Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(3): 1978-1984
Received: 07-03-2020
Accepted: 09-04-2020

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Mutation induced alterations in agronomic traits of M_1 generation chickpea

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DOI: https://doi.org/10.22271/phyto.2020.v9.i3ag.11606

Abstract

Present investigation was aimed to identify the response of mutagenic treatments on agronomical traits of M_1 generation mutant chickpea. Present study was carried out under legumes pathology division, ICRISAT during rabi 2018-19. Results revealed that chickpea varieties BG 212 and JG 11 showed significant reaction for mutagenic treatments i.e 100GY, 150 GY, 200 GY, 400 GY, 0.2% EMS and 0.3%. Among 7 treatments including control (wild type) two treatments (100 GY and 150 GY) have significant impact on agronomical traits of experimental material, i.e. change in seedling height, decrease in germination percentage and decrease in plant height and variation in growth habbits as compared to control. While, both the EMS treatments found to be non-significant in generating any kind of impact on agronomical traits, except reducing the seed germintaion.

Keywords: Biological parameters, ethyl methane sulphonate, gamma irradiation and wild types

Introduction

Chickpea is a cool season pulse crop and is grown in several countries worldwide as a food source. It is the third most important food legume crop and India is the largest producer contributing to 65% of world's chickpea production. The improvement of chickpea using conventional breeding approaches has been hampered due to lack of sufficient genetic variability. Mutagenesis is a common and efficient tool to create new desirable genetic variability in chickpea (Micke, 1988) [8]. The use of ionizing radiation such as, x-rays, gamma rays, and neutrons and chemical mutagens for inducing variation is well established. Induced mutation have been used to improve major crop which are seed propagated. Mutation can be linked to changes in DNA sequences for some plant traits and to establish molecular maps in structural and functional genomics of crop plants. These in turn would lead to a rapid enhancement of crop yields and quality (Maluszynski et. al., 1995) [7]. The utilization of mutation breeding is a simple, less cost full and time saving method proved in many cases to be useful in breeding new lines (Chobe *et al*., 2016, 2017, Pawar *et al*., 2018) [2, 3, 9] The aim of the present investigation is to use gamma rays and EMS as a nuclear technique to induce mutation for identifying response of mutagenic treatments on agronomical traits of M_1 generation mutant chickpea.

Material and Methods

Present investigation was aimed to identify on agronomical traits of M_1 generation mutant chickpea carried out under legumes pathology division ICRISAT during rabi 2018-19. A total of 7-treatments (including control) were evaluated separately for each variety planted in Randomized Block Design with three replications.

Material

Ethyl Methane Sulphonate, Pre-soaked seeds of selected genotypes (BG 212 and JG 11), Gamma chamber, Distilled Water, Conical Flask, Presoaked gamma irradiated seeds of selected genotype, Rotary shaker, Magnetic stirrer, gamma chamber, handpicked uniform sized seeds of selected genotypes etc.

Collection of chickpea seed

Chickpea seeds of BG 212 and JG 11 varieties were collected from healthy plants at maturity stage of the crop from the seed multiplication plot of Division of Legumes Pathology, ICRISAT.

Methodology

Gamma irradiation (Physical mutagens) (Seed treatment)

Gamma irradiation was performed (Nuclear Research Laboratory IARI, New Delhi) in gamma chamber by exposing the seeds to the gamma rays from ^{60}Co source. A sample of 120 seeds was packed in butter paper covers and placed in 100 curie ^{60}Co gamma cells where the treatments were given for various duration depending on the doses required (100,150G, 200 and 400 Gray) with the dose rate of 54.05 rads/sec (Chobe *et al*., 2016) [2].

Ethyl Methane Sulphonate (EMS Chemical mutagen) (Seed treatment)

Set of seeds soaked in distilled water (12 hrs.) of selected genotypes (BG 212 and JG 11) were treated with ethyl methane sulphonate at different concentration by using magnetic stirrer (0.2, and 0.3) for 6 hrs. with constant intermitted shaking in shaker. Further it was washed under running tap water and used for sowing (Chobe *et al*., 2017) [3].

Agronomical traits and observations recorded in M_1 generation mutant Chickpea

<table>
<tr><th>Biological parameters</th><th colspan="3">Observations</th></tr>
<tr><td>Seed germination (25 DAS)</td><td colspan="3">Chickpea seeds germinate at an optimum temperature (28-33 °C) and moisture level in about 5-6 days. Hence observations are taken after 25 days of sowing.</td></tr>
<tr><td>Plant height (cm)</td><td colspan="3">Height is measured at the time of maturity from the base of the plant to the top of the main shoot. (Average of 5 plants)</td></tr>
<tr><td>No. of pods plant^{-1}</td><td colspan="3">Pods of 5 plants selected randomly from the net plot are counted and the average number of pods plant-1 calculated.</td></tr>
<tr><td>No. of seeds plant^{-1} (g)</td><td colspan="3">Seeds from 5 plants were counted to compute the average number of seeds plant^{-1}.</td></tr>
<tr><td>Seed yield plant^{-1}</td><td colspan="3">Yield plant-1 is recorded for use in biometrical or genetic studies in which individual plant data are required. The yield is recorded for each plant or as an average of 5-10 plants.</td></tr>
<tr><td rowspan="7">Plant type (spread) (cm)</td><td colspan="3">The angles of primary branches are recorded in the sixth week after sowing on a 1-5 scale.</td></tr>
<tr><td>Score</td><td>Plant type</td><td>Description</td></tr>
<tr><td>1</td><td>Erect</td><td>0-15° from the vertical</td></tr>
<tr><td>2</td><td>Semi Erect</td><td>15-25° from the vertical</td></tr>
<tr><td>3</td><td>Semi Spreading</td><td>25-60° from the vertical</td></tr>
<tr><td>4</td><td>Spreading</td><td>60-80° from the vertical</td></tr>
<tr><td>5</td><td>Prostrate</td><td>Branches flat on the ground</td></tr>
</table>

Treatment and Doses (M_1 Generation)

Treatment Code.	**Treatments**	**Time (min)**
T_1	100GY	5.15
T_2	150GY	7.53
T_3	200GY	10.31
T_4	400GY	21.03
T_5	EMS 0.2%	360
T_6	EMS 0.3%	360
T_7	Wild type (Control of each variety)	NIL

Result and Discussion

It was found that mutation breeding has contributed significantly to varietal development, as it is an efficient and potent approach to create novel genetic variation for difficult traits to breed. Seeds treated by ethyl methane sulfonate (EMS) or physical mutagens such as gamma-ray irradiation was used to induce mutations and deletions in the genome, (Ahloowalia *et al*. 2004) [1] and to create desired genetic variablity. Nowaday's gamma radiation from radioactive ^{60}Co is widely used in developing new mutant varieties as it has high penetrating potential. Also, it has an advantage of being used for irradiating whole plants, pollen grains, seeds and tubers.

From the present investigation it was found that mutagenic treatments generated a significant impact on the seed germination of both the varieties. Lower doses of mutagenic treatments caused lower lethality in seedling damage as compared to higher doses. Results revealed that, mutagenic treatment of 100GY and 150GY found to be good and less lethal on chickpea. While the percent of seed germination was found to be very low in EMS treatments. Also, the mutagenic treatments had a variable impact on plant height characters in both the varieties (BG 212 and JG 11) (Table 1. Fig.1). It was found that high doses of gamma rays and EMS treatments caused higher reduction in plant height (Table 2. Fig.2). Haq and his co-workers (1992) observed similar results of mutagen sensitivity, where the germination percent and plant height of chickpea genotypes ICL 6104 and ICL 3279 were reduced in mutant population.

According to Yamaguchi *et al*., 2003 both physical and chemical mutagens offers varying degree of accuracy and reproducibility. Similarly, from the present study highest numbers of variable growth habits were observed in 100GY and 150GY of mutagenic treatments in both the varieties. While, compared to EMS treatments gamma treatments were found to be significant in producing highest number of spread types in both the varieties. It was also found that no variation occurred in wild type (Control BG 212 and JG 11 control treated with sterilized deionized water) plants in producing spread types, as both the varieties were produce spread types according to their varietal characters (Table. 3 Fig.3). Also the overall analysis of pods per plants suggested that all mutagenic treatments had significantly reduced the pod setting in both the varieties when compared to their wild type (Table. 4. Fig.4). Similarly, EMS treatments showed poor seed setting due to less pod formation. Reduction in seed yield in mutagenic treatments suggested that these treatments were responsible for the reduction in seed size. Kharkwal (2003) [6] conducted a similar study in chickpea to induce polygenic variability in the form of micro mutation and observed a wide range of variability for quantitative characters and found a different response to different varieties of M_1 generation. The study revealed that characters like grain yield, number of pods, grain per plant, grain weight and biological yield showed a higher response to mutagenic treatments. The present findings of study was found to be confirmatory with the above mentioned study (Table 5 and 6, Fig. 5 and 6). While similar kind of studies was conducted by Javed *et al*. (2014) [5] to study the interrelationship and genetic diversity in Vigna radiata in response to gamma radiation.

Table 1: Effect of mutagenic treatments on seed germination

Variety	Treatment	Replication			Germination (%)
		R1	R2	R3	
BG 212	100GY	67	60	35	54.0
	150GY	34	40	38	37.3
	200GY	46	44	45	45.0
	400GY	28	24	26	26.0
	EMS 0.2%	24	22	26	24.0
	EMS 0.3%	32	30	34	32.0
	Wild type (Treated with sterilized deionized water)	93	89	88	90.0
JG 11	100GY	60	65	70	65.0
	150GY	50	55	61	55.3
	200GY	29	20	39	29.3
	400GY	27	22	30	26.3
	EMS 0.2%	28	22	30	26.7
	EMS 0.3%	33	20	22	25.0
	Wild type (Treated with sterilized deionized water)	70	77	75	74.0
	SEM	2.44			
	CD	7.11			

Table 2: Effect of mutagenic treatments on plant height

Variety	Treatment	Plant Height			Mean
		R1	R2	R3	
BG 212	100GY	25.5	23.4	27.3	25.40
	150GY	19.1	17.7	22.2	19.68
	200GY	23.2	23.4	28.2	24.94
	400GY	22.3	22.5	25.6	23.47
	EMS 0.2%	18.2	16.3	15.4	16.62
	EMS 0.3%	18.1	15.2	13.2	15.48
	Wild type (Treated with sterilized deionized water)	45.6	47.5	44.3	45.80
JG 11	100GY	24.6	22.3	21.2	22.70
	150GY	23.6	25.6	27.3	25.51
	200GY	29.3	28	33	30.11
	400GY	25.1	31.2	21	25.75
	EMS 0.2%	20.4	18.2	15.4	18.01
	EMS 0.3%	20.4	13.5	21.3	18.41
	Wild type (Treated with sterilized deionized water)	35.6	33.2	40.3	36.37
	SEM	1.62			
	CD	4.70			

Table 4: Effect of mutagenic treatments on growth habits (plant type)

Variety	Treatment	Total number of plants observed	S	E	SE	P	SS
BG 212	100GY	54	7	8	3	5	31
	150GY	37	5	1	4	1	26
	200GY	45	5	6	4	4	26
	400GY	26	4	1	3	5	13
	EMS 0.2%	24	2	3	0	1	18
	EMS 0.3%	32	2	3	0	1	26
	Wild type (Treated with sterilized deionized water)	90	0	0	0	0	90
JG 11	100GY	65	2	3	6	2	52
	150GY	55	1	2	1	2	49
	200GY	29	2	2	2	2	21
	400GY	26	5	3	4	1	13
	EMS 0.2%	27	2	1	2	2	20
	EMS 0.3%	25	4	4	3	2	12
	Wild type (Treated with sterilized deionized water)	74	0	0	0	0	74

S-spreading, SE- Semi erect, E- Erect, P- prostate and SS- Semi Spread

Table 5: Effect of mutagenic treatments on number of pods per plant

Variety	Treatment	No. of pods plant^{-1}			Mean
		R1	R2	R3	
BG 212	100GY	11	18	13	14.00
	150GY	38	42	40	40.00
	200GY	17	20	19	18.67
	400GY	25	22	38	28.33
	EMS 0.2%	5	3	3	3.67
	EMS 0.3%	2	6	5	4.33
	Wild type (Treated with sterilized deionized water)	45	42	30	39.00
JG 11	100GY	26	21	22	23.00
	150GY	21	20	22	21.00
	200GY	10	5	11	8.67
	400GY	24	21	25	23.33
	EMS 0.2%	13	6	17	12.00
	EMS 0.3%	11	4	5	6.67
	Wild type (Treated with sterilized deionized water)	44	35	42	40.33
	SEM	2.45			
	CD	7.11			

Table 6: Effect of mutagenic treatments on number of seeds per plant

Variety	Treatment	No. of seeds plant^{-1}			Mean
		R1	**R2**	**R3**	
BG 212	100GY	11	12	11	11.33
	150GY	40	43	55	46.00
	200GY	23	25	32	26.67
	400GY	28	33	27	29.33
	EMS 0.2%	3	2	3	2.67
	EMS 0.3%	2	8	10	6.67
	Wild type (Treated with sterilized deionized water)	60	64	69	64.33
JG 11	100GY	30	35	36	33.67
	150GY	32	37	38	35.67
	200GY	14	7	12	11.00
	400GY	21	25	36	27.33
	EMS 0.2%	11	10	17	12.67
	EMS 0.3%	10	5	4	6.33
	Wild type (Treated with sterilized deionized water)	50	46	44	46.67
	SEM	2.24			
	CD	6.52			

Table 7: Effect of mutagenic treatments on seed yield per plant (g)

Variety	Treatment	No. of seeds yield plant^{-1} (g)			Mean
		R1	**R2**	**R3**	
BG 212	100GY	2.8	2.6	3.0	2.79
	150GY	2.1	1.9	2.4	2.16
	200GY	2.6	2.6	3.1	2.74
	400GY	2.5	2.5	2.8	2.58
	EMS 0.2%	2.0	1.8	1.7	1.83
	EMS 0.3%	2.0	1.7	1.5	1.70
	Wild type (Treated with sterilized deionized water)	5.0	5.2	4.9	5.04
JG 11	100GY	5.7	5.1	4.9	5.22
	150GY	5.4	5.9	6.3	5.87
	200GY	6.7	6.4	7.6	6.93
	400GY	5.8	7.2	4.8	5.92
	EMS 0.2%	4.7	4.2	3.5	4.14
	EMS 0.3%	4.7	3.1	4.9	4.23
	Wild type (Treated with sterilized deionized water)	8.2	7.6	9.3	8.36
	SEM	0.33			
	CD	0.96			

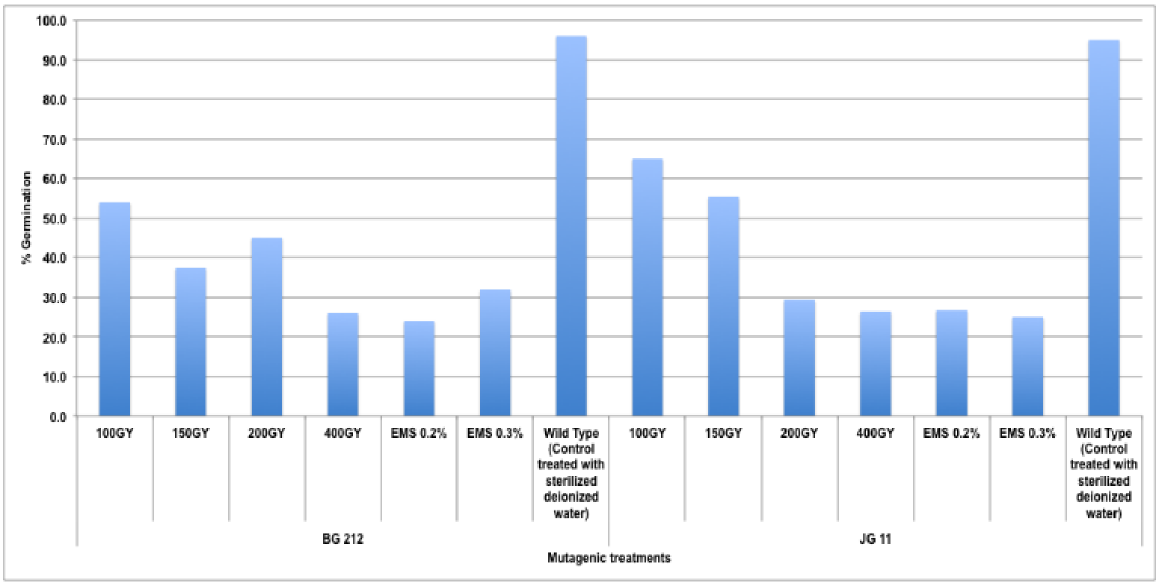

Fig 1: Effect of mutagenic treatments on seed germination

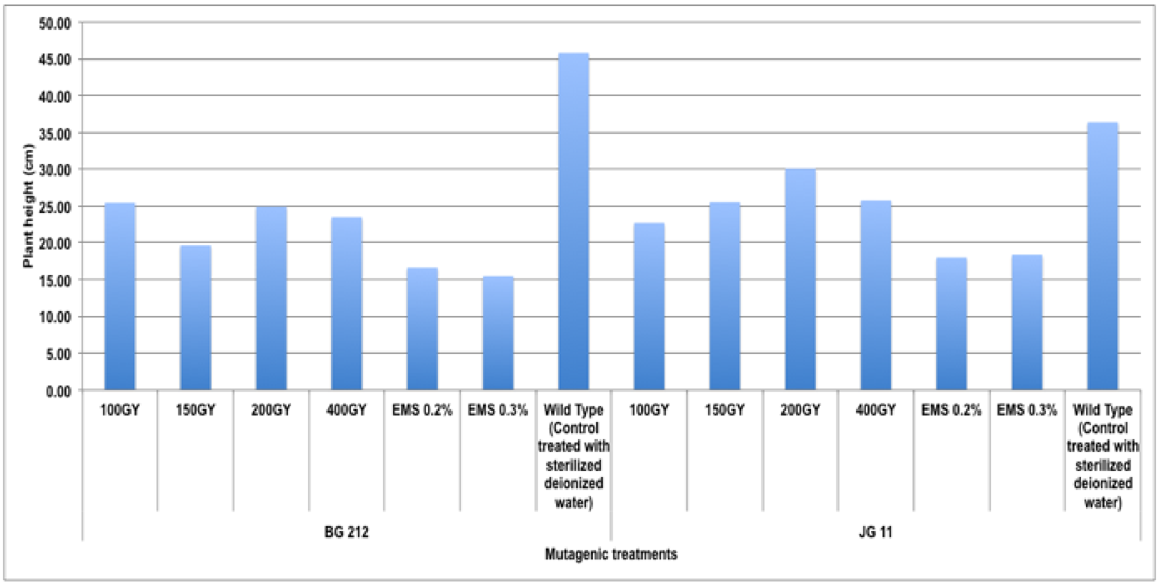

Fig 2: Effect of mutagenic treatments on plant height

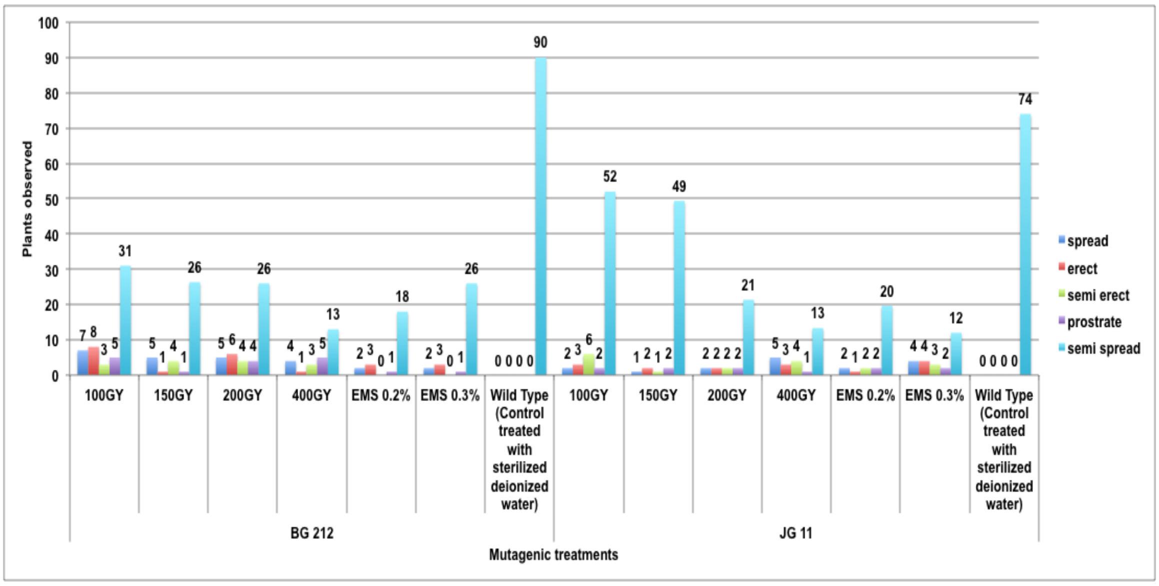

Fig 3: Effect of mutagenic treatments on growth habits (plant type)

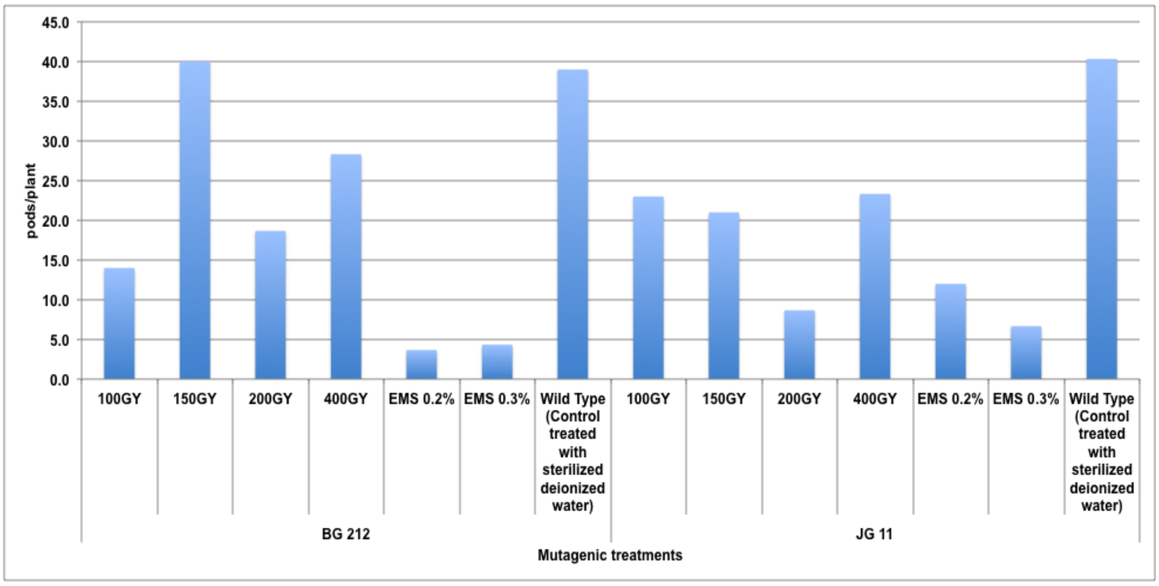

Fig 4: Effect of mutagenic treatments on number of pods per plants

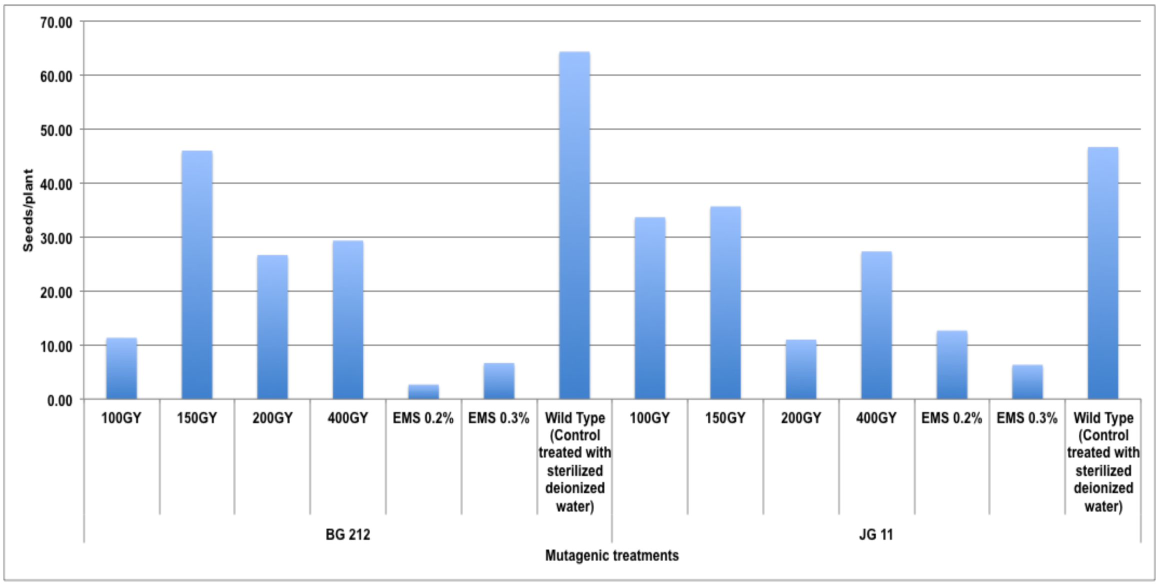

Fig 5: Effect of mutagenic treatments on number of seeds per plant

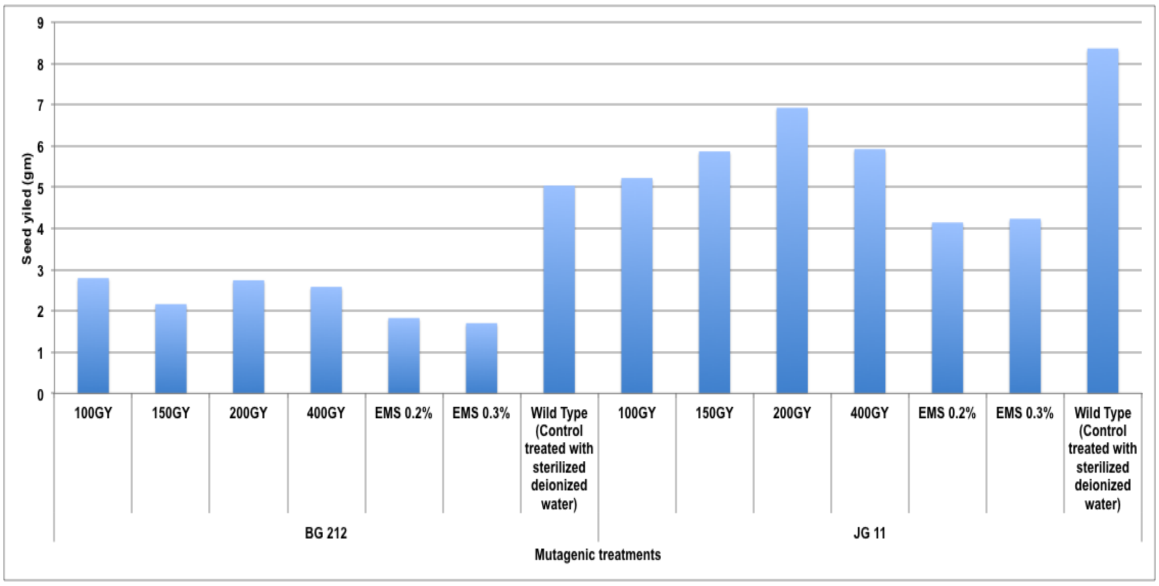

Fig 6: Effect of mutagenic treatments on seed yield per plant (g)

Conclusion
Mutagenic treatments (100 GY, 150 GY, 200 GY, 400 GY 0.2 and 0.3%) showed significant impact on agronomical traits of M_1 generation chickpea mutants. Mutagenic treatment of 100GY and 150GY found to be good and less lethal on chickpea and created more number of variations in plants, whereas the EMS treatments were found to be non significant in producing any impact on agronomical traits.

Acknowledgements
The authors express thanks to head Department of Plant Pathology, RVSKVV Gwalior, Legumes Pathology Division ICRISAT and Nuclear research laboratory, Indian Agriculture research institute, New Delhi, for providing the necessary facilities for the derived study. We are also thankful to our technical staff Mr. Balakrishna, Mr. K. Ramulu, Mr. Satish Chandra and John Bosco for their help to conduct an experiment.

References
1. Ahloowalia BS, Maluszynski M, Nichterlein K. Global impact of mutation derived varieties. Euphytica. 2004; 135:187–204
2. Chobe DR, Gupta OM, Pawar M. Radiation induced mutation for resistance against races/pathotypes of *Fusarium oxysporum* f.sp *ciceris* in chickpea (*Cicer arietinum* L.) Ind. Phytopath. 2016; 69(4s):260-265.
3. Chobe DR, Sharath Chandran US, Tarafdar A, Ghosh R, Singh R, Sharma M. Exploring the response of mutant germplasms against dry root rot of chickpea". ISMPP International conference on Plant Health for Human Welfare, Jaipur 1-4 Nov, 2017, 237.
4. Haq MA, Singh K, Abidin Z, Ahmad MS. Mutation studies in chickpea (*Cicer arietinum* L.) Pak. J Agri. Sci. 1992, 29(4).
5. Javed I, Hafiz MA, Qurban A, Muhammad UG, Muhammad SI, Muhammad R, Hafiza NA. Induced genetic variability by gamma radiation and traits association study in mungbean (*Vigna radiata* L.). Life Science Journal. 2014, 11(8s).
6. Kharakwal MC. Induced mutations in chickpea (*Cicer arietinum* L.) VI. Significance of Induced altered correlations. Indian Journal of Genetics. 2003; 63(3):219-224.
7. Maluszynski M, Van Zanten l, Asir A, Brunner H, Ahloowalia B, Zapata FJ, Weck E. Mutation techniques in plant breeding. proc. induced mutations and molecular techniques for crop improvement. Fao/iaea, Austria, Vienna, June 19-23, 1995, 489-504.
8. Micke A. Genetic improvement of grain legumes using induced mutations. An overview. In Improvement of grain legume production using induced mutations. IAEA, Vienna. 1988; 1:50-51.
9. Pawar M, Gupta O, Chobe DR. Radiation induced mutation for resistance against dry root rot in chickpea (*Cicer aeriatinu*m Linn.). Int. J Curr. Microbiol. App. Sci, 2018, 7. doi: 10.20546/ijcmas.2018.707.xx
10. Yamaguchi H, Morishita T, Degi K, Tanaka A, Shikazono N, Hase Y. Effect of carbon-ion beams irradiation on mutation induction in rice. Plant Mut Rep. 2006; 1:25-27.