Research Paper

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Determination of Lethal Dose For Gamma Rays and Ethyl Methane Sulphonate Induced Mutagenesis In Cassava (*Manihot Esculenta Crantz.*)

Agriculture

KEYWORDS : Lethal dose, gamma irradiation, ethyl methane sulphonate, survival, cassava.

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ABSTRACT

Chemical and physical mutagenesis has been used to raise the genetic variation in crop plants. Chemical mutagens such as EMS, MMS and sodium azide and physical mutagens such as Gamma rays, X-rays and fast neutrons have been broadly used to cause a majority of practical variations in several crops. In this research, an attempt was made to find out the effects of gamma ray and EMS on survival, shoot length, leaf length and width to identify the Lethal Dosage (LD) in cassava. The stem cuttings of potential genotype of the popular cassava cultivar H226 was exposed to different doses of gamma radiations (10-100 Gy) using 60Co as the radiation source. Also the cuttings treated using EMS with concentrations (25-200 mM). Based on the probit curve from survival of treated material the LD50 dose/concentration for Gamma rays and EMS were 27.5 Gy and 122 mM respectively. The increase in concentration of EMS, a decrease in survival, shoot length, leaf length and width was observed. In addition, shoot length, leaf length and width decreased with the increase in Gamma Dose and an abnormal decrease in survival was observed. The Lethal Dose was determined by different measurements on treated material compared to untreated control. The effective dose observed based on the reduction of growth parameters after treatment that was between 20 and 30 Gy of gamma exposure and between 75 and 125 mM in the EMS mutagenesis for the cassava cultivar H226.

INTRODUCTION

Mutation breeding has been widely used for the improvement of potential traits of various crop plants. The prime strategy in mutation breeding was to upgrade the well-adapted plant varieties by altering one or two major agronomic metrical traits which limit their productivity or enhance their quality and potential source of creating variability (Novak and Brunner, 1992). It is a powerful and effective tool and being used to study the nature and function of genes which are the building blocks and basis of plant growth and development, thereby producing raw materials for genetic improvement of economic crops (Adamu and Aliyu, 2007). Mutation induction offers significant increase in crop production (Kharkwal and Shu, 2009) and the possibility of inducing desired attributes that either cannot be found in nature or have been lost during evolution.

The role of mutation breeding in increasing the genetic variability for desired traits in various crop plants have been proved beyond doubt by a number of scientists (Tah, 2006; Adamu and Aliyu, 2007; Khan and Goyal, 2009; Kozgar *et al*., 2011; Mostafa, 2011). Mutagenic agents have been used to induce useful phenotypic variations in plants for more than seventy decades (Anitha *et al.* 2005) and more than 2543 mutant cultivars from 175 plant species have been officially released in 50 countries all over the world (Chopra, 2005; Bhat *et al*., 2007). In any mutation breeding programme, selection of an effective and efficient mutagen is very essential to produce high frequency of desirable mutation. Several factors such as properties of mutagens, duration of treatment, pH, pre and post treatment and temperature etc. influence the effect of mutagens.

Mutations are induced by physical and chemical mutagen treatment of both seed and vegetative propagated crops. The mechanism of mutation induction is that the mutagen treatment breaks the nuclear DNA and during the process of DNA repair mechanism, new mutations occur randomly and are heritable. It is a simple, efficient, rapid and cheap option for obtaining desired genotypes from recalcitrant species. Chemical mutagens and ionizing radiation have been used for long time as the plant mutagens in breeding research and genetic studies (Guenet, 2004). Production of mutants by chemical or irradiation mutagenesis is fairly economical. Chemicals cause mainly point mutations, therefore are perfect for production of missense and nonsense mutations. However, ionizing radiations normally cause chromosomal rearrangements and deletions (Bhat *et al*., 2007). The use of physical and chemical mutagens help to improve many traits of agronomical importance in major crops such as wheat, rice, barley, cotton, cassava and beans (Hassan, 1986; Ahloowalia and Maluszynski, 2001; Amenorpe, 2010). Induced mutations are necessary to enhance the rate of genetic variability. A specific advantage of mutation induction is to develop a range of mutant lines and identify trait specific genes in order to set up molecular gene database and study molecular functional genomics for future to develop plant variety to grow the existing arable land under climate change to feed rapid human population growth (Albokari *et al.,* 2012).

Alkylating agents such as mustard gas, methyl methane sulphonate (MMS), ethyl methane sulphonate (EMS), and nitroso guanidine have several effects on DNA. Among the alkylating agents, EMS is the most commonly used chemical mutagen in plants. EMS alkylates guanine bases and leads to mispairing-alkylated G pairs with T instead of C, resulting in primarily G/C to A/T transitions (Bhat *et al*., 2007). Therefore, EMS may likely be the mutagen of choice for TILLING (Target Induce Local Lesion in Genome) in plants. However, the toxicity of EMS may vary depending on the species and other mutagens or post-treatments with antioxidants may be worth considering (Henikoff and Comai, 2003). The dose assessment for chemicals is determined by varying the concentration and duration of treatment, solvent used or pH of the solution (Jain, 2010).

Gamma irradiation (from a 60Co source) accounts for 61% of more than 200 direct-use mutant varieties released in Japan (Hitoshi, 2008). In rice it was successfully attempted to extensively induce low amylose content for a better taste and more adhesive, moist and soft (Sowbhagya *et al*., 1987). The LD_{50} for cassava stem cuttings was determined in previous research as 32 Gy (Amenorpe *et al*., 2004). 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. 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 material survive) doses before massive irradiation of similar materials are accepted. Lethal dose, the percentage of test organisms

that killed by a specific dosage (of chemicals or radiation), half will die at LD_{50} and is considered as a dose at which highest frequency of mutation occurs. The present study is aimed at determining the optimum lethal dose (LD_{50}) for two mutagens Gamma rays as physical mutagen and EMS as chemical mutagen in cassava.

MATERIAL AND METHODS

The commercial cultivar H_{226} was chosen for inducing mutations which is derived from a cross between Ethakkakaruppan x Malaya M_4. It has a semi-branching type with medium cooking quality having 10 months duration. The cultivar was released in 1971 and has been widely grown for industrial uses in Southern states of India especially Kerala and Tamil Nadu. The yield potential is about 30-35 tonnes per hectare. A chemical mutagen, Ethyl Methane Sulphonate (EMS) and a physical mutagen, gamma rays (^{60}Co) were used in the present investigation to induce mutations in the selected plant material and to achieve genetic variability.

The Dry, uniform stem cuttings of cassava hybrid H_{226} irradiated with 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 Gy of gamma rays. Gamma irradiation was conducted 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, Tamil Nadu, India. Also for EMS mutagenesis, healthy and mature stem cuttings were selected for their uniformity in size and soaked in distilled water for 8 hours. Water was decanted and dry in shed for 6 hours. Fresh solution of Ethyl Methane Sulphonate (Hi-media, Mumbai) was prepared in phosphate buffer at pH 7.0 in different concentrations (25, 50, 75, 100, 125, 150, 175 and 200 mM). Cuttings were incubated for 4 hours at room temperature followed by decanting of the EMS and rinsing with 0.1 M sodium thiosulphate. Finally rinse with running tap water for 1 hour to wash out the chemical residues. Based on the Gamma radiation and EMS mutagenesis, ten cuttings were planted for each treatment in polythene container filled with red soil: FYM: sand (1:1:1) with untreated cuttings as control. The percentage survival, shoot length, leaf length and breadth were measured after four weeks of planting. The experiment was organized in triplicate. Data obtained was subjected to analysis of variance (ANOVA) at the significant level of 5% ($\alpha \leq 0.05$) using AGRIS software. When statistical differences were found, the least significant difference (LSD) was used to compare means at the 5% significance level.

RESULTS AND DISCUSSION

Determination of Lethal dose

The prime strategy in mutation breeding has been to upgrade the well-adapted plant varieties by altering one or two major agronomic metrical traits which limit their productivity or enhance their quality. The success of mutation breeding greatly depends on the rate of mutation, the number of screened plants and the mutation efficiency. 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 material survive) doses before massive irradiation of similar materials are accepted. In the present study, popular and well adapted cassava genotype, H_{226} was chosen to study the effect of physical mutagen Gamma rays (Fig. 1) and chemical mutagen EMS on various parameters *viz.,* survival, shoot length, leaf length and width. LD_{50} values were determined with the help of probit analysis based on their survival rate of the stem cuttings after treatment with different doses/concentration of Gamma rays and EMS compared with untreated control. Optimum dose is the dose that cause maximum of mutation with minimum of damage to the plant. In the present study, from the probit curve analysis the LD_{50} value for Gamma rays and EMS were 27.5 Gy and 122 mM respectively (Fig. 2 and 3).

Figure 1. Effect of Gamma rays on survival of cassava cuttings

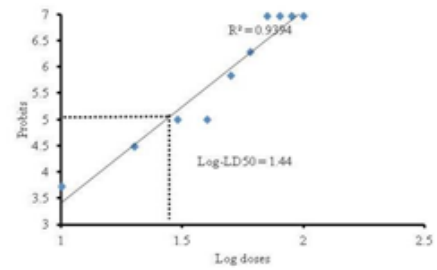

Figure 2. Plot of log-doses versus probits for calculation of LD_{50} in cassava for gamma irradiation

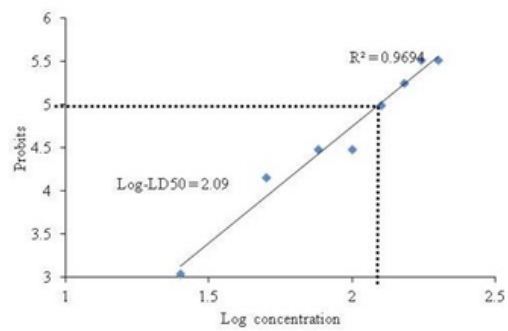

Figure 3. Plot of log-doses versus probits for calculation of LD_{50} in cassava for Ethyl methane Sulfonate

Asare and Safo-Kantanka, (1997) was given a series of irradiations in cassava cuttings and determined 25 and 30 Gy as appropriate doses to induce mutation in cassava. The LD_{50} for cassava stem cuttings was determined in previous research as 32 Gy (Amenorpe *et al.*, 2004). In concert with a previous study on radiation mutation (Kiong *et al.*, 2008), survival of plants to maturity depends on the nature and extent of chromosomal damage.

Impact of Mutagenesis on survival, shoot length, leaf length and width

Survival percentage of H_{226} under different dose concentrations were calculated based on the survival of cuttings after treatment and compared with control. There was an abnormal reduction in the survival of stem cuttings with the raise of Gamma dose. Conversely, there was an increase the survival reduction in higher doses compared to lower doses (Table 1 and Fig. 4).

Table 1. Mean value of survival, shoot length, leaf length and width in gamma irradiation

Treatment (Gy)	Survival		Shoot length		Leaf length		Leaf width	
	Actual	% of control	Actual	% of control	Actual	% of control	Actual	% of control
Control	10	100	15.23^a	100	5.43^a	100	2.10^a	100
10	9	90	11.74^{ab}	88.75	4.10^b	75.52	1.70^b	80.95
20	7	70	9.09^{bc}	68.64	3.40^c	62.63	1.31^c	62.38
30	5	50	7.57^{bc}	57.23	3.40^c	62.63	1.12^d	53.33
40	5	50	7.21^c	54.51	2.79^d	51.39	1.09^d	51.91
50	2	20	5.59^d	42.26	2.70^d	49.73	0.93^e	44.29
60	1	10	3.72^e	28.12	2.45^{de}	45.13	1.04^d	49.52
70	0	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0	0
90	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0
CV%			23.65		11.37		6.81	

*Mean value with the same superscript are not significantly different at 5%

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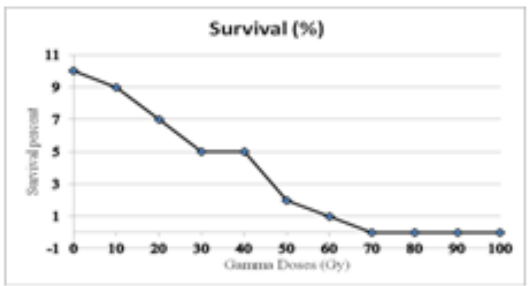

Figure 4. Effect of Different Doses Gamma Irradiation on survival

Data analysis on number of cuttings that survived showed an attendant decrease in survival significantly with applied increases in concentration of EMS. According to Figure 5 and Table 2,

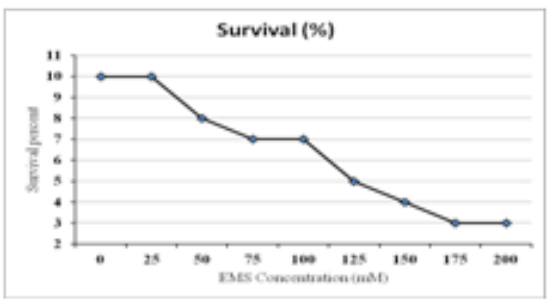

Figure 5. Effect of Different concentrations of EMS on survival

Table 2. Mean value of survival, shoot length, leaf length and width in EMS treatment

Treatment (mM)	Survival		Shoot length		Leaf length		Leaf width	
	Actual	% of control	Actual	% of control	Actual	% of control	Actual	% of control
Control	10	100	15.10^{a}	100	6.35^{a}	100	1.93^{a}	100
25	10	100	14.70^{a}	97.31	5.56^{a}	87.57	1.48^{a}	76.68
50	8	80	12.68^{b}	83.94	5.10^{ab}	80.33	1.15^{b}	59.58
75	7	70	12.15^{c}	80.43	4.25^{bc}	66.94	1.40^{a}	72.54
100	7	70	9.95^{d}	65.87	3.97^{c}	62.53	1.02^{a}	52.85
125	5	50	8.42^{e}	55.74	3.44^{c}	54.18	1.03^{a}	53.36
150	4	40	7.76^{ef}	51.37	3.16^{cd}	49.77	1.10^{a}	56.99
175	3	30	7.56^{f}	50.05	2.92^{d}	45.99	0.91^{c}	47.15
200	3	30	6.41^{g}	42.43	2.88^{d}	45.36	1.06^{a}	54.92
CV%			4.94		12.59		2.42	

*Mean value with the same superscript are not significantly different at 5%

the results obtained indicate that significant reduction in survival occurred with corresponding increase in EMS concentration. Although the reduction in survival was high in gamma radiation as compared to EMS treatment. This is evident that chemicals produce only point mutations, whereas radiations normally cause chromosomal rearrangements and deletions (Bhat *et al*., 2007). Reduction in germination inability, plant growth and survival was due to increasing frequency of chromosomal harm with increasing radiation dose or mutagen concentration (Kiong *et al.,* 2008). The lethal values (LD_{30} and LD_{50}) observed based on the reduction of survival of cuttings after treatment was between 20 and 30 Gy for gamma radiation and 75 and 125 mM for EMS.

According to results obtained in this study (Fig. 6 and 7), shoot length, leaf length and width decreased significantly in proportion with increase in applied either Gamma dose or EMS as compared to non-treatment control. The least shoot length and leaf length has been recorded when either 60 Gy of gamma dose or 200 mM concentration of EMS has been applied (Table 1 and 2).

The results showed that the differences among mutation treatments considerably influence the survival, shoot length, leaf length and width. There were a significant decrease in the level of survival, shoot length, leaf length and width with the increase of the either Gamma rays or concentration of EMS comparing to non -treatment control. Moreover, with the raise in the doses of gamma or amount of EMS mutagenesis, shoot length, leaf length and width plant height and root length approximately decreased as a liner chart (Fig. 6 and 7). Similar results of decrease in the level of survival and seedling length with increase in the Gamma dose or concentration of EMS was reported in rice (Talebi et al., 2012a and 2012b).

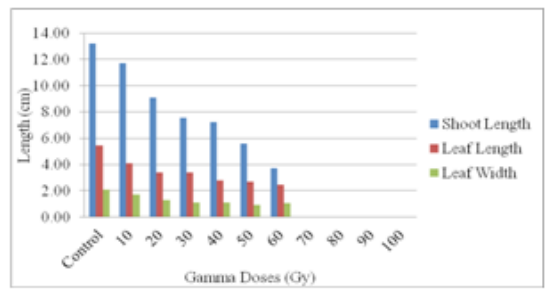

Figure 6. Effect of Different Doses Gamma Irradiation on shoot length, leaf length and width

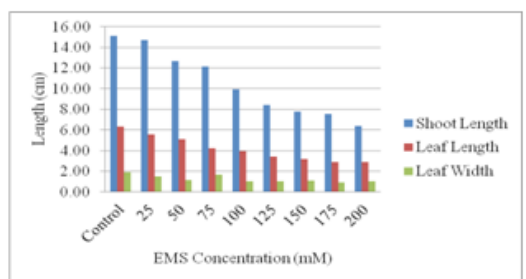

Figure 7. Effect of Different concentrations of EMS on shoot length, leaf length and width

To identify the biological influences of different physical and chemical mutagens, seedling height is mostly utilized as an index (Bhat *et al*., 2007). It has been shown that a linear dependency exists between seedling height and the dosage of physical or chemical mutagens. In concept with this observation, our findings show that decreases in shoot length were because of increases in EMS concentration or gamma radiation. The results of radio sensitivity study were confirmed by the results obtained by Wi et al., 2007.

CONCLUSION

Determination of LD_{50} value for any mutagen is necessary to produce maximum viable mutants with minimum damage to the plant. The LD50 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 cassava cultivar H_{226}. In addition, the optimum dose determined for based on the reduction in survival and growth parameters were 75-125 mM of EMS concentration and 20-30 Gy of gamma rays to creates maximum variability with minimum numbers of undesirable mutants. These optimum mutagen doses determined for the cassava genotype could be useful while formulating cassava mutation breeding programme for improvement of specific traits in cassava.

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