



Effects of gamma rays on germination and growth in *Jatropha curcas* L.

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Abstract: The present investigation was carried out at the Forestry Research Farm, Navsari Agricultural University, Navsari to evaluate the gamma rays at 10 kR, 20 kR and 30 kR induced variability in *Jatropha curcas* L. on germination, growth and yield for seven *Jatropha* genotypes (Phule J-1, Urlikanchan, Hansraj, SKN Big, Chhatrapati, Hansot and MPJ-55). The significantly maximum germination percentage (66.96%), seedling survival (74.18%), seedling collar diameter (0.958cm), shoot length (49.442cm), number of leaves per seedling (7.757) and leaf area (37.58) was observed in Chhatrapati genotype during nursery stage. While low rate of gamma rays treatment (10 kR) had stimulatory effect for germination percentage, seedling survival, seedling collar diameter, shoot length, number of leaves per seedling and leaf area. However, higher gamma rays doses (30 kR) drastically reduced all studied characters. The interaction effect of genotype and gamma rays were significant for number of leaves per seedling. Further, it was not significant in other traits like germination percentage, seedling survival, seedling collar diameter, shoot length and leaf area.

Keywords: Gamma rays, Genotype, *Jatropha curcas*, Morphological traits

INTRODUCTION

Increasing prices of fossil fuel and diminishing resources as well as its contribution in global warming have stimulated the interest toward alternate source of energy. The solar energy is the only source for harvesting that can contribute substantially to transportation fuel needs at costs competitive with fossil fuel; is captured by photosynthesis and stored in biomass (Sommerville, 2006). The fast-growing trees allocating large quantities of carbon belowground (Christian and Ryan, 2002) as well as accumulate more carbon in plant biomass (Chen *et al.*, 2015). These plants could be one of the best available alternative sustainable solutions to the fuel industry. *Jatropha curcas* L. belongs to Euphorbiaceae family has similar potential as fast growing bio-fuel crop in India. Meanwhile, currently available planting materials is lack of high yielding varieties or hybrids for oil content and yield; characterized by one or two flowering flushes with large variation for female flowers per inflorescence, capsules per plant, seeds per capsules, seed filling and oil content. Several reports suggest that very little variability of desired characters is available in natural populations of *J. curcas* (Divakara *et al.*, 2010, Nayak *et al.*, 2010 and Nayak *et al.*, 2012); hence, it is necessary to develop variable populations artificially. In the past, the major breakthrough in castor (Family: Euphorbiaceae) improvement was achieved through mutation breeding.

Using fast neutrons, Hybrid Castor-6, a variety (Aruna) was developed with reduced plant height and earliness with increased number of spikes per plant, increased yield as compared to the parent genotype (Kulkarni and Ankineedu, 1966). In Thailand *J. curcas* dried seeds from the Mukkadham (*Jatropha*) clone were treated with ⁶⁰CO gamma ray doses of 0 to 20 kR to identify the variables. In M₃ generation dwarf and/or early flowering mutants of *jatropha* were isolated (Sakaguchi and Somabi, 1987). Pandey and Datta, 1995 reported the cotyledonary variability in *J. curcas* L using gamma radiation. In African violets (*Saintpauia ionantha* H. Wendl.), gamma radiation at 15 Gy improved plant architecture of the mutants (Seneviratne and Wijesundra, 2007). Till 2000, the FAO/IAEA Mutant Varieties Database (MVD) had collected information on 2252 cultivars obtained by mutation and officially released in 59 countries worldwide including 59 mutants of oil crops. Of 1585 directly developed mutant varieties, the great majority (1411) were selected from mutated generations following the use of radiation, mainly gamma rays, as the mutagen (Maluszynski *et al.*, 2000). Hence, mutation breeding could be one of the best available options. Looking to the above prospects and future promises, present investigation was under taken to utilize the gamma ray induced variability as a potential tool to enhance the ontogenic variations through the passage of phylogenetic variation in the improvement programme.

MATERIALS AND METHODS

This investigation on the gamma rays induced variability in selected genotypes of *J. curcas* were conducted at Forestry Research Farm, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, Gujarat, India.

Location: The experiment was carried out at Green House Complex. Geographically, Navsari is situated at 20°95' North latitude and 70°90' East longitude at 10 meters above mean sea level. The experimental site is located 13 km away from Arabian seashore of the historical place Dandi, famous for Salt movement initiated by Father of India-The Mahatma Gandhi.

Climate and weather condition: Climatically, this region is typically tropical, characterized by fairly hot summer, moderately cold winter and more humid warm monsoon with heavy rainfall. The average annual rainfall is approximately 1355 mm. Monsoon commences mostly from the third week of June and retreats by the end of September. Most of the precipitation is received from South West monsoon, concentrating in the month of July to August. The winter season sets in usually towards the end of October with mild cold and lasts upto February. December and January are the coldest months of the season (10°-23°C). Summer commences from mid February and lasts upto mid June. April and May are the hottest months of the year.

Source of planting materials: Seven genotypes were taken for the present studies. These genotypes/accessions were collected from different research institutes/universities across the country. The accessions Hansraj, Urlikanchan, SKN Big and SDAUJ-1 (Chhatrapati) were collected from Shardar Krishinagar Dantiwada Agricultural University, Gujarat; Phule J-1 from Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra and MPJ-55 from Maharana Pratap University of Agriculture and Technology, Rajasthan. One accession named Hansot was included from Navsari Agricultural University, Gujarat in the present experimentation.

Treatment with gamma rays: The dry seeds of *Jatropha* were irradiated in Gamma Cell-200 (Cobalt-60 source emitting 3600 rads per minute) at Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Center, Trombay, Mumbai (Maharashtra), India. About 200g *jatropha* seeds of uniform size/average seed weight of each genotype in each treatment were irradiated with 10, 20 and 30 kR doses. Among them, one is control (untreated) genotype. So, there were total twenty eight treatment combinations for all seven genotypes.

Sowing of seeds in nursery: All treated seven genotypes/accessions including one control seeds were soaked in cold water for 24 hours before sowing in the 200 gauge size polythene bags filled with soil: sand: farmyard at the ratio of 1:1:1 respectively. One seed each was sown in each polythene bag. The seedlings were regularly watered with a roscan. The complete

emergence of shoot above the ground was treated as germination. The germination count was recorded from 7 DAS (Days After Sowing) up to 25 DAS on a daily basis. The emergence of shoot above ground level was considered as germinated and it was converted into percentage.

Germination percentage =

$$100 \times (\text{Number of seeds germinated} / \text{Number of seeds sown})$$

The seedlings were maintained till 150 DAS in the nursery for collecting different observations. Shoot length was measured from the collar region up to the apex of the leading shoot to the nearest mm at 150 DAS. The collar diameters of seedling from each treatment and replicate were measured at the collar region with the help of vernier caliper to the nearest mm. Average values was worked out for each treatment. Area of each leaf was calculated by using leaf area meter CI-202 (CID Bioscience Inc. USA) for 10 leaves and the average leaf area was multiplied with the total number of leaves per plant and thus total leaf area was calculated and expressed in cm². Similarly the number of leaves per seedling was counted from each treatment for all 3 replications at 150 DAS and the average number of leaves is expressed as number of leaves per seedling.

Statistical analysis: The data were statistically analyzed using Complete Randomized Design with Factorial Concept (FCRD) as suggested by Panse and Sukhatme (1967). The appropriate standard error of mean (S.E.m.) and the Critical Difference (C.D.) were calculated at 5% level of probability.

RESULTS AND DISCUSSION

The large wasteland areas can be utilized by planting of *Jatropha* into a productive land. Several properties of the plant stemming from its hardiness, rapid growth, easy propagation and wide ranging usefulness have resulted in spread of *Jatropha* for beyond its original distribution. However, the full potential underlying *Jatropha* is far from being realized. Mutation breeding would be a valuable supplementary approach in plant breeding under situation as is prevailing in oilseeds, specifically in Physic nut, where the variability existing in natural gene pool is not enough to meet the requirements of present genetic information (Nayak, *et al.*, 2010). The genetic diversity in the natural population of *J. curcas* appeared narrow, since no remarkable morphological and molecular differences have been observed (Mahroliya, 2006). Mutation in tree crops is considered attractive because of lacunae in conventional breeding like time consuming, unpredictable results, long juvenile phase, high heterozygosity and fear of loss of the unique genotype (Divakara *et al.*, 2010). Gamma radiation has been reported to have beneficial effects on many crops. In African violets (*Saintpaulia ionantha* H. Wendl.), gamma radiation at 15 Gy improved plant architecture of the mutants (Seneviratne and Wijesundra, 2007). The mutation on

major gene causes distinct changes whereas, minor genes causes quantitative variation which could be realized. The combination of both types of mutations in one individual may also occur. Considering the importance of macro and micro mutations, simultaneous utilization may prove to be better approach with breeding point of view where improvement work is at juvenile stage. Hence, to induce artificial variation, present exploration was done using gamma rays for different characters at M₁ generation among seven accessions (Hansraj, Phule J-1, Urlikanchan, SKN Big, SDAUJ-1 (Chhatrapati), Hansot, MPJ-55).

Effect of genotypes: The germination percentage was significantly influenced by genotypes. The Table 1 shows that the maximum germination percentage (66.96) was observed in genotype Chatrapati which was *at par* with Hansot (65.13), Hansraj (65.18), SKN Big (65.03), Phule J-1 (63.45) and MPJ 55 (64.26) genotype, while genotype Urlikanchan showed minimum germination percentage (59.61). The significantly maximum seedling collar diameter (0.958 cm) was recorded in Chatrapati genotype which was *at par* with MPJ 55 (0.936 cm) while minimum seedling collar diameter was recorded in Urlikanchan genotype (0.904 cm). Similarly, significantly maximum shoot length (49.442 cm) was recorded in Chhatrapati genotype which is *at par* with Phule J-1 (49.083 cm), Urlikanchan (48.292 cm), SKN Big (48.867 cm), Hansot (48.392 cm), MPJ 55 (48.950 cm). Also, significantly highest number of leaves per seedling (7.757) was recorded in Chhatrapati genotype. In the same way, leaf area was significantly affected due to effect of genotypes. Maximum leaf area (37.58 cm²) was recorded under genotype Chhatrapati. Significantly maximum seedling survival percentage (74.18) was observed under genotype Chhatrapati while minimum survival percentage (68.48) was observed under MPJ-55 genotype. From the above results, it revealed that significant genotypic variation was observed in seed germination and seedling growth parameters (survival percentage, seedling height, collar diameter, leaves/seedling, and leaf area). Horn and Shimelis (2013) found that the effects of different doses of gamma radiation depended also on genotypes. Dhakshanamoorthy *et al.* (2010) found seeds treated with 50 Gy of gamma rays revealed stimulatory effect except for germination, whereas 250 Gy dose showed inhibitory effects on traits compared to other treatments. This could be due to the damage in seed tissues and the severity of the damage depending on the dosage used. Ginwal *et al.*, (2004) found that variance registered in *J. curcas* for seed germination, seedling growth and biomass parameters showed considerable variation among different seed sources of *J. curcas*. The small value of error or environmental variances of the seedling growth traits suggested that majority of characters were under genetic control. It is suggested that the plant have strong genetic control which can be exploited for improvement of this species

(Ginwal *et al.*, 2005).

Effect of treatments : The effect of treatments on genotype is presented in table-1. The germination percentage was significantly affected by gamma radiation treatments. The results revealed that significantly maximum germination percentage was noted at 10 kR (74.21) over no radiation treatment (0 kR), indicating stimulatory effects of the lower doses (Nayak *et al.*, 2012). In other treatments germination percentage was adversely affected with the increase in the dosage of irradiation. The germination of the treated plants had shown a sharp dose rate relationship, which decreased with increase in the doses of gamma rays treatment. Percent reduction/stimulation in seed germination might have been due to the effect of mutagen on meristematic tissue of the seed (Dhakshanamoorthy *et al.*, 2010; Dhakshanamoorthy *et al.*, 2011). Wang *et al.* (2010) reported that low-dose laser irradiation induced low-frequency but significant alterations in DNA methylation level and pattern in sorghum plants. The decrease in seed germination at higher doses of the mutagen may be attributed to disturbances at cellular level caused either at physiological (or) physical level (Dwimahyani and Ishak, 2004; Pandey and Datta, 1995 and Lehtiniemi, 1977). Higher doses of gamma radiation (> 200 Gy) resulted in the significant reductions in germination percentage in *J. curcas* L. (Nayak *et al.*, 2012; Songsri *et al.* 2011). Also, high germination percentages (>90%) were observed in Broccoli seeds irradiated at < 4 kGy, but the sprout length decreased with increased irradiation dose (Waje *et al.*, 2009). However, in the present investigation lower exposure is induced seed germination. In *Leucaena leucocephala* exposed to a dose of 2 kR gamma radiation increased the germination frequency in comparison to control. This may attributed to the origin of these stimulations are acceleration in cell division rates (Zaka *et al.*, 2004) as well as activation of auxin (Gunckel and Sparrow, 1991).

Significantly highest seedling survival (100%) was recorded in no irradiation (0 kR) while lowest seedling survival (50.34%) was recorded in 30 kR treatment. The survival of *J. curcas* seedlings decreased with increasing doses of gamma rays and usually shows inhibitory effect of higher dose of gamma rays on seeds of angiosperms and gymnosperms (Akhaury and Singh, 1993; Thapa, 1999). Similar findings were reported by Ignacimuthu and Babu (1988) in *Vigna mungo* where plant survival decreased with increasing doses of gamma rays and EMS.

Gamma rays imposed significant impact on seedling height. The highest seedling length (59.74 cm) was recorded under 10 kR which is *at par* with no irradiation (58.41 cm). It was noticed that the growth of irradiated plants were strikingly vigorous than that of control (Bandyopadhyaya *et al.*, 1987). However, higher doses have inhibitory effect of mutagens on the length of seedling was evident from the decrease in length of root and shoot with increasing dose / concentration of

gamma rays and EMS (Dhakshanamoorthy *et al.*, 2010). Induced-dwarfism was noticed in the nursery stage when the seeds of *Anacardium occidentale* Cv. Madakkathata-1 were treated with gamma rays at 10, 20, 30 and 40 kR doses using Cobalt-60 source (Mareen *et al.*, 2008). Significant decreased in shoot length in *Albizia lebbek* was also recorded due to induced mutation by Hanumatha *et al.*, (2002). Significant decrease in shoot length in *A. lebbek* was also recorded due to induced mutation by Hanumatha *et al.*, (2002).

The effect of gamma rays on seedling collar diameter was found to be significant. The significantly maximum seedling collar diameter (0.992 cm) was recorded over control while higher doses of gamma rays reduces seedling collar diameter. Nayak *et al.* (2012) reported that seedling collar diameter significantly decreased with the increased rate of gamma rays. It shows that higher exposures of gamma rays caused injury to seeds and affected seedling development. Higher doses adversely affected the seedling height and shoot length which was inversely proportional to the radiation intensity.

The gamma irradiation has significant effect on number of leaves/seedling as well as on leaf area between treatments (Table 1). The treatment 10 kR enhanced number of leaves per seedling (7.647), which was followed by no irradiation treatment (6.701). Significantly maximum reduction in number of leaves were noted at highest gamma rays treatment *i.e.* 30 kR (5.428). It suggests that low rate of gamma rays has stimulatory effects for number of leaves per seedling. But, increase in radiation intensity beyond 20 kR, adversely affected number of leaves per seedling in *J. curcas* (Dhakshanamoorthy *et al.*, 2011). The increase in number of leaves might have direct contributory effects on collar diameter of seedling and origin of these stimulations, are acceleration in cell

division rates (Zaka *et al.*, 2004) as well as activation of auxin (Gunckel and Sparrow, 1991).

Leaf area is a desirable morpho-economic trait for any fast growing crop which influences on total photosynthetic space. There were significantly decreased leaf area (28.95 cm²) observed at higher level at 30 kR treatment, however low doses at 10 kR increases leaf area (42.28cm²). The present findings are supported by Rafiullah and Hasan (1994) who reported gradual decrease in leaf area in *Brassica* species with the increase intensities of radiation. Generally, 10 kR dose produced a stimulatory effect in most of the characters while 25 kR decreased in brassica. Ramesh *et al.*, (2012) reported that significantly decreased in leaf area of mulberry when treated at 10 kR using gamma rays where as low doses (1 kR) showed slightly bigger leaf area as compared to control. Similar results on growth parameters have been reported in *A. lebbek* by Singh and Paliwal (1987). Gamma rays irradiation with the dose of 10 Gy found desirable genetic variability on plant development of *J. curcas* (Dwimahyani and Ishak, 2004). Pandey and Datta (1995) reported that higher doses of gamma rays have strong variation on size, shape and stomata number in *J. curcas*. In soybean plant leaves developed from gamma irradiated seeds showed reductions in stomatal density and stomatal number compared to the control plants (Celik *et al.*, 2014). However, in *Embllica officinalis* grafts; the leaf area showed inconsistent relationship between the dosage of gamma rays and genetic variability which indicates a better scope for success in further improvement of desirable traits in the existing commercial variety of the crop (Selvi *et al.* 2007).

Effect of interaction: The interaction of genotypes and gamma rays treatments were found non-significant in influencing on germination percentage, shoot length,

Table 1. Effect of gamma rays on germination and seedling survival percentage of *J. curcas* .

Treatments	Germination percentage	Survival percentage	Seedling collar diameter (cm)	Shoot length (cm)	Number of leaves/seedling	Leaf area (cm ²)
Genotypes (G)						
G1: Phule J-1	63.45	71.74	0.913	49.083	6.047	36.85
G2: Urlikanchan	59.61	71.43	0.904	48.292	6.500	36.68
G3: Hansraj	65.18	71.24	0.930	45.467	6.107	36.40
G4: SKN Big	65.03	71.72	0.927	48.867	6.292	35.25
G5: Chhatrapati	66.96	74.18	0.958	49.442	7.757	37.58
G6: Hansot	65.13	73.61	0.934	48.392	6.747	35.51
G7: MPJ 55	64.26	68.48	0.936	48.950	6.278	35.45
S. Em. +	1.21	0.99	0.008	0.694	0.100	0.15
C. D. at 5 %	3.51	2.80	0.023	2.010	0.289	0.433
Irradiation (I)						
I0: No irradiation	71.78	100.00	0.992	58.41	6.701	40.98
I1: 10 kR	74.21	74.49	0.947	59.74	7.647	42.28
I2: 20 kR	61.07	62.26	0.914	44.97	6.355	32.76
I3: 30 kR	49.87	50.34	0.861	30.30	5.426	28.95
S. Em. +	00.90	00.75	0.006	0.525	0.075	0.085
C. D. at 5 %	02.60	2.12	0.018	1.520	0.218	0.247
Interaction (G x I)						
S. Em. +	2.37	0.75	0.016	0.439	0.199	0.60
C. D. at 5 %	NS	NS	NS	NS	0.578	NS
C. V. %	6.39	4.77	3.009	4.97	5.287	4.94

Table 2. Interaction effect of gamma rays on number of leaves per seedling in *J. curcas* .

Interaction effects (Genotype X Irradiation)	Number of leaves/seedling				
	I ₀ : 0 kR	I ₁ : 10 kR	I ₂ : 20 kR	I ₃ : 30 kR	Mean
G1: Phule J -1	5.863	7.623	5.580	5.120	6.047
G2: Urlikanchan	6.743	7.753	6.393	5.110	6.500
G3: Hansraj	6.367	7.093	5.847	5.120	6.107
G4: SKN Big	5.880	7.933	6.240	5.113	6.292
G5: Chhatrapati	8.070	8.343	7.797	6.817	7.757
G6: Hansot	7.353	7.827	6.123	5.684	6.747
G7: MPJ 55	6.633	6.957	6.503	5.017	6.278
Mean	6.701	7.647	6.355	5.426	
Source	S. Em. ±	C.D. at 5 %		C.V. %	
G	0.100	0.289		5.287	
I	0.075	0.218			
G x I	0.199	0.578			

G: Genotype, I: Irradiation

seedling collar diameter and leaf area (Table 2). However, significant variations of number of leaves due to variety and different treatments with their interactions were noticed. Chatrapati accession significantly enhanced mean number of leaves (8.343) was noted under 10 kR gamma rays treatment, which was followed by 0 kR (8.24). Minimum number of leaves per seedling (5.017) was recorded in 30 kR treatment under MPJ 55 genotype. The low rate of gamma rays has stimulatory effects for number of leaves per seedling. The biological effect of gamma-rays is based on the interaction with atoms or molecules in the cell. The low radiation dose used could have produced its long-term effects in part by means of the stimulation of lipid degradation, possibly mediated through the action of free radicals that are known to be generated after irradiation (Katsaras *et al.*, 1986; Voisine *et al.*, 1991). The stimulating effects of gamma ray on germination may be attributed to the activation of RNA synthesis (Kuzin *et al.*, 1975) on coater bean, or protein synthesis (Kuzin *et al.*, 1976) which occurred during the early stage of germination after seeds irradiated with 4 K-rad. Hence, these interaction factors may be responsible for the contribution of total number of leaves in the seedlings.

Conclusion

The significantly maximum germination percentage, seedling survival, seedling collar diameter, shoots length, number of leaves per seedling and leaf area was observed in Chhatrapati genotype during nursery stage. The low rate of gamma rays treatment (10 kR) has stimulatory effect; however higher gamma rays doses drastically reduced seedling germination, seedling survival, seedling collar diameter, shoot length, number of leaves per seedling and leaf area. The interaction effect of genotype and gamma rays were significant for number of leaves per seedling. While, it was not significant in other traits like germination percentage, seedling survival, seedling collar diameter, shoot length and leaf area. The present study on

induced variability of *J. curcas* by gamma rays with respect to germination percentage, seedling survival, seedling collar diameter, shoots length, number of leaves per seedling, leaf area were maximum in Chhatrapati genotype during the germination and seedling growth stage. The application of 10 kR gamma rays radiation had stimulatory effect which may be best for producing wide desired variant in *Jatropha* crop improvement.

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