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Determination of LD_{50} Value of *Vigna radiata* (L.) Wilczek Cultivar- Naval

Amol Chandrakant Vikhe[1,*], Janardhan Namdeo Nehul[2]

[1]Department of Botany, New Arts, University of Pune, India
[2]Department of Botany, University of Pune, India

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Abstract *Vigna radiata* (L.) Wilczek is an important legume crop in India for Food as well as Fodder Purpose, enriched with good Proteins, Carbohydrates, Vitamins and Minerals. The demand of *Vigna radiata* is high in market today, so there is a need to enhance the productivity and area under cultivation. The Mutation Plant Breeding is the most popular, widely used and globally accepted method to improve the cultivars for high yield, early maturity, drought, salinity, heat, insect, disease tolerant/resistant, high nutritional and low antinutritional factors. Mutagens like; Ethyl Methane Sulphonate, Sodium Azide, Gamma Irradiation are used in Mutation Breeding to create variability. The present study has been carried out on the *Vigna radiata*, Cultivar-Naval. The first stage in the research was the determination of LD_{50} Value of the Cultivar Naval. It was carried out with different concentrations and time of intervals with mutagens like; EMS, SA and GR with including control as nil (without treatment). The parameters were studied in accordance with the International Seed Testing Association (ISTA) rules. The results showed variation in the studied parameters as compared to control.

Keywords *Vigna Radiata*, EMS, SA, Gamma Radiation, LD_{50} Value

1. Introduction

Vigna radiata (L.) Wilczek (2n=22), is the member of Leguminosae family, cultivated throughout the world including India, Pakistan, China, Nepal, Bangladesh, Myanmar, Malaysia, Thailand, Indonesia and Srilanka. Recently its cultivation has been started in USA and Africa. India is the largest producer in the world to cultivate Mungbean. Day by day, the area under Mungbean Cultivation is increasing in the world. *Vigna radiata* is cultivated throughout the year, i.e. Kharif, Rabi and Summer season.

During 2017-2018, the total coverage under mungbean was about 41 Lha with a production of 19 Lt. There has been phenomenal increase in area of mungbean in the country from 2015-2016 onwards. Rajasthan with more than 42 percent area and 39 percent of production in the total mungbean contribution in the country. According to report, more than 80 percent of mungbean production comes from 10 states of Rajasthan, Madhya Pradesh, Maharashtra, Bihar, Karnataka, Tamilnadu, Gujarat, Andhra Pradesh, Odisha and Telangana *(DES, Ministry of Agri. &FW (DAC&FW), Govt. of India; 2017-18-IIIrd Adv. Est.*

Genetic improvement through Mutation Breeding has broad scope in agriculture sector to develop new varieties which are resistant/ tolerant to disease, insect, drought, salinity, heat, pest with high nutritional and low antinutritional factors. EMS, SA and GR mutagens create great variability in plant materials as compared to other mutagens. Therefore, we have selected these mutagens for this study. The cultivar Naval is commercially and widely cultivated in Maharashtra. It is not suitable in summer season, susceptible to drought and salinity.

The Gamma rays has been widely used for the improvement of various traits of crops (Khatri *et al.*, 2005; Tah, 2006; Songsri *et al.*, 2011; Aney, 2013). The Sodium azide concentrations affect the biological parameters (G. Roopa Lavanya *et al.*, 2011). Chlorophyll mutations can be induced by Ethyl Methane Sulphonate (Bhal and Gupta 1982).

The effect of Mutation rates depends on the dose of that chemical concentration and time for soaking which is applied on seed material. The minimum lethal dose is 50 percent; i.e. out of 100 seeds 50 percent of seeds should be germinated and mortality is 50 percent. Hence we must to know the LD_{50} value of that species. A dose that causes 50% mortality and 50% of seeds can be survived.

The LD50 value is different between species and varieties in a species. For example, the LD_{50} of K-851 Mungbean cultivar is 54.06kr and for Sona is 53.20 (Tah, 2006) for Gamma Irradiation. Therefore, this study was carried out to determine the LD_{50} of *Vigna radiata* Cultivar-Naval. In M2 Generation mutation frequency of Chlorophyll mutants and Leaf morphological changes should be observed. Quantitative traits like; Plant Height, Number of pods per plant, Pod Length, Seeds per pod should be observed. The transmitted characters in M3 Generation should be confirmed for Morphological and Biochemical Characterization.

2. Materials and Methods

Seeds selected for the present study of *Vigna radiata* (L.) Wilczek, commonly known as Mungbean were procured from Nirmal Seeds Pvt. Ltd. Pachora, Jalgaon. The cultivar is determinate in habit and not suitable for cultivation in summer season and widely cultivated in Maharashtra. Naval cultivar has distinguishing characters like medium time of flowering, dark green leaf colour, straight curvature of mature pod, long pod length, large seed size and number of seed/ pod is 12-14.

Ethyl Methane Sulphonate and Sodium Azide- Source of Chemicals EMS and SA (Sigma- Aldrich) were availed from Research Center Department of Botany, New Arts, Commerce and Science College Ahmednagar. The doses employed were 5mM, 10mM, 15mM, 20mM and 25mM of EMS, 1mM, 2mM, 3mM, 4mM and 5mM of SA. Gamma Irradiated seeds were treated with (Co^{60})150GY, 250GY, 350GY, 450GY and 550GY from BARC Mumbai. Seeds without treatment were used as control. The experiment was conducted in the year 2018-2019 at Department of Botany, New Arts, Commerce and Science College Ahmednagar.

3. Experimental Setup

The seeds were pre-soaked in Distilled water for 2 hours. The pre-soaked seeds were dried and deepened in the prepared concentrations of EMS (5mM, 10mM, 15mM, 20mM and 25mM(2) and SA (1mM, 2mM, 3mM, 4mM and 5Mm) for 6 hours(4), Gamma Irradiated seeds with 150GY, 250GY, 350GY, 450GY and 550GY(2) seeds as a dry. Untreated seeds were kept as a control. After treatment period seeds were removed and washed 3 times with running distilled water and dried. The 200 seeds in each concentration including control were kept for germination in between paper method and paper with seeds were rolled with plastic paper covering sheet to maintain moisture content. The rolls were placed in an upright position and kept in germination chamber at 25± 1°C temperature with relative humidity 80±1%. The germination percentage was recorded on 5^{th} day after treatment period.

The parameters were calculated as per ISTA rules. The final percentage of seed germination and parameters were calculated using formula.

$$\textbf{Percent Seed Germination} = \frac{\text{Number of Seeds Germinated (Normal Seedlings)}}{\text{Total Number of Seeds Kept for Germination}} \times 100$$

4. Result and Discussion

The experiment on *Vigna radiata* Cultivar-Naval, to determine the LD_{50} Value of EMS, SA and GR treated seeds was carried out. The doses were prepared and seeds were treated with given concentrations. The EMS, SA and GR showed the significant effect on the seed germination. The categories of seedlings fall into normal seedlings, abnormal seedlings, fresh Ungerminated seeds, hard seeds and dead seeds. The control showed highest seed germination 83% normal seedlings (Table-1., Fig.1). Less number of abnormal seedlings 11% (Table-1., Fig.2), were observed in control as compare to all other treatments. EMS-5mM dose Showed 76% normal seedlings which is highest in the lower dose as compare to other treatments 70, 64, 54, 46% in 10mM, 15mM, 20mM and 25mM (Table-1., Fig.1) respectively which is in decreasing order, similar results were obtained by Giri S. P. (2014). 46% normal seedlings were observed in 25mM dose which is lethal i.e. the dose at which minimum 46% of seeds with normal seedlings. The Abnormal seedlings were observed high in 25mM dose is 34% (Table-1., Fig.2). The SA treatment with 1mM to 5mM showed normal seedlings 75, 70, 65, 58 and 49%(Table-1., Fig.1) respectively. Same results were obtained by Lavanya R. G et *al*. (2011). The abnormal seedlings were observed more in 5mM dose is 34% (Table-1., Fig.2). The same results were obtained in Gamma Irradiated seeds, according to increasing order of treatment decreased the normal seedlings and increased abnormal seedlings. The dose 150GY, 250GY, 350GY, 450GY and 550GY (Table-1., Fig.1) showed normal seedlings 73, 66, 61, 55, and 49% respectively; the similar results were obtained by A. G Rukesh et.*al.* (2017). The highest abnormal seedlings were observed in dose 550GY is 26% (Table-1., Fig.2). All the treatments showed the minimum number of normal seedlings and high number of abnormal seedlings as compare to control.

Table1 1. Determination of LD50 Value of *Vigna radiata* (L.) Wilczek Cultivar- Naval

Determination of LD_{50} Value of *Vigna radiata* (L.) Wilczek Cultivar- Naval							
Sr. No	**Treatment**	**Conc.**	**Normal Seedlings**	**Abnormal Seedlings**	**Fresh Ungerminated Seeds**	**Hard Seeds**	**Dead Seeds**
1	**Control**	Nil	83	11	5	0	1
2	**Ethyl Methane Sulphonate**	5mM	76	19	1	0	4
		10mM	70	21	3	0	6
		15mM	64	25	3	0	8
		20mM	54	32	4	1	9
		25mM	46	34	5	0	15
3	**Sodium Azide**	1mM	75	16	6	0	3
		2mM	70	18	6	0	6
		3mM	65	21	4	0	10
		4mM	58	27	2	0	13
		5mM	49	34	1	0	16
4	**Gamma Radiation**	150GY	73	16	10	1	0
		250GY	66	18	14	1	1
		350GY	61	18	16	3	2
		450GY	55	23	19	0	3
		550GY	49	26	20	2	3
		***SD*±**	***10.887***	***6.772***	***6.356***	***0.894***	***5.106***
		***SE*±**	***2.72***	***1.69***	***1.59***	***0.22***	***1.28***

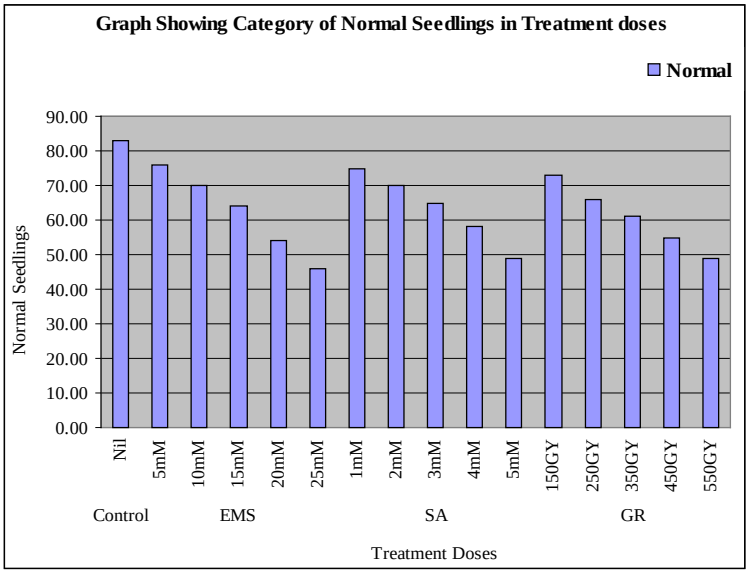


Figure 1. Normal Seedlings

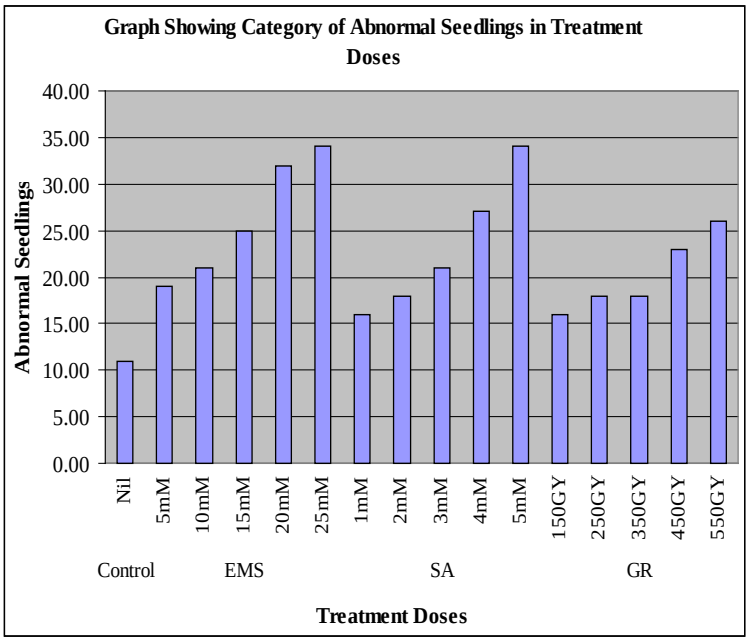


Figure 2. Abnormal Seedlings

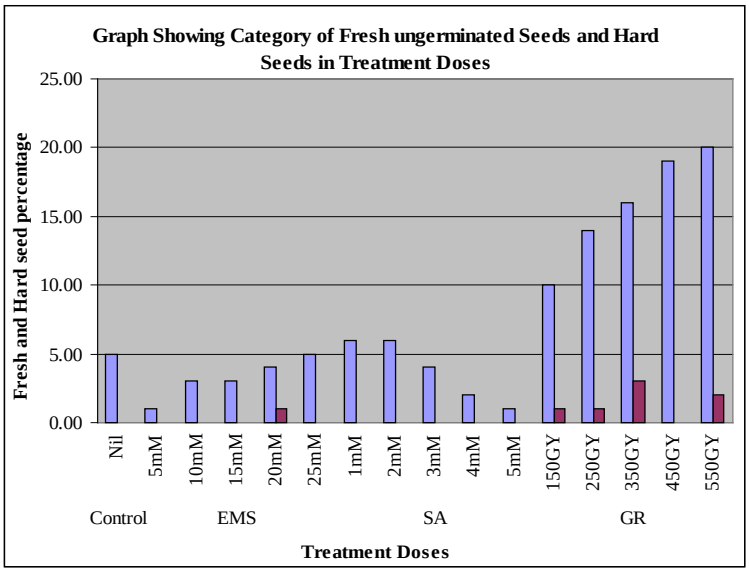


Figure 3. Fresh Ungerminated and Hard seeds

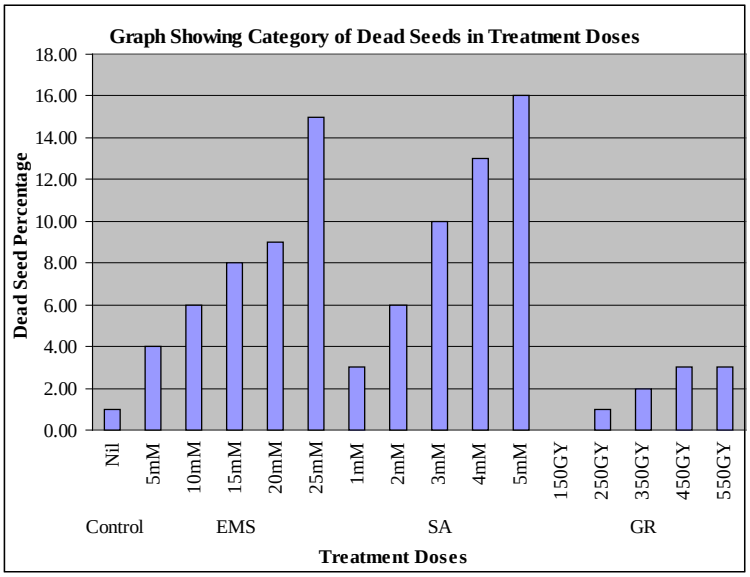
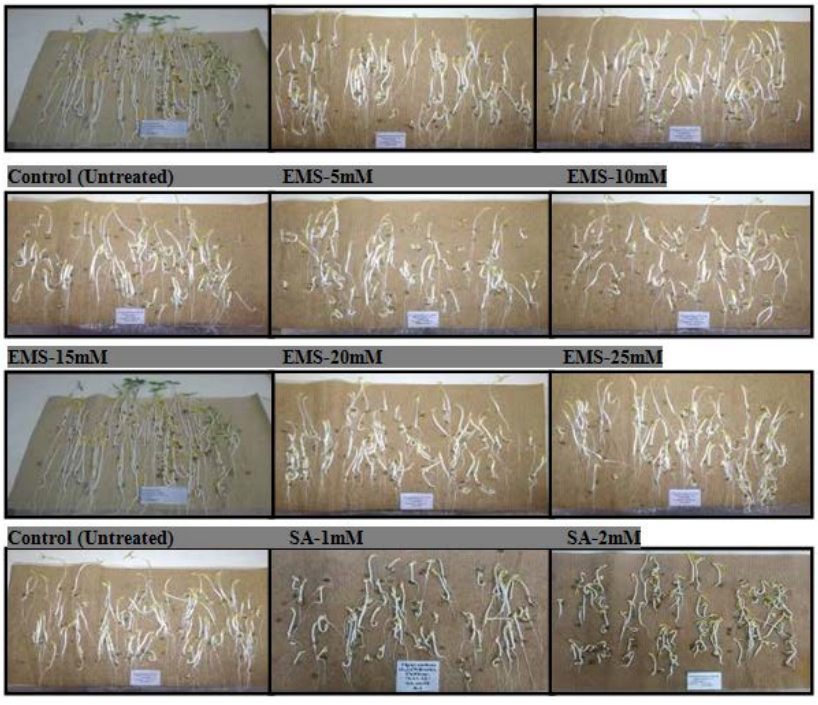


Figure 4. Dead Seed

5. Conclusions

In given experimental studies, LD_{50} Value of *Vigna radiata* Cultivar-Naval based on the survival of 50% of plants. The optimum dose was determined and these doses were selected for treatment. The sensitivity of cultivar-Naval to EMS, SA and GR were studied for germination as well as growth parameters including control. All the parameters were found significantly in all the doses of chemical treatments were studied. The study reveals that the EMS-10, 15 and 20mM dose were significant, SA-2, 3 and 4mM dose were significant and GR-250, 350 and 450GY dose were significant; hence we have selected these doses for treatment of seeds to investigate for further study.

Photoplate



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