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# $M_1$ generation studies in urdbean (*Vigna mungo* (L.) Hepper)

**U. P. Bhosale[1*], B. V. Hallale[2] and S. V. Dubhashi[1]**

[1]*Department of P.G. Studies & Research in Genetics, Walchand College of Arts and Science, Solapur(MS), India*
[2]*Department of Botany Dnyanopasak College of Arts, Commerce and Science, Parbhani(MS), India*

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**ABSTRACT**

*Seeds of urdbean were treated with various doses/ concentrations of gamma radiation (10 to 50kR), EMS (0.1 to 0.5%) for studying seed germination, seedling injury, pollen sterility, survival of plants at maturity and lethality. Seed germination and survival of plants at maturity decreased with increasing concentration/dose of the mutagens, while seedling injury, pollen sterility and lethality were found increased with increasing concentration / dose of the mutagens in the present variety of Urdbean.*

**Key words:** urdbean, $M_1$ generation, EMS and gamma rays

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## INTRODUCTION

Black gram or urid is one of the important pulse crop in India. Black gram (*Vigna mungo* (L.) Hepper) reported to be originated in India. It is the important food grain legume and rich in protein. In spite of its importance, the crop however has very limited genetic variability [1]. Therefore, development of new plant types for different situations is required. Mutation breeding has been widely used for the improvement of plant characters in various crops. It is a powerful and effective tool in the hands of plant breeders especially for autogamous crops having narrow genetic base [2]. Biological damage caused by mutation to germination, seedling injury, pollen sterility and survival at maturity may be considered as an indication of mutagenic effect.[3]. The present study was conducted to understand the effect of two mutagens namely ethyl methane sulphonate and gamma radiation on above parameters. It is helpful in determining the effect and mechanism of action of the mutagen for further mutation breeding programme.

## MATERIALS AND METHODS

The authentic seeds of urdbean was procured from the Pulses Improvement Division of Mahatma Phule Krishi Vidyapeeth (MPKV) Rahuri, District Ahmednagar, Maharashtra state, India. Healthy dry seeds of the variety of black gram were selected for their uniformity in size. For the treatment of EMS, the healthy seeds were surface sterilized with 0.1% mercuric chloride solution for about one minute, washed thoroughly and soaked in distilled water for 6 hours followed by treatment in freshly prepared solutions of mutagens for 6 hours. Fresh solution of Ethyl Methane Sulphonate of 0.1, 0.2, 0.3, 0.4, and 0.5 % concentrations was prepared in phosphate buffer (pH 7.0). The chemical mutagen treatments were given at 25+2°C with intermediate shaking. The volume of chemical mutagen was about 5 times as that of seeds for uniform absorption. The seeds treated with chemical mutagens were thoroughly washed under running tap water for an hour to terminate the reaction of the chemical before sowing in the field. For physical mutagen treatments the facility available at the Bhabha Atomic Research Institute, Trombay, Mumbai, (M.S. India) was availed. The doses employed were 10, 20, 30, 40 and 50 kR. Dry, uniform seeds of black gram were exposed to different doses of gamma rays (10, 20, 30, 40 and 50 kR). Seeds not exposed to gamma rays were used as control. Each treatment was carried out for 300 seeds. The treated seeds along with control were sown in the field at spacing of 15 cm in row apart and 45 cm between rows in randomized block design (RBD) with three replications to rise $M_1$ generation during *kharip* season.100 seeds of each treatment along with control were germinated in four replications on moist blotting papers in Petri-dishes using distilled water for germination and

seedling injury study. The germination was observed on the 5 day after treatment while seedling injury was recorded on the 10 day. The chlorophyll mutations were scored in the field by the methods of Blixt [4]. The pollen sterility was observed at flowering stage on 30 plants per treatment selected randomly. Survival of plants at maturity and morphological changes in different treatments for each cultivar were recorded in the field.

## RESULTS AND DISCUSSION

Effect of different treatments of Ethyl Methane Sulphonate and Gamma rays on biological parameters such as seed germination, seedling injury, pollen sterility, survival of plant at maturity and lethality were studied. The results obtained are as follows-

The percent seed germination in black gram, subjected to treatment with different concentrations of EMS and gamma rays is less than that of their Control. It clearly indicates that the mutagens have clearly exerted an inhibitory effect on seed germination. Percent seed germination has decreased with an increase in concentration or dose of the mutagens. The decrease in germination was more conspicuous with Gamma rays than that of EMS treatments. The percent seed germination was 91.66% in Control of black gram. The percent seed germination decreased up to 36% EMS and 38.33% in gamma rays. Maximum decrease in percent seed germination was observed with EMS (0.5%). Thus 0.5% dose of EMS seemed very effective in reducing percent seed germination in black gram. The results also revealed that the black gram cultivar was more sensitive to both the mutagens (EMS and Gamma rays). Among the two mutagens, EMS was proved to be more effective in reducing percent seed germination than the Gamma rays. Similar inhibitory effect on seed germination by the mutagens has also been reported earlier in chickpea [5] and [6], pea [7] and [8], cowpea [9] and [10], urdbean [11], mungbean [12], [13] and [14].

Seedling injury is widely used as an index of determining biological effects of various physical and chemical mutagens in $M_1$ generation [15]. In the present investigation it was observed that all mutagenic treatments showed clear inhibitory effect on the height of seedlings. Both the mutagens, EMS and Gamma rays, were almost equally highly effective in black gram in causing seedling injury. The seedling injury increased with an increase in concentration or dose of mutagenic treatment in black gram. Maximum seedling injury (26.33%) was observed in the treatment with 0.5% of EMS (Table 1). In case of Gamma rays, maximum seedling injury (25%) was observed at a concentration of 50 kR. From this it can be concluded that black gram is more sensitive to EMS than the Gamma rays with respect to seedling injury. Similar increase in seedling injury with increased concentrations of mutagens has been reported in *Vigna mungo* [16], mungbean [14].

Both the mutagens, employed in the present investigation, were effective in inducing pollen sterility in black gram at $M_1$ generation. Gamma rays were found to be more effective than the EMS in inducing pollen sterility. The rate of pollen sterility increased with an increase in the concentration or dose of the mutagens in black gram. The highest pollen sterility (34.66%) was recorded in the plants raised from the 50 kR of gamma rays treated seeds. Minimum pollen sterility (9.33%) was observed in the plants raised from 0.1% EMS treated seeds (Table 1). These results confirmed that black gram is more sensitive to Gamma rays as compared to EMS with respect to pollen sterility. These results are in agreement with those of the earlier researchers in red gram [17], in *Solanum nigrum* [18], in urdbean [11] and [19], in mungbean [14] and in cowpea [10].

**Table.1 Effects of mutagens on seed germination, seedling injury, pollen sterility, survival at maturity and lethality in Urdbean variety TPU-4 in M generation**

| Mutagen/ Control | Conc./ dose | % Seed germination | %Seedling injury | % Pollen sterility | % Survival | % Lithality |
|---|---|---|---|---|---|---|
| Control | -- | 91.66 | -- | -- | 85.42 | -- |
| EMS | 0.1% | 81.33 | 06.66 | 09.33 | 79.45 | 09.83 |
| | 0.2 % | 70.33 | 11.66 | 12.66 | 75.91 | 18.00 |
| | 0.3 % | 51.00 | 15.66 | 19.00 | 67.42 | 26.14 |
| | 0.4% | 44.00 | 21.33 | 24.00 | 58.73 | 34.32 |
| | 0.5 % | 36.00 | 26.33 | 30.33 | 54.61 | 40.00 |
| Gamma Rays | 10kR | 80.33 | 07.33 | 10.66 | 78.11 | 11.61 |
| | 20kR | 71.33 | 11.00 | 14.33 | 73.21 | 14.88 |
| | 30kR | 48.66 | 16.33 | 21.00 | 66.73 | 24.79 |
| | 40kR | 45.00 | 20.33 | 25.66 | 56.57 | 31.11 |
| | 50kR | 38.33 | 25.00 | 34.66 | 49.18 | 41.73 |

It is evident that the percent survival of plants at maturity decrease with the increased in concentration of the mutagens in black gram. Reduction in percent survival of plants at maturity was more pronounced in Gamma rays as compared to the EMS treatments. The percent survival of plants at maturity with 50 kR of Gamma rays 49.18% where as it was 54.61% in the 0.5% EMS. Thus it was proved that black gram is more sensitive to gamma rays, as

compared to EMS. Such inverse relation between concentration of the mutagen and survival of plants at maturity has also been reported in urdbean [19], in chick pea [20] and [21].

The rate of lethality increased with an increased in the concentration/dose of the mutagens. Maximum lethality was observed in 50 kR of Gamma rays (40.73%) and 0.5% EMS (40.00%) while minimum lethality was recorded in 0.1% EMS (09.83) and 10 kR of Gamma rays (11.61%). Black gram was found to be more sensitive to Gamma rays than EMS with respect to lethality. Lethality has also been reported in urdbean [11] and [19], in chickpea [20] and [21], and in cowpea [10].

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