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Efficacy of gamma rays, ethyl methane sulphonate and their combination treatment for resistance/tolerance against *Fusarium oxysporum* f. sp. *ciceri*

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Abstract
Chickpea (*Cicer arietinum* L.) is the third most important pulse crop grown throughout the world. About 65 per cent of the global area and 68 per cent of global production of chickpea is contributed by India. *Fusarium oxysporum* f. sp. *ciceri* is a soil-borne as well as seed-borne fungus. During the past few decades, modern techniques such as mutation breeding by radiation and chemical mutagens have been tried to identification/creation of novel genes that impart disease resistance/tolerance in cultivars of many crop plants. Present investigation entitled "Radiation induced mutation for resistance against *Fusarium oxysporum* f. sp. *ciceri* (*Cicer arietinum* L.)" was aimed at identification of suitable mutant or a combination of mutants influencing resistance/tolerance to Fusarium wilt in chickpea. The experimental material was consisted of the population of four selected cultivars of chickpea (JG 62, BDNG 798, JAKI 9218 and Vijay) grown in randomized block design at College of Agriculture Golegaon, VNMKV, Parbhani during *Rabi* 2018-19. Among 11Mutagenic treatments seven treatments (20 KR, 30 KR, 0.2% EMS, 0.3% EMS, 20 KR+0.2% EMS, 30 KR+0.2% EMS) showed significant impact on various morphological and biological parameters of the plant habit. Efficacy of Gamma rays, EMS and their combination treatment screened for resistance/tolerance in M_2 progeny mutant chickpea against two isolates of *Fusarium oxysporum* f. sp. *ciceri* by using root dip inoculation technique. Present finding revealed that combination treatment of 30 KR+0.2% EMS showed resistance/tolerance against both the isolates of *Fusarium oxysporum* f. sp. *ciceri* in the three experimental genotypes *i.e.*, BDNG 798, JAKI 9218 and Vijay whereas, 0.3% EMS shows resistance/tolerance against both the isolate of *Fusarium oxysporum* f. sp. *ciceri* for Vijay genotype.

Keywords: Gamma rays, *Fusarium oxysporum*, *Cicer arietinum* L.

Introduction
Chickpea (*Cicer arietinum* L.) is the third most important pulse crop in the World after dry bean (*Phaseolous vulgaris* L.) and dry pea (*Pisum sativum* L.) (Nikam *et al*., 2007). It is a self-pollinating and diploid (2n=2x=16), *Rabi* season legume crop that belongs to kingdom *Plantae*, order *Fabales*, family *Fabaceae*, genus *Cicer* and species *arietinum* with genome size of ~738 Mb (Varshney *et al*., 2013) [10]. It is composed of 9 annual and about 34 perennial wild species. Among the 9 annual species, chickpea (*C. arietinum* L.) is the only cultivated species (Singh *et al*., 2008). *Fusarium* wilt is one of the important limiting factors of chickpea. Wilt caused by *F. oxysporum* f. sp. *ciceri* was first reported from India by Butler (1918) [1]. Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceri* is one of the major disease causes up to 90% losses depending on weather conditions. The incidence is more if the crop is subjected to sudden temperature rise and water stress (Venkataramanamma *et al.*, 2018) [9]. Wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri* which one of the most prevalent disease in India. In India, the four high-yielding, *Ascochyta* blight-resistant and wilt disease-resistant chickpea mutant varieties, *viz*. Pusa 408 (Ajay), Pusa 413 (Atul), Pusa 417 (Girnar) and Pusa 547, developed at Indian Agriculture Research Institute, New Delhi (Kharkwal and Shu 2009) [5]. Induced mutagenesis is a promising technology to overcome the pest and disease resistance in cereals, legumes and economically important crops. Both physical mutagens such as ionising radiations and chemical mutagens that show resistance to pathogen and disease outbreak utilized for the development of elite crop varieties. Mutagenesis enables identification of wild genes or the creation of novel genes that impart disease resistance.

Materials and Methods

Efficacy of Gamma rays, ethyl methane sulphonate and their combination treatment for resistance against isolates of *Fusarium oxysporum* f. sp. *ciceri* was studied by root dip method. Among 11 treatments seeds of 7 treatments (20 KR, 30 KR, 0.2% EMS, 0.3% EMS, 20 KR+0.2% EMS, 30 KR+0.2% EMS) from all the four genotypes (JG 62, BDNG 798, JAKI 9218 and Vijay) showing better germination and other morphological characters in M_1 generation were used to test the wilt resistance against under net house at VNMKV, Parbhani. M_2 progeny seeds selected from above treatments were screened for resistance to Fusarium wilt under laboratory conditions using root dip method. Percent mortality due to wilt was recorded up to 25 days. The data on disease incidence for each selected mutant variety was calculated by using the following formula.

$$\text{Wilt incidence (\%)} = \frac{\text{No. of wilted plants}}{\text{Total number of plants}} \text{ X } 100$$

Observations on wilt were recorded in percentage of disease incidence for the level of resistance/susceptibility for each M_2 progeny mutant chickpea genotypes was recorded by applying 0-9 point disease rating scale (Mayee and Datar, 1986; IIPR, 1999) [7, 4] and the genotypes were categorized as, highly resistant (HR), resistant (R), moderately resistant (MR) moderately susceptible (MS), susceptible (S) and highly susceptible (HS) as described by (IIPR, 1999) [4].

Table 1: Disease rating Scale

Scale	Description
0	No wilting.
1	1% or less plant wilted.
3	1 to 10% plants wilted.
5	11 to 20% plants wilted.
7	21 to 50% plants wilted.
9	51% or more plants wilted.

(Mayee and Datar, 1986) [7]

Table 2: Grade for accounting per cent mortality of *Fusarium oxysporum* f. sp. *Ciceri*

Grade	Percent wilting (mortality)	Disease reactions
0	No disease	Highly resistant (HR)
1	1 to 10	Resistant (R)
2	10.1 to 20	Moderately resistant (MR)
3	20.1 to 30	Moderately susceptible (MS)
4	30.1 to 50	Susceptible (S)
5	50 and above	Highly susceptible (HS)

(IIPR, 1999) [4].

Ten seeds were sown of each genotypes consisting seven treatment along with untreated control were surface-sterilized with 2% sodium hypochlorite solution for 2 minutes, rinsed in sterilized water 2-3 times in order to wash off sodium hypochlorite and germinated for eight days in earthen pots containing sterilized sand. Obtain a culture of Foc from infected chickpea plants and purify by single spore isolation on potato dextrose agar (PDA). For multiplication of inoculum, a 7 mm disk of actively growing culture of Foc is inoculated into 100 ml of potato dextrose broth (PDB) in 250 ml flasks.

Eight days old seedlings were grown in sterile sand are carefully uprooted, and the roots were washed under running water to remove excess sand. Root tips around 0.5 cm long were cut off to facilitate the entry of the pathogen into the roots. The roots of the seedlings were then dipped separately of each treatment in the inoculum for each isolate for 1-2 minute to enable conidia to adhere the roots. Inoculated seedlings were transplanted in pre-irrigated sterile vertisol and sand (3:1) mixture filled in pots and incubated at 25 ± 30°C. Inoculated seedlings were observed regularly to see the mortality due to wilt.

Results and Discussion

Seedling reaction of M_2 progeny on four chickpea mutant genotypes was studied against the isolates representing *Fusarium oxysporum* f. sp. *ciceri.* On the basis of criteria 7 mutagenic treatments among the 11 treatments were selected to raise the M_2 progeny using root dip method. Results revealed that in JG 62, 20 KR +0.2% EMS resulted moderately resistant reaction towards FOC 2, whereas 30 KR Gamma rays treatment showed moderately susceptible reaction against FOC 1 and FOC 2 followed by 0.2% EMS, 20 KR+0.2% EMS and 0.3% EMS showed moderately susceptible reaction against FOC 1 and FOC 2 respectively, similarly 30 KR+0.2% EMS shows susceptible reaction against both FOC 1 and FOC 2 followed by 0.3% EMS and 20 KR, 0.2% EMS shows susceptible reaction to FOC 1 and FOC 2 respectively, 20 KR treatment shows highly susceptible to FOC 1, whereas the control which shows highly susceptible and susceptible to *Fusarium oxysporum* f. sp. *ciceri*. Similarly in BDNG 798 treatment *viz.*, 30 KR +0.2% EMS showed resistant reaction towards FOC 1 and FOC 2, whereas 0.2% EMS, 0.3% EMS and 20 KR+0.2% EMS combination treatment exhibited moderate resistance against both the isolates of *Fusarium oxysporum* f. sp. *ciceri*, whereas 20 KR and 30 KR shows moderately resistant to FOC 1 and FOC 2 respectively. 30 KR shows resistant to FOC 1 while 20 KR moderately susceptible to FOC 2, whereas the control shows moderate resistant reaction against FOC 1 but susceptible against FOC 2.

Table 3: Seedling reaction of M_2 progeny mutant chickpea of different genotypes against isolates of *Fusarium oxysporum* f. sp. c*iceri* by root dip method.

Genotypes/Doses	*Fusarium oxysporum* f. sp *ciceri* isolates	
	FOC 1	FOC 2
JG 62		
20 KR	HS	S
30 KR	MS	MS
0.2% EMS	MS	S
0.3% EMS	S	MS
20 KR+0.2% EMS	MS	MR
30 KR+0.2% EMS	S	S
CONTROL	HS	S
BDNG 798		

20 KR	MR	MS
30 KR	R	MR
0.2% EMS	MR	MR
0.3% EMS	MR	MR
20 KR+0.2% EMS	MR	MR
30 KR+0.2% EMS	R	R
CONTROL	MR	S
JAKI 9218		
20 KR	MR	MR
30 KR	R	R
0.2% EMS	MR	R
0.3% EMS	MR	MR
20 KR+0.2% EMS	MR	MR
30 KR+0.2% EMS	R	R
CONTROL	MR	MR
VIJAY		
20 KR	R	HR
30 KR	MR	MR
0.2% EMS	MR	R
0.3% EMS	R	R
20 KR+0.2% EMS	R	MR
30 KR+0.2% EMS	R	R
CONTROL	MR	R
HR- Highly Resistant MS- Moderately Susceptible		
R- Resistant S- Susceptible		
MR- Moderately Resistant HS- Highly Susceptible		

The efficacy of Gamma rays, ethyl methane sulphonate and their combination treatment for resistance/tolerance against *Fusarium oxysporum* f. sp. *ciceri* using root dip inoculation technique led to conclusion that mutagenic treatments *viz.*, 20 KR, 30 KR, 0.2% EMS, 0.3% EMS, 20 KR+0.2% EMS, 30 KR+0.2% EMS results obtained after phenotypic evaluation in M_1 generation had significant results on reaction of genotypes. It was found that combination of 30 KR+0.2% EMS treatment revealed resistance/tolerance against isolates of FOC 1 and FOC 2 of *Fusarium oxysporum* f. sp. *ciceri*. in three genotypes, whereas, 0.3% EMS showed resistance against both the isolates of *Fusarium* only for Vijay genotypes, and 30 KR dose of Gamma rays was resistant to JAKI 9218 and moderately resistant for both the isolates in chickpea cultivar BDNG 798 and Vijay.

Conclusions

The effectiveness of EMS treatments initially increased with an increase in concentration but at the higher concentration treatment the effectiveness of EMS treatments initially reduced. The efficacy of Gamma rays, ethyl methane sulphonate and their combination treatment for resistance/tolerance against *Fusarium oxysporum* f. sp. *ciceri* using root dip inoculation technique in selected M_2 progeny of four genotypes summarizes combination treatment of 30 KR+0.2% EMS showed resistance/tolerance against both the isolates of *Fusarium oxysporum* f. sp. *ciceri* in the three experimental genotypes *i.e.*, BDNG 798, JAKI 9218 and Vijay whereas, 0.3% EMS shows resistance/tolerance against both the isolate of FOC for Vijay genotype.

Efficacy of gamma rays, ethyl methane sulphonate and their combination treatment for resistance/tolerance against *Fusarium oxysporum* f. sp. *ciceri.*

The present results were also supported by the findings of Hassan *et al.* (2001) [3] studied gamma rays induced high yielding kabuli type chickpea mutant variety "Hassan-2K" and confirmed its resistance to blight and wilt in M_3 and M_4 generation. Wani (2009) [11] stated that the mutagenic treatments proved to be effective in producing morphological mutations along with improved tolerance to *Fusarium wilt and Ascochyta blight*. The findings of present investigation revealed further scope of research for development of resistance against multiple diseases in crops by the mutation breeding technique.

Chobe (2016) [2] reported that the efficacy of Gamma rays, EMS and their combination treatment screened for resistance in M_2 progeny mutant chickpea against race 2 and race 4 of *Fusarium oxysporum* f. sp. *ciceri* by using root dip inoculation technique. 400 GY treatment of physical mutagen and combination treatment of 200GY
+ 0.3% EMS exhibited resistance against both the races of *Fusarium* in all the three-selected genotypes whereas, 0.4% EMS showed resistance against both the races of *Fusarium* for two genotypes i.e., JG 63, JG130.

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