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IMPACT OF STIMULATION THROUGH GAMMA IRRADIATION ON SEEDLINGS OF LATHYRUS (*LATHYRUS SATIVUS* L.)

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ABSTRACT

A study was conducted on induced mutagenesis using two varieties of Lathyrus, namely Mahateora and Prateek. The mutagen employed was Gamma rays, with experiments conducted at the Bhabha Atomic Research Centre, Mumbai, and field studies carried out under the AICRP on forage crop and utilization, Research cum Instructional Farm, Department of Genetics and Plant Breeding, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, (C.G.). Laboratory evaluations were conducted at the Chhattisgarh States Seed Certification Agency. Variability was observed between varieties in their tolerance to the mutagens.

Different doses of Gamma radiation were administered, including Control, 75 Gy, 100 Gy, 150 Gy, 200 Gy, 250 Gy, 300 Gy, 350 Gy, 400 Gy, 450 Gy, 500 Gy, 550 Gy, 600 Gy, and 75 Gy + EMS. The impact of doses was assessed on traits of the varieties under laboratory and field conditions. Parameters such as germination percentage, seedling height (mm), plumule length (mm), and radicle length (mm) were recorded in laboratory conditions. Field conditions were evaluated based on plant survival percentages at 30 days, 60 days, and at the time of crop harvesting.

The dose of 350 Gy was the most lethal, significantly reducing the percentage of surviving plant varieties. However, gamma irradiation exhibited a positive effect (GR 50) on the biological traits of varieties. Interestingly, under laboratory conditions, gamma rays have stimulatory effects on parameters like germination percentage, seedling height, plumule length, and radicle length. Both varieties were observed more sensitive to radiation. Furthermore, it was observed that low (100 Gy and 150 Gy) and medium doses (300 Gy and 350 Gy) had minor biological effects on Lathyrus in both laboratory and field conditions in the M1 generation, respectively.

Keywords: Induced mutagenesis, Lathyrus, Gamma rays, Biological traits, Radiation dose, Varietal sensitivity, Laboratory evaluation, Field study.

Introduction

Lathyrus (*Lathyrus sativus* Linn.) is an important crop of economic significance in India, China, Bangladesh, Pakistan, Nepal and Ethiopia. Qualities of this grain legume like sturdiness, drought and flood tolerance and adaptability to a wide range of agro-climatic conditions (Campbell, 1997) make this otherwise neglected crop popular among the farmers and attracted them to grow this crop. Despite its high

protein content (21.8-40%) and stress resistance, no significant breakthrough has been achieved about genetic rectification for its neurotoxin content (β -ODAP: β -N-oxalyl- α,β -diaminopropionic acid) present in seed and other plant parts (Mondal and Puteh, 2014). Induced mutagenesis plays an important role in improvement of crops like lathyrus where predominant presence of self-pollination and inter specific incompatibility hindered its improvement through conventional breeding which results in

existence of narrow genetic variation. Spontaneous or induced mutants, with desirable character changes have been a vital material for gene discovery, mapping, functional genomics and breeding in many crops including Lathyrus. Induced mutagenesis can be used to create additional genetic variability that may be utilized by plant breeders in the development of cultivars for specific purposes or with specific adaptations (Rybinski, 2003). Improvement in the frequency and spectrum of mutations in a predictable manner and thereby achieving desired plant characteristics for their either direct or indirect exploitation in the breeding programme is an important goal of mutation research (Patil *et al.* 2017). Several researches worked in lathyrus involving induced mutagenesis (Talukdar, 2009), (Kumar *et al.*, 2011), (Tripathy *et al.*, 2012) and (Ramezani and More, 2014) to improve genetic variability in this orphan crop. A systematic and comparative study on mutagenic efficiency and effectiveness of gamma rays, ethyl methane sulphonate (EMS) and their combinations based on frequency mutations in M_1 generation in two genotypes of lathyrus has been undertaken in the present investigation.

Materials and Methods

Two varieties, Mahateora and Prateek of lathyrus formed the materials for the present investigation. Dry (9-12%) moisture and healthy seeds were obtained from National seed project, Indira Gandhi Krishi

Vishwavidyalaya, Raipur and exposed to (Control, 75 Gy, 100 Gy, 150 Gy, 200 Gy, 250 Gy, 300 Gy, 350 Gy, 400 Gy, 450 Gy, 500 Gy, 550 Gy 600 Gy and 75 Gy + EMS). Irradiation was done using the Cobalt 60 sources in Gamma chamber at Bhabha Atomic and Research Centre, Mumbai. In *Rabi* 2017, one hundred (100) treated seeds of two varieties of lathyrus Mahateora and Prateek irradiated with twelve (12) different doses of γ -rays (75 Gy, 100 Gy, 150 Gy, 200 Gy, 250 Gy, 300 Gy, 350 Gy, 400 Gy, 450 Gy, 500 Gy, 550 Gy 600 Gy) were conducted at Seed testing laboratory under Chhattisgarh State seed certification agency, Raipur for laboratory condition and plant survival percentage studies were sown along with untreated control seeds with treated seed in the single plot design at Research cum Instructional Farm, Department of Genetics and Plant Breeding, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, (C.G.).

Data were recorded at laboratory condition on germination%, seedling height, plumule length and radicle length and field condition date are recorded on plant survival % in 30 days, 60 days and at the time of crop harvesting. The plant survival % (30 days after sowing and at crop harvesting) and injury percentage. Likewise variations in quantitative characters were also observed in untreated control as well as treated plants throughout the life span of the plant.

Table 1: Details of Mutagenic Treatment Given to Lathyrus

Mutagen used	Dose/conc.	Duration of presoaking (hrs)
Control	DDW	12
Gamma rays (GY)	75 Gy	12
	100 Gy	
	150 Gy	
	200 Gy	
	250 Gy	
	300 Gy	
	350 Gy	
	400 Gy	
	450 Gy	
	500 Gy	
	550 Gy	
600 Gy		
Gamma rays (GY) + EMS	75 Gy + EMS	12

Results and Discussion

Laboratory condition

Detailed observation on all the M_1 plants of γ -rays treated seeds 75 Gy, 100 Gy, 150 Gy, 200 Gy, 250 Gy,

300 Gy, 350 Gy, 400 Gy, 450 Gy, 500 Gy, 550 and Gy 600 Gy with control of both varieties Mahateora and Prateek were recorded for lethality percent and injury percent. Observations for quantitative characters were also recorded.

Germination percentage (%):

Germination percentage was recorded after 5th days, 7th days and 9th days of sowing in irradiated seeds of the varieties Mahateora and Prateek in M₁ (Table-2, Fig.1 and Fig.2 At the 5th day, the germination rates for Mahateora and Prateek were as follows: Control (100% and 100%), 75 Gy (92% and 96%), 100 Gy (88% and 96%), 150 Gy (82% and 91%), 200 Gy (79% and 88%), 250 Gy (75% and 78%), 300 Gy (69% and 62%), 350 Gy (60% and 54%), 400 Gy (54% and 48%), 450 Gy (49% and 41%), 500 Gy (47% and 38%), 550 Gy (41% and 34%) and 600 Gy (33% and 31%).

On the 7th day, the germination rates for Mahateora and Prateek were: Control (100% and 100%), 75 Gy (93% and 96%), 100 Gy (89% and

97%), 150 Gy (84% and 93%), 200 Gy (80% and 88%), 250 Gy (75% and 88%), 300 Gy (70% and 63%), 350 Gy (62% and 55%), 400 Gy (54% and 48%), 450 Gy (49% and 42%), 500 Gy (48% and 38%), 550 Gy (42% and 33%) and 600 Gy (33% and 33%).

By the 9th day, the germination rates for Mahateora and Prateek were: Control (100% and 100%), 75 Gy (93% and 96%), 100 Gy (89% and 97%), 150 Gy (85% and 93%), 200 Gy (80% and 89%), 250 Gy (75% and 79%), 300 Gy (70% and 63%), 350 Gy (62% and 55%), 400 Gy (54% and 47%), 450 Gy (49% and 42%), 500 Gy (48% and 38%), 550 Gy (42% and 35%) and 600 Gy (33% and 30%).

Table 2 : Germination percentage in difference doses of gamma rays treated seed in Lathyrus at laboratory condition.

Germination %						
Doses	5th Day		7th Day		9th Day	
	Mahateora	Prateek	Mahateora	Prateek	Mahateora	Prateek
Control	100	100	100	100	100	100
75 Gy	92	96	93	96	93	96
100 Gy	88	96	89	97	89	97
150 Gy	82	91	84	93	85	93
200 Gy	79	88	80	88	80	89
250 Gy	75	78	75	79	75	79
300 Gy	69	62	70	63	70	63
350 Gy	60	54	62	55	62	55
400 Gy	54	48	54	46	54	47
450 Gy	49	41	49	42	49	42
500 Gy	47	38	48	38	48	38
550 Gy	41	34	42	33	42	35
600 Gy	33	31	33	32	34	30

Result observed in both the varieties Mahateora and Prateek indicated that seed germination percent decreased with an increasing dose of gamma rays clearly indicating that gamma rays as mutagen have induced an inhibitory effect on seed germination. (Chaudhary and Singh, 1980), (Satpute and Fultambkar, 2012) reported similar results in Soybean. A similar result of dose dependent germination percent reduction in other crops was reported by different

workers *viz.*, (Kumar *et al.*, 2009) in Cowpea, (Sagade and Apparao, 2011) and (Bhosale *et al.*, 2013) in Urdbean. The reduction in germination may be either due to genetic cause or inhibition of physiological process in cell by mutagen. In present investigation the variety Mahateora was found more sensitive to gamma rays as compared to variety the Prateek. Similar mutagenic sensitivity has been reported by (Kothekar and Kothekar, 1992) in Mothbean.

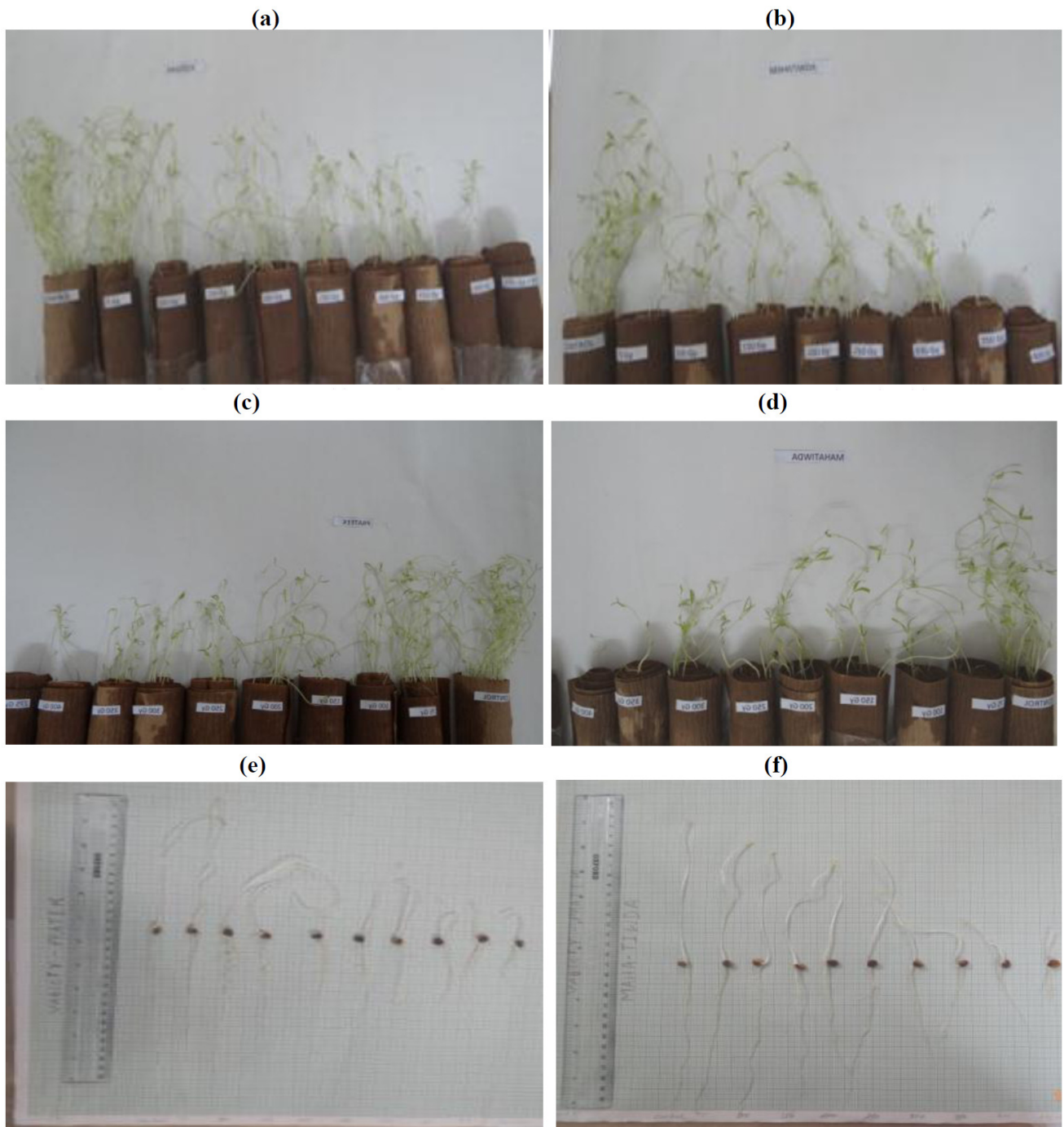


Fig. 1: (a, b, c, d, e and f) Germination of M_1 generation gamma rays treated seed in *Lathyrus* of laboratory condition.

Fig.:2a

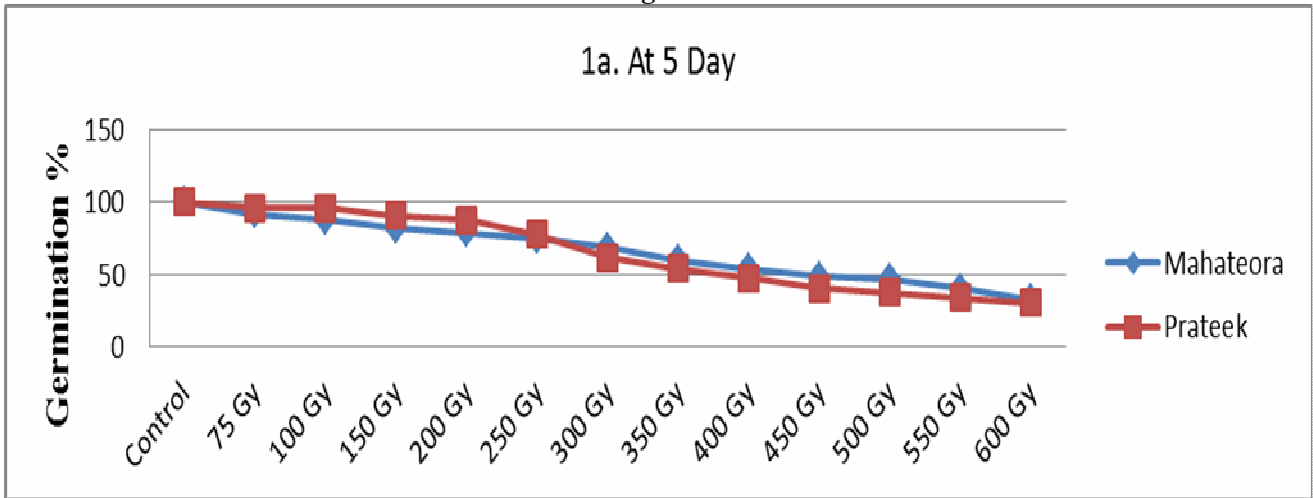


Fig.:2b

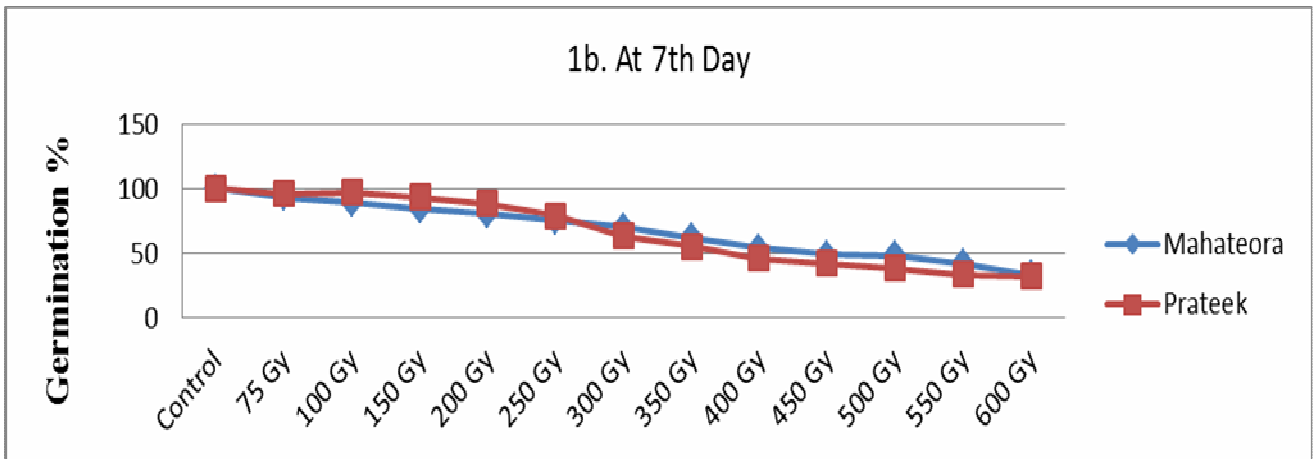


Fig.:2c

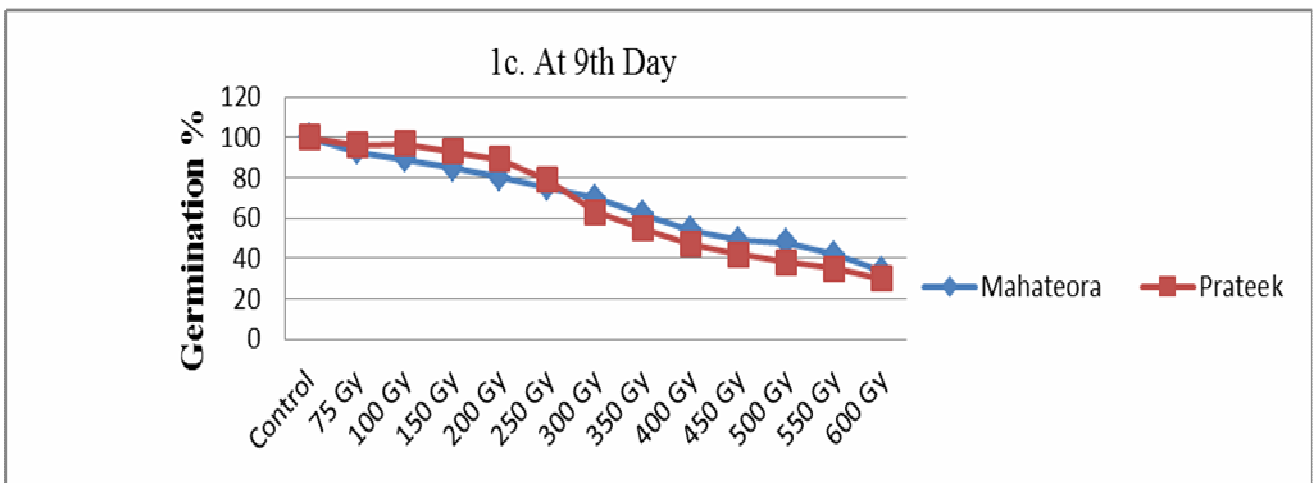


Fig. 2 (a, b, c): Germination percentage of M₁ generation for difference doses of gamma rays treated seed in Lathyrus of laboratory condition.

Seedling height (mm)

Seedling height was recorded after 5th days, 7th days and 9th days of sowing in irradiated seeds of the varieties Mahateora and Prateek in M₁ (Table-3) and Figure-3 (a, b, c). At 5th days of Control (170mm and 250mm), 75 Gy (210mm and 220mm), 100 Gy (190mm and 190mm), 150 Gy (170mm and 190mm), 200 Gy (200mm and 150mm), 250 Gy (130mm and 130mm), 300 Gy (120mm and 110mm), 350 Gy (100mm and 100mm), 400 Gy (80mm and 80mm), 450 Gy (60mm and 75mm), 500 Gy (45mm and 65mm), 550 Gy (20mm and 60mm) and 600 Gy (10mm and 45mm).

At 7th days of Control (290mm and 350mm), 75 Gy (270mm and 340mm), 100 Gy (260mm and 260mm), 150 Gy (240mm and 240mm), 200 Gy

(215mm and 230mm), 250 Gy (145mm and 200mm), 300 Gy (180mm and 150mm), 350 Gy (140mm and 125mm), 400 Gy (120mm and 110mm), 450 Gy (100mm and 80mm), 500 Gy (85mm and 75mm), 550 Gy (70mm and 65mm) and 600 Gy (55mm and 50mm).

At 9th days of Control (430mm and 510mm), 75 Gy (430mm and 430mm), 100 Gy (360mm and 460mm), 150 Gy (400mm and 380mm), 200 Gy (390mm and 470mm), 250 Gy (280mm and 500mm), 300 Gy (260mm and 190mm), 350 Gy (230mm and 270mm), 400 Gy (180mm and 240mm), 450 Gy (140mm and 90mm), 500 Gy (115mm and 80mm), 550 Gy (90mm and 70mm) and 600 Gy (65mm and 55mm).

Table 3: Seedling height (mm) for difference doses of gamma rays treated seed in Lathyrus at Laboratory condition

Seedling height (mm)						
Doses	5th Day		7th Day		9th Day	
	Mahateora	Prateek	Mahateora	Prateek	Mahateora	Prateek
Control	170	250	290	350	430	510
75 Gy	210	220	270	340	430	430
100 Gy	190	190	260	260	360	460
150 Gy	170	190	240	240	400	380
200 Gy	200	150	215	230	390	470
250 Gy	130	130	245	200	280	500
300 Gy	120	110	180	150	260	190
350 Gy	100	100	140	125	230	270
400 Gy	80	80	120	110	180	240
450 Gy	60	75	100	80	140	90
500 Gy	45	65	85	75	115	80
550 Gy	20	60	70	65	90	70
600 Gy	10	45	55	50	65	55

The result of this study demonstrated that increasing doses from Control–600 Gy had effect on seedling height even though there were slight decrease in heights with increase in dosage but the decrease was not proportional to the increase in dosage for both lathyrus varieties. This indicated that the varieties did not differ in radio sensitivity with respect to seedling height as the dose of 350 Gy causing 50% seedling height reductions was in both varieties. However, at

550 Gy and 600 Gy above the effected seedling height. Similar studies on rice have been documented by (Cheema and Atta, 2003) and (Smith, 1972). However, a linear dependency of seedling height of rice on the dosage of physical and chemical mutagens have been reported by (Katoch *et al.*, 1992), (Wang *et al.*, 1995) and (Goyal *et al.*, 2009) on Mungbean and on Pigeon pea by (Biradar *et al.*, 2004).

Fig.-3a

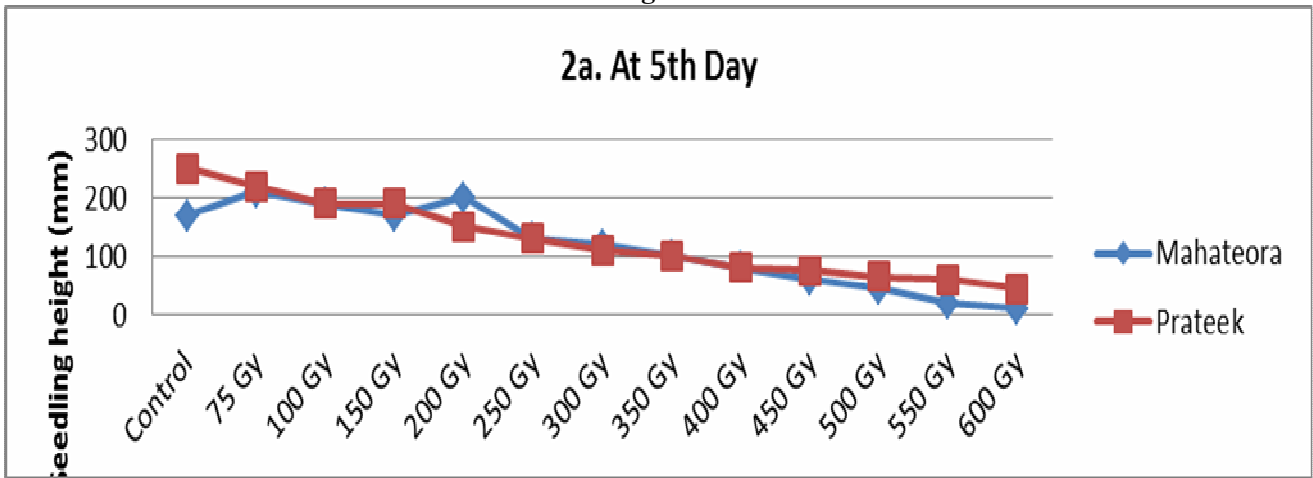


Fig.-3b

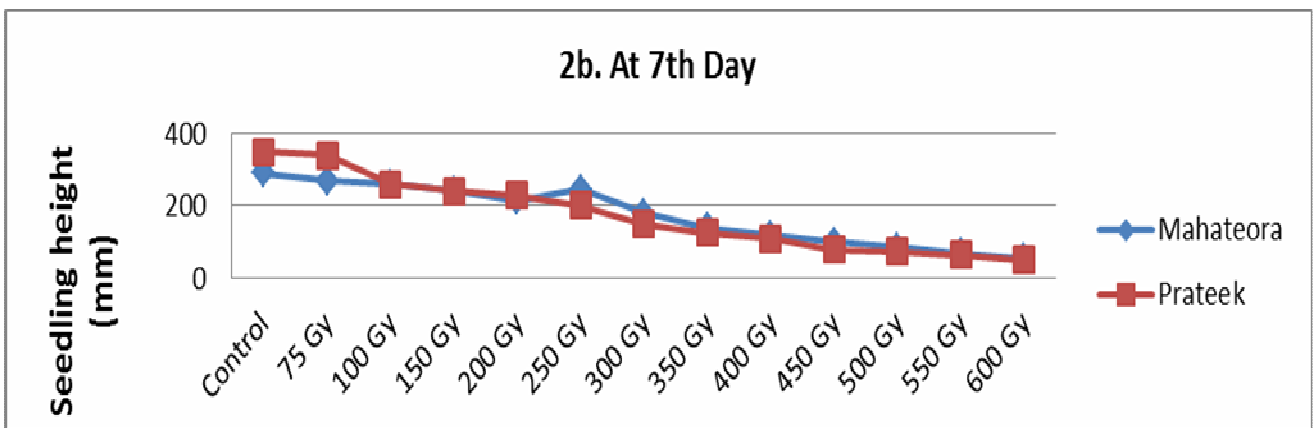


Fig.-3c

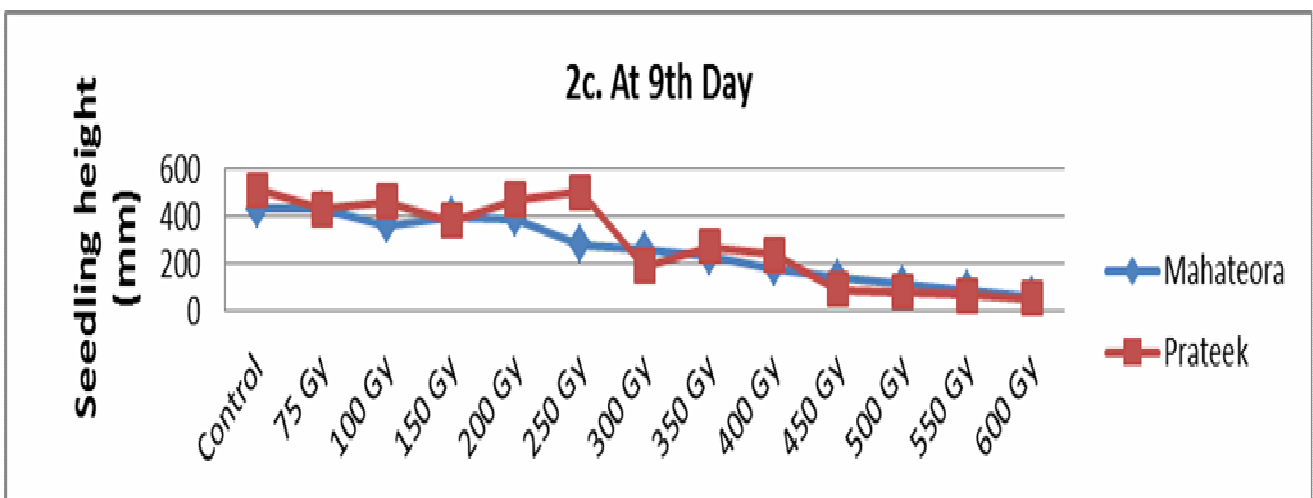


Fig. 3 (a, b, c): Seedling height of M₁ generation for difference doses of gamma rays treated seed in lathyrus of Laboratory condition

Plumule length (mm)

Plumule length was recorded after 5th days, 7th days and 9th days after sowing in irradiated seeds of the varieties Mahateora and Prateekas in M₁ Population (Table-4) and Figure-4(a, b, c). At 5th days of Control (70mm and 100mm), 75 Gy (115mm and 95mm), 100 Gy (80mm and 65mm), 150 Gy (65mm and 75mm), 200 Gy (90mm and 65mm), 250 Gy (45mm and 40mm), 300 Gy (50mm and 45mm), 350 Gy (50mm and 30mm), 400 Gy (20mm and 25mm), 450 Gy (35mm and 20mm), 500 Gy (20mm and 15mm), 550 Gy (15mm and 10mm) and 600 Gy (10mm and 5mm).

At 7th days of Control (140mm and 150mm), 75 Gy (120mm and 100mm), 100 Gy (110mm and 115mm), 150 Gy (110mm and 120mm), 200 Gy

(105mm and 80mm), 250 Gy (115mm and 85mm), 300 Gy (75mm and 70mm), 350 Gy (65mm and 45mm), 400 Gy (50mm and 40mm), 450 Gy (60mm and 30mm), 500 Gy (45mm and 25mm), 550 Gy (25mm and 15mm) and 600 Gy (15mm and 10mm).

At 9th days of Control (230mm and 290mm), 75 Gy (220mm and 280mm), 100 Gy (200mm and 210mm), 150 Gy (220mm and 180mm), 200 Gy (220mm and 200mm), 250 Gy (150mm and 260mm), 300 Gy (160mm and 120mm), 350 Gy (140mm and 110mm), 400 Gy (110mm and 100mm), 450 Gy (120mm and 50mm), 500 Gy (90mm and 35mm), 550 Gy (75mm and 25mm) and 600 Gy (50mm and 15mm).

Table 4: Plumule length for difference doses of gamma rays treated seed in Lathyrus at laboratory condition

Plumule length (mm)						
Doses	5th Day		7th Day		9th Day	
	Mahateora	Prateek	Mahateora	Prateek	Mahateora	Prateek
Control	70	100	140	150	230	290
75 Gy	115	95	120	100	220	280
100 Gy	80	65	110	150	200	210
150 Gy	65	75	110	120	220	180
200 Gy	90	65	105	80	220	200
250 Gy	45	40	115	85	150	260
300 Gy	50	45	75	70	160	120
350 Gy	50	30	65	45	140	110
400 Gy	20	25	50	40	110	100
450 Gy	35	20	60	30	120	50
500 Gy	20	15	45	25	90	35
550 Gy	15	10	25	15	75	25
600 Gy	10	5	15	10	50	15

The result of Plumule length demonstrated that increasing doses from Control–600 Gy had effect on Plumule length even though there were slight decrease in heights with increase in doses but the decrease was not proportional to the increase in dosage for both lathyrus varieties. Plumule length of both varieties had not growth at all doses compare to control. The increase Plumule length so seedling height increasing and Plumule length small so seedling height reduced.

This indicated that the varieties are differ in radio sensitivity with respect to seedling height and Plumule length as the dose of Gy 75 (115 mm), Gy 200 (90 mm) and Gy (80 mm) Plumule length increasing was in Mahateora varieties. Similar observation had been reported in Pigeonpea (Toker *et al.*, 2004), Cicerseeds (Toker *et al.*, 2005) and Lathyrus (Mahadavi *et al.*, 2007).

Fig.-4a

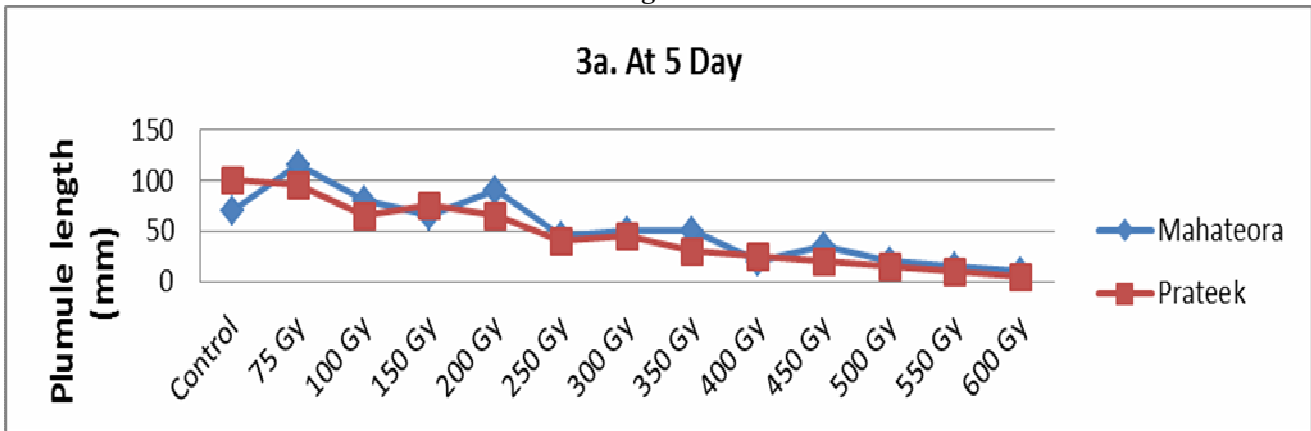


Fig.-4b

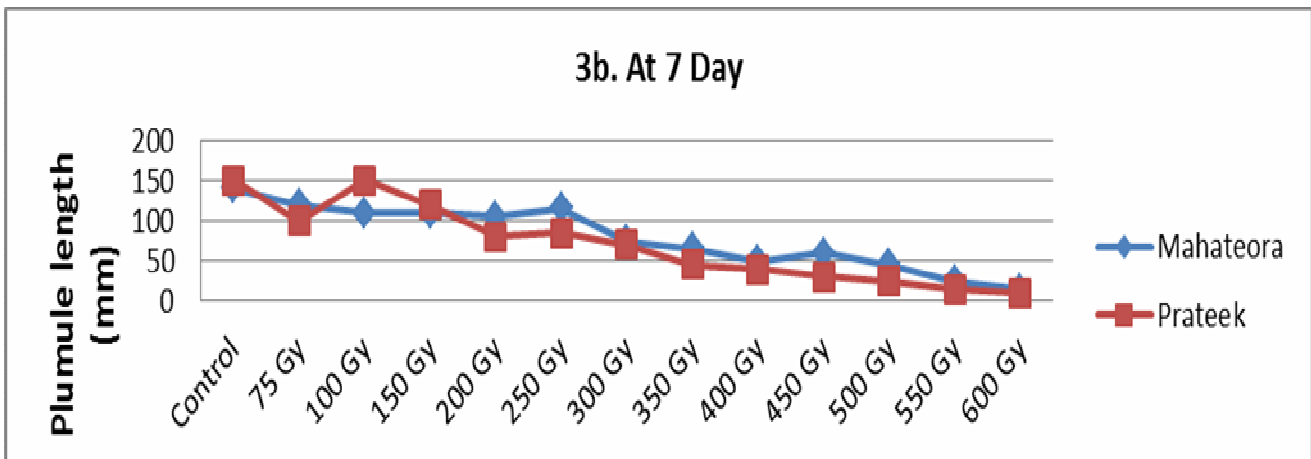


Fig.-4c

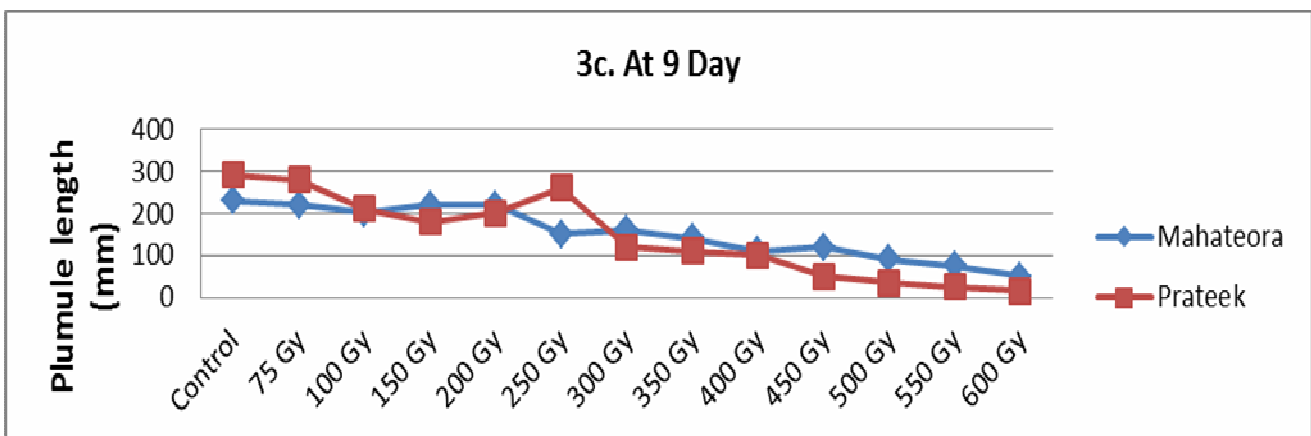


Fig. 4(a, b, c): Plumule length of M₁ generation for difference doses of gamma rays treated seed in lathyrus of laboratory condition.

Radicle length (mm)

Radicle length was recorded after 5th days, 7th days and 9th days of sowing in irradiated seeds of the varieties Mahateora and Prateek in M₁ (Table-5) and Figure-5 (a,b,c). At 5th days of Control (100mm and 150mm), 75 Gy (105mm and 135mm), 100 Gy (110mm and 125mm), 150 Gy (105mm and 115mm), 200 Gy (110mm and 85mm), 250 Gy (105mm and 90mm), 300 Gy (70mm and 65mm), 350 Gy (50mm and 70mm), 400 Gy (60mm and 55mm), 450 Gy (50mm and 50mm), 500 Gy (40mm and 40mm), 550 Gy (25mm and 35mm) and 600 Gy (15mm and 30mm).

At 7th days of Control (100mm and 200mm), 75 Gy (105mm and 145mm), 100 Gy (150mm and

160mm), 150 Gy (130mm and 140mm), 200 Gy (115mm and 150mm), 250 Gy (135mm and 115mm), 300 Gy (105mm and 80mm), 350 Gy (85mm and 80mm), 400 Gy (70mm and 70mm), 450 Gy (65mm and 65mm), 500 Gy (50mm and 50mm), 550 Gy (40mm and 40mm) and 600 Gy (30mm and 20mm).

At 9th days of Control (200mm and 220mm), 75 Gy (210mm and 150mm), 100 Gy (160mm and 250mm), 150 Gy (180mm and 200mm), 200 Gy (170mm and 270mm), 250 Gy (140mm and 240mm), 300 Gy (110mm and 70mm), 350 Gy (90mm and 160mm), 400 Gy (70mm and 140mm), 450 Gy (60mm and 120mm), 500 Gy (55mm and 100mm), 550 Gy (40mm and 80mm) and 600 Gy (30mm and 40mm).

Table 5 : Radicle length for difference doses of gamma rays treated seed in Lathyrus at laboratory condition

Radicle length (mm)						
Doses	5th Day		7th Day		9th Day	
	Mahateora	Prateek	Mahateora	Prateek	Mahateora	Prateek
Control	100	150	100	200	200	220
75 Gy	105	135	150	145	210	150
100 Gy	110	125	150	160	160	250
150 Gy	105	115	130	140	180	200
200 Gy	110	85	115	150	170	270
250 Gy	105	90	135	115	140	240
300 Gy	70	65	105	80	110	70
350 Gy	50	70	85	80	90	160
400 Gy	60	55	70	70	70	140
450 Gy	50	50	65	65	60	120
500 Gy	40	40	50	50	55	100
550 Gy	25	35	40	40	40	80
600 Gy	15	30	30	20	30	40

In this investigation, reduction in radicle length of seedling occurred with each corresponding increase in gamma radiation doses not in a linear fashion. Higher doses of gamma irradiation such as 550 Gy and 600 Gy showed drastic suppressing effect on radicle length in both lathyrus varieties. Radiation doses of 350 Gy and 400 Gy for Mahateora and Prateek varieties of lathyrus

which showed less than 50% growth reduction (GR50) in radicle length of seedlings at 5th day, 7th day and 9th day. Such a conclusion gets supported by (Shah *et al.*, 2008) mentioned that higher doses gamma irradiation causing 35-50% reduction in radicle length of seedlings under the laboratory conditions.

Fig.-5a

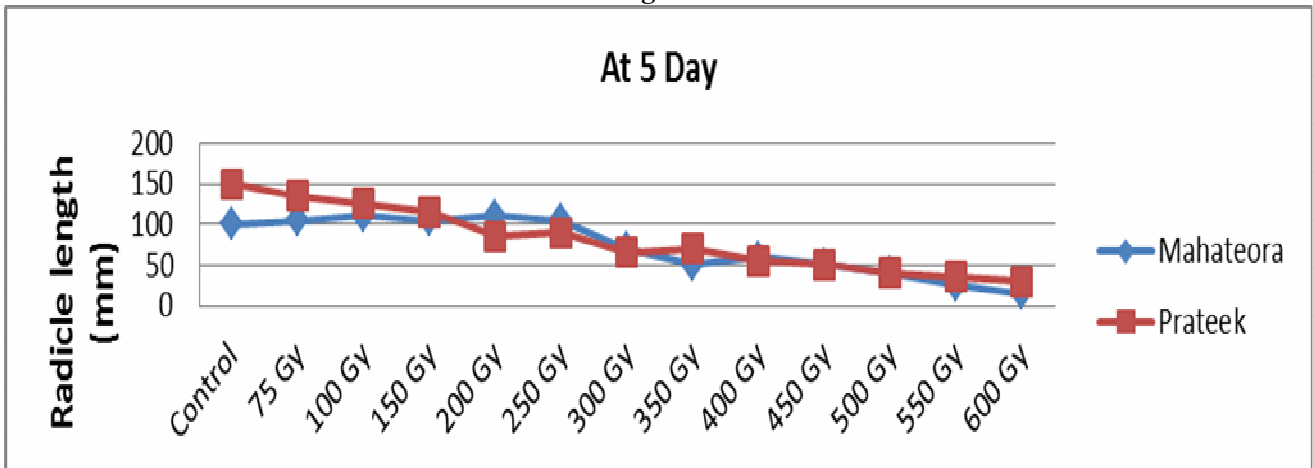


Fig.-5b

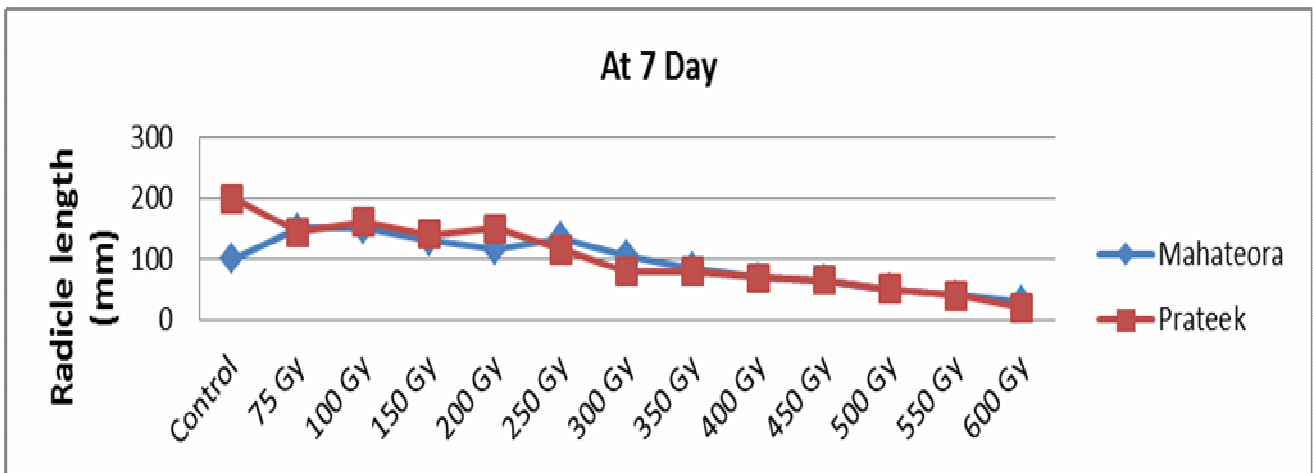


Fig.-5c

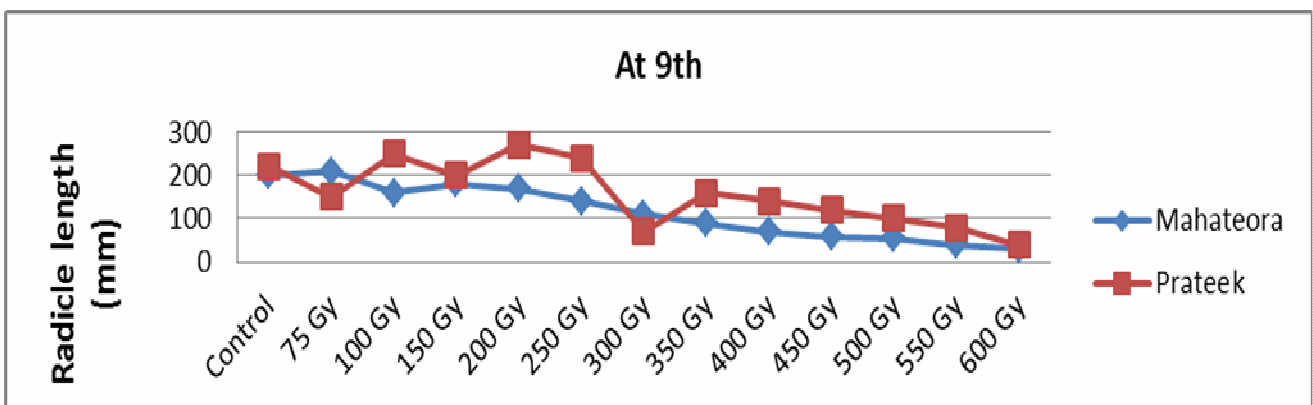


Figure-5 (a,b,c): Radicle length of M₁ generation for difference doses of gamma rays treated seed in lathyrus of laboratory condition.

Field condition:**Plant survival percentage:**

Plant survival percentage recorded at 30 days, 60 days and crop maturity of sowing in irradiation seed of both varieties Mahateora and Prateek in M_1 are presented in Fig.-6. At 30 of control (98.00% and 100%), 300 Gy (49.49% and 52.51%), 350 Gy (51.20% and 45.29%), 400 Gy (29.51% and 29.51%) and 75Gy + EMS (04.08% and 04.12%), At 60 days of control (97.00% and 96%), 300 Gy (41.09% and 45.46%), 350 Gy (44.60% and 44.60%), 400 Gy (28.49% and 26.77%) and 75Gy + EMS (02.88% and 02.72%) and At crop harvesting of control (92.00% and 95%), 300 Gy (39.23% and 40.44%), 350 Gy (24.34% and 37.00%), 400 Gy (22.26% and 20.31%) and 75Gy + EMS (00.92% and 00.76%) days Maximum plant survival percentage was found at 300

Gy in all most three stages are both varieties. There was significant difference in plant survival percentage at 350 Gy in radiation doses within each variety of GR 50. Both varieties all most similarly responded with increasing doses of irradiation. Similar results have been reported in *Vigna sesquipedalis* (Kon *et al.*, 2007) and chickpea (Khan and Wani, 2005) and (Toker *et al.*, 2005). The number of surviving plants decreased with increase radiation doses in an approximately linear fashion (Grif-2) in both varieties. Radiation dose with EMS proved most lethal dose are reduced the percentage of survived plants in lathyrus varieties Mahateora and Prateekin. Reduction in plant survival due to mutagenic treatments is a common phenomenon in induced mutation experiments (Toker and Cagigran, 2004), (Khan and Wani, 2005) and (Toker *et al.*, 2005).

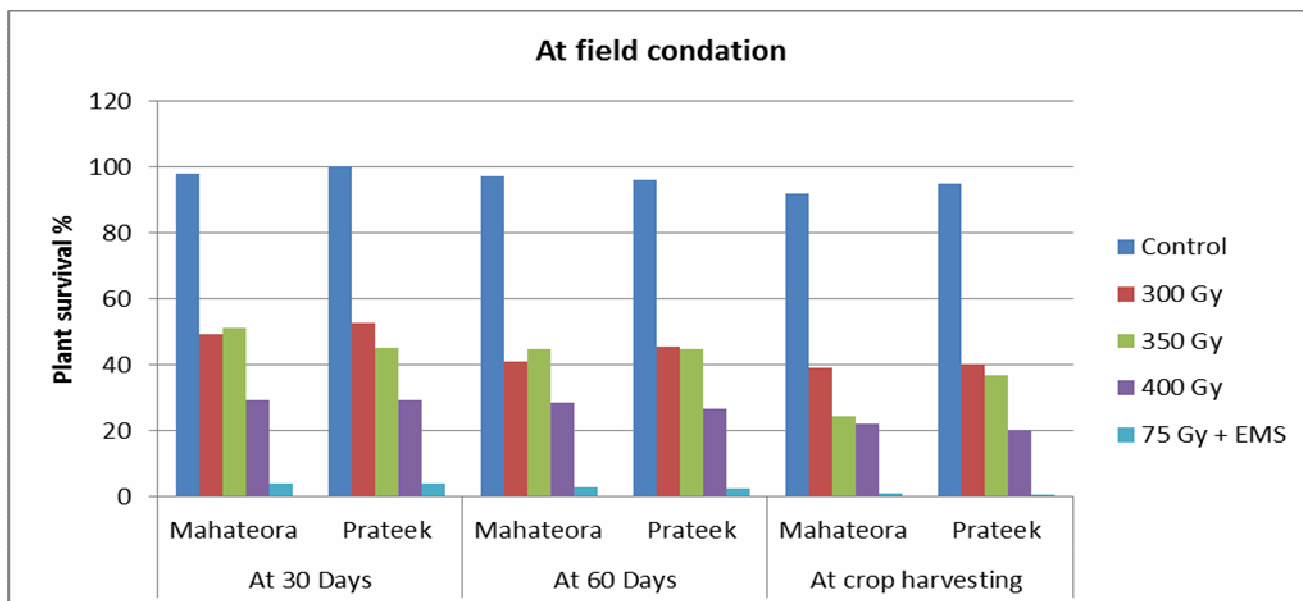


Fig. 6: Plant survival percentage of M_1 generation for difference doses of gamma rays and EMS treated seed in Lathyrus of field condition

Conclusion

From this investigation we concluded that gamma irradiation has a GR50 are positive effect on biological traits of lathyrus varieties. In contrast, gamma rays had some stimulatory effects at laboratory condition on germination%, seedling height, Plumule length and Radicle length and Field condition date are recorded on plant survival % in 30 days, 60 days and at the time of crop harvesting. The variety Mahateora and Prateekin have to be found more sensitive to gamma radiation. The results indicated that low radiation (100 Gy and 150 Gy) and medium radiation (300 Gy and 350 Gy)

doses have small biological effects on Lathyrus in both condition.

Acknowledgement

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