The Pharma Innovation Journal 2022; 11(2): 1160-1164

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(2): 1160-1164

www.thepharmajournal.com
Received: 17-11-2021
Accepted: 24-12-2021

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Effect of mutagenic treatments on seed germination, seedling growth and survival of pigeon pea [*Cajanus cajan* (L.) Mill. Sp.]

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Abstract
Pigeonpea [*Cajanus cajan* (L.) Millsp], belongs to family Fabaceae, is the most important food legume crop. Pigeonpea is an economic source of not only protein but of carbohydrate, minerals and B-complex vitamins particularly in vegetarian diet (Salunkhe *et al.*, 1985). Mutation breeding technique is the best method to enhance the genetic variability of crop with in short time. In the present investigation the seeds of Pigeon pea (*Cajanus cajan* (L.) Millsp) were treated with different doses of gamma radiation (100 Gy, 200 Gy, 300 Gy, and 400 Gy) and concentration of Ethyl Methane Sulphonate (0.05, 0.1, 0.2 and 0.3 per cent) and their combinations with 100 Gy, 200 Gy, 300 Gy and 400 Gy were immersed in freshly prepared 0.2 per cent aqueous solution of Ethyl methane sulfonate for studying seed germination, shoot length, root length, germination index, mean daily germination and vigour index under laboratory condition and germination percentage, survival percentage and mortality percentage under field condition. Decreasing trend was observed in all mutagenic treatments as per the increased dose of mutagen under laboratory as well as under field condition. Considering laboratory and field observation on per cent germination and other related parameters among the various mutagenic treatments, LD_{50} dose was optimized. For gamma rays mutagenic treatment 300Gy, for EMS mutagenic treatment 0.20% EMS and for combination treatment 300Gy+ 0.20% EMS doses were optimized as a LD_{50} doses which were close to LD_{50} for pigeonpea.

Keywords: Pigeon pea, gamma rays, EMS; LD_{50}, mutagen, germination percentage, survival percentage and seedling vigour index

Introduction
Pigeon pea or red gram is an important crop in India, belongs to the family fabaceae, where it is next important pulse crop after chickpea. It is mainly eaten in the form of split pulse as 'dal'. Seeds of pigeon pea are rich in iron, iodine, essential amino acids like lycine, threonine, cystine and arginine etc. Pigeonpea seeds are also rich in potassium, phosphorus, magnesium, calcium and iodine. Pigeon pea is an important grain legume crop of rainfed agriculture in the semi-arid tropics. It plays an important role in food security, balanced diet and alleviation of poverty because of its diverse usages as a food, fodder and fuel. Mutation breeding is effective tool in the hands of plant breeder for evolving a new better forms increasing genetic variability. Although ionizing radiations are a patent for creating mutation, but after the discovery of several chemical mutagenes, it was found that the combination of these two mutagenes proved to be more efficient. Mutation breeding has been found to be effective means for inducing desired mutations and developing isolines in a short time without much altering polygenically controlled economic characters which is not normally possible by hybridization. Mutation breeding is very effective to improve one or two desired characters those are lacking in the well adapted variety. Gamma radiation can be useful for the alteration of physiological characters (Chaudhuri, 2002), (Kiong *et al*., 2008).
These radicals can damage or change important components of plant cells. They have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants depending on the radiation dose (Ashraf *et al*., 2003) [2]. Ethyl Methane Sulphonate (EMS) is mutagenic and carcinogenic organic compound, it produces random mutations in genetic material by nucleotide substitution; particularly by guanine alkylation and it is reported to be the most effective and powerful mutagen (Hajara, 1979) [7] and typically produces only point mutations (Okagaki *et al*., 1991) [11]. Pigeonpea cultivar KonkanTur-1 was selected for the present study has important feature like semi-spreading and suitable to cultivate on rice bunds during *kharif* season.

The present investigation was undertaken with view to study the effect of physical, chemical and combination of physical and chemical mutagenic treatment on seed germination and other seedling parameters.

Material and Methods

For the present investigation dry, healthy and genetically pure seeds of pigeonpea (*Cajanus cajan*) cultivar Konkan Tur-1were obtained from Botany Farm, Dept. of Agril. Botany, College of Agriculture, Dapoli.

Seed treatment

Seeds were treated with gamma rays, EMS and combination treatments of these as given below.

Gamma rays

Four sets of 250 dry seeds (moisture content around 10%) of Pigeonpea cultivar Konkan Tur-1were irradiated with gamma rays of 100 Gy, 200 Gy, 300 Gy and 400 Gy doses, respectively at Bhabha Atomic Research Centre, Mumbai.

Ethyl Methane sulfonate (EMS)

Four sets of 250 dry seeds (moisture content around 10%) of pigeonpea cultivar Konkan Tur-1were immersed in freshly prepared 0.05, 0.1, 0.2 and 0.3 per cent aqueous solution of ethyl methane sulfonate for 12 hours with intermittent shaking at room temperature.

Combination treatments

Two hundred fifty seeds of each gamma rays irradiated treatments *viz*., 100 Gy, 200 Gy, 300 Gy and 400 Gy were immersed in freshly prepared 0.2 per cent aqueous solution of Ethyl methane sulfonate for 12 hours with intermittent shaking at room temperature. Some of the mutagen treated seeds (100) were used to study the germination parameters like germination%, germination index, mean daily germination, root length, shoot length and vigour index. Under field condition, the treated seeds were immediately sown on 31st May 2019. Germination%, survival% and mortality% were also recorded under field condition.

Results and Discussion

Germination percentage (Laboratory condition)

It was observed that germination percentage was reduced as per the increased dose of mutagen. Among the four gamma rays treatments maximum germination percentage was recorded by the treatment 100 Gy (91.7%) and substantial decreased germination percentage was observed in treatment 400 Gy (32.3%). Within EMS treatments, range of germination percentage was 41.3% (0.30% EMS) to 84.7% (0.05% EMS). Among the four combination treatments, treatment 100 Gy+0.20% EMS showed maximum germination percentage (88.7%) and minimum in 400 Gy+0.20% EMS (43.3%). After the mutagenic treatments, an inhibitory effect on seed germination could be distinctly seen in pigeon pea. Mutagenic treatments revealed a gradual decreasing trend in germination from lower to higher doses (Sunil *et al*., 2011). The results supported by the works done by Datir *et al*. (2007) [4] in horsegram, Potdukhe and Narkhede (2002) [12] in pigeon pea.

Germination index

Among all mutagenic treatments, it was observed that germination index was reduced as per the increased dose of mutagen. Within gamma rays treatments, treatment 100 Gy (18.2) recorded maximum germination index while minimum in treatment 400 Gy (6.3). Among the EMS treatments, maximum germination index was observed in treatment 0.05% EMS (16.9) and minimum in treatment 0.30% EMS (7.7). Within combination treatments, highest germination index was reported in treatment 100 Gy +0.20% EMS (17.4) and lowest in treatment 400 Gy + 0.20% EMS (8.1).

Mean daily germination

Reduction in mean daily germination was observed in all mutagenic treatments as compared to control (14.2). It was observed that, mean daily germination was reduced as per the increased dose of mutagen. Among all mutagenic treatments, maximum mean daily germination was observed in treatment 100 Gy (13.1) and minimum in treatment 400 Gy (4.6).

Shoot Length (cm)

Mean values and per cent reduction in shoot length was recorded after 7 days of treatment with gamma rays, EMS and their combinations treatments. In present investigation, range of this parameter was from 3.0 cm (400Gy) to 8.2 cm (0.05% EMS). Increasing trend of per cent reduction in shoot length as per the increased dose of mutagen was observed in all mutagenic treatments. Among the all mutagenic treatments, maximum per cent reduction in shoot length was reported in treatment 400 Gy (64.29) and minimum in treatment 0.05% EMS (2.38).

Root Length (cm)

In present findings, root length ranged from 3.5 cm (400 Gy + 0.20% EMS) to 12.7 cm (0.05% EMS). The reduction in root length occurred with each corresponding increase in the strength of mutagenic treatment. Increased per cent reduction in root length was reported as per the increased dose of mutagen. Within all mutagenic treatments, maximum per cent reduction in root length was recorded by treatment 400 Gy + 0.20% EMS (72.66) and minimum in treatment 0.05% EMS (0.78). The reduction in length of root and shoot was attributed to the effects of mutagens on the physiological system (Gaul, 1977) [5] such a reduction in length of root and shoot arising out of mutagenic treatments was previously reported in crop plants (Reddy and Gupta, 1989; Amarnath and Prasad 1998 and Uma and Salimath, 2001) [13, 1].

Vigour index

Vigour index was decreased progressively with increasing mutagenic doses. In all mutagenic treatments, it was higher at lower dose and lower at higher dose. Among the all mutagenic treatments, treatment 0.05% EMS (1769.5) showed maximum seedling vigour and minimum in treatment 400 Gy (250.0).

Germination percentage (Field condition)

It was revealed that germination in all the treated seed was reduced as compared to control (94.00%). It was also observed that germination percentage was reduced as per the increasing the doses of mutagenic agents. Maximum germination percentage was recorded in treatment 100Gy (91.49%) whereas minimum in treatment 400Gy+ 0.20% EMS (5.11%). Similar inhibitory effects on seed germination observed by Mundhe & Borse (2012) [10], and Khan & Wani

(2006) [8].

On the basis of germination percentage among the various mutagenic treatments, LD_{50} dose was optimized. For gamma rays mutagenic treatment 300Gy, for EMS mutagenic treatment 0.20% EMS and for combination treatment 300Gy+ 0.20% EMS doses were optimized as a LD_{50} doses which were close to LD_{50} for pigeonpea.

Mortality percentage

It was observed that mortality percentage in various mutagenic treatments were maximum as compared to control (8.09%). The range of mortality percentage among the various treatments was in between 9.14% (100Gy + 0.20% EMS) and 73.08% (400Gy).

Survival percentage

Effect of various mutagenic treatments used under study on survival of pigeon pea cultivar Konkan Tur 1shows reduced survival in all treated seeds as compared to control (91.91%). Among all the mutagenic treatments maximum survival was observed in treatment 100Gy +0.20% EMS (90.86%) whereas minimum survival was observed in treatment 100Gy (26.92%). The decrease in survival of plant at maturity is due to rapid injection of chemical mutagen and their ability to produce chromosomal abstractions (Sharma *et al*., 2005) [16]. Similar results were also obtained by Bashir *et al*., 2013 in Fenugreek. Kulkarni and Mogle, 2013 [9] in Horse gram, Sangale *et al*., 2011 [15] and Giri and Apparao, 2011 [6] in Pigeonpea.

Table 1: Per cent Germination, Germination index, Mean daily germination, shoot length, root length and Vigour index in M_1 generation of pigeon pea (Lab.) (Paper Towel Method)

Treatment code	Treatment	Germination% 7 DAT		Germination index	Mean daily germination	Shoot Length (cm) 7 DAT		Root Length (cm) 7 DAT		Vigour index
		Mean*	% Reduction			Mean	% Reduction	Mean	% Reduction	
T0	Control	99.3		19.6	14.2	8.4		12.8		2105.9
T1	100 Gy	91.7	7.65	18.2	13.1	7.0	16.67	11.8	7.81	1723.3
T2	200 Gy	85.3	14.10	16.8	12.2	6.8	19.05	10.5	17.97	1479.1
T3	300 Gy	58.3	41.29	11.2	8.3	5.5	34.52	8.3	35.16	805.0
T4	400 Gy	32.3	67.47	6.3	4.6	3.0	64.29	4.8	62.50	250.0
T5	0.05 EMS	84.7	14.70	16.9	12.1	8.2	2.38	12.7	0.78	1769.5
T6	0.10 EMS	58.7	40.89	11.3	8.4	8.0	4.76	12.1	5.47	1179.2
T7	0.20 EMS	44.7	54.98	8.7	6.4	7.6	9.52	12.3	3.91	888.9
T8	0.30 EMS	41.3	58.41	7.7	5.9	7.3	13.10	10.4	18.75	733.0
T9	100 Gy + 0.20 EMS	88.7	10.67	17.4	12.7	7.1	15.48	11.0	14.06	1599.0
T10	200 Gy + 0.20 EMS	74.7	24.77	14.3	10.7	5.9	29.76	8.7	32.03	1090.1
T11	300 Gy + 0.20 EMS	55.0	44.61	10.7	7.9	5.0	40.48	5.9	53.91	597.7
T12	400 Gy + 0.20 EMS	43.3	56.39	8.1	6.2	3.5	58.33	3.5	72.66	303.3
	S.E.	0.58				0.25		0.35		
	C.D. @1%	2.23				0.98		1.34		
	C.V.	1.53				6.95		6.34		

Mean germination percentage was calculated on the basis of control mean

Table 2: Per cent germination, Mortality and Survival in M_1 generation of Pigeon pea (Field)

Treatment	Germination%	Mortality%	Survival%
T_0- Control	94.00	8.09	91.91
T_1- 100 Gy	91.49	12.56	87.44
T_2- 200 Gy	85.11	18	82.00
T_3- 300 Gy	55.32	20.77	79.23
T_4- 400 Gy	22.13	73.08	26.92
T_5- 0.05% EMS	80.00	12.24	87.76
T_6- 0.10% EMS	57.45	19.26	80.74
T_7- 0.20% EMS	44.68	24.77	75.23
T_8- 0.30% EMS	39.57	30.11	69.89
T_9- 100Gy+0.20% EMS	83.83	9.14	90.86
T_{10}- 200Gy+0.20% EMS	73.62	16.77	83.23
T_{11}- 300Gy+0.20% EMS	46.81	40.00	60.00
T_{12}- 400Gy+0.20% EMS	5.11	41.67	58.33
Total of T_0 to T_{12}	779.12	326.46	973.54
Average of T_1 to T_4	63.51	31.1	68.89
Average of T_5 to T_8	55.42	21.5	78.4
Average of T_9 to T_{12}	52.34	26.8	73.1
Overall average of T_1 to T_{12}	57.09	26.5	73.4

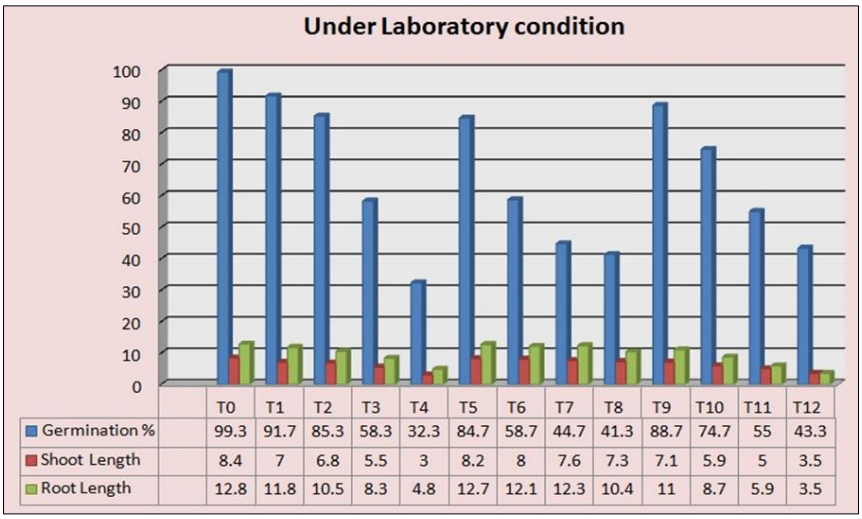

Fig 1: Per cent germination, Shoot Length and Root Length

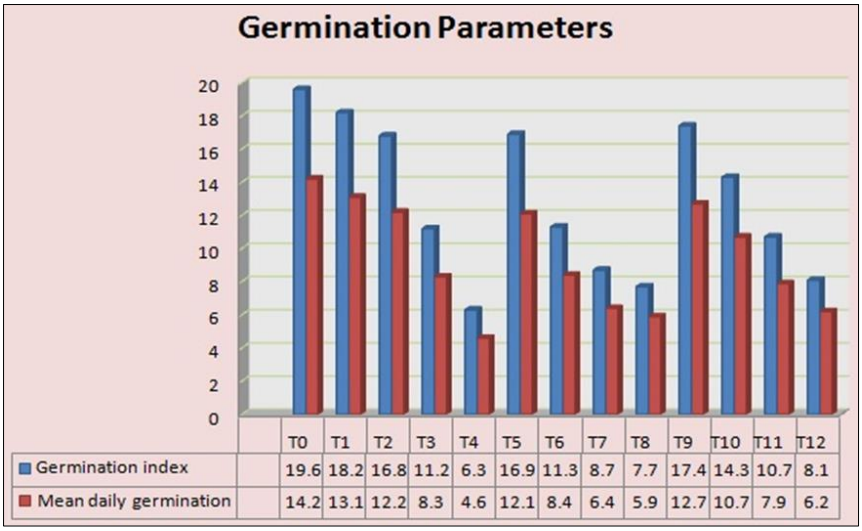

Fig 2: Germination index and Mean daily germination

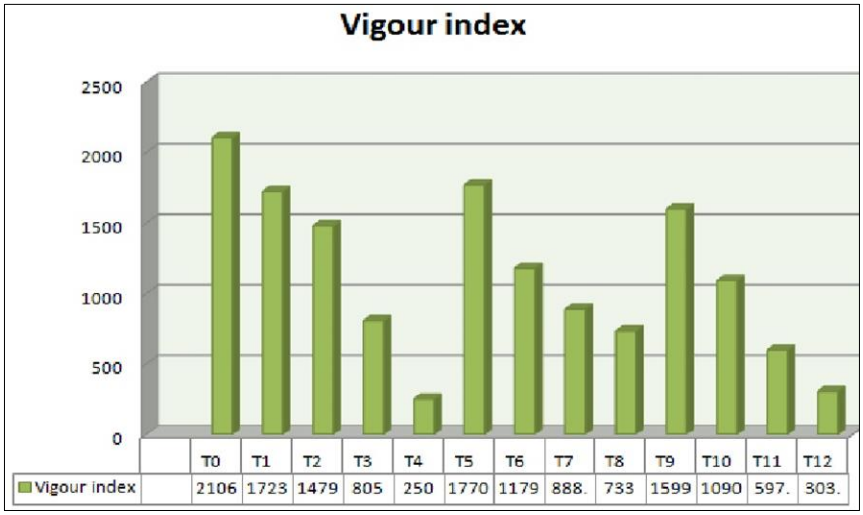

Fig 3: Vigour index

Conclusion

Percent seed germination and seedling growth was inhibited due to increasing doses/ concentrations of mutagens. The germination parameters like per cent germination, root length, shoot length, germination index, mean daily germination, Vigour index showed decreasing trend as per the increased dose of mutagen under laboratory condition. Similar result was also reported under field condition for per cent germination and survival percentage.

Acknowledgement
I acknowledge the support given by the Bhaba Automic Research Centre, Mumbai, for supporting my Ph.D. research program. I would like to extend my most heartfelt thanks to my major and minor guide, also to the faculty of the Genetics and Plant Breeding Department for their valuable guidance and support.

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