



# Mutagenesis in Horsegram [*Macrotyloma uniflorum* (Lam.) Verdc.]

<sup>1</sup>Bolbhat Sadashiv and <sup>2</sup>Wagh Kajal

<sup>1</sup>Faculty and U. G. Student<sup>2</sup>

Department of Botany and Ph. D. Research Center in Botany,

Rayat Shikshan Sanstha's Annasaheb Awate Arts, Commerce and Hutatma Babu Genu Science College,  
Manchar, Tal. Ambegaon, Dist- Pune- 410503 (MS) India.

## ABSTRACT:

Induced mutagenesis has been used to create desired genetic variability, the base of crop improvement. Induced genetic variability in crop plants is an important resource from which plant breeders can select and combine different desired characteristics to produce best crop plants. The desirable characters which have been bred through induced mutations. In present investigation the physiological effects on seed germination as well as seedling height on 7<sup>th</sup> day after sowing were investigated. Gradual reduction in seed germination and seedling height was recorded with increase in concentration /dose of mutagens. Majority of the mutagenic treatments caused decrease in seed germination, seedling height and survival of plants at maturity. While pollen sterility increased with increasing dose/conc. of mutagens.

**Keywords-** Mutagenesis, horsegram, mutants, variability, seedling height.

## INTRODUCTION

Horsegram [*Macrotyloma uniflorum* (Lam.) Verdc.] is locally known as *hulga*. It is an important minor, rainfed pulse crop. Horsegram is drought tolerant and having good nitrogen fixing ability [1]. With protein supplement in human diet, it has medicinal value. It also provides concentrated feed for cattle & domestic animals. Horsegram is grown both in *kharif* as well as *rabi* seasons as main crop, or as a mixed crop with tur, bajra or finger millet [2]. Pulses are major sources of proteins among the vegetarians, and also provides essential amino acids, vitamins and minerals. It provides significant nutritional, health benefits. It reduces diseases like colon cancer and cardiovascular diseases [3]. Horsegram is used for the treatment of heart problems, asthma, bronchitis, leucoderma, treatment of kidney stones and for the urinary discharge [4]. Horsegram has the great potential for further utilization as nutraceuticals and pharmaceuticals [5,6]. It plays a major role in antioxidation and antimicrobial activities [7]. It is the best unconventional feedstuff and low cost poultry feed [8]. The major pulses of the world are peas, beans, gram, cowpeas, urbeans, and mungbeans while the minor ones include horsegram. Horsegram is an important legume crop but under exploited. The induction of physical or chemical mutations is the quickest way to produce the varieties [9].

## MATERIALS AND METHODS

**Seed material :** The seeds of Horsegram [*Macrotyloma uniflorum* (Lam.) Verdc.] were obtained from local market of Manchar, Tal. Ambegaon, Dist Pune-410503 (M.S.) India.

**Mutagens used :** Gamma rays (GR), ethyl methane sulphonate (EMS) and sodium azide (SA) were used in present experiment for the treatments of horsegram seeds. Gamma radiation from <sup>60</sup>Co source fixed in the gamma cell 200 installed at Chemistry Department, Savitribai Phule, Pune University, Pune was used in the present work. Dry and uniform healthy seeds of horsegram with moisture content of 11-12 % were treated with 300, 400 and 500 Gy. Ethyl methane sulphonate (CH<sub>3</sub>SO<sub>2</sub>OC<sub>2</sub>H<sub>5</sub>) molecular weight 124.16, and 8% soluble in water, manufactured by Sigma chemical Co. Ltd. USA was used for the seed treatments of horsegram. Different concentrations of EMS (0.3% to 0.5%) were prepared in distilled water. Sodium Azide is an inorganic compound. It is a colorless salt, ionic compound, soluble in water and is highly toxic. Mol. Wt. is 65.0099g/mol. It is a chemical mutagen and used for induction of mutations in the crop plants. Different concentrations of SA (0.01%, 0.02% and 0.03%) were prepared in distilled water.

**Treatment details :** The pilot experiments were conducted to determine the lethal dose (LD<sub>50</sub>), suitable concentrations of EMS, SA and duration of seed treatment. The doses of gamma rays, 300, 400 and 500Gy, EMS 0.3, 0.4 and 0.5% while SA 0.01, 0.02 and 0.03% were finally selected for the seed treatment and the duration fixed was two hours. Selected seeds were soaked in distilled water for 12 hours and the wet seeds were treated with different concentrations of EMS (0.3, 0.4 and 0.5%) and SA 0.01, 0.02 and 0.03% for four hours. The untreated seeds served as control. For each treatment 180 seeds were used.

The seeds treated with different concentrations of SA and EMS were washed thoroughly under tap water for one hour. It terminate the reaction of chemical mutagen which leach out the residual chemicals. A total of 30 seeds from each treatment was used for seed germination in laboratory. Three replications with 10 seeds / replication kept in petri dishes, having seed germination paper, were used for recording seed germination percentage, root and shoot length, on 7<sup>th</sup> day. The remaining lot of treated seeds (150) from each treatment was used for raising M<sub>1</sub> generation in field.

**Experimental site :** Present investigation was carried out at experimental field, Department of Botany, Annasaheb Awate Arts, Commerce and Hutatma Babu Genu Science College, Manchar, Tal. Ambegaon, Dist- Pune (410503) (M.S.). The soil type of the experimental field was slightly deep and calcareous with good drainage. The average minimum temperature was recorded as 18.65°C and maximum 34.63°C with average annual rainfall 671.09mm.

**Experimental design for field experiments:** The field experiments were conducted on the experimental field at Department of Botany. The crop of horsegram was grown in *Kharif* season under uniform conditions. All the experiments were carried out in triplicate followed RBD design. Each plot with single treatment had 50 plants. The distance between two rows and two plants was 45X 30 cm and the distance between two adjacent plots was one meter. A total of 10 treatment combinations in M<sub>1</sub> generation with untreated seeds were used as control. Treated and control seeds were sown in field in randomized block design.

### Observations on M<sub>1</sub> generation

**Germination percentage:** The number of seeds showing emergence of the radical and plumule was counted from the seeds kept in petri plates with moist germination paper, data was used to calculate percent seed germination.

**Root and shoot length:** On 7<sup>th</sup> day of sowing, 10 seedlings from control and each treatment were selected randomly for measuring the root & shoot length and the average values were recorded in table.

**Seedling injury:** Seedling height was recorded on 7<sup>th</sup> day. Reduction in the mean seedling height as compared to the control was regarded as seedling injury and expressed as percentage. The seedling injury was calculated as follows

$$\text{Seedling injury\%} = \frac{\text{Control seedling height} - \text{Treatment seedling height}}{\text{Control seedling height}} \times 100$$

**Pollen sterility :** Pollen sterility was determined from 10 randomly selected plants per treatment, along with control. The pollen grains from freshly dehisced anthers were stained with aceto-carmin (1%). Pollen grains stained as uniform deep red colour were counted as fertile and others as sterile.

**Survival of plants at maturity :** Survival percentage was calculated by scoring the total number of plants attaining maturity (45 DAS) in each treatment and expressed as percentage over the control.

**Harvesting of seeds from M<sub>1</sub> plants :** All the surviving M<sub>1</sub> plants were harvested individually and bulked seeds of plant from each treatment were kept separately for raising M<sub>2</sub> generation.

### Statistical analysis

The data were summarized as the means of three replicates with standard deviation as the measures of variability. One-way ANOVA test was performed to determine significant differences due to various treatments. Fisher's LSD (Least significant difference) was used as post hoc test to as certain significant differences among treatments at p= 0.05. Statistical analysis and graphical data presentations were carried out by using IBM SPSS (ver.25).

### RESULTS AND DISCUSSION:

Results obtained in the present investigation on seed germination, seedling injury, pollen sterility and survival of plant at maturity in M<sub>1</sub> generation of horsegram are given in Table- 1. Data obtained on mean percent seed germination in control and mutagen treatments presented in Table-1 clearly indicated that the percent seed germination was decreased in all the treatments as compared to control. It has clearly indicated that the mutagens had exerted negative effects on seed germination. Seed germination percent was decreased with the increase in doses/ conc. of the mutagens. The percent seed germination decreased from 70.12% to 45.61% in gamma radiation, 75.41% to 48.17% in EMS and 80.16% to 54.67% in SA. The maximum (50%) decrease in percent seed germination was observed with gamma radiation treatment 500Gy (45.61%), EMS 0.5% (48.17%) and in SA 0.03% (54.67%). Thus 500Gy treatment was very effective in reducing percent seed germination in horsegram to almost 50%.

**Table 1 : Mutagenic effect on seed germination, seedling height, seedling injury, pollen sterility and plant survival at maturity in M<sub>1</sub> generation of horsegram.**

Treatments	Germination (%)	Shoot length (cm)	Root length (cm)	Seedling height (cm)	Seedling injury (%)	Pollen sterility (%)	Plant survival at Maturity (%)
Control	82.47±11.55	4.33±0.61	4.22±0.59	8.55±1.20	00.00±0.00	05.21±0.73	73.43±10.28
300Gy	70.12±5.61	4.08±0.33	4.17±0.33	8.25±0.66	03.51±0.28	15.14±1.21	64.19±5.14
400	54.23±5.97	3.36±0.37	3.05±0.34	6.41±0.71	25.03±2.75	20.32±2.24	56.11±6.17
500	45.61±5.93	3.11±0.40	3.00±0.39	6.11±0.79	28.54±3.71	27.08±3.52	47.28±6.15
0.3 % EMS	75.41±10.56	4.17±0.58	4.03±0.56	8.20±1.15	4.09±0.57	18.43±2.58	68.07±9.53
0.4	61.20±4.28	3.75±0.26	3.47±0.24	7.22±0.51	15.56±1.09	24.50±1.72	57.14±4.00
0.5	48.17±3.85	3.42±0.27	2.90±0.23	6.32±0.51	26.08±2.09	30.02±2.40	49.59±3.97
0.01%SA	80.16±12.02	4.23±0.63	3.65±0.55	7.88±1.18	7.84±1.18	13.36±2.00	68.33±10.25
0.02	62.67±8.77	3.89±0.54	3.16±0.44	7.05±0.99	17.54±2.46	17.79±2.49	59.36±8.31
0.03	54.67±4.92	3.84±0.33	2.97±0.27	6.81±0.60	21.75±1.96	25.27±2.27	52.72±4.74
SEM±	6.46	0.37	0.34	0.71	1.60	1.83	5.93
F-value	8.24	2.44	4.77	3.18	8.53	3.26	4.29
P-value	0.01	0.05	0.01	0.02	0.01	0.01	0.01
LSD <sub>0.05</sub>	14.08	0.81	0.74	1.55	3.49	3.99	12.92

Data are means of three replicates ± standard deviation. Significant difference due to treatments was assessed by Fisher's LSD as a post-hoc test.

Results indicate that, percent seed germination decreased with increasing doses/ conc. of GR, EMS and SA in horsegram. This clearly indicates that the mutagens have exerted an inhibitory effect on seed germination. Similar inhibitory effect on seed germination reported earlier by, [1,2,9,10,11,12] in horsegram, [13] in sesame, [17] in black beans. The reduction in germination may be due to genetic as well as physiological processes inhibited by the mutagens resulting in cell maturity. Presoaking of seeds increases the sensitivity to chemical mutagens. EMS and GR are potent mutagens well known for their action in inducing point mutations, chromosomal aberrations and enzyme inhibitions.

Data presented in table indicated that doses of GR, conc. of EMS and SA treatments showed inhibitory effect on seedling height. Maximum decrease in seedling height (6.11cm) was noted in 500Gy, 0.5%EMS (6.32cm) and 0.03%SA (6.81cm). Data on the effect of GR, EMS and SA on seedling injury at M<sub>1</sub> shown in table revealed that all mutagenic treatments were highly injurious to the seedlings. The seedling injury increased with the increase in doses/ conc. of mutagenic treatments. Maximum seedling injury (28.54%) was observed in 500Gy.

In the present investigation it was reported that the seedling injury increased with the increase in conc. or dose of mutagenic treatments in horsegram. Similar increase in seedling injury with increased dose/conc of mutagens has been reported by [14,15] in urdbean, [13] in sesame, [17] in black beans. According to [6] reduction in seedling growth with higher dose may be due to the gross injury caused at cellular level either due to gene controlled biochemical process or acute chromosomal aberrations.

Data on pollen sterility given in table 1, it is clear that all mutagens were effective in inducing pollen sterility in horsegram. EMS was the most effective of all the three mutagens in inducing pollen sterility, followed by the GR and SA. The highest pollen sterility was recorded in 0.5%EMS (30.02%). The rate of pollen sterility increased with increase in the conc. or dose of the mutagens. Similar inhibitory effect on pollen sterility reported earlier by [1,2,9,10,11,12] in horsegram [13] in sesame [17] in black beans. [16] proposed that gross injury at cellular level is due to chromosomal aberrations. Various types of chromosomal abnormalities like translocation, anaphase bridges and laggards were found in the progenies obtained from treated seeds.

The results on the effects of GR, EMS and SA revealed that in all the mutagenic treatments, survival % was decreased than the control (Table-1). There was decrease in the survival % with increasing conc. /dose of GR, EMS, and SA. The lowest survival % at the higher treatments was noted (47.28%) in 500Gy, 0.5%EMS (49.59%) and 0.03%SA (52.72%) as compared to control (73.42%). All mutagens reduced the rate of survival at maturity [1,2,9] in horsegram, [13] in sesame, [17] in black beans supported the above findings.

## CONCLUSION

Seed germination percent and seedling growth was inhibited due to increasing doses/ conc. of mutagens. All mutagens were effective in inducing pollen sterility in M<sub>1</sub> generation. The rate of pollen sterility increased with increase in dose/ concs. of the mutagens and the survival rate was highly reduced with increasing dose/concs. of mutagens.

## ACKNOWLEDGEMENT

Authors are thankful to Principal Dr. N.S. Gaikwad for providing library, laboratory and research field facilities.

## REFERENCES

- [1] Bolbhat, S. N. (2011). Studies on Induced mutations in horsegram (*Macrotyloma uniflorum* (Lam.) Verdc). Ph.D. Thesis. University of Pune, Pune (MS), India.
- [2] Awte, P.A. and Bolbhat, S.N. (2014). Effect of EMS on seed germination, seedling height, and plant survival of horsegram cv. Rayat-1. *JECET*, 1:272-276.
- [3] Jukanti, A.K., Gaur, P.M., Gowda, C.L.L., and Chibbar, R.N. (2012). Nutritional quality and health benefits of chickpea.
- [4] Ghani, A. (2003). Medicinal plants of Bangladesh: Chemical constituents and uses. 2<sup>nd</sup> Edn. Asiatic Society of Bangladesh, Dhaka, ISBN: 9-845123481:5-16.
- [5] Morris, J.B. (2008). *Macrotyloma axillare* and *M. uniflorum*: Descriptor analysis, anthocyanin indexes and potential uses. *Genetic Resour. Crop Evolu.* 55: 5-8.
- [6] Kawsar, S. M.A., Huq, E., Nahar, N. and Ozeki, Y. (2008). Identification and quantification of phenolic acids in *Macrotyloma uniflorum* by reversed phase HPLC. *Am. J. Plant Physiol.* 3:165-172.
- [7] Reddy, A.A. (2009). Pulses Production Technology: Status and Way Forward. Review of agriculture vol. xlv no 52. (<http://ssrn.com/abstract=1537540>). Last viewed on 31<sup>th</sup> July 2010).
- [8] Ravindran, R. and Bino Sundar, S.T. (2009). Nutritive value of horsegram (*Dolichos biflorus*) for egg- type chicks and growers. *Tamilnadu J. Veterinary & Animal Sciences.* 5 (4): 125-131.
- [9] Shirsat, R.K., Mohrir, M.N., Kare, M.A. and Bhuktar, A.S. (2010). Induced mutations in horsegram: mutagenic efficiency and effectiveness. *Recent Research in Science and Technology*, 2 (7): 20-23.
- [10] Kulkarni, R.N. (1978). Mutagenic effects of 5
- [11] Rudraswami, P. (1983). Induced mutagenesis in horsegram (*Macrotyloma uniflorum* (Lam.) Verdc) using gamma-rays and EMS. *Mysore J. of Agri. Sci.* 21 (1): 95.
- [12] Datir S.S., Dhupal, K.N. and Pandey, R.N. (2007). Gamma radiation and EMS induced variation in seed germination & plant survival in horsegram. *J. Arid Legumes* 4 (1): 15-17.
- [13] Uttarde, R.B. and Bolbhat, S. N. (2020). Induced mutations in sesame (*Sesamum indicum* L.). *IJCRT.* 8 (3) : 3171-3176.
- [14] Sharma, S.K., Sood, R., and Pandey, D.P. (2005). Studies on mutagen sensitivity effectiveness and efficiency in urdbean (*Vigna mungo* (L.) Hepper), *Indian J. Genet.* 65 (1): 20-22.
- [15] Sagade, A.B. (2008). Genetic improvement of urdbean (*Vigna mungo* L. Hepper) through mutation breeding. Ph.D. Thesis. University of Pune, Pune (MS), India.
- [16] Gunkel, J. E. (1957). Symposium on the effects of ionizing radiation on plants. Morphological effects. *Quart. Rev. Biol.* 32: 32-56.
- [17] Bolbhat, S.N., Lande, A.B., Naik, P.D., Gaikwad, S.S. and Shaikh, S. (2020). Induced Genetic Variability in Black Turtle Bean (*Phaseolus vulgaris* L.), 8(4) :258-262.