistant
3	11-20% plant mortality	Resistant
5	21-30% plant mortality	Moderately Resistant
7	31-50% plant mortality	Susceptible
9	More than 50% plant mortality	Highly Susceptible

DATA ANALYSIS

The analysis of variance (ANOVA) and the differences among means were analyzed by applying LSD test at 5% level of probability (Steel *et al.,* 1997).

RESULTS

Response of sixty four advance lines along with the three check varieties were assessed against the disease. The data revealed that twenty two advance lines

(TGx1203, TG1309, TG1305, T4x205, TGx1207, TGx1315, TGx214, TGx1221, TGx1228, TGx220, TGx1107, TGx228, TG1129, CM2006, CM72, CM44, TGx1213 , TGx1209, TGx12K10, TGx1101 ,TGx1313, TGx1210) behaved as highly susceptible with more than 50% plant mortality. Among the highly susceptible lines thirteen showed 1 to 24%higher disease incidence as compared to check varieties. Profuse growth of fungus could also be observed on dead and wilted plants with mummified or no seed formation in pods. Some of these lines also showed the early wilting at seedling stage. Seventeen Advance lines (TGx1222, TGx1312, TGx1317, TGx1401, TGx1403, TGx1303, TGx1205, TGxK04, TG1310, TGx12Ko9, TG12K13, TGx1402, 09AK055, TGxKo9, TGx1301, TGx1204 and TGx1304) were rated as susceptible with31to 50% plant mortality.While10 lines (TGx1308, TGx12K01, TGx1318, TGx12Ko7, 16x201, TG1123, TG1117, TGx12K04, TGx12K05, TGx12Ko6) were found to be moderately resistant with 21 to 30 % plant mortality. Other 11 lines (TGx12K02, TGx1112, TGx1400, TGx1302, TGx1306, TG1105, TGK1319, TGx1311, TGx1314, TGx1108 and TG225) were ranked as resistant against the pathogen with 11-20% plant mortality. Further seven lines (TGxKo1, TGx1110, TGx1219, TG1307, TGx1232, TGx1218 and TGx1113) were found highly resistant towards disease with 0 to 10% plant mortality. Disease plants in resistant and highly resistant advance lines showed the same symptoms as described in moderately resistant plants but their number was less as compared to the moderately resistant lines.

DISCUSSION

Evaluation of chickpea germplasm against wilt disease caused by *Fusarium oxysporum* is a common practice and already have been carried out by different researchers all over the world. Ahmad *et al*., 2010;Chaudhry*et al*., 2007; Infantino, 2006; Elfatih *et al*., 2002.Sick plot method has been widely used and was found efficient and also provided natural fluctuating environmental condition between pathogenic and non-pathogenic microorganisms. The present experiment was conducted in field (sick plot) by keeping in view above mentioned fact and the results of Ahmad *et al*., 2010 which indicates that incidence and severity of chickpea wilt is higher in field and as compared to the green house. None of the test line was found immune or highly resistant by the Chaudhry *et al*., 2007 when 196 chickpea lines/cultivars were screened to determine the resistance against *Fusarium* wilt. Chaudhry *et al*., 2006 evaluated 414 varieties/ lines against the disease and found 35 test lines. Chickpea germplasm originating from national and international research institutes was evaluated by Iqbal *et al*., (2005) against *Fusarium* wilt. Identified 14 chickpea lines having resistant against wilt at seedling stage but no line was found to be resistant at reproductive stage. The results of present study also not only coincide with Iqbal *et al*.,2005 but also with the Ahmad *et al*., 2010,Haware 1996; the reason might be that high temperature favor the disease development as compared to low temperature . High temperature prevails for a short time at seedling stage due to onset of winter while at reproductive stage due to onset of summer season it prevailed for a long time. That's why most of the varieties that shows resistance to the disease at seedling stage become susceptible a reproductive stage.

Ayyub *et al*., 2003 found high level of resistance in chickpea germplasm originating from different sources. A similar study was conducted by Bajwa *et al*., 2000 who evaluated 32 genotypes of chickpea against *F. oxysporum* f. sp. *ciceris* and found that only 1 line (97021) was resistant, 4 lines (97019, 97020, 97022 and 97023) were tolerant and 27 lines were susceptible to highly susceptible. Gurha *et al*., 2002 found that out of 570 chickpea lines 22 genotypes were resistant to *Fusarium* wilt. Ayyub *et al*., 2001 stated that under field conditions, among 101 lines,9 lines could not show any expression of the disease symptoms. While, 8 lines behaved as resistant and 7 lines as moderately resistant. All the remaining lines were found to be susceptible to highly susceptible.

Resistant lines of chickpea have been reported from many other countries but their success has been limited due to location specific races of *Fusarium oxysporum* (Singh and Reddy, 1991).

Use of resistant cultivars is the most ideal and economical way of managing *Fusarium* wilts which are not common in the existing chickpea advance lines. The present study revealed some useful advance lines in the chickpea, which exhibiting resistance. These lines should be incorporated in breeding programme for evolution of new genetic material against chickpea wilt.

Table 2. Evaluation of chickpea germplasm against *Fusarium oxysporum sp. ciceris.*

Sr. No.	Advance lines	Disease incidence (%)	Disease response
1	TGx1203	92.55 A	HS
2	TG1309	88.08 Ab	HS
3	TG1305	84.88 Abc	HS
4	T4x205	82.11 Abc	HS
5	TGx1207	81.05 Abcd	HS
6	TGx1315	79.75 Abcd	HS
7	TGx214	79.51 Abcd	HS
8	TGx1221	76.95 Bcde	HS
9	TGx1228	74.78 Bcdef	HS
10	TGx220	73.41 Cdefg	HS
11	TGx1107	69.51 Defgh	HS
12	TGx228	69.21 Defgh	HS
13	TG1129	69.08 Defghi	HS
14	CM2006	68.08 Efghi	HS
15	CM72	65.26 Fghi	HS
16	CM44	65.10 Fghij	HS
17	TGx1213	64.65 Fghij	HS
18	TGx1209	61.05 Ghijk	HS
19	TGx12K10	58.51 Hijkl	HS
20	TGx1101	56.61 Ijklm	HS
21	TGx1313	55.35 Jklm	HS
22	TGx1210	51.05 Klmn	HS
23	TGx1222	46.55 Lmno	S
24	TGx1312	45.68 Lmnop	S
25	TGx1317	44.15 Mnopq	S
26	TGx1401	43.65 Mnopq	S
27	TGx1403	41.41 Nopqr	S
28	TGx1303	39.98 Nopqrs	S
29	TGx1205	39.75 Nopqrst	S
30	TGxK04	39.75 Nopqrst	S
31	TG1310	38.28 Nopqrst	S
32	TGx12Ko9	37.01 Opqrstu	S
33	TG12K13	36.71 Opqrstu	S
34	TGx1402	36.15 Opqrstu	S
35	09AK055	33.31 Opqrstuv	S
36	TGxKo9	32.91 Pqrstuv	S
37	TGx1301	32.08 Qrstuvw	S
38	TGx1204	31.35 Qrstuvw	S
39	TGx1304	30.88 Qrstuvw	S
40	TGx1308	29.68 Rstuvw	MR
41	TGx12K01	28.35 Rstuvwx	MR
42	TGx1318	27.95 Rstuvwx	MR
43	TGx12Ko7	27.41 Stuvwx	MR
44	16x201	26.41 Tuvwxy	MR
45	TG1123	24.58 Uvwxyz	MR
46	TG1117	23.58 Uvwxyz	MR
47	TGx12K04	21.85 vwxyzA	MR
48	TGx12K05	21.81 vwxyzA	MR
49	TGx12Ko6	21.21 wxyzAB	MR
50	TGx12K02	19.95 wxyzABC	R
51	TGx1112	18.91 wxyzABCD	R
52	TGx1400	16.78 xyzABCDE	R
53	TGx1302	16.68 xyzABCDE	R
54	TGx1306	15.48 xyzABCDE	R
55	TG1105	14.88 xyzABCDE	R
56	TGK1319	14.21 yzABCDE	R
57	TGx1311	13.98 yzABCDE	R
58	TGx1314	13.05 yzABCDE	R
59	TGx1108	12.41 zABCDE	R
60	TGx225	11.78 ABCDE	R
61	TGxKo1	8.51 ACDE	HR
62	TGx1110	8.11 BCDE	HR
63	TGx1219	6.65 CDE	HR
64	TG1307	5.98 DE	HR
65	TGx1232	5.88 DE	HR
66	TGx1218	4.65 E	HR
67	TGx1113	4.41 E	HR

LSD = 10.29 Level of probability 0.05

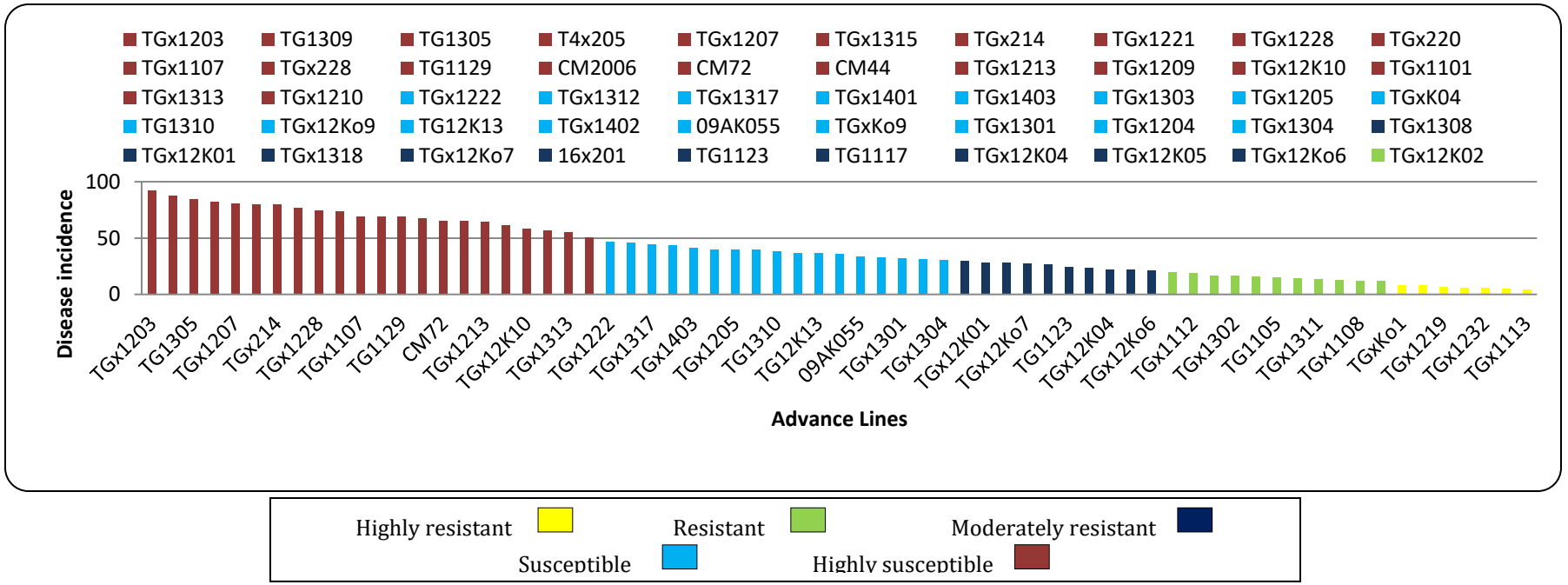

Figure 1. Graphical representation for the Incidance of *Fusarium oxysporum .sp ciceris* on different advance lines of chickpea

Table 3: Summary of disease incidence data of chickpea germplasm against *Fusarium oxysporum* f. sp. *ciceris*

Disease Incidence %	Reaction		No. of varieties lines	varieties/lines
0-10% plant wilted	Highly resistant	HR	7	TGxKo1, TGx1110, TGx1219, TG1307, TGx1232, TGx1218, and TGx1113
11-20% plant mortality	Resistant	R	11	TGx12K02, TGx1112, TGx1400, TGx1302, TGx1306, TG1105, TGK1319, TGx1311, TGx1314, TGx1108, and TG225
21-30% plant mortality	Moderately resistant	MR	10	TGx1308, TGx12K01, TGx1318, TGx12Ko7, 16x201, TG1123, TG1117, TGx12K04, TGx12K05, TGx12Ko6
31-50% plant mortality	Moderately susceptible	S	17	(TGx1222, TGx1312, TGx1317, TGx1401, TGx1403, TGx1303, TGx1205, TGxK04, TG1310, TGx12Ko9, TG12K13, TGx1402, 09AK055, TGxKo9, TGx1301,TGx1204, and TGx1304)
More than 50%% plant mortality	Susceptible	HS	22	TGx1203, TG1309, TG1305, T4x205, TGx1207, TGx1315, TGx214, TGx1221, TGx1228, TGx220, TGx1107, TGx228, TG1129, CM2006, CM72, CM44, TGx1213 , TGx1209, TGx12K10, TGx1101 ,TGx1313, TGx1210
		Total	67	

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