



Studies on germination and LD₅₀ by induced chemical mutagenesis in urdbean (*Vigna mungo* (L.) Hepper)

V Vijayakanth*, L Mullainathan, M Sathya

Department of Botany, Annamalai University, Annamalai Nagar, Tamil Nadu, India

Abstract

Black gram (*Vigna mungo* (L.) Hepper) is a crucial crop in our country. It belongs to the family Leguminosae and subfamily papilionaceae. Mutation breeding is the tool in the hand of breeder to discover variability in crop population and to make option in the population with the view to bring about more improvement in crop. Genetic improvement through mutation breeding has extension in Agriculture sector to establish new varieties. The two mutagens are employed, chemical mutagens namely Diethyl Sulphate (DES) and sodium Azide (SA) (10 mM to 50 mM) were used at different concentration to induce mutagenesis. The LD₅₀ value and germination percentage was noted to each treatment. According to results the LD₅₀ was observed in 40 mM of DES and 30 mM of SA.

Keywords: black gram, DES, SA, mutation breeding, seed germination and LD₅₀ value

Introduction

Black gram (*Vigna mungo* (L.) Hepper) is a crucial crop in our country. It belongs to the family Leguminosae and subfamily papilionaceae. The chromosome number of black grams is $2n=22$ (Bhatnagar *et al.*, 1974) [7]. The crop is resistance to detrimental climatic conditions and development the soil fertility by fixing atmospheric nitrogen in the soil. It includes about 26% protein which is almost three times that of cereals and other minerals and vitamins. It is essential source of human and animals' meal. The essential amino acids composition of urdbean seed is Tryptophan, Lysine, Methionine, Phenyl alanine, Threonine, Valine, Leucine Isoleucine (Kanade, 2006) [12]. Although legume seeds are conclusive as protein source in human nutrition in many parts of the universal (Brohult and Sandegren, 1954) [6].

It is known as poor man's meat since it is contributing a large share of the dietary protein of the vegetation population they are universally grown in the Indian subcontinent followed by Thailand, Australia, and other Asian and south pacific countries on a meagre scale (Harouna, 2020) [9]. It covers of around 3.24 million hectares and production 1.4 million tonnes. Its productivity is only 526 kg per ha. In Tamilnadu black gram covers an area of 3.41 lakh hectares with production of 1.21 lack tonnes and 355 kg per ha (Jayamani *et al.*, 2012) [11]. In Indian black gram involve the fourth position in the area covered by cultivation and production after chickpea, pigeon pea and green gram (Singh and Ahlawat, 2005) [18]. The increasing food demand of the fast-growing population expected to ascent to 9 billion by 2050 (Annual Report, 2016-2017). Mutagenesis has been universally used as a great method of enhancing variability for crop improvement (Singh and Singh, 2001). Mutagens may cause genetic change in an organism, crack linkages and crop many new promising traits (Shah *et al.*, 2008; Usharani and Kumar, 2015) [17, 22]. Mutation breeding is the tool in the hand of breeder to discover variability in crop population and to

make option in the population with the view to bring about more improvement in crop. In general mutation breeding has been playing a key role self-pollinated crop with maximum variability has been announced by many workers in black gram (Thilagavathi and Mullainathan, 2009) [20]. Genetic improvement through mutation breeding has extension scope in Agriculture sector to establish new varieties which are resistant/tolerant to disease, insect, drought, salinity, heat, pest with high nutritional and low antinutritional cause EMS and SA (Khatri *et al.*, 2005; Tah, 2006; Songsri *et al.*, Aney, 2013) [8, 3]. Induced mutation has already been used in many crop species to develop agronomical and quality traits affecting plant size, flower time, fruit ripening and fruit colour (IAEA, 2020) [10]. EMS successfully has been used to advance new cultivars of a few agronomic and horticultural crops including black gram (Rao and Jana, 1976) [15]. Artificial induction of mutation brings raw materials for the genetic improvement of economic crops (Adamu and Aliyu., 2007) [1]. For any mutagenesis, it is very essential a suitable mutagen dose. Lethal dose is the percentage of test organisms that killed by a definite dosage of chemicals, half will die at LD₅₀. The mutagen dose administrated should be acceptable to kill 50 percentage of the seed to obtain the maximum number of mutations. The LD₅₀ used by most of the researcher to regulate the lethal dose mutagens (Anbarasan *et al.*, 2013). The LD₅₀ value is different among species and varieties in a species (Tah, 2006) [3].

Materials and Methods

The healthy seeds of *Vigna mungo*. L (vamban-3) were purchased from Tamil Nadu Agricultural University (TNAU), Coimbatore. Mutagens employed, chemical mutagens namely Diethyl Sulphate (DES) and sodium azide (SA) were used at various concentration to be induced mutagenesis. The seeds were pre-soaked in distilled water for 6 hours in regulation to make them relatively also sensitive to mutagenic action. The solution of EMS

prepared in phosphate buffer of P^H 7, the room temperature of 25 °C. Pre-soaked seeds were treated with different concentration viz., 10, 20, 30, 40 and 50mM of diethyl sulphate (DES) and sodium azide (SA) solution for 4 hours with redone stirring. After treatment, seeds were thoroughly washed in running tap water for 8-10 times to remove the particle of the chemicals. Untreated seeds of *vigna mungo* L. Vamban-3 variety was pre-soaked in distilled water for 4 hours and used as control. After pre-soaking, the seeds were sown in the petri plates.

LD₅₀ values and percentage seed germination

The LD₅₀ is measure of the lethal dose of chemicals. The LD₅₀ was observed by the measure of material, given all at once, which caused the death of 50% of plants treated with chemical mutagen in seedlings.

Seed germination (%)

The seeds of each treatment along with control were fixed on absorbent cotton-wet petridishes. For each treatment three replicates were studied, and the number of seeds germinated on the 7th day was embodied, the germination percentage was calculated. Based on the reduction of 50% seed germination, the LD₅₀ value were placed and two treatment of DES and SA around LD₅₀ value for further studies.

$$\text{Germination (\%)} = \frac{\text{No. of seeds germinated}}{\text{No. of seeds sown}} \times 100$$

Results and Discussion

Seed Germination

The plant that grows from seed was considered seed germination. The seed germination data on *Vigna mungo*. Vamban-3 variety is given in the Table1. The present analysis focused on induced chemical mutagenesis on *Vigna mungo* L. Variety vamban-3 treated with two particular mutagens in different concentrations for induction of mutation. The seed germination percentage of different mutagenic treatments under the laboratory, *in-vitro* condition discovered that, the seed germination percentage was decreased with increasing concentration of DES and SA.

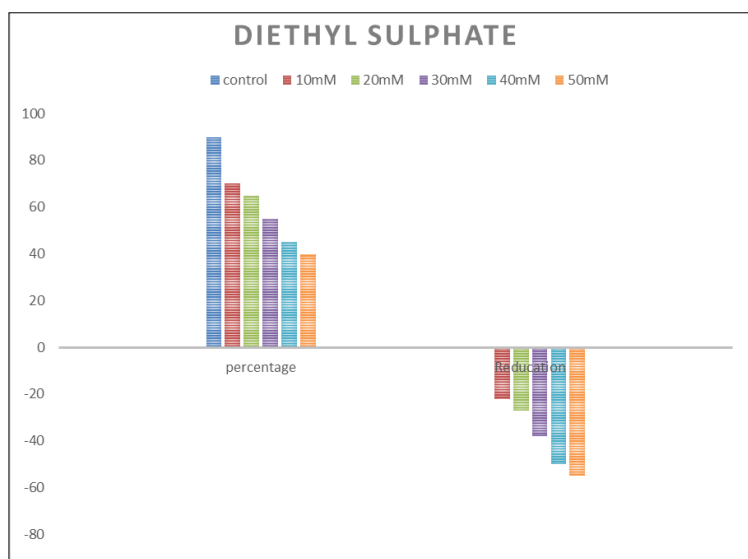
The percentage of seed germination was high in DES (40 mM 45%) and SA (30 mM 50%). Based on the seed germination percentage on the 7th Day, the LD₅₀ values were fixed at 40 mM of DES and 30 mM of SA.

The role of mutation breeding in increasing the genetic variability for quantitative traits in different crop plants have been tested beyond doubt (Khan *et al*, 1979). They all decreased in ethyl methane sulphonate treatment than in control in M₁ generation of *Vigna mungo* (Thilagavathi and Mullainathan, 2011) [20].

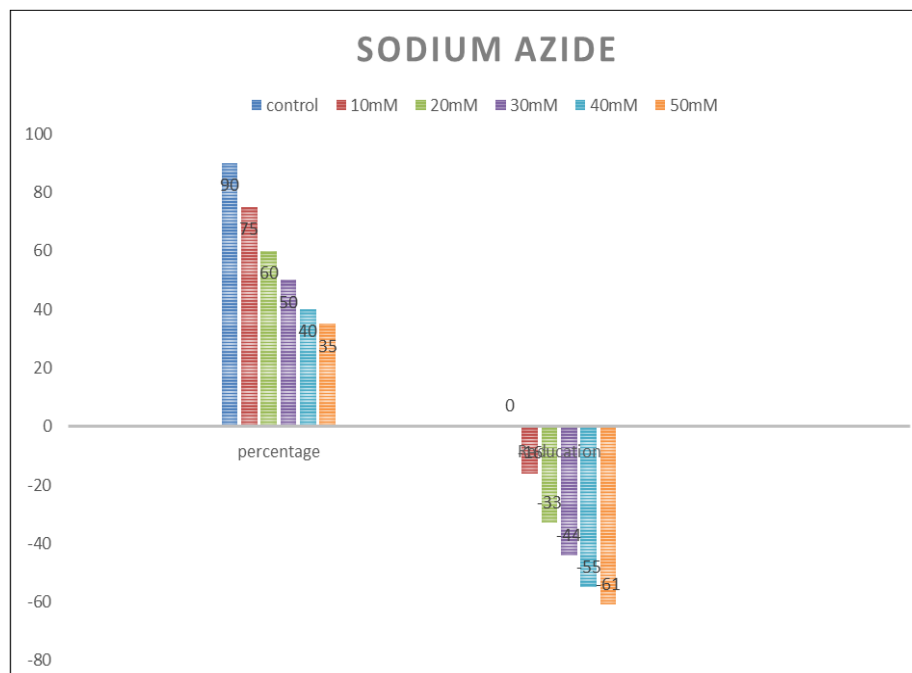
The reduction in plant survival due to the mutagenic analysis has also been reported in *Dianthus* (Roychowdhury *et al.*, 2012). SA treatment reported in various crops such as soybean, Mung beans and LD₅₀ value was observed in 30 mM of SA in Tomato (Norfdzin *et al.*, 2007)

Table 1: Determination of LD₅₀ for DES and SA on *Vigna mungo*. L

Mutagens	Treatments Con. (mM)	Number of Seeds Sown	Number of Seeds Germination	Percentage of Seeds Germination	Percentage of Reduction over control
	Control	20	18	90	00
DES	10 mM	20	14	70	-22
	20 mM	20	13	65	-27
	30 mM	20	11	55	-38
	40 mM	20	9	45	-50
	50 mM	20	8	40	-55
SA	10 mM	20	15	75	-16
	20 mM	20	12	60	-33
	30 mM	20	10	50	-44
	40 mM	20	8	40	-55
	50 mM	20	7	35	-61



Graph 1: Determination of LD₅₀ for DES on *vigna mungo* L.



Graph 2: Determination of LD₅₀ for SA on *vigna mungo* L.

Conclusion

Among the mutagen treatment for *Vigna mungo*, the LD₅₀ was found in 40 mM of DES and 30 mM of SA this concentration is suitable for these studies.

References

1. Adamu AK, Aliyu H. Morphological effects of sodium azide to tomato (*Lycopersicon esculentum* Mill). *Science world journal*,2007:2(4):9-12
2. Anbarasan K, Sivalingam D, Rajendran R, Anbazhagan M, Chidambaram AA. Studies on the mutagenic effect of EMS on seed germination and seedling characters of sesame (*Sesamum indicum* L.) var. TMV3. *International Journal of Research in biological Sciences*,2013:3(1):68-70
3. Tah A PR. Studies on Gamma Ray Induced Mutations in Mungbean [*Vigna radiata* (L.) Wilezek]. *Asian Journal of Plant Sciences*,2006:5(1):61-70.
4. Annual Report. In; Government of India, Ministry of Agriculture and Farmers Welfare, Department of agriculture, Cooperation and Farmers Welfare, Directorate of Pulses Development, Vindhyachal Bhavan, India, 2016-17. <http://dpd.gov.in/web%20Annual%20Report%202016-17%20-pdf>. Accessed.
5. Blixt S (1972) Mutation genetics in Pisum. *Agric Hort Genet*,1972:31:1-293
6. Brohult S, Sandegren C. The proteins, Academic press, New York,1954:2A:487
7. Bhatnagar A, Singh HB. Chromosome number is an okra from Ghana. *Indian J. Genet. Plant breed*,1975:36:26-27.
8. Draghici S, Khatri P, Eklund AC, Szallasi Z. Reliability and reproducibility issues in DNA microarray measurements. *TRENDS in Genetics*,2006:22(2):101-109.
9. Harouna DV. Biochemical and agro-morphological characterization of wild, under-exploited *Vigna* species and their utilization. Doctoral dissertation, NM-AIST.
10. IAEA, 2020. [https://mvd.iaea.org/#!/Search?Criteria\[0\]\[val\]=onion](https://mvd.iaea.org/#!/Search?Criteria[0][val]=onion)
11. Jayamani P, Kumaravadivel N, Nadarajan N, Muthiah AR, Durairaj C, Kamalakannan A, *et al.* TNAU Blackgram CO6: A High Yielding Short Duration Variety. *MADRAS Agric. J*,2012:99(1-3):34-36.
12. Kanade RS. Post harvest profile of blackgram. Ministry of Agric. Sep 8th, 2006, 2.
13. Khan AU, Kasha M. Direct spectroscopic observation of singlet oxygen emission at 1268 nm excited by sensitizing dyes of biological interest in liquid solution. *Proceedings of the National Academy of Sciences*,1979:76(12):6047-6049.
14. Norfadzrin F, Ahmed OH, Shaharudin S, Rahman DA. A preliminary study on gamma radiosensitivity of tomato (*Lycopersicon esculentum*) and okra (*Abelmoschus esculentus*). *Int. J. Agric. Res*,2007:2(7):620-625.
15. Rao SA, Jana MK. Leaf mutations induced in black gram by X-rays and EMS. *Environmental and Experimental Botany*,1976:16(2-3):151-154.
16. Roychowdhury R, Ferdousul Alam MJ, Bishnu S, Dalal T, Tah J. Comparative study for effects of Chemical Mutagenesis on Seed Germination, Survivability and Pollen Sterility in M₁ and M₂ generations of Dianthus. *Plant Breeding and Seed Science*,2012:65:29-38.
17. Shah TM, Mirza JI, Haq MA, Atta BM. Induced genetic variability in chickpea (*Cicer arietinum* L.). II. Comparative mutagenic effectiveness and efficiency of physical and chemical mutagens. *Pak. J. Bot*,2008:40(2):605-613.
18. Singh DP, Ahlawat IPS. Greengram (*Vigna radiata*) and blackgram (*V. mungo*) improvement in India: past, present and future prospects. *Indian journal of Agricultural science*,2005:75(5):243-250.
19. Gupta S, Gupta SR, Dikshit HK, Singh RA. Variability and its characterization in Indian collections of blackgram [*Vigna mungo* (L.) Hepper] (No. Research). IPGRI AND FAO, 2001.
20. Thilagavathi C, Mullainathan L. Isolation of macro mutants and mutagenic effectiveness, efficiency in

- black gram [*Vigna mungo* (L) Hepper]. Global Journal of Molecular Sciences,2009:4(2):76-79.
21. Thilagavathi C, Mullainathan L. Influence of physical and chemical mutagens on quantitative characters of *Vigna mungo* (L. Hepper). International Multidisciplinary Research Journal, 2011, 1(1)
 22. Usharani KS, Kumar CA. Induced viable mutants in urdbean (*Vigna mungo* (L.) Hepper). The Bioscan,2015:10(3):1103-1108.