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Determination of mutagenic sensitivity of chrysanthemum (*Dendranthema grandiflora* Tzvelev) cv. Pusa Chitraksha to physical and chemical mutagens

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Abstract
An experiment was conducted to determine LD_{50} does of physical and chemical mutation in cv. Pusa Chitraksha. In a mutation breeding experiment, LD_{50} gives an indication of the response on different types of explants to mutagen, so that the right dose(s) for the main field experiment can be fixed to obtain beneficial mutants with minimal population loss. In the present investigation, herbaceous cuttings (7 - 10 cm long with 4- 5 nodes) of chrysanthemum cv. Pusa Chitraksha were treated with twenty one different dosages of Gamma rays and EMS ranging from 5.00 to 50.00 Gray and 0.10 to 0.50 per cent respectively. The results revealed a gradual and significant reduction based on survival rates of the treated cuttings using probit analysis as well as the growth rate in different dosage of both mutagens. All the treatments with gamma rays and EMS revealed a dose dependent negative linear relationship between dose and survival of treated cuttings. The survival percentage of variety Pusa Chitrakshain gamma irradiated population ranged from 85.00 per cent in 5 Gy to 10.00 per cent in 45 Gy among the gamma ray treatment. In case of EMS treatments, survival was ranged from 80.00 per cent in 0.10 per cent of EMS and 10.00 per cent in 0.40 per cent of EMS and none of the plants were survived in 0.45 and 0.50 percent ethyl methane sulphonate. Based on the survival of treated cuttings probit curve analysis was exhibited that, LD_{50} determined as 23.00 Gray for gamma radiation and 0.27 per cent for EMS.

Keywords: Chrysanthemum, Eethyl methane Sulphonate, gamma rays, mutagenesis, survival and sensitivity

Introduction
Chrysanthemum (*Dendranthema grandiflora* Tzvelve.) is one of the most important cut flower, loose flower and ornamental (pot and garden flower) crop in the world. The genus chrysanthemum belong to family Asteraceae and basic chromosome number is n=9. Wide variation exhibited in respect of growth, habit, size, colour and shape of bloom make the chrysanthemum suitable for every purpose for a flower crop. It is one of the most important loose flower crop grown commercially in many part of the country. Chrysanthemum is very popular and important cut flower crop grown all over the world in Japan, China, USA, France, UK, and India. In India, it is commercially cultivated in Karnataka, Maharashtra, Tamilnadu, Andhra Pradesh, and West Bengal. Flowers are used for garland making, wreath as religious offering in hall decoration etc.

The present complex genetic hetrozygosity make the cultivated chrysanthemum an unlimited source of new flower form and cultivars. Genetic variation is essential in any plant breeding programme for crop improvement. Mutation breeding is efficient way to produce heritable change particular for the flower colour. Increasing demand to new form of chrysanthemum lead to research for obtaining new varieties. Mutation techniques are used because chrysanthemum is hexaploid plant and vegetative propagated which make it difficult to conduct the hybridization (Dwimahyani and Widiarsih, 2010) [10].

The major objective of any mutation breeding programme is to obtain new and better genotype through the creation of genetic variability in the existing gene pool. Induction of mutations employing physical mutagens offers some spectacular possibilities for the improvement of chrysanthemum cultivar of commercial values. Commercially important traits in horticulture plants have been altered in as positive way by the various physical mutagens. Among the physical mutagens, gamma rays are widely used for inducing mutations in flowering plants

due to their easy application and high efficiency. However, chemical mutagens are not widely used in vegetative propagated plants due to their low penetration into plant tissue. Induced mutations have attributed to the development of new cultivars of chrysanthemum.

The main advantage of mutagenesis in chrysanthemum is the ability to change one or a few characters of an excellent cultivar without changing rest of the genotype. The gamma rays have been used effectively for induction of mutation in chrysanthemum and the optimum dose range from 1.0 to 3.0 Krads depending upon the genotypes (Dilta *et al*., 2003) [9]. While going for mutation breeding programmed various factors like choice of material, character to be improved, type of mutagens and its dose to be used, experimental procedure to be chosen should be considered. Thus through mutation breeding it is possible to induce a genetic variation for quantitative and qualitative characters that is heritable of sufficient magnitude and frequency of interest in the breeding program.

Induced mutagenesis is an essential plant breeding tool which involved in the generation of allelic variants of genes that modulate the expression of traits. Both spontaneous as well as induced mutations have played an important role in origin of many new forms of chrysanthemum and many of the present day cultivars are the end result of them. Many mutagens can be employed to induce mutagenesis in this plant. Mutation breeding, which leads to altered phenotypes after permanent heritable change in the structure of the genetic material (Rego and Faria, 2001) [25], is now established as a time-saving and inexpensive approach for flower improvement (Datta and da Silva, 2006) [7].

However, mutation breeding by physical mutagens has been extensively used for the development of new cultivars with improved characteristics in vegetatively propagated ornamental plants (Kumar *et al.* 2006) [17]. The frequency and saturation of mutations can be regulated by varying the mutagen dose (Jander *et al.* 2003; Menda *et al.* 2004) [14, 24] and mutagenic agents can induce different extensions of genomic lesions, ranging from base mutations to larger fragment insertions or deletions (MacKenzie *et al.* 2005; Kim *et al.* 2006) [22, 19]. Gamma irradiation is considered to be more suitable for obtaining mutants with less radiation damage (Yamaguchi *et al*. 2010) [29]. In case of chemical mutagens, alkylating agents such as mustard gas, Methyl Methane Sulphonate (MMS), Ethyl Methane Sulphonate (EMS) and nitroso guanidine have been reported to have several effects on DNA (Anon, 1977) [22]. However, EMS is the most commonly used chemical mutagen in plants because of its potency and ease with which it can be used. The basic requirement for an effective use of any mutagen is to analyze its efficacy and the effective dose for inducing viable mutation. Chemical mutagens produced point mutations, whereas radiations normally caused chromosomal aberrations and deletions and EMS is alkylates guanine bases and leads to mispairing of alkylated G which pairs with T instead of C, resulting in primarily G/C- to-A/T transitions (Bhat *et al.* 2007) [4].

The success of mutation breeding greatly depends on the rate of mutation, the number of screened plants and the mutation efficiency. The mutation rate is affected by the total dose of the mutagen employed and can be modified by physical and biological factors. Higher doses inevitably bring about mortality, high pollen and seed sterility and deleterious mutations (Amenorpe *et al*. 2004) [1]. To avoid excessive loss of actual experimental materials, radio-sensitivity tests must be conducted to determine LD_{50} (the safe dose at which half of the planting materials survive) doses before massive irradiation of similar materials are accepted.

Since there are differences in radiation tolerance among species (Sparrow, 1966) [26] and even among genotypes of the same species (Kwon and Im, 1973) [20], the present study was performed to fix the LD_{50} of gamma radiation and EMS in the chrysanthemum *cv*. Pusa Chitraksha.

Materials and methods

The experimental material was obtained from the chrysanthemum germplasm collection maintained in Division of Floriculture, IARI, New Delhi. Chrysanthemum cv. Pusa Chitraksha, the variety released from IARI, with magenta coloured spray type cultivar was used in the present explants. The plants are vigorous growing suitable for potted plant and garden display. Herbaceous cuttings (7 - 10 cm long with 4- 5 nodes) of chrysanthemum cultivars 'Pusa Chitraksha' were employed as biological material were treated with eleven different doses of gamma radiation (0.00, 5.00, 10.00, 15.00, 20.00, 25.00, 30.00, 35.00, 40.00, 45.00, 50.00 Gy) including control. Gamma irradiation was given using ^{60}Co gamma source (Gamma Chamber 1200, Board of Radiation and Isotope Technology (BRIT), Mumbai, India) at Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. In case of chemical mutagenesis, uniform sized herbaceous cuttings were soaked soaked in EMS solution (freshly prepared in phosphate buffer at pH 7.0) of different concentrations *viz*., 0.00 (Control), 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, 0.50 for 3 hours. After incubation, cuttings were thoroughly rinsed with running tap water for 1 hour to wash out the chemical residues. The above dosages of Gamma irradiation and concentrations of EMS were chosen for preliminary experiments. Twenty cuttings for each treatment were planted in polythene bag filled with red soil: FYM: sand (2:1:1) along with untreated cuttings as control to arrive at semi-lethal dose (LD_{50}) based on survival percentage as well as to study the effect of mutagen on morphological traits were measured after 30 days of planting. The experiment was organized as completely randomized design (CRD) with three replicates. The LD_{50} value was calculated based on probit analysis for both the mutagens using the survival percentage (30 days after planting) and mortality per cent of treated cuttings to that of control.

Probit analysis

The LD_{50} values of gamma radiation and EMS were determined based on the probit analysis (Finney, 1971, 1978) [11, 12]. The probit function is the inverse cumulative distribution function (CDF) or quantile function associated with the standard normal distribution.

Results and Discussion

Data pertaining to the response of chrysanthemum genotype Pusa Chitraksha to gamma irradiation and EMS treatments in relation to survival on 30th days after planting are presented in Table 1. All the treatments with gamma rays and EMS revealed a dose dependent negative linear relationship between dose and survival of treated cuttings.

In gamma-ray treatment, a survival rate varies from 90.00 to 0.00 per cent, the highest (85.00 %) survival per cent was recorded 5.00 Gy and lowest (10.00 per cent) was recorded in 45.00 Gy. Survival over reduction (5.56 per cent) was observed in 5.00 Gy and highest reduction (100.00 per cent)

was observed in 50.00 Gy. Dose rate is also an important factor in gamma ray treatment, and its effect has been investigated by evaluating various responses including lethality (Sripichtt *et al*. 1988) [28], growth (Bottino *et al.* 1975 and Yamashita 1964) [5, 30]. Where as in EMS it was observed that there was complete inhibition of survival of cuttings in 0.45 per cent concentrations and above. The highest survival (80.00 per cent) was observed in 0.10 per cent and the lowest (10.00 per cent) of survival recorded in 0.40 per cent concentration among the treatments except control. Based on reduction over control 15.79 per cent was observed in 0.10 per cent concentrations and 100.00 per cent in 0.50 per cent concentration. But when considering these two mutagens, higher diminution of survival percentage was noticed in case of gamma irradiation than EMS.

Significant reduction in survival after exposure to gamma rays was also observed by Kapadiya *et al.* (2014) [16] in chrysanthemum variety 'Maghi' and Kumari *et al.* (2013) [18] reported similar results while treating rooted cuttings of chrysanthemum variety 'Otome Pink' with gamma rays and EMS in which the minimum survival (28.33 %) was noted with 0.04 % of EMS. Reduction in survival after exposure to gamma rays was explained due to inactivation and/or decrease in auxin content that affect cell division, it resulting in poor establishment and survival (Gordon, 1957; Mahure *et al.* 2010) [13, 23] or lethal effect of gamma rays caused due to chromosomal aberration (Datta and Banerji, 1993) [6]. Dilta *et al.* (2003) [9] reported that higher concentrations of EMS reduced the plant survival per cent in chrysanthemum.

Mortality per cent in respect to gamma rays and EMS treated population of cultivar Pusa Chitraksha are presented (Table 2 and 3) based on probit analysis to assess LD_{50} value. Sensitivity tests must be conducted to determine LD_{50} which is the safe dose at which half of the planting materials survive with maximum recovery of plant materials. LD_{50} is of great importance to know the sensitivity of the genotype to the critical dose of the mutagen causing 50 percent mortality. LD_{50} values were determined with the help of probit analysis based on the survival rate of the stem cuttings after treatment with different dosages/concentrations of gamma rays and EMS compared with untreated control to avoid excessive loss of actual experimental materials. In the present study, LD_{50} value for gamma rays as assessed from the probit curve analysis (Fig. 1) was 23.00 Gy. The gamma rays being more potent and highly penetrating in nature might have impacted the cells undergoing meiotic division in the bud region (Deshpande *et al.* 2010) [8]. But in case of EMS treatment, 0.27 per cent concentration assessed from the probit curve analysis (Fig. 2) was fixed as a LD_{50}. Lamseejan *et al.* (2000) [21] studied the effect of gamma rays on purple color clone of spray type chrysanthemum and the LD_{50} dose was determined as 14 Gy based on the survival of treated plants. According to Berenschot *et al*. (2008) [3] in *Petunia,* the LD_{50} corresponded to 100 Gy for gamma radiation and 0.1% for EMS treatment. The LD_{50} dose based on the reduction in survival after treatment with different doses of gamma rays and different concentration of EMS were 27.5 Gy and 122 mM for the stem cuttings of cassava cultivar H_{226} (Kanagarasu *et al.* 2014) [15].

Conclusion

Determination of LD_{50} dose is an essential pre-requisite in mutation studies, as excess dosage leading to sterility or even lethality. For induction of desired mutations by radiation/chemical treatment being by chance, it is safer to choose the doses that can cause less damage and give higher multiplication and survival rates and also produce some useful mutations, which might be not possible by lower dosage. In the present study, based on the survival and growth rates, LD_{50} dose for gamma irradiation and EMS treatment have been fixed

as 23.00 Gy and 0.27 per cent respectively. These are regarded as the optimal doses for induced mutagenesis in chrysanthemum cv. Pusa Chitraksha for the achievement of optimum mutation frequency with the least possible unintended damage.

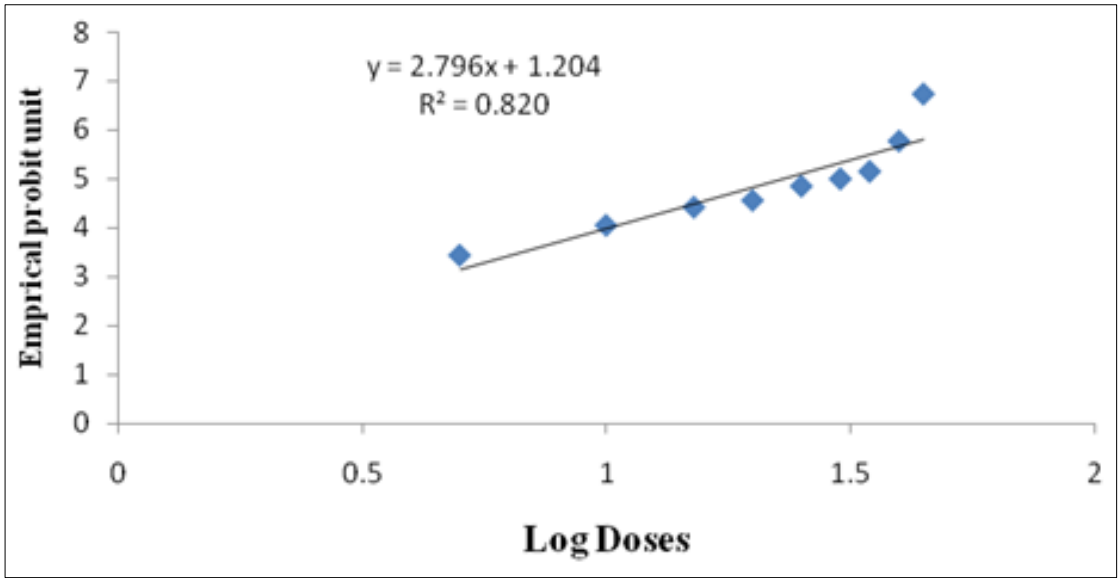

Fig 1: Probit curve for the determination of LD_{50} for gamma radiation in Pusa Chitraksha

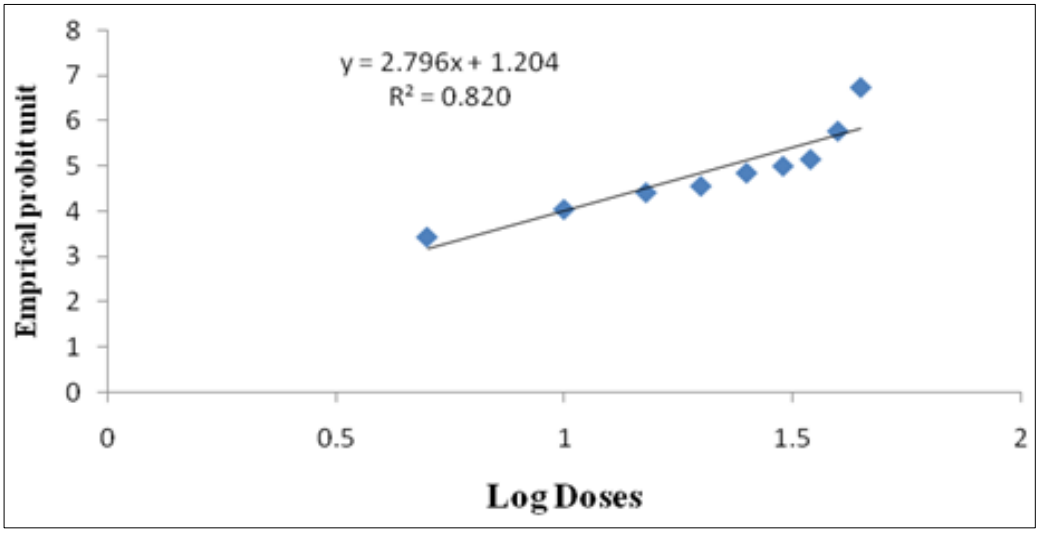

Fig 2: Probit curve for the determination of LD_{50} for gamma radiation in Pusa Chitraksha

Table 1: Effect of mutagens on survival of cuttings of chrysanthemum var. Pusa Chitraksha on 30 days after planting

Gamma rays					Ethyl Methane Sulphonate (EMS)				
Dose (Gy)	**Survival (nos.)**	**Survival (%)**	**% over control**	**% reduction over control**	**Concentration (%)**	**Survival (nos.)**	**Survival (%)**	**% over control**	**% reduction over control**
0	18.00	90.00	100.00	-	0.00	19.00	95.00	100.00	-
5	17.00	85.00	94.44	5.56	0.10	16.00	80.00	84.21	15.79
10	15.00	75.00	83.33	16.67	0.15	13.00	65.00	68.42	31.58
15	13.00	65.00	72.22	27.78	0.20	10.00	50.00	52.63	47.37
20	12.00	60.00	66.67	33.33	0.25	8.00	40.00	42.11	57.89
25	10.00	50.00	55.56	44.44	0.30	7.00	35.00	36.84	63.16
30	9.00	45.00	50.00	50.00	0.35	4.00	20.00	21.05	78.95
35	8.00	40.00	44.44	55.56	0.40	2.00	10.00	10.53	89.47
40	4.00	20.00	22.22	77.78	0.45	0.00	0.00	0.00	100.00
45	2.00	10.00	11.11	88.89	0.50	0.00	0.00	0.00	100.00
50	0.00	0.00	0.00	100.00					

(Number of cuttings treated per treatment = 20)

Table 2: Probit analysis for calculating LD_{50} for gamma rays in chrysanthemum var. Pusa Chitraksha on 30 days after planting

Dose (Gy)	**Log_{10} of doses**	**Survival (nos.)**	**Survival (%)**	**% survival over control**	**% reduction over control**	**Observed mortality %**	**Corrected mortality %**	**Emprical probit unit**	**LD_{50} Value**
0	0	18.00	90.00	100.00	-	10	0	0.00	
5	0.70	17.00	85.00	94.44	5.56	15	6	3.44	
10	1.00	15.00	75.00	83.33	16.67	25	17	4.05	
15	1.18	13.00	65.00	72.22	27.78	35	28	4.42	
20	1.30	12.00	60.00	66.67	33.33	40	33	4.56	
25	1.40	10.00	50.00	55.56	44.44	50	44	4.85	**23 Gy**
30	1.48	9.00	45.00	50.00	50.00	55	50	5.00	
35	1.54	8.00	40.00	44.44	55.56	60	56	5.15	
40	1.60	4.00	20.00	22.22	77.78	80	78	5.77	
45	1.65	2.00	10.00	11.11	88.89	90	89	6.73	
50	1.70	0.00	0.00	0.00	100.00	100	100	0.00	

(Number of cuttings treated per treatment = 20)

Table 3: Probit analysis for calculating LD_{50} for EMS in chrysanthemum var. Pusa Chitraksha on 30 days after planting

Dose (%)	**Log_{10} of doses**	**Survival (nos.)**	**Survival (%)**	**% survival over control**	**% reduction over control**	**Observed mortality %**	**Corrected mortality %**	**Emprical probit unit**	**LD_{50} Value**
0.00	0.00	18	90	100	-	10	0	0.00	
0.10	2.70	18	90	100.00	0.00	10	0	0.00	
0.15	3.18	15	75	83.33	16.67	25	17	4.05	
0.20	3.30	12	60	66.67	33.33	40	33	4.56	
0.25	3.40	10	50	55.56	44.44	50	44	4.85	**0.27 %**
0.30	3.48	9	45	50.00	50.00	55	50	5.00	
0.35	3.54	6	30	33.33	66.67	70	67	5.44	
0.40	3.60	4	20	22.22	77.78	80	78	5.77	
0.45	3.65	2	10	11.11	88.89	90	89	6.23	
0.50	3.70	0	0	0.00	100.00	100	100	0.00	

(Number of cuttings treated per treatment = 20)

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