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Effect of gamma irradiation and ethyl methane sulphonate in annual moringa (*Moringa oleifera* L.) variety PKM-1

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Abstract
The main aim of the plant breeding is to change and improve the genetic structure of plants to meet the demands of farmers. Alternatively, breeders have been using mutation breeding to overcome the problem of low genetic variation. Gamma rays and EMS, is widely employed to induce genetic diversity on many plant species and hence they were used to induce mutations in Moringa variety PKM - 1. The observations were made on seed germination and survivability in M1 generation. The study revealed that germination percentage and survivability were decreased by increasing dose/concentration of the mutagens when compared to the untreated control. Gamma rays and EMS were effective in creating mutations with lower biological damage in Moringa and they can be used as potential mutagens that can induce desirable mutations in Moringa.

Keywords: EMS, gamma rays, LD_{50}, moringa, mutation breeding

Introduction
Moringa (*Moringa oleifera* Lam.,) is an incredible plant to mankind, because of its Pharmacognostical and Nutritionl properties. *Moringa oleifera* is shown in the scientific division belong to Kingdom: Plantae, Division: Magnoliphyta, Class: Magnoliopsida, Order: Brassicales, Family: Moringaceae, Genus: *Moringa*, Species: *M. oleifera* (Fahey, 2005) [15]. With the 13 known species, Moringa is the single genus of the family Moringaceae. (Khawaja *et. al.,* 2010) [21], and *Moringa oleifera* is the most exploited species among them. Moringa is native to India particularly foot hills of Himalayas. At present it is widely distributed in Ghana, Philippines, Nigeria, Kenya, Rwanda, Niger, Mozambique, Cambodia and Haiti are predominantly cultivating moringa and its products (Moringa India, 2015) [24]. All the plant parts of moringa has been utilized in for various purposes; the leaves are considered as nutritionally superior vegetables (Prabhakar and Hebbar, 2008) [27]. The dried moringa leaf powder is a concentrated source of nutrients and phytochemicals. About 6 teaspoon of leaf powder can fulfil woman's daily requirement of iron and calcium, during pregnancy (Titi *et al.,* 2013) [33].These leaves could be a great boon to people who do not get protein from animal source such as milk and egg. It also contains arginine and histidine, the two amino acids especially important for infants (Zaku *et al.,* 2015) [35].

Moringa with an annual production of 2.2 million tonnes of tender fruits leading to the productivity of 51 tonnes per ha. Tamil Nadu is the largest producers of Moringa with an annual production of 6.71 lakh tonnes of tender fruits from an area of 13042 ha.

Among the export of moringa products, moringa leaf /powder stands second with the export value of 1.1 billion US$ next to moringa seeds (US$ 1.6 billion). India is the largest exporter of moringa leaf powder. European countries stands first in importing the moringa leaf powder (Global Moringa meet, 2015) [24]. Since, it is a cross pollinated crop which often leads to genetic variation, it is very difficult to maintain superior qualities. Seedling trees are often large, therefore comparatively expensive to maintain in orchard, and perform any regular conventional breeding practices. In order to improve leaf yield and other polygenic characters, mutation breeding can be effectively utilized (Deepalakshmi and Ananda Kumar, 2004) [11].

Mutation breeding is one of the most effective ways of inducing genetic variability available to the plant breeder (Muhammed *et al*., 2016) [25]. The main advantage of mutational breeding is the possibility of improving one or two characters without changing the rest of the genotype (Aruna *et al.,* 2010) [6]. Artificial induction of mutation provides raw materials for the genetic improvement of economic crops (Adamu and Aliyu, 2007) [1] and also used to create genetic

variability in quantitative traits of various crop plants within the shortest possible times (Devi and Mullainathan, 2012; Aruldoss *et al*., 2015) [14, 5]. Physical and chemical mutagens were used widely for producing mutations in agricultural and horticultural crops (Bind *et. al.,* 2016) [9]. For any mutagenesis, it is very important to determine a suitable mutagen dose. Lethal dose is the percentage of test organisms that killed by a specific dosage of physical or chemicals, half will die at LD_{50}. The mutagen dose administrated should be sufficient to kill about 50 percent of the seed to obtain the maximum number of mutation. The LD_{50} used by most of the researcher to determine the lethal dose of mutagens (Anbarasan *et al.,* 2013) [3].

On the light of above facts, the present investigation was carried out to evaluate the effect of physical mutagen (Gamma rays) and chemical mutagen (Ethyl Methane Sulphonate) on seed germination and survivability of moringa. These parameters are helpful in determining the dose/concentration of mutagens for further mutation breeding programme.

Materials and Methods

Plant material

Annual Moringa variety PKM-1 was used for the present experiment. The details of the variety, including the morphological features are furnished in Table – 1.

Table 1: Description of Annual Moringa Variety PKM – 1

Characters	Description
Parentage	: Selection from a local type 'Eppothum Vendran'
Duration	: Main crop- One year, ratoon crop- two years.
Mean plant height	: 4.64m
Stem colour	: Grayish white
No. of primary branches	: 6-12
Leaves	: Tripinnate, imparipinnate, 40cm long
Inflorescence	: Auxillary raceme in cluster of 25- 150 florets.
Days to flowering	: 90-100 days
Days to maturity	: 160-170 days
Mean pod length	: 69.2 cm
Mean pod circumference	: 6.3 cm
Mean pod weight	: 158.3 g
Yield	: 220 pods/tree, 58 t/ha

Mutagenic treatments

Physical mutagen (Gamma rays) and chemical mutagen (EMS) were used in the present experiment. For physical mutagen treatment, the seed material was irradiated with different doses of gamma radiation at BARC, Mumbai. The source of Gamma irradiation used in the present study was 60Co. The doses employed were 100 Gy, 200 Gy and 300 Gy of gamma rays. Healthy, well-matured and untreated seeds were used as control. For chemical mutagen treatment, EMS ($CH_3SO_2OC_2H_5$), an alkylating agent having molecular weight 124.16 was used in the present study. For the treatment of EMS, the seeds were pre-soaked in distilled water for 6 hours in order to make them relatively more sensitive to mutagenic action. Pre-soaked seeds were treated with different concentrations of EMS (0.15%, 0.20% and 0.25%) for 4 hours with repeated stirring. After the chemical treatment, the treated seeds were washed thoroughly in running tap water to remove the residues of the chemicals. Healthy, well-matured and untreated seeds were used as control.

Observations

The M_1 generation (the treated seeds) and the control were sown in the polybag and maintained in shade net. Each treatment encompassed of 400 seeds. Observations on germination per cent and survival per cent were made. The germination per cent was worked out by observing the number of seeds germinated on 14 days after sowing from the following formula,

$$Germination\ per\ cent = Number\ of\ seeds\ germinated \times 100 / Total\ number\ of\ seeds$$

Survival per cent was calculated from the number of plants survived on 30th day after sowing by using the following formula.

$$Survival\ per\ cent = Number\ of\ plants\ survived \times 100 / Number\ of\ seeds\ germinated$$

Results and Discussion

Germination per cent

The data on seed germination for aforesaid mutagens in Annual moringa (*Moringa oleifera* L.) variety PKM -1 is given in the Table 2. The germination per cent varied from 57.5 (300Gy) to 83.5 (100Gy) per cent in gamma ray treatment and from 60 (0.15%) to 81.5 (0.25%) per cent in Ethyl methane sulphonate treatment. The percentage of seed germination was highest in lower dose of Gamma rays (100 Gy = 83.5%) and in EMS (0.15% = 81.5%). The germination percentage of all the treatments were lower than the control which fits well with the previous reports on Onion (Joshi *et al.,* 2011) [20], Grasspea (Ramezani and More, 2013) [28], *Vinca rosea* (Murugan and Dhanvel, 2015) [26], Coriander (Sarada *et al.,* 2015) [30] and Cluster bean (Deepika *et al.,* 2016) [12]. Reduction in seed germination may be due to the effect of mutagen on meristematic tissues of the radical/plumule (Deepika *et al.,* 2016) [26]. One of the physiological effects caused by treatment of these mutagens particularly chemical mutagens might be due to the disturbances in the formation of enzymes involved in the germination process (Kulkarni, 2011) [22].

The mutagen dose that causes 50% reduction in seedling viability are likely to be the most effective and efficient ones in creating mutants. The per cent reduction in germination over control ranged from 9.23 (100Gy) to 37.5 (300Gy) per cent in gamma ray treatment and from 15.10 (0.15% EMS) to 37.5 (0.25% EMS) per cent in Ethyl methane sulphonate treatment. Based on the seed germination percentage and per cent reduction in seed germination over control on the 14th day, the LD_{50} values could be fixed at doses higher than 300 Gy for Gamma rays and 0.25% for EMS since all the treatments under study were not capable of causing 50 per cent germination reduction.

Table 2: Effect of Gamma rays on Seed germination of (*Moringa oleifera* L.) variety PKM-1 at 14 days after sowing

Treatments	Germination per cent	Percent reduction over control
Gamma rays (Gy)		
Control	92	
100Gy	83.5	9.23
200Gy	75.5	17.93
300Gy	57.5	37.5
Mean	77.13	21.55
Ethyl methane sulphonate (%)		
Control	96	
0.15%	81.5	15.10
0.20%	74.5	22.39
0.25%	60	37.5
Mean	78.00	24.99

Survival percentage

Per cent plant survival on 30th day after sowing is presented in Table 3. The survival percentage ranged 51.75 (100 Gy) to 83.50 (300 Gy) per cent in Gamma ray treatment and in EMS treatments the survivability ranged from 92.00 (0.15% EMS) to 95.00 (0.25% EMS). The survivability was higher at lower doses of treatment compared with higher doses in both gamma ray and EMS treatments. All the treatments recorded lesser survival per cent than the control. The per cent reduction in survival rate over control was found to be lesser in EMS treatments (1.04 to 4.16 per cent) compared to gamma ray treatments (2.33 to 39.76 per cent) indicating the property of EMS mutagen as a point mutant that produces less biological damage. Increasing frequency of chromosomal harm with increasing radiation dose may be responsible for reduction in plant survival (Talebi *et al.*, 2012) [32]. The reduction in plant survival due to the mutagenic treatments has also been reported in *Dianthus* (Rajib Roychowdhury *et al.*, 2012) [29], Ashwagandha (Bharathi *et al.*, 2013) [8], Pigeon pea (Ariraman *et al.*, 2014) [4], Garden pea (Monica *et al.*, 2016) [23] and Okra (Baghery *et al.*, 2016) [7].

Table 3: Effect of Gamma rays and EMS on Plant survival on 30th day in M_1 generation of (*Moringa oleifera* L.) variety PKM-1

Treatments	Survival per cent	Percent reduction over control
Gamma rays (Gy)		
Control	85.5	-
100Gy	83.5	2.33
200Gy	73	14.61
300Gy	51.5	39.76
Mean	73.37	18.90
Ethyl methane sulphonate (%)		
Control	96	-
0.15%	95	1.04
0.20%	93	3.12
0.25%	92	4.16
Mean	94	2.77

Conclusion and future prospects

The gamma ray and EMS treatments were successful in creating mutation in PKM 1 Annual moringa. To fix the LD_{50} value, studies involving higher doses of Gamma ray and EMS mutagens are essential. The present study provides a base to conduct future mutation breeding studies in Annual Moringa variety PKM - 1 variety at doses higher than 300 Gy for gamma rays and 0.25% for EMS.

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