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Determination of effective dose of gamma irradiation for lentil seed mutagenesis

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Abstract
Mutation breeding is key method for generating huge number of variation. In less diverse crops lentil has potential for generating variation. Effective dose need to standardize for inducing variation. Gamma irradiation using standard LD_{50} dose will generate sufficient variation undergone several mutation. This study identifies the actual effective LD_{50} dose by evaluating several dosage of irradiation and considering morphological effects of physical irradiation. Result of this study revealed that 300Gy is the effective LD_{50} for giving irradiation to lentil seeds. Effective dose for irradiation will help to develop desirable mutation in succeeding generation after irradiation treatment.

Keywords: ld50, morphological effects, effective dose, irradiation

Introduction
Lentil is an autogamous diploid cool season annual food legume crop with a haploid genome size of an estimated 4063 Million base pair (Arumuganathan & Earle, 1991) [8] cultivated globally. Though flowers are self-pollinated, a 0.8% cross pollination by thrips or small insects but not by wind or honey bee has been reported. It is one of the important and most nutritious among *Rabi* pulses. They are moderately resistant to drought because of its tap root system which usually grows to a depth of 15 inches (Ansari and Sharma, 2015) [9]. Originating from Mediterranean region or Middle East, variation of lentil germplasm is not so diverse, overall population consists of very less number of sub population. Inducing variation through different methods like crossing between diverse population, physical irradiation, and chemical mutagenesis can be exploited whereas probability of getting extreme variants beyond parental class is very less in conventional cross breeding programme. Mutation breeding programme is beneficial for lentil crop for generating desirable mutant with yield advantage and other traits (Singh *et al*., 2000) [1]. Various physical mutagens have been used since long year ago for various crops as in rice for generation of variation for different traits (Singh *et al.*, 1998) [3]. Gamma ray is most effective electromagnetic radiation now a days for crop improvement (Im *et al*., 2018) [7]. For different crops lethal dose has been standardized like in Rice 229 Gy has been fixed from laboratory method and 235 Gy from probit analysis (Rajarajan *et al*., 2016) [1]. Standardization of most effective mutagenic dose and efficient mutagens is very essential to attain good amount of variation for gaining desirable mutation (Solanki *et al.,* 1994) [2] Improvement in different lentil variety through mutation breeding programme has been done as in variety DPL 62, Pant L 406 (Laskar *et al*., 2017) but stable line development with desirable traits need more time.

Materials and Methods
Dry and cleaned seed of WBL-77 (Motri), a popular variety of eastern India, released from PORS, Beharampur, and West Bengal with moisture percent (10-12%) have been collected from ICARDA-BCKV legume research development programme from Dept. of Agronomy. Seeds were treated with gamm ray with 100Gy, 200Gy, 300Gy, 400Gy, 500Gy doses @6.761 KRad/hour by GC-5000 system with Cobalt-60, sources at Regional Nuclear Agriculture Research Centre (RNARC) of BARC-BRNS at BCKV, Mohanpur campus. For each doses irradiation was applied separately on 50 seeds with four replications. Immadiate after irradiation seeds were kept for germination on petriplates inside the net chamber facility of Department of Genetics and Plant Breeding. Sufficient air, light was confirmed during

germination of irradiated seeds. Non-irradiated seeds with same amount and replication were also taken as control treatment for comparison. Days to germination for different dosage of irradiation including control seed was noted till 14 days from zero days of germination as similar with one study in rice (Rajarajan *et al*., 2016) [1]. For calculation of LD_{50}, shoot length, shoot weight, root length, root weight was measured with sufficient replication.

Results and Discussion

Table 1: Effect of irradiation on germination of lentil seeds

	No. of avg. seed germinated			
	After 5days	***SD***	**After 10 days**	***SD***
Control	16.3	0.6	34.0	4.6
100Gy	14.7	1.2	27.0	2.6
200Gy	16	3.5	32.0	1.0
300Gy	20.7	1.2	34.3	3.2
400Gy	16.3	1.2	36.3	1.5
500Gy	17.3	0.6	34.7	0.6

Optimal dose for mutagenesis is LD_{50} where growth of the plant material will be reduced to half and will lead maximum mutation in the favorable direction, dosage beyond the LD_{50} will lead to higher chances of getting mutation (Rajarajan *et al*., 2016) [1] and highest amount of mutation with very minimum damage can be generated only by applying the dose as LD_{50} (Suresh *et al*., 2017) [10]. Germination was recorded after 5 and 10 days from irradiation, it revealed that in both the cases germination trends were similar to control and no unique trends was seen with accordance to irradiation dose. Germination was consistent in control condition. It is showing 300Gy where most number of seed germinated. But this observation regarding germination is genotype and environment dependent. Different seed size and varietal difference will also influence the germination phenomenon. In other variety like DPL 62 and Pant L 406, effect of radiation on seed germination is steady (Laskar *et al*., 2017) [4] and revealed LD50 as 400Gy for lentil. Seed germination initiated first in irradiated seeds in all the treatments than control, but in another study by Laskar *et al*., 2017 [5] found contrasting results.

Table 2: Mean and standard deviation of traits under different dosage of irradiation

	Shoot length (cm.)		**Root length (cm.)**	
	Mean	*SD*	*Mean*	*SD*
Control	13.53	0.981	6.561	3.28
100Gy	11.34	2.604	4.81	1.86
200Gy	12.7	2.901	6	1.25
300Gy	7.29	1.316	4.03	0.80
400Gy	2.57	1.182	2.26	0.86
500Gy	0.79	0.074	0.745	0.16

Table 3: Mean and standard deviation of traits under different dosage of irradiation

	Shoot weight (mg.)		**Root weight(mg.)**	
	Mean	SD	Mean	SD
Control	58.8	0.010	46.07	3.906
100Gy	55.55	0.013	36.6	6.755
200Gy	45.47	0.176	19.47	0.007
300Gy	36.88	0.009	15.18	0.002
400Gy	16.17	0.007	6.8	0.002
500Gy	6.84	0.001	4.98	0.002

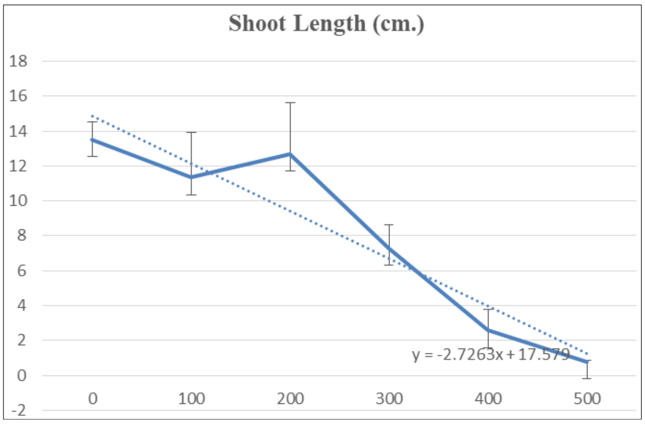

Fig 1: Shoot length variation with different gamma irradiation dosage

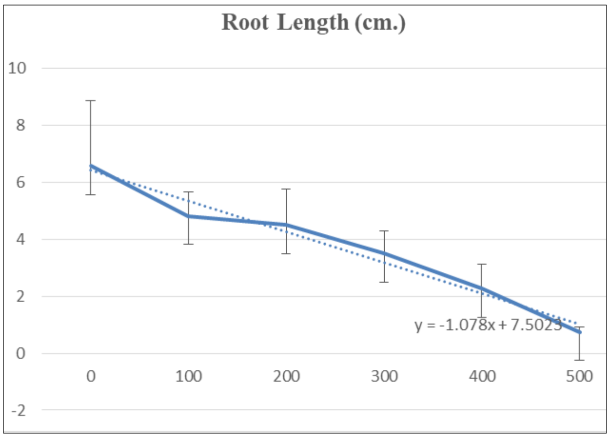

Fig 2: Root length variation with different gamma irradiation dosage

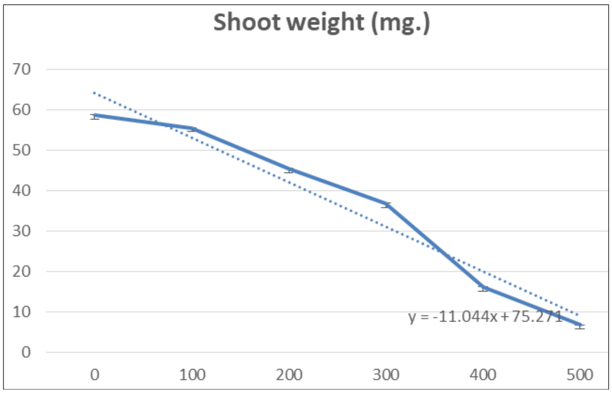

Fig 3: Shoot weight variation with different gamma irradiation dosage

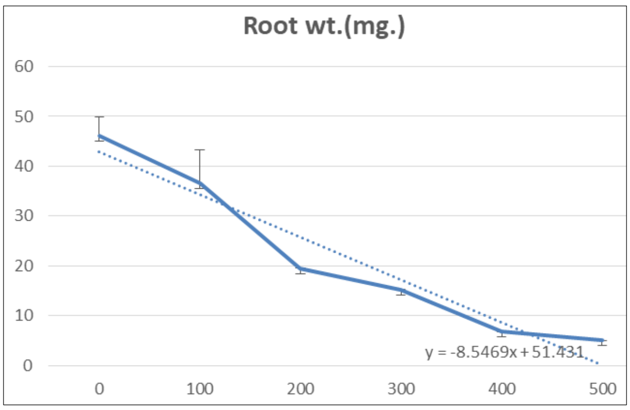

Fig 4: Root weight variation with different gamma irradiation dosage

From the above figure LD50 has been derived as effective dose when the shoot or root length reduced to 50% compared to control. For shoot length control value i.e. supposed to 0 Gy it is near 14cm, and 50% value i.e.7cm is under 280Gy. For root length 6.5 cm control value falls to 50% value of around 3.25 at around 280Gy. Shoot weight varies near 58 mg for control condition, where this value falls to 50% at near 300Gy. Root weight varies near 48mg for control where around 250 Gy is the LD50 for the root weight. From these entire phenotypic trait it is showing 280Gy as LD50 as effective dose. Effective dose has been standardized as 30 kR with combined chemical agents like 0.3% EMS (Ahirwar *et al*., 2014) [6] for lentil.

Conclusion

From these study it can be stated that for small seeded lentil variety can be gamma irradiated for inducing mutagenesis @280Gy as LD_{50} dose as effective mutagenic dose for lentil seed. For dissecting effect on germination detailed study need to be undertaken as it is environment and genotype dependent event.

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