Pak. J. Bot., 41(4): 1945-1955, 2009.

SCREENING OF CHICKPEA (*CICER ARIETINUM*) INDUCED MUTANTS AGAINST *FUSARIUM* WILT

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Abstract

Two hundred and forty nine chickpea mutants in M_4 generation developed through gamma irradiation and Ethyl methane sulphonate (EMS) were screened along with their respective parents and susceptible check Aug-424 for resistance to *Fusarium* wilt in natural wilt sick plot during 2003-2004 seasons. All the 4 parent genotypes showed highly susceptible reaction to *Fusarium* wilt. Out of a total of 249 morphological mutants of 4 genotypes, 75 mutants exhibited highly resistant reaction (less than 10 %) followed by 31 mutants resistant (11 to 20%), 34 mutants moderately resistant / tolerant (21 to 30%), 35 mutants susceptible (31 to 50%) and 75 mutants were highly susceptible (50 to 100%). The mutagenic treatments proved to be effective in producing morphological mutations along with improved tolerance to *Fusarium* wilt. These mutants with resistant to tolerant reaction for *Fusarium* wilt could be used in hybridization program for transferring of resistance genes into high yielding elite cultivars/ producing better recombinants.

Introduction

Chickpea (*Cicer arietinum* L.) is the most important food legume crop of Pakistan. It is cultivated on an area of 1073 thousands hectares with 785 kg^{-1} yield and 842 thousand tones production (Anon., 2006-07). The average yield of chickpea in Pakistan is lower than the other leading countries of the world and has been unreliable and low amounting to only about 10% of the world's production (Auckland & Van-der-Maesan, 1980). One of the factors responsible for low yield is the incidence of diseases mostly the wilt caused by *Fusarium oxysporum* Schlecht. Emend Snyd. & Hans. f.sp. *ciceri* Padwick. The yield losses due to this disease may fluctuate from 10-90% (Jimenez-Diaz *et al.*, 1989; Ratnaparkhe *et al*., 1998; Akhtar, 2008). Approximately, the loss of one million dollar annually may be caused by this disease in Pakistan (Sattar *et al.*, 1953). The wilt has reduced the share of chickpea from 50% in 1950s to 10% in 1990s on irrigated lands in Pakistan (Hanif *et al*., 1999).

Fusarium oxysporum f.sp. *ciceris* is the second most severe problem after blight in Pakistan (Khan, 1980), particularly in Thal area *i.e.,* districts of Jhang, Layyah, Khushab, Bhukkar and Mianwali. The disease is a vascular pathogen that travel in seed and soil (Kraft *et al*., 1994; Pande *et al.*, 2007) and consequently is difficult to handle by the use of chemicals and through crop rotation. The pathogen can stay alive in the soil in the absence of the host for at least 6 years (Stevenson *et al.*, 1995; Haware *et al.*, 1996). The wilt can be observed in susceptible genotype within 25 days after sowing in the field. The pathogens attack the roots of plants and cause wilting as a result the whole plant shows drooping of leaves and paler color than healthy plants. The plant finally collapses and dies. Such plants do not show external rotting and look healthy, when cut vertically downward from the collar region, show brown streak of the internal tissues.

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Since most of the commercial cultivars in the country have been found to be susceptible, there is therefore urgent need for an extensive screening of germplasm for the identification of resistant sources. But screening program of chickpea germplasm has abortive to identify stable and high level resistance against a number of diseases (Singh & Reddy, 1993; Singh *et al*., 1994). Limited germplasm of chickpea resistant to *Ascochyta* blight and *Fusarium* wilt is found in existing chickpea species so it is, necessary to search out new sources of resistance to this disease (Reddy & Singh, 1984).

The use of induced mutation appears to be the best management option for the disease. Mutation breeding does not disturb co-adapted linkages of agronomically important commercial varieties and can create new and complex loci for resistance that can confer durable resistance. In view of above facts, it was planned to conduct the screening of advance promising morphological mutants in M_3 and M_4 generation for the identification of mutant (s) having increased level of resistance to *Fusarium* wilt.

Materials and Methods

Screening for *Fusarium* wilt: Genetic variability was induced in two desi (Pb2000 and C44), one kabuli (Pb-1) and one desi x kabuli recombinant genotype (CH40/91) through gamma irradiation and Ethyl methane sulphonate (EMS) and 249 morphological mutants were selected from M_2 population. A set of 249 true breeding morphological and blight tolerant mutants from Pb2000, C44, Pb-1 and CH40/91, in M_4 generation and their respective parents were screened for resistance to *Fusarium* wilt in natural wilt sick plot by applying the sick plot technique developed by Nene *et al*., (1981). The field was highly infested causing 100% wilt to all lines of the susceptible check AUG-424. The wilted plants were uprooted and plated on PDA (Potato Dextrose Agarose) medium. All the wilted plants produced *Fusarium oxysporum* f.sp. *ciceris* isolates with 98% as a pure colony, thus confirming that the field is sick for *Fusarium* wilt. The mutants were sown in this field in the third week of October. Sixty seeds per test mutant were sown in a two row, 4 meter long with inter and intra row spacing of 30 and 15 cm respectively with 3 replications. The susceptible check (Aug 424) was sown after every second-test line so that the performance of test lines could be evaluated and at the same time fungus inoculums maintained in the plot. Weeding was performed manually. The wilt incidence was noted at 10-day intervals starting from 30 days after sowing till seed maturity and harvest (Haware *et al*., 1992). The data on the number of wilted seedlings in each row for each mutant was calculated for each mutant line by using the following formula:

$$\text{Wilt incidence (\%)} = \frac{\text{No. of plants wilted}}{\text{Total number of plants}} \text{ x100}$$

The level of resistance/susceptibility of each mutant line was determined by using the rating scale of Iqbal *et al*., (1996). Plants wilted in these nurseries were taken to laboratory and the pathogens were isolated to confirm that the diseased caused were of *Fusarium oxysporum* f.sp. *ciceris*.

Disease incidence	**Response**
0-10 percent	Highly resistant
11-20 percent	Resistant
21-30 percent	Moderately resistant/ Tolerant
31-50 percent	Susceptible
51-100 percent	Highly susceptible

Promising lines were critically evaluated and mutants that showed less than 20% mortality in the field were selected for further studies.

Results and Discussion

The susceptible check variety (Aug 424) along with the susceptible mutant lines were uniformly killed throughout in the natural wilt sick plot during 2003-2004. There was no variability in inoculum distribution in the field as in all cases the pathogens isolated were found to be *F. oxysporum* f.sp *ciceris* containing more than 98% as pure isolates. The results of field reaction of M_4 mutants of Pb2000, C44, Pb1 and CH40/91 to *Fusarium* wilt are presented in Table 1 and some important morphological mutants discussed in this paper are included in Table 4. All the 4 parents showed highly susceptible reaction to *Fusarium* wilt. Out of a total 249 morphological mutants of 4 genotypes, 75 mutants (30.1%) had less than 10 ratings (highly resistant), 31 mutants (12.5%) had 11 to 20% (resistant), 34 mutants (13.7%) had 21 to 30% (moderately resistant), 35 mutants (14.1%) had 31 to 50% (susceptible) and 75 mutants (30.1%) had 50 to 100 (highly susceptible) rating. Among the desi genotype Pb2000, 30 mutants (30.6%) exhibited highly resistant reaction followed by 16 mutants (16.3%) resistant and moderately resistant, 22 mutants (22.5%) susceptible and 14 mutants (14.3%) highly susceptible reaction against *Fusarium* wilt. The 43 mutants (45.3%) of desi genotype C44 rated as highly resistant followed by 13 mutants (13.7%) as resistant, 12 (12.6%) as moderately resistant, 6 mutants (6.3%) as susceptible and 22 mutants (23.2%) as highly susceptible. In kabuli genotype Pb-1, 35 mutants (85.4%) showed highly susceptible and 3 mutants (7.3%) susceptible reaction while only one mutant was found to be highly resistant and two were moderately resistant. The mutants of desi x kabuli introgression genotype CH40/91 showed mixed reaction against *Fusarium* wilt. Out of 15 mutants, only one was highly resistant while two were resistant. The remaining 12 mutants equally showed moderately resistant (26.7%), susceptible (26.7%) and highly susceptible (26.7%) reaction to this disease.

Overall among the 4 genotypes (Pb2000, C44, Pb-1 and CH 40/91), the induction of resistance/susceptibility was higher in mutants of desi genotypes Pb2000 (39.4%) and C44 (38.2%) followed by kabuli genotype Pb-1 (16.5%) and desi x kabuli genotype CH40/91 (6.0%) (Table 2). Overall the higher number of resistant/susceptible mutants in the doses of gamma irradiation treatments were observed in desi x kabuli genotype CH40/91 (100%) followed by desi genotypes Pb2000 (68.4%), C44 (53.7%) and kabuli genotype Pb-1 (43.9%) (Table 2). The pooled data of physical and chemical treatments revealed that the higher number of resistant/susceptible mutants was induced by gamma rays (60.4%) than EMS (39.6%) treatments (Table 3).

The (ANOVA) table revealed that the variation among 249 mutants were highly significant. The mean disease scores and their standard errors (SE) for all mutants tested in the screening nursery are given in the Table 4. Mutants possess significantly lower (at $p \geq 0.05$ and $p \geq 0.01$) mean disease scores than that of cultivar Aug-424 (susceptible check). These results indicated that mutagenic treatments were effective in inducing genetic variability for *Fusarium* wilt resistance in addition to promising morphological mutants with higher level of resistance in 4 chickpea genotypes.

Table 1. Disease reaction of M_4 mutants of four chickpea genotypes to *Fusarium* wilt at wilt sick plot.

Genotypes	Dose	No. of plants with disease reaction					Total
		HS*	S**	MR†	R‡	HR††	
Pb.2000	Control						
	300Gy	3	8	4	10	20	**45**
	400Gy	4	6	7	3	2	**22**
	0.3%EMS	2	6	4	2	2	**16**
	0.4%EMS	5	2	1	1	6	**15**
	Total	**14 (14.3%)**	**22 (22.5%)**	**16 (16.3%)**	**16 (16.3%)**	**30 (30.6%)**	**98 (39.4%)**
C44	Control						
	500Gy	3	0	2	0	10	**15**
	600Gy	0	1	5	10	20	**36**
	0.3%EMS	9	3	4	2	10	**27**
	0.4%EMS	10	2	1	1	3	**17**
	Total	**22 (23.2%)**	**6 (6.3%)**	**12 (12.6%)**	**13 (13.7%)**	**43 (45.3%)**	**95 (38.2%)**
Pb.1	Control						
	200Gy	9	3	1	0	1	**14**
	300Gy	4	0	0	0	0	**4**
	0.2%EMS	13	0	0	0	0	**13**
	0.3%EMS	9	0	1	0	0	**10**
	Total	**35 (85.4%)**	**3 (7.3%)**	**2 (4.9%)**	**0 (0%)**	**1 (2.4%)**	**41 (16.5%)**
CH40/91	Control						
	200Gy	4	3	1	1	1	**10**
	300Gy	0	1	3	1	0	**5**
	0.2%EMS	0	0	0	0	0	**0**
	0.3%EMS	0	0	0	**0**	0	**0**
	Total	**4 (26.7%)**	**4 (26.7%)**	**4 (26.7%)**	**2 (13.3%)**	**1 (6.7%)**	**15 (6.0%)**
	G. Total	**75 (30.1%)**	**35 (14.1%)**	**34 (13.7%)**	**31 (12.5%)**	**75 (30.1%)**	**249**

HS* =Highly susceptible, S** =Susceptible, MR† =Moderately resistant, R‡ =Resistant, HR†† =Highly resistant

Double poddedness is considered an advantage (6-11% yield advantage) over single poddedness in yielding ability (Sheldrake *et al*., 1978). However, all double-podded accessions in the chickpea germplasm at International Crops Research Institute for Semi Arid Tropics (ICRISAT) were reported to be highly susceptible to *Fusarium* wilt (Kumar & Haware, 1983). In our present study, 9 double poded mutants (CM418-1/01, CM446-1/01, CM499/01, CM499-1/01, CM499-2/01, CM554-1/01, CM554-2/01, CM557-2/01 and CM557-4/01) were highly resistant, 5 (CM557-5/01, CM557-6/01, CM557-7/01, CM557-8/01 and CM499-5/01) were resistant and only one (CM506-2/01) was moderately resistant to *Fusarium* wilt indicating that it is now possible to breed wilt resistant double podded with two or more seeded per pod for the improvement of yield in chickpea.

Table 2. The overall pooled data showing disease reaction vs mutagenic treatment in four chickpea genotypes for screening against *Fusarium wilt*.

Genotypes	Dose	No. of plants with disease reaction					Total
		HS*	S**	MR†	R‡	HR††	
Pb.2000	Gamma rays	7	14	11	13	22	**67 (68.4%)**
	EMS	7	8	5	3	8	**31 (31.6)**
	Total	**14 (14.3%)**	**22 22.5%)**	**16 (16.3%)**	**16 (16.3%)**	**30 (30.6%)**	**98 (39.4%)**
C44	Gamma rays	3	1	7	10	30	**51 (53.7%)**
	EMS	19	5	5	3	13	**44 (46.3%)**
	Total	**22 (23.2%)**	**6 (6.3%)**	**12 (12.6%)**	**13 (13.7%)**	**43 (45.3%)**	**95 (38.2%)**
Pb.1	Gamma rays	13	3	1	0	1	**18 (43.9%)**
	EMS	22	0	1	0	0	**23 (56.1%)**
	Total	**35 (85.4%)**	**3 (7.3%)**	**2 (4.9%)**	**0 (0%)**	**1 (2.4%)**	**41 (16.5%)**
CH40/91	Gamma rays	4	4	4	2	1	**15 (100%)**
	EMS	0	0	0	0	0	**0**
	Total	**4 (26.7%)**	**4 (26.7%)**	**4 (26.7%)**	**2 (13.3%)**	**1 (6.7%)**	**15 (6.0%)**
	G. Total	**75 (30.1%)**	**35 (14.1%)**	**34 (13.7%)**	**31 (12.5%)**	**75 (30.1%)**	**249**

HS* =Highly susceptible, S** =Susceptible, MR† =Moderately resistant, R‡ =Resistant, HR†† =Highly resistant

Table 3. The overall pooled data of resistance of mutants over genotypes for gamma radiation and EMS treatments of screening against *Fusarium wilt*.

Treatments	No. of plants with disease rating					Total
	HS*	S**	MR†	R‡	HR††	
Gamma rays	27	22	23	25	54	**151 (60.4%)**
EMS	48	13	11	6	21	**98 (39.6)**
Total	**75**	**35**	**34**	**31**	**75**	**249**

HS* =Highly susceptible, S** =Susceptible, MR† =Moderately resistant, R‡ =Resistant, HR†† =Highly resistant

In the present study, chickpea mutants reactions against *Fusarium* wilt observed were some what comparable to those reported earlier by Iqbal *et al*., (2005), Zote *et al*., (1983, 1993), Dandnaik & Zote (1988). Sharma *et al.*, (2004) and Dandnaik & Zote (1988) screened 400 genotypes for resistance against wilt in wilt sick plot. Of them 6 lines were reported as resistant (10% mortality) against chickpea wilt. Gurha *et al*., (2002) screened 570 chickpea genotypes for resistance to isolate (Race-2) of *F.*

oxysporium and reported 21 cultivars exhibited stable resistance against *Fusarium* wilt. At Pulses Research Institute, Faisalabad, 414 varieties/germplasm accessions were evaluated for *Fusarium* wilt in a wilt sick plot developed during the year 2002-03 and 2003-04 by Munir *et al*., (2006). Thirty-five test lines were found resistant, 208 intermediate/tolerant, 77 susceptible and 94 were highly susceptible. Ahmad *et al*., (2007) were evaluated 158 genotypes under artificial disease condition. At seedling stage the disease incidence ranged from 0% to 57.2% and at reproductive stage it varied from 0% to 100%. At seedling stage, 107 genotypes exhibited resistant response, 29 were tolerant and 22 were susceptible. Only 3 genotypes with disease incidence 0%, 6.7% and 8.3% were resistant, 4 with disease incidence of 18.2 to 20% were tolerant and 151with disease incidence of 25% to 100% were susceptible at reproductive stage. Neupane *et al.*, (2007) screened 77 chickpea cultivars in wilt sick plot during 2003/04 and 2004/05 in Nepal. Of the 77 genotypes, 37 genotypes were resistant (≤10.0%), 13 moderately resistant (10.1-20.0%), 19 susceptible (20.1-50.0%) and 8 highly susceptible (>50.0%) to *Fusarium* wilt. Two genotypes ICCV 95432 and ICCV 03405 showed complete resistance (0% plant mortality) to FW in both the years. Recently developed 117 desi chickpea genotypes at ICRISAT, India were evaluated against *Fusarium* wilt in wilt sick plot. Three genotypes (ICCV 05526, ICCV 05530, ICCV 05533) were found to be asymptomatic (0% mortality), 11 resistant and 4 moderately resistant (Pande *et al*., 2007).

Several workers have recognized sources of resistance to *Fusarium* wilt (Nene & Haware, 1980; Halila *et al*., 1984; Jim´enez-Diaz *et al*., 1991; Bhatti & Kraft, 1992) but most of these were of the 'desi' type and very few of the 'kabuli' type. Halila & Strange, (1997) screened a total of 1915 kabuli chickpea lines in a wilt sick plot and complete resistance was only observed in only 110 lines. Nene *et al*., (1989) also reported several 'desi' chickpea lines with broad-based and stable resistance to wilt. Haware *et al*., (1992) screened over 13,500 accessions of chickpea germplasm for resistance to race 1 of *Fusarium oxysporum*. They found 160 were resistant but only 10 of these were of the 'kabuli' type. Desi types are considered as a good source of resistance to *Fusarium* wilt. In the present study, out of 249 mutants of desi, kabuli and desi x kabuli, 73 desi, and only one of each kabuli and desi x kabuli introgression mutant was found to be highly resistant to *Fusarium* wilt and confirmed the findings of above workers. Because 'kabuli' chickpeas are susceptible to most of the *F. oxysporum ciceris* races (Jim´enez-D´ıaz & Trapero-Casas, 1990), therefore, efforts must be addressed toward developing new alternatives for more effective disease management.

Some white flowered and white seeded mutants developing from desi genotypes (CM27/02 from Pb2000 and CM553/01, CM 430/01 from C44) were highly resistant to *Fusarium* wilt. These white seeded mutants having inbuilt wilt resistance is good addition in kabuli chickpea germplasm; because most of the natural germplasm of white seeded is susceptible to wilt (Haware *et al*., 1992; Jim´enez-D´ıaz & Trapero-Casas, 1990). By the use of induced mutations in desi chickpea, the scarcity of resistance in the kabuli germplasm could be enhanced and the world kabuli germplasm may be improved for wilt resistance.

In contrast to desi genotype, the pink flowered mutants, CM1715/01, CM1411/01, CM2278/01 induced in kabuli chickpea were highly susceptible to wilt. These results indicated that pink flower mutants in kabuli chickpea have no practical and commercial value.

Early type mutants are normally wilt susceptible but in this study some wilt resistant and early mutants (CM51/01, CM72/01, CM461/02, CM517/02) were isolated. These mutants may be used as releasing early type varieties for green vegetable (Chollia) which may fetch higher price as compared to other late chickpea varieties.

Table 4. Disease score of some selected M_4 morphological mutants against *Fusarium* wilt.

Sr. No.	Mutant	Mutagenic dose	Character	Wilt rating (Mean±SE)	Class
	Aug 424	**Check**		**95±1.53**	**H.S**
	Pb2000	**Control**		**75±1.37***	**H.S**
1.	CM27/02	300Gy	White flower	13.7±0.72**	R
2.	CM51/01	300Gy	Early flower	4±0.72**	H.R
3.	CM72/02	300Gy	Early flower	13.3±0.72**	R
4.	CM96/01	300Gy	Early flower	37±1.74**	S
5.	CM137/01	300Gy	Early flower, gigas	4±0.72**	H.R
6.	CM321/01	0.4% EMS	Early flower	68±1.30*	H.S
7.	CM461/02	0.4% EMS	Early flower	1.0±0.47**	H.R
8.	CM517/02	0.4% EMS	Early flower	1.0±0.47**	H.R
	C44	**Control**		**63±0.55***	**H.S**
9.	CM418-1/01	500Gy	Double flower, double pod	4±0.59**	H.R
10.	CM430/01	500Gy	White flower, white seed	5±0.59**	H.R
11.	CM446-1/01	500Gy	Double flower, double pod	7±0.89**	H.R
12.	CM499/01	600Gy	Double Pod	2±0.59**	H.R
13.	CM499-1/01	600Gy	Double flower, double pod	4±0.72**	H.R
14.	CM499-2/01	600Gy	Double flower, double pod	8±0.72**	H.R
15.	CM499-5/01	600Gy	Double flower, double pod	14±1.52**	R
16.	CM506-2/01	600Gy	Double Pod	29±1.09**	MR
17.	CM553/01	600Gy	White flower, white seed	9±1.09**	H.R
18.	CM554-1/01	600Gy	Double flower, double pod	8±0.72**	H.R
19.	CM554-2/01	600Gy	Double flower, double pod	5±0.89**	H.R
20.	CM557-2/01	600Gy	Double flower, double pod	6±0.72**	H.R
21.	CM557-4/01	600Gy	Double flower, double pod	4±0.72**	H.R
22.	CM557-5/01	600Gy	Double flower, double pod	13±1.30**	R
23.	CM557-6/01	600Gy	Double flower, double pod	11±1.09**	R
24.	CM557-7/01	600Gy	Double flower, double pod	15±1.09**	R
25.	CM557-8/01	600Gy	Double flower, double pod	15±0.72**	R
26.	CM1020-2/01	0.4% EMS	Early flower	73±3.58*	H.S
27.	CM1106/01	0.4% EMS	Early flower	93±1.30 ^{NS}	H.S
28.	CM1732/01	300Gy	Early flower	96±2.19 ^{NS}	H.S
29.	CM1715/01	200Gy	Pink flower	94±1.52 ^{NS}	H.S
30.	CM1411/01	0.2% EMS	Pink flower	88±1.52 ^{NS}	H.S
31.	CM2081/01	0.2% EMS	Pink flower	77±1.74*	H.S
32.	CM2278/01	0.3%EMS	Pink flower	87±1.96 ^{NS}	H.S
	CH40/91			72±0.65*	H.S.
33.	CM1534/01	200Gy	Early	49±1.09**	S
34.	CM1590/01	300Gy	Early	27±1.96**	MR

Classification: R= Resistant, S= Susceptible, HS= Highly susceptible, MR= Moderately resistant, T= Tolerant, HR= Highly resistant

*****Mean disease score is significantly different at p≥0.05 from cv. Aug4-24 control

******Mean disease score is highly significantly different at p≥0.01 from cv. Aug-424 control; NS non-significant

Table 5. Detail of chickpea mutants having multiple tolerance/resistance against *Ascochyta* blight and *Fusarium* wilt.

Sr. No.	Mutant	Mutagenic dose	Character	Blight rating (Mean±SE) †	Class	Wilt rating (Mean±SE)	Class
1.	CM54-5/02	300Gy	Semi-spreading	3.9±0.06**	R	13.3±0.98**	R
2	CM 59-1/02	300Gy	Semi-spreading	3.3±0.26**	R	24.3±0.98**	T
3.	CM 72/02	300Gy	Early	5.1±0.38*	T	13.3±0.72**	R
4.	CM86-2/02	300Gy	Semi-spreading	4.7±0.25**	T	8.0±0.94**	H.R
5.	CM 86-5/02	300Gy	Semi-spreading	4.8±0.19**	T	13.3±0.72**	R
6.	CM94-1/01	300Gy	Bold seed	4.7±0.25**	T	28±1.30**	T
7.	CM94-2/01	300Gy	Bold seed	5.0±0.45*	T	22±0.89**	T
8.	CM128/01	300Gy	Compact	14±1.09**	R	14±1.09**	R
9.	CM149/01	400Gy	Open canopy	5.4±0.28**	T	13±1.09**	R
10.	CM176-2/01	400Gy	Broad leaf	4.7±0.12**	T	8±0.72**	H.R
11.	CM188/01	400Gy	Tall, Broad leaf	4.8±0.19**	T	29±1.09**	T
12.	CM191/01	400Gy	Extra vigorous	5.3±0.31*	T	13±1.30**	R
13.	CM236/01	400Gy	Extra broad leaf	4.3±0.37**	T	24±1.09**	T
14.	CM269/01	400Gy	Round pod	4.4±0.22**	T	29±0.89**	T
15.	CM303/01	0.3% EMS	Blue flower	4.7±0.22**	T	28±1.09**	T
16.	CM359/01	500Gy	Vigorous	3.7±0.45*	R	26±2.19**	T
17.	CM393-1/01	500Gy	Spreading,.vig.	4.5±0.26**	T	28±1.30**	T
18.	CM542/01	600Gy	Spreading	5.1±0.33*	T	18±1.52**	R
19.	CM575-1/01	0.3% EMS	Thick stem, compact	4.9±0.26**	T	26±2.42**	T
20.	CM609/01	0.3% EMS	Wilt resistant	5.1±0.33*	T	19±1.09**	R
21.	CM891/01	0.3% EMS	S. pod, compact	4.4±0.28**	T	26±2.42**	T
22.	CM1127/01	0.4% EMS	Broad leaf	5.1±0.43*	T	27±1.74**	T
23.	CM2283-2/01	0.3%EMS	Bold seed	4.9±0.48*	T	28±1.52**	T
24.	CM1511/01	200Gy	Semi spreading	3.8±0.34**	R	27±1.96**	T
25.	CM1590/01	300Gy	Early	4.4±0.24**	T	27±1.96**	T
26.	CM1631/01	300Gy	Bold pod	5.1±0.38*	T	14±1.09**	R

Classification: R= Resistant, T= Tolerant, HR= Highly resistant

*****Mean disease score is significantly different at p≥0.05 from cv. K-850 control

******Mean disease score is highly significantly different at p≥0.01 from cv. K-850 and Aug-424 controls † (Shah *et al*., 2007)

Multiple disease resistance is not common phenomenon in chickpea. The genotypes having resistance to both diseases (*Ascochyta* blight and *Fusarium* wilt) is valuable and positive feature. In another study, these mutants were screened against *Ascochyta* blight and only 79 mutants showed highly resistant reaction to blight (Shah *et al.*, 2007). In the present research, out of 249 mutants, only 26 mutants have multiple tolerance/resistance (Table 5). Multiple resistant mutants may be helpful in stabilizing the yield of country and they are equally good for drought, barani as well as irrigated environments. The genetic variability showing resistance to both diseases could be used in hybridization program for transferring multiple resistance traits into high yielding elite cultivars. The promising mutants with resistance to blight and wilt would be a good source for transferring resistance and making desirable recombinants or may be used directly as a variety.

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(Received for publication 22 October 2008)