DOI: 10.15740/HAS/AU/12.TECHSEAR(5)2017/1345-1349 *Agriculture Update*
Volume **12** | TECHSEAR-5 | 2017 | 1345-1349
Visit us : *www.researchjournal.co.in*

RESEARCH ARTICLE :

Determination of LD_{50} for induced mutagenesis through Gamma and Ethyl Methanesulphonate in Periwinkle [*Catharanthus roseus* L.] .) cv. LOCAL

■ **K. KANNABIRAN, K. RAJAMANI, J. SURESH, R. JOHN JOEL AND D. UMA**

ARTICLE CHRONICLE :
Received :
15.07.2017;
Accepted :
30.07.2017

SUMMARY : The present study was conducted during 2016-17 to determine the lethal dose (LD_{50}) of physical and chemical mutagens on periwinkle (*Catharanthus roseus* L.) cv. Local. The mutagens utilized for this study were physical mutagen *i.e.*, gamma radiation dose ranging from 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 kR and chemical mutagen *i.e.*, chemical mutagen, Ethyl methanesulphonate (EMS) ranging from 10, 20, 30,40, 50 and 60 mM. The germination percentage (94.00 %) and survival (20.00 %) for gamma and (65.00 %) and survival (35.00 %) for EMS was greatly influenced by mutagenic treatments, respectively. The mutagenic doses or concentrations are determined by the LD_{50} value for the mutagens used. The results of probit analysis revealed that the seeds treated with gamma rays 40 kR and EMS 30 mM showed 50 per cent mortality over other mutagenic treatments. The other traits *viz.*, shoot length (4.1 cm) and root length (5.5 cm) for gamma and for EMS shoot length (3.8 cm) and root length (4.9 cm) ultimately exhibited a linear reduction with every increasing treatment doses.

KEY WORDS :
Mutagenesis, Gamma rays, Ethyl methane sulphonate, LD_{50} , Periwinkle

How to cite this article : Kannabiran, K., Rajamani, K., Suresh, J., Joel, R. John and Uma, D. (2017). Determination of LD_{50} for induced mutagenesis through Gamma and Ethyl Methanesulphonate in Periwinkle [*Catharanthus roseus* L.] .) cv. LOCAL. *Agric. Update*, **12**(TECHSEAR-5) : 1345-1349; **DOI: 10.15740/HAS/AU/12.TECHSEAR(5)2017/1345-1349.**

Author for correspondence :

K. KANNABIRAN
Department of Medicinal and Aromatic Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, COIMBATORE (T.N.) INDIA
Email : kannabiranhort@gmail.com

See end of the article for authors' affiliations

BACKGROUND AND OBJECTIVES

Periwinkle (*Catharanthus roseus* L.) G. Don belongs to the family Apocyanaceae commonly known as 'Sadabahar', 'ShavamNaari' 'Nithyakalyani', 'Sudukattumalli' in Tamil. It is a perennial tropical glycophyte plant, native of Madagascar and from there; it has spread to India, Indonesia, Indo-china, Philippines and other parts of the world. However, it is also widely cultivated and naturalized in the tropical and subtropical areas in the world (Lewis and Elvin, 1977). It is found to be an evergreen sub-shrub or the herbaceous plant that grows to about 1 m tall with dark pink flowers (Frode and Mediros, 2008). The Mutation Breeding is a tool which can be easily used for creation of genetic variability Venkateswarlu *et al.* (1988); Bhattacharjee (1998) and Chatterjee *et al.* (1978) has also studied the mutagenesis approach for the crop improvement in *C. roseus*.

Among the commercially important medicinal plants in India periwinkle play an important role because of its ability to synthesize a wide range of terpenoids indole alkaloids (TIAs), export value, and consistent demand in other countries. Tamil Nadu leads in periwinkle cultivation with nearly 2000 ha spread in dry districts of Virudhunagar, Madurai, Trinelveli districts. It is known to biosynthesize more than 130 alkaloids (Van Der Heijden *et al*., 2004). Periwinkle contains dozens of alkaloids, among them vincristine and vinblastine are the major which plays an important role in western medicine as potent anticancer agents. Vinca alkaloids are the second-most-used class of cancer drugs and will stay among the original therapies (Moudi *et al.*,2013). These anticancer alkaloids are expensive (Fonttanel and Tabata 1986). Vinbalstine has been extensively used in combination with cis platinum and bleomycin to treat testicular and ovarian cancers (Pratt, 1994).

Various breeding methods are followed to improve the crop growth, yield and disease resistance. Among the various type of breeding methods, induced mutation plays an important role in crop improvement. In plant breeding, induction of mutation has become an effective way of supplementing existing germplasm and improving cultivars (Micke, 1987). Production of mutants by physical and chemical mutagenesis is impartially economical. Chemicals mainly cause point mutations and ionizing radiations normally cause chromosomal rearrangements and deletions (Bhat *et al.,* 2007). Induced mutations are essential to enhance the rate of genetic variability. The normal term LD_{50} refers to the amount of material given to test organisms which causes 50 per cent death (either physical or chemical mutagens) and it is considered which high frequency of mutation in the plant. Therefore this study was undertaken to optimize the lethal dose for gamma and ethyl methane sulphonate in periwinkle cv. LOCAL.

RESOURCES AND METHODS

The present investigation was conducted at Botanical Garden, Department of Medicinal and Aromatic Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore during the year 2016. Physical mutagen (gamma ray) was employed, which was applied to seeds pre-soaked for 24 hrs in water and then subjected to exposure dosage fro 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 kR (krad) in the gamma chamber installed at the orchard of Horticultural College and Research Institute, Tamil Nadu Agricultural University Coimbatore, Similarly for EMS the dosage range from 10, 20, 30, 40, 50, 60mM. Gamma ray source was Cobalt - 60 in 1000 Ci, emitting 5000 rads per minute at the time of irradiation.

The LD_{50} values of gamma and Ethyl methane sulphonate were determined based on the probit analysis using SPSS version 17 (Finney, 1971 and 1978). Under field conditions, the observations like survival on 30th day, plant height on 30th day and at maturity (Six month approx.), were calculated for each treatment and expressed as per cent over control. The percentage in respect to germination on 15 days, survival (15 days), were analyzed and subjected to arcine transformation (Hayes *et al.*,1970). The significant difference among characters was brought out by resorting to method as proposed by Panse and Sukhatme (1967).

OBSERVATIONS AND ANALYSIS

The germination percentage and plant survival rate were found to be greatly affected by mutagenic treatments. LD_{50} is of great importance to know the sensitivity of different genotypes to the critical dose of mutagens causing 50 per cent mortality. Effects of gamma rays and EMS on seed germination and survival (observed on 15th and 30th day, respectively) are presented in the Table 1.

The response of seeds for gamma rays and EMS are presented in Table 3 and 4. In both the mutagens reduction in germination and survival was noticed, with increasing dose of mutagens. The germination percentage ranged from 28.00 to 94.00 per cent and 32.00 to 65.00 per cent compared to control (98.00 %) in physical (gamma rays) and chemical (EMS) treatments, respectively. The maximum lethality was observed in gamma 60 kR (28.00) followed by 55 kR (32.00) and 50kR (39.00), similarly in EMS the maximum lethality was observed 60 mM (32.00) followed by 50 mM (36.00) and 40 mM (42.00). The mutation effect showed a similar effect as reported by Verma *et al.* (2013) decrease with increase in the dose or concentration of mutagens.

In the present study lethal dose (LD_{50}) was determined based on survival of plants derived from radiated seeds (Fig. 1 and 2). The germination percentage of periwinkle seedlings ranged from 28 per cent (60 kR) to 94 per cent for the treatment of gamma ray(5 kR),

For EMS the seed germination percentage ranged from 32 per cent (60 mM) to 65 per cent (5 kR). The lowest survival of plants were observed at 55- 60 Kr (Table 3 and 4). The mortality of plants increased linearly with the increase of gamma ray doses. The LD_{50} of periwinkle was observed 40 kR in gamma radiation (Table 1). Subjecting the plants propagules to the higher dosage lead to the deleterious effect. The similar trend in the lethal dose was observed by Mangaiyarkarasi *et al.*, 2014 in *Cathranthus roseus*, Jayakumar and Selvaraj (2003) in sunflower, and Jabee and Ansari (2005) in chickpea.

The results showed that differences among the radiation doses considerably influenced the shoot and root length. Critical dose that prevented the root elongation

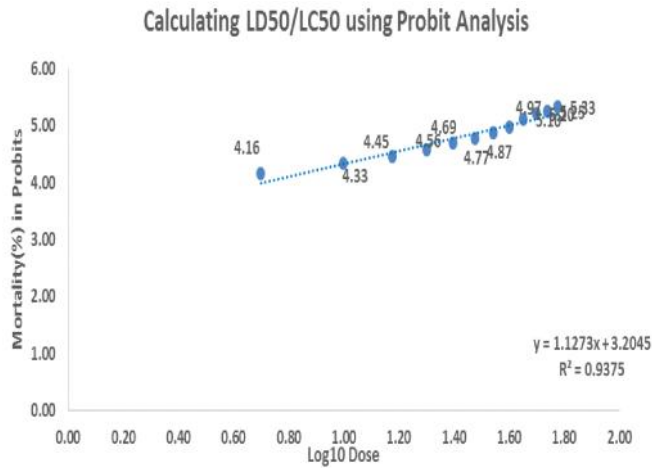

Fig. 1 : Probit analysis calculation for LD_{50} of Gamma irradiation

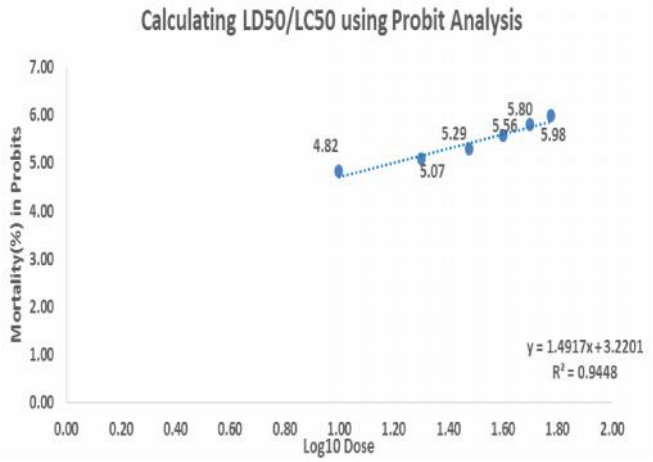

Fig. 2 : Probit analysis calculation for LD_{50} of EMS

Table 1: Probit analysis for the determination LD_{50} of gamma radiation in *Catharanthus roseus* cv. LOCAL

Treatments	No. of seeds sown	Number of seeds germinated	Mortality percentage (%)	Probit value
5 Kr	100	98	20 %	4.16
10 Kr	100	94	25 %	4.33
15 Kr	100	89	29 %	4.45
20 Kr	100	85	33 %	4.56
25 Kr	100	79	38 %	4.69
30 Kr	100	73	41 %	4.77
35 Kr	100	68	45 %	4.87
40 Kr	100	62	49 %	4.97
45 Kr	100	51	54 %	5.10
50 Kr	100	46	58 %	5.20
55 Kr	100	39	60 %	5.25
60 Kr	100	32	63 %	5.33

Table 2 : Probit analysis for LD_{50} of EMS in *Catharanthus roseus* cv. LOCAL

Treatments	No. of seeds sown	Number of seeds germinated	Mortality percentage (%)	Probit value
10 mM	100	65	35 %	4.82
20 mM	100	57	43 %	5.07
30 mM	100	50	50 %	5.29
40 mM	100	42	58 %	5.56
50 mM	100	36	64 %	5.80
60 mM	100	32	68 %	5.98

Table 3 : Effect of Gamma on germination percentage and growth parameters in *Catharanthus roseus* cv. LOCAL

Treatments	Number of seeds sown	Germination percentage (%)	Shoot length	Root length
Control	100	98.00	4.1	5.5
5 Kr	100	94.00	3.8	4.9
10 Kr	100	89.00	3.7	4.4
15 Kr	100	85.00	3.4	4.1
20 Kr	100	79.00	3.1	3.9
25 Kr	100	73.00	2.9	3.7
30 Kr	100	68.00	2.5	3.4
35 Kr	100	62.00	2.1	3.1
40 Kr	100	51.00	1.8	2.8
45 Kr	100	46.00	1.6	2.5
50 Kr	100	39.00	1.3	2.1
55 Kr	100	32.00	1.1	1.8
60 Kr	100	28.00	0.8	1.5
Mean	-	1.78	2.47	3.36
C.D. (P=0.05)	-	0.01	0.09	0.15
S.E.±	-	0.02	0.04	0.07

Table 4 : Effect of EMS on germination percentage and growth parameters in *Catharanthus roseus* cv. LOCAL

Treatments	Number of seeds sown	Germination percentage	Shoot length	Root length
Control	100	81 %	3.8	4.9
10 mM	100	65 %	3.5	4.5
20 mM	100	57 %	3.1	4.1
30 mM	100	50 %	2.7	3.6
40 mM	100	42 %	2.3	3.2
50 mM	100	36 %	2.1	2.7
60 mM	100	32 %	1.7	2.4
Mean	-	1.69	2.74	3.62
C.D. (P=0.05)	-	0.01	0.10	0.13
S.E. ±	-	0.02	0.04	0.06

varied from 0.1 to 0.5 kR. In higher radiation dose, there were the reduction in the germination comparing to non-treatment control (Chaudhuri, 2002). Subsequently seeds are treated with physical and chemical mutagens based on the survival percentage at 40 kR of gamma rays and 30 mM of EMS (Table 2). The further selection of desirable mutants at later stages based on specific traits can be used to develop trait could be useful as a selectable marker for indirect selection for several traits which are needed to be studied in periwinkle (Kulkarni and Baskaran, 2014).

Conclusion :

Therefore, the determination of lethal dose$_{50}$ by calculating the germination of seed, plant height, root length under the M_1 generation was obtained. Based on the probit analysis from survival of treated materials, the LD_{50} doses for gamma irradiation and Ethyl Methane Sulphonate were 40 kR and 30mM, respectively. The data recording to root and shoot length and germination percentage were collected and recorded and also variable means considered. Based on lethal dose obtained from the above experiment, the higher and lower dose treatment of both EMS and Gamma were subjected to raising for M_1 generation in the main field. Subsequently the selected plants and their seeds were separately transferred to M_2 generation after harvesting M_1. The M_2 generation plants were being further will be observed for variability based on qualititative and quantitative traits of major concern.

Acknowledgement :

The author is highly thankful to the Department of Medicinal and Aromatic Crops, HC and RI, TNAU Coimbatore for facilitating and the financial assistance by UGC-Fellowship for conducting research.

Authors' affiliations :

K. RAJAMANI, Department of Medicinal and Aromatic Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, COIMBATORE (T.N.) INDIA

J. SURESH, Department of Spices and Plantation Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, COIMBATORE (T.N.) INDIA

R. JOHN JOEL, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, COIMBATORE (T.N.) INDIA

D. UMA, Department of Biochemistry, Tamil Nadu Agricultural University, COIMBATORE (T.N.) INDIA

REFERENCES

Bhat, R.S., Upadhyaya ,N., Chaudhary, A., Raghavan, C., Qui, F., Wang, H., Wu, J., McNally, K., Leung, H. and Till, B. (2007). *Chemical and irradiation induced mutants and TILLING. Rice Functional Genomics*. ISBN 978-0-387-48903-2, Chapter 8. 148-180.

Bhattacharjee, R. (1998). Mutagenic effectiveness and efficiency of gamma rays ethyl methane sulphonate and nitroso-methyl urea in periwinkle, *Catharanthus Roseus. J. Nuclear Agric. & Biol.*, **27** (1):61-64.

Chatterjee, S.K. (1978). The cultivation of *Catharanthus roseus* in India: In: Alfermann AW and Reinhard E (eds), Production of natural compounds by cell culture methods. Proc. Int. Symp. Plant Cell Culture, Munchen FRG. 74-85.

Chaudhuri, S.K. (2002). A simple and reliable method to detect gamma irradiated lentil (*Lens culinaris* medik.) seeds by germination efficiency and seedling growth test. *Radiation Physics & Chem.*, **64**(2): 131-136.

Finney, D.J. (1971). *Probit analysis*. Cambridge University Press 3rd Ed., **60**(9):1432pp.

Finney, DJ. (1978). *Statistical Method in Biological Assay*. Charles Griffin & Co.

Frode, T.S. and Mediros, Y.S. (2008). Animal models to test drugs potential antibiotic activity. *J. Ethanopharmacol.*, **115** : 173-183.

Hayes, H.K., Immer, F.R. and Smith, D.C. (1970). *Methods of Plant Breeding*. Mc GrawHil Book Co., New York:171-187.

Jabee, F. and Ansari, M.Y.K. (2005). Mutagenic effectiveness and efficiency of hydrazine sulphate (HS) in indusing cytomorphological mutation in *Cicer arietinum* L. var. K. 850. *J.Cytol. Genet.*, **6**(2) : 161-166.

Jayakumar, S. and Selvaraj, R. (2003). Mutagenic effectiveness and efficiency of gamma rays and ethylmethane sulphonate in sunflower (*Helianthus annuus* L.). *Madras Agric. J.*, **90**(7-9): 574-576.

Kulkarni, R.N. and Baskaran, K. (2014). Increasing total leaf alkaloid concentrations in periwinkle (*Catharanthus roseus*) by combining the macro-mutant traits of two induced leaf mutants ('necrotic leaf' and 'nerium leaf'). *J. Hort. Sci. & Biotech.*, **89** (5) : 513–518.

Lewis, WH. and Elvin Lewis, M.P.H. (1977). Medicinal botany plants affecting mans health.John Wiley & Sons, New York, U.S.A.

Mangaiyarkarasi, R., Girija, M. and Gnanamurthy, S. (2014). Mutagenic effectiveness and efficiency of gamma rays and ethyl methane sulphonate in *Catharanthus roseus*. *Internat. J. Curr. Microbiol. App. Sci.*, **3**(5): 881-889.

Micke, A. (1987). Induced mutations for crop improvement – a review, trop. Agri.(Trinidad), **4**: 259-278.

Moudi, M., Go, R., Yien, C.Y. and Nazre, M. (2013). 'Vinca alkaloids'. *Internat. J. Prev. Med.*, **4**(11): 1231-1235.

Panse, V.G. and Sukhatme (1967). *Statistical methods for agricultural workers*. ICAR, New Delhi: pp. 83.87.

Pratt, W.B. (1994). The Anticancer Drugs. 2nded. New York: Oxford Press. Inc.p. 2189-191.

Van Der Heijden, R., Jacobs, D. I., Snoeijer, W., Hallared, D. and Verpoorte, R. (2004). The *Catharanthus* alkaloids; pharmacognosy and biotechnology. *Curr. Med. Chem.*, **11** : 607-628.

Venkateswarlu, M., Susheelamma, Kumar, B.N. and Subha, P.K. (1988). Studies on induced mutation frequency in *Catharanthus roseus* (L.) G. Don. By gamma rays and EMS individually and in combination. *Indian J. Genet.*; **48** : 313-316.

Verma, A.K., Singh, R.R. and Singh, S. (2013). Mutation breeding in *Catharanthus roseus* (L.) G. Don: An Overview *J. Pharmacognosy & Phytochem.*, **2**(1):334-337.