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Research Article

Morphological spectrum of gamma rays and ems induced viable mutants in cowpea (*Vigna unguiculata* (L.) Walp)

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Abstract
Seeds of cowpea (*Vigna unguiculata* (L.) Walp) variety CO 7 and Tirunelveli local were treated with gamma ray doses at 150, 200, 250, 300 and 350 Gy and EMS (Ethyl Methane Sulphonate) doses at 5, 10, 15, 20 and 25 mM. The total of 248 mutants from gamma irradiated population and 345 mutants from EMS treatments were identified. More number of viable mutants were recorded for EMS than gamma ray treatment in both the varieties. In variety CO 7, the total of 165 mutants were observed in the gamma ray and 251 mutants in EMS treated plants. In Tirunelveli local, the total of 83 and 94 mutants were observed for gamma ray and EMS treatment respectively. The gamma ray dose of 200 Gy and EMS dose of 10 mM recorded the highest frequency of viable morphological mutants.

Keywords
Cowpea; CO 7; Tirunelveli local; gamma ray; EMS; viable mutants

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is an annual herbaceous legume from the genus *Vigna*. Due to its tolerance for sandy soil and low rainfall, it is an important crop in the semi-arid regions across Africa and Asia. Being a fast growing crop, cowpea curb erosion by covering the ground fixes atmospheric nitrogen and its decaying residues contribute to soil health. Cowpea is consumed in many forms: the young leaves, green pods, green seeds as vegetables and dry seeds are used in various recipes . Cowpea is a versatile kharif pulse crop because of its smothering nature, drought tolerant characters, soil restoring properties and multipurpose uses (Deepa *et al.,* 2010).

Despite the rich germplasm collection available in various breeding programs the genetic base for the cowpea is narrow for economic traits such as grain yield, yield components, drought and insect pest tolerance (Horn and Shimelis, 2013). Various breeding methods like recombination breeding, mutation breeding and transgenic breeding, each with its unique way of generating variation and selecting target line, are utilized in the crop improvement programme. Crop Improvement of pulses through hybridization and recombination is very difficult, because of their autogamous nature. Due to their autogamous nature, lack genetic variability. The spontaneous mutation rate is pretty low and can't be exploited for breeding and that is why artificially mutations are induced with physical and chemical mutagen treatment. According to Micke *et al* (1993) only eight out of 1000 improved mutant varieties of different crops released up to 1989 in over 48 countries were cowpea. It is an established fact that mutagen, besides causing changes in major genes, also induces mutations at loci governing the quantitative characters. Mutagen agents, including gamma rays, offered great possibilities for increasing genetic variability of quantitative traits such as yield. Since induced mutations are useful to produce new genetic variation and select favourable mutants, systematic study of induced mutagenesis by physical mutagens (Gamma ray) and chemical mutagens (EMS) in cowpea was attempted.

MATERIALS AND METHODS

Two cowpea varieties *viz*., CO 7 and Tirunelveli local were treated with Gamma ray in a ^{60}Co chamber available at Sugarcane Breeding Institute (SBI), Coimbatore. 300 dried and healthy seeds of above varieties were treated with gamma rays at five different doses from 150 to 350 Gy with an interval of 50 Gy and Ethyl Methane Sulphonate (EMS) at five levels of doses from 5 to 25 mM with an interval of 5mM. The treated seeds were sown along with control seeds of both the varieties in Randomized Block Design (RBD) at three replications with the spacing of 45 x 20 cm during Rabi season of 2014. The recommended agronomic practices and plant protection measures were followed uniformly for all the treatments. The M_2 generation was raised from individual M_1 plant following plant to the progeny method in both the varieties namely Co 7 and Tirunelveli local. A total set around 100 (75 from CO 7 and 25 from Tirunelveli Local) M_1 plants seeds from two varieties were forwarded to M_2 generation. The M_2 seeds of each M_1 plants were sown during February 2015 without replication with the spacing of 45 x 25 cm. The standard agronomic practices were followed throughout the period of crop growth as like that of M_1 generation. In M_2 generation, the occurrence of chlorophyll mutants was observed in the nursery when the seedlings were with 2-3 leaves just to assess the effect of mutagen on the biological materials. The number of M_2 families evaluated in CO 7 and Tirunelveli local are furnished in **Table 1**. The induced mutations in the plant morphology of the two varieties were categorized into six major phenotypic categories *viz*. plant size, growth habit, leaf, flower, pod and seed.

Each category includes various mutant phenotypes related to that particular morphology and frequencies of the mutation in each morphological category out of the total morphological mutations were calculated throughout the growing season of M_2 generations. (**Table 4 and 5**).

Table 1. Number of M_2 families evaluated for CO 7 and Tirunelveli Local

Variety	Gamma rays	150 Gy	200 Gy	250 Gy	300 Gy	350 Gy	Total
CO 7		15	15	15	15	15	**75**
Tirunelveli Local		5	5	5	5	5	**25**
	EMS	5 mM	10 mM	15 mM	20 mM	25 mM	
CO 7		15	15	15	15	15	**75**
Tirunelveli Local		5	5	5	5	5	**25**

RESULT AND DISCUSSION

In the present investigation, viable macro mutations with changes in attributes like stature, duration, cotyledon, stem, leaf, pod, flower and seed mutants were recorded. Stature mutants namely dwarf, tall, and duration mutants like early and late mutants were observed in both the varieties *viz.,* Co 7 and Tirunelveli local. (**Fig.1**). Plants in control did not produce any morphological mutant. As given in **Table 2 and 3**, the frequency of morphological abnormalities increased with increase in the dose of gamma rays till 200 Gy and 10 mM in chemical treatment followed by a decline. Most of the induced mutants were found to be fall under leaf mutations (27%) and pod mutations (22%) category followed by a duration (16%) and plant height (8%) in CO 7 variety whereas leaf mutations (21%) and stem mutations (29%) category followed by pod (12%) and duration mutations (11%) in Tirunelveli Localrespectively. (**Fig. 4 and 5**).

Table 2. Percentage mutated plant in CO7

Treatment	No of plants scored	Morphological mutant types of CO7											TMP	% Mutated plants
		Plant Height	Durations	Sterile	Leaf	Single Cotyledon	Stem	Flower	Pod	Seed Colour	Chimera	Others		
Gamma														
150Gy	620	4	9	0	10	0	3	1	7	3	0	0	37	5.97
200Gy	602	7	11	0	12	1	4	2	10	4	0	0	51	8.47
250Gy	583	1	3	1	10	0	4	1	10	5	0	0	34	5.83
300Gy	446	2	3	0	9	0	2	1	6	2	0	0	25	5.61
350Gy	248	1	2	0	5	0	3	0	5	2	0	0	18	7.26
EMS														
5mM	553	6	10	0	12	0	4	2	14	4	0	0	52	9.40
10mM	550	8	13	0	14	2	8	4	18	4	1	0	72	13.09
15mM	536	4	5	2	16	0	8	2	7	4	0	2	48	8.96
20mM	502	2	6	0	13	0	8	3	8	3	0	0	43	8.57
25mM	476	0	5	0	12	0	10	0	5	2	0	0	34	7.14

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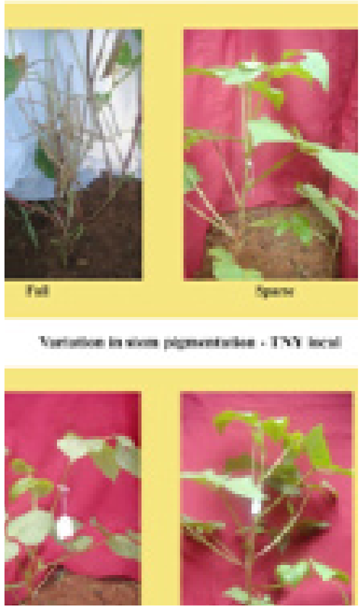
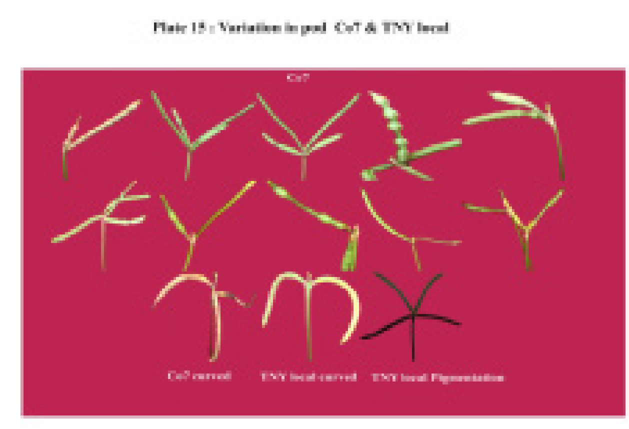
A. Tall and Dwarf

B. Flower color

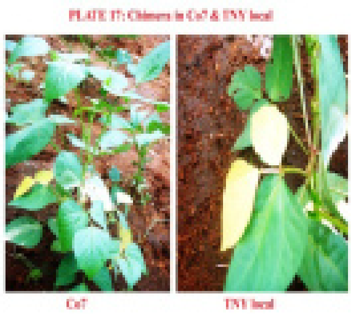


C. Stem pigmentation

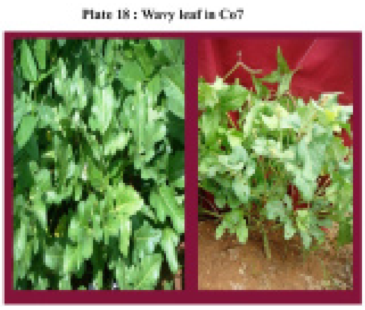


D. Pod Variation

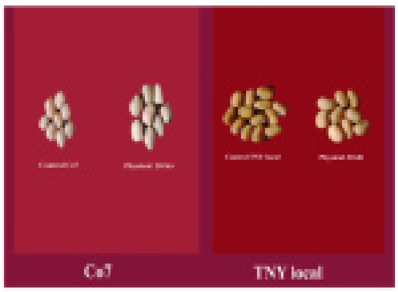
E. Sterile plants



F. Chimeras



G. Wavy leaf margins



H. Seed coat colour

Fig. 1. Different types of morphological mutants observed in CO 7 and Tirunelveli Local in M_2 generation

Table 3. Percentage mutated plant in Tirunelveli Local

Treatment	No of plants scored	Morphological mutant types of Tirunelveli Local: Plant Height	Durations	Sterile	Leaf	Single Cotyledon	Stem	Flower	Pod	Seed Colour	Chimera	Others	TMP	% Mutated plants
Gamma														
150Gy	425	1	2	1	3	0	5	1	3	2	0	0	18	4.24
200Gy	418	2	3	0	4	1	7	3	6	3	0	0	29	6.94
250Gy	350	0	1	2	2	0	4	1	1	3	0	0	14	4.00
300Gy	332	0	1	1	3	0	5	0	0	2	1	0	13	3.92
350Gy	300	0	2	0	4	0	2	0	2	1	0	0	11	3.67
EMS														
5mM	410	3	2	2	4	0	7	1	2	1	0	0	22	5.37
10mM	402	6	3	0	6	1	10	1	5	2	0	0	34	8.46
15mM	343	0	2	1	4	0	6	0	1	1	0	0	15	4.37
20mM	321	0	2	1	2	0	4	0	3	1	0	0	13	4.05
25mM	305	0	1	0	5	0	3	0	2	1	0	0	12	3.93

Table 4. Frequency and spectrum of morphological mutants induced by various mutagens in M_2 generation of CO 7

TYPE OF MUTANT		MUTAGEN DOSE OF CO7: GAMMA RAYS (Gy)		EMS		Total		Grand Total	
		N	F%	N	F%	N	F%	N	F%
Plant Height	Tall	4	0.16	6	0.23	10	0.20	35	**0.68**
	Dwarf	8	0.32	8	0.31	16	0.31		
	Erect	3	0.12	6	0.23	9	0.18		
Duration mutants	Early mutant	21	0.84	27	1.03	48	0.94	67	**1.31**
	Late mutant	7	0.28	12	0.46	19	0.37		
Sterile mutants	Sterile	1	0.04	2	0.08	3	0.06	3	**0.06**
Cotyledonary abnormalities	Single cotyledon	1	0.04	2	0.08	3	0.06	3	**0.06**
Leaf modification	Variation in leaflet number	35	1.40	47	1.80	82	1.60	113	**2.21**
	Other leaf mutants	10	0.40	20	0.76	30	0.59		
Stem Pigmentation	Narrow	1	0.04	0	0.00	1	0.02	52	**1.02**
	Spares	7	0.28	10	0.38	17	0.33		
	Entire	7	0.28	28	1.07	35	0.68		
Flower modification	White with blue stripes	2	0.08	3	0.11	5	0.10	16	**0.31**
	Light blue colour	3	0.12	8	0.31	11	0.22		
Pod modification	Small pods	8	0.32	7	0.27	15	0.29	90	**1.76**
	Long pods	5	0.20	14	0.53	19	0.37		
	Colour variation	1	0.04	1	0.04	2	0.04		
	Constriction	24	0.96	30	1.15	54	1.06		
Changes in seed character	Bold seed	2	0.08	0	0.00	2	0.04	34	**0.66**
	Seed coat colour	15	0.60	17	0.65	32	0.63		
Others	Chimeric mutant	0	0.00	1	0.04	1	0.02	1	**0.02**
Other		0	0.00	2	0.08	2	0.04	2	**0.04**
Total		165	6.60	251	9.59	416	8.13	416	**8.13**

Table 5. Frequency and spectrum of morphological mutants induced by various mutagens in M_2 generation of Tirunelveli local

TYPE OF MUTANT		MUTAGEN DOSE OF TIRUNELVELI LOCAL							
		GAMMA RAYS (Gy)		EMS		Total		Grand Total	
		N	F%	N	F%	N	F%	N	F%
Plant Height	Tall	2	0.11	5	0.28	7	0.19	12	**0.33**
	Dwarf	0	0.00	2	0.11	2	0.06		
	Erect	1	0.05	2	0.11	3	0.08		
Duration mutants	Early mutant	6	0.33	7	0.39	13	0.36	19	**0.53**
	Late mutant	3	0.16	3	0.17	6	0.17		
Sterile mutants	Sterile	4	0.22	4	0.22	8	0.22	8	**0.22**
Cotyledonary abnormalities	Single cotyledon	1	0.05	1	0.06	2	0.06	2	**0.06**
Leaf modification	Variation in leaflet number	14	0.77	18	1.01	32	0.89	37	**1.03**
	Other leaf mutants	2	0.11	3	0.17	5	0.14		
	Narrow	0	0.00	0	0.00	0	0.00		
Stem Pigmentation	Spares	10	0.55	13	0.73	23	0.64	53	**1.47**
	Entire	13	0.71	17	0.95	30	0.83		
Flower modification	White with blue stripes	1	0.05	0	0.00	1	0.03	7	**0.19**
	Light blue colour	4	0.22	2	0.11	6	0.17		
Pod modification	Small pods	0	0.00	0	0.00	0	0.00	21	**0.58**
	Long pods	3	0.16	4	0.22	7	0.19		
	Colour variation	3	0.16	0	0.00	3	0.08		
	Constriction	4	0.22	7	0.39	11	0.31		
Changes in seed character	Bold seed	0	0.00	0	0.00	0	0.00	17	**0.47**
	Seed coat colour	11	0.60	6	0.34	17	0.47		
Others	Chimeric mutant	1	0.05	0	0.00	1	0.03	1	**0.03**
Total		83	4.55	94	5.28	177	4.91	177	4.91

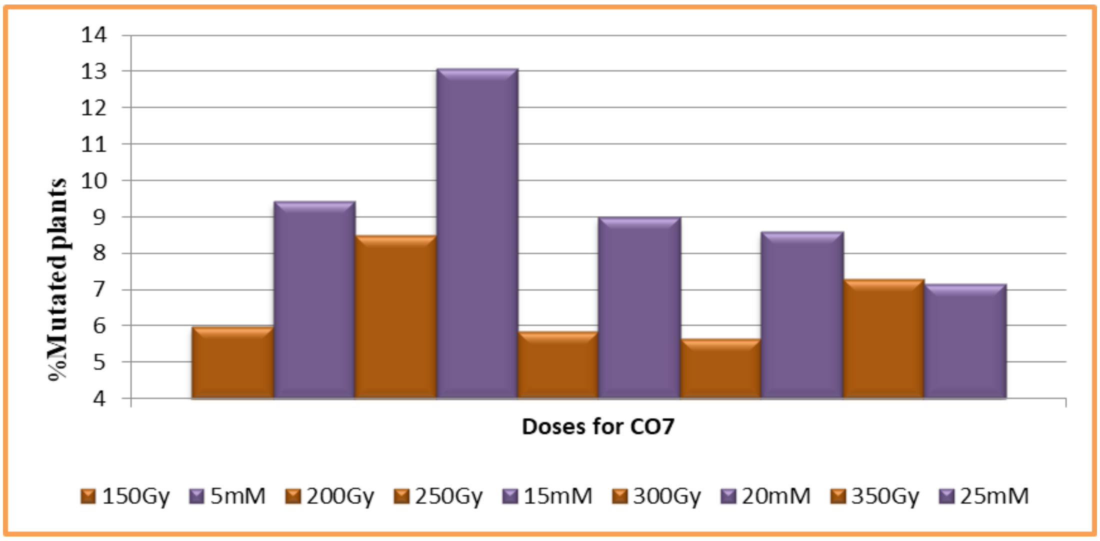


Fig.2. The comparative estimation of phenotypic mutants induced by Gamma radiation and EMS in CO7

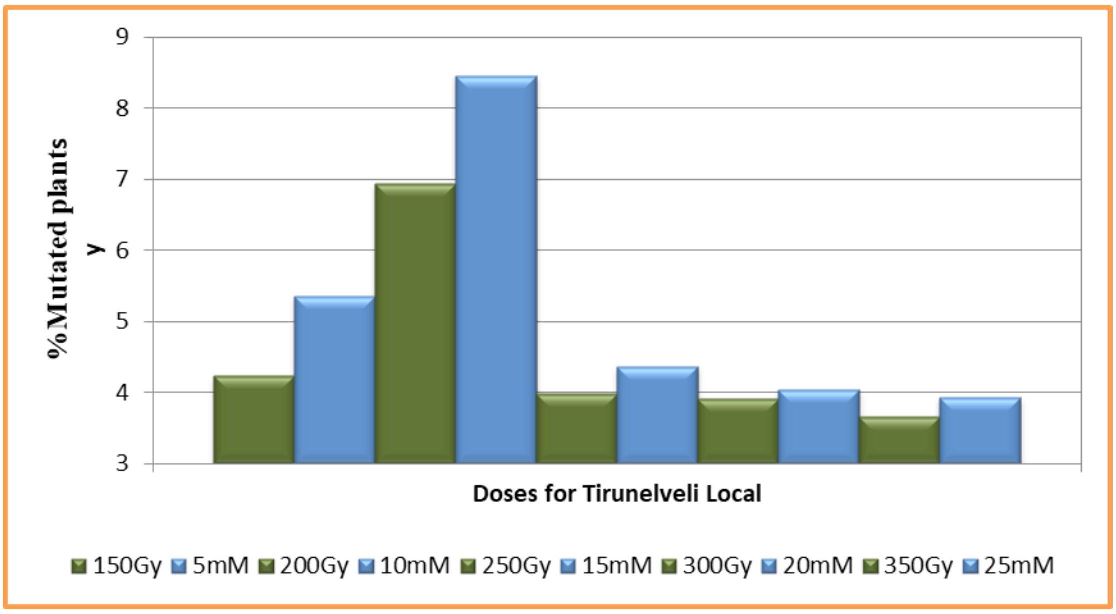


Fig.3. The comparative estimation of phenotypic mutants induced by Gamma radiation and EMS in Tirunelveli Local

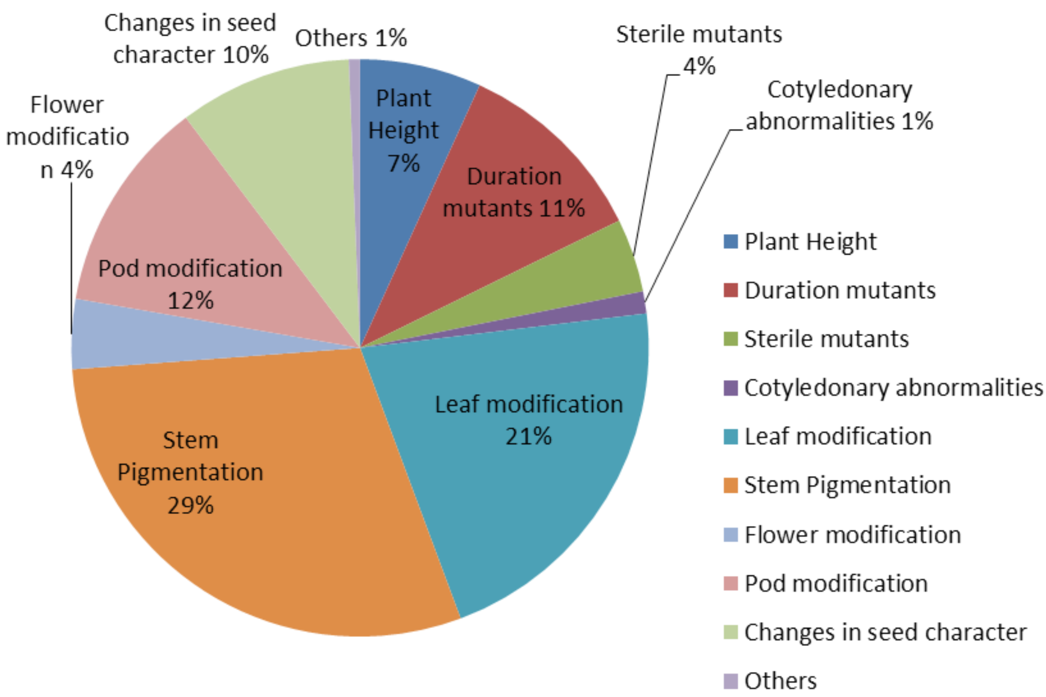


Fig. 4. Spectrum of viable mutants in Tirunelveli local

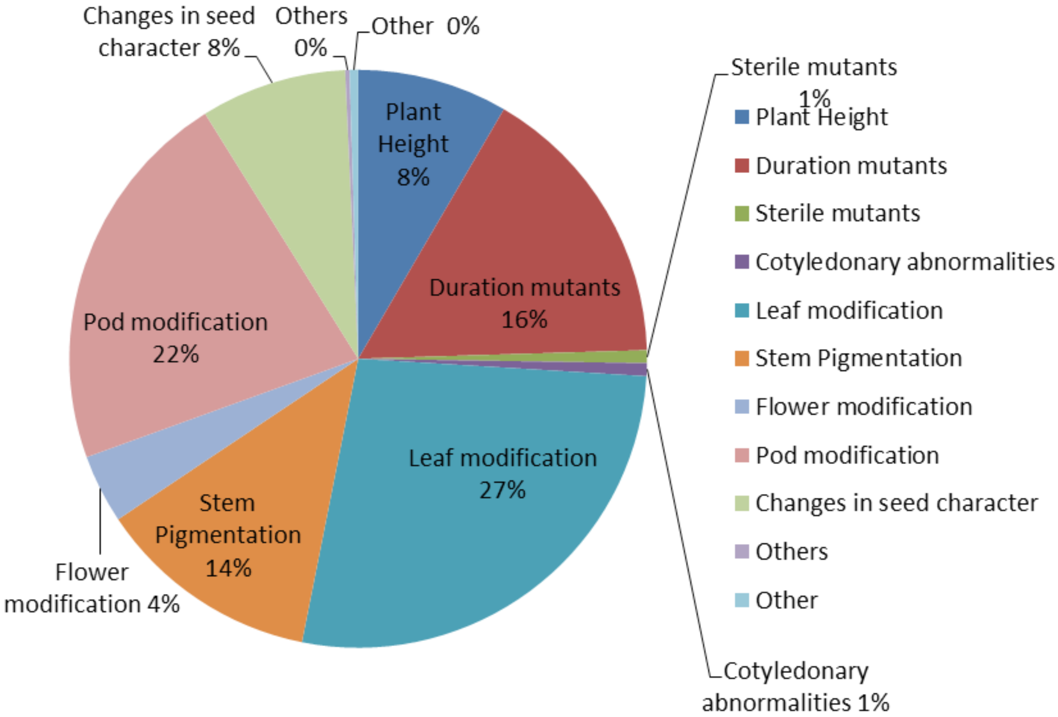


Fig. 5. Spectrum of viable mutants in CO 7

In the present study, different frequencies of occurrence were observed not only between the two varieties but also within the mutagenic treatments thereby signifying the mutagen type and concentration dependency for inducing macromutations. Based on morphological mutation frequency, the variety Tirunelveli Local was found to be comparatively less mutable than the variety CO 7. (**Fig.2 and 3**)

Tall mutants were characterized by long internodes. These mutants appeared more frequently in EMS treatments. Tall mutants, as observed in the present study, were also reported earlier by Solanki *et al.* (2005) in lentil, Kumar *et al.* (2009) in black gram and Goyal *et al* (2019) in black gram. Early and late mutants have been reported by Dhanavel *et al.* (2012) in cowpea; Rudraswami *et al.* (2006) and Dhumal and Bolbhat (2012) in horse gram. Sterile mutants were found to be induced by both gamma ray and EMS treatments. A similar type of mutants has been reported by Adekola and Oluleye, (2007) for gamma ray induced mutation in cowpea. Seedlings with single cotyledon were reported by Banu (2000) in cowpea.

Leaf abnormalities are attributed to a disruption in mineral metabolism, accumulation of free amino acids, disturbance in auxin synthesis and chromosomal abnormalities (Gnanamurthy and Dhanavel, 2014). The leaf mutants observed in M_2 generation included variations in leaflet number (tetra foliate leaf and penta foliate leaf), large thick leaf, small, narrow leaf mutants were Similar with mutants reported by Banu (2000) Nair and A.K. Mehta (2014) in cowpea.

Various types of mutants were observed for pod modification, which included, more number of pods per plant, long and succulent pods, pods with less number of seeds, single seed pod *etc.* as already reported by Banu (2000) and Mishra and Chand, (2003) in cowpea. The mutants exhibited brownish white seed coat colour in Tirunelveli local was also reported by Singh and Yadav (1991) in greengram and Ashok *et al* (2010). Chimeric mutants were identified in gamma ray treatments in Tirunelveli local and in EMS treatments in CO7 which consonances with Thakur (2004) and Gnanamurthy *et al.* (2012) in cowpea. Bhat *et al.* (2006) in *Vicia faba* (L.).

The variety Co 7 produced 165 and 251 viable mutants in gamma rays and EMS treatment respectively. In Tirunelveli local, gamma rays and EMS treatment produced 83 and 94 viable mutants respectively. Total morphological mutation frequency was more prominent in EMS (9.59%) (5.28%) than gamma radiation (6.60 %) (4.55%) in CO 7 and Tirunelveli local respectively. This finding revealed the better efficiency of alkylating agents in inducing point mutations than irradiation. The frequency of viable mutants was higher in EMS treatments on M_2 plant basis was corroborated with Nair and A.K. Mehta (2014) in cowpea, Dhumal and Bolbhat (2012) in Horsegram and

Ramesh *et al* (2019) in barnyard millet whereas, this was in contrast to the findings by (Senapati *et al* , 2008; Khursheed *et al* 2017) and Ramchander *et al* (2017) stating gamma radiation the efficient one than EMS.

Among the ten doses of treatment in gamma rays and EMS, the dose 200 Gy and 10 mM registered the highest frequency of viable mutants in M_2 generation in both the varieties.(**Table.2 and 3**).

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