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RESEARCH ARTICLE

INDUCED PHYSICAL AND CHEMICAL STUDIES IN M_1 GENERATION OF PEARL MILLET (*PENNISETUM TYPHOIDES*) (BURN.)STAPF.VAR.CO (CU)-9

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ABSTRACT

Pearl millet is an important food and feed crops grown mostly in semi-arid regions of the world. Induced mutations are highly effective in enhancing natural genetic resources and have been used in developing beneficial variations for practical plant breeding. In the present study, C(cu)-9 variety of *Pennisetum typhoides* were treated with different concentration of Gamma rays such as 10, 20,30,40,50 and 60kR and 10,20,30,40 and 50mM of EMS along with control. The present investigation was carried out to find out the LD_{50} value, 7th and 15th day seedling characters, Plant height, Days to first bloom, Number of leaves, Number of nodes, Length of earhead, Breadth of earhead, 1000 grains weight and yield per plant. The survival percentage and mean value of M_1 generation were decreased increasing doses/ Concentrations of treatment. Mean performance of different quantitative traits were better in control when compared with the treated plant.

INTRODUCTION

Pearl millet (*Pennisetum typhoides*) (Burn.)Stapf. is a drought-tolerant cereal crop used for grain and forage. It is a diploid, sexual species with large chromosomes (2n=14). Pearl millet is grown on about 31 million hectares in the world, primarily in India, Africa, United States and Australia. It is estimated that Pearl millet occupies 46% of the total millet area and represents about 40% of the total millet production in the world (Rachie and Majmudar,1980). The grain is used as food in India and Africa. This plant is used as fodder and fuel. It is usually grown in dry areas. Pearl millet grows best on light-textured, well-drained soils.

Variability in the population creates the chance of selection for desirable improvement. Induced mutagenesis can be used to create variability as the rate of spontaneous mutation is very low. The use of induced mutation has been widely accepted by plant breeders as a tool in crop improvement. The induction of mutation in plant materials can be achieved either through physical or chemical mutagens (Karim *et al*.,2008).

Ionizing radiations have been effectively utilized in inducing genetic variability in pearl millet (Smith, 1972).

Ethyl methane sulfonate (EMS) has recently received much effective mutagenic agent in higher plants known today. Studies reveal that EMS is an effective mutagen and has been used to induce genetic variability in a number of crop plants(Kumar and Rai,2005) and (Jabeen and mirza, 2002).

Induction of mutations in this crop using either Chemical or Physical mutagens (Vijay Laxmi & Rao 1960, Jagathesan 1977, Jagathesan and Ratnam 1978, Balasundaram 1981, Hrishi *et al*., 1968 and Hrishi & Marimuthamal 1968). However, simultaneous study with two types of mutagens are very rare. In this present investigation therefore, mutagenic effects of Physical (gamma rays) and Chemical (ethyl methane sulphonate) mutagens in separate treatments have been studied on pearl millet M_1 generation.

MATERIALS AND METHODS

In this research, the seeds of cultivar (*Pennisetum typhoides* (Burn.)Stapf. Var.C (u)-9 have been selected to induced mutagenesis. The seeds of C(u)-9 Variety from Tamilnadu Agricultural University, Coimbatore was used for the present study. The seeds irradiated with different doses (10, 20, 30, 40, 50 and 60kR) of gamma rays from ^{60}CO from The Sugarcane Breeding Institute, Coimbatore. For EMS treatment, healthy seeds were treated with different concentrations of (10, 20, 30, 40 and 50mM). The treated seeds were carefully removed from the solution and they were thoroughly washed in tap water for two to three times. Untreated dry seeds were presoaked in distilled water for 4 hours and used as control.

Raising M_1 generation

For raising M_1generation, the seeds were treated with different doses/concentrations of Gamma rays and EMS were sown along with controls at the Botanical garden of Botany Department, Annamalai University, Annamalai nagar in a complete Randomized Block Design (CRBD). The spacing was maintained at 30cm (Plant to plant in a row) and 15cm (between the rows) in the field. The panicle was harvested separately and randomly from healthy individual of M_1 plants. Germination of seeds was observed and investigated the differences in average of all tested parameters between treatment and non- treatment plants. 7th and 15th day seedling characters of Root length and shoot length, Days to first bloom, Plant height ,Number of leaves, number of nodes,

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Table 1 Mutagenic effect of gamma rays and EMS on seed germination, plant survival on 7th and 15th day shoot and root length

Mutagen	Treatments	Percentage of seed germination 7th day	Plant survival at 30th day	7th day shoot length mean± SE	7th day root length mean± SE	15th day shoot length mean± SE	15th day root length mean± SE
Control	-	92	88	13.05±0.71	6.70±0.45	19.58±0.83	7.69±0.46
Gamma Rays	10kR	76	74	12.98±0.71	6.38±0.44	18.13 ±0.89	7.33±0.43
	20kR	48	48	11.75±0.54	6.14±0.43	16.52±0.83	7.09±0.48
	30kR	42	40	11.69±0.70	5.82±0.43	16.17±1.06	6.77±0.44
	40kR	36	32	9.61±0.59	5.47±0.33	14.20±0.66	6.49±0.46
	50kR	30	30	8.81±0.44	5.02±0.27	13.54±0.72	6.14±0.39
	60kR	24	20	7.08±0.42	4.59±0.32	12.56±0.67	5.80±0.35
EMS	10mM	78	72	11.81±0.55	6.08±0.38	17.86±0.76	7.29±0.38
	20mM	68	68	11.43±0.62	5.78±0.37	16.74±0.87	6.95±0.41
	30mM	50	50	9.52±0.66	5.45±0.38	15.07±0.87	6.61±0.43
	40mM	38	34	8.45±0.27	4.89±0.29	12.17±0.66	6.12±0.53
	50mM	30	26	7.03±0.54	4.36±0.36	11.07±0.80	5.28±0.33

Table 2 Mutagenic effect of gamma rays and EMS on Days to first bloom, Plant height, Number of leaves, number of nodes, Length of ear head, Breadth of ear head, 1000 grains weight and yield per plant

Mutagen	Treatments	Days to first Bloom	Plant height(cm)	Number of leaves	Number of nodes	Length of ear head(cm)	Breadth of earhead(cm)	1000 grains weight(gm)	Yield per plant(gm)
Control	-	44.75±0.21	198.04±6.86	8.05±0.50	10.05±0.59	30.86±1.58	6.77±0.48	10.43±0.71	11.34±0.45
Gamma rays	10kR	46.25±0.22	195.40±13.09	7.80±0.47	8.60±1.02	28.12±1.63	6.52±0.35	10.34±0.57	10.64±0.45
	20kR	49.20±0.41	172.78±7.47	6.70±0.41	8.10±0.63	26.89±1.81	6.47±0.36	10.13±0.50	10.40±0.35
	30kR	49.55±0.45	164.95±7.66	6.60±0.33	8.00±0.55	26.41±1.81	5.99±0.34	9.41±0.33	10.05±0.34
	40kR	51.45±0.53	149.80±5.78	6.10±0.45	7.00±0.55	23.92±1.15	5.84±0.33	8.95±0.56	9.64±0.24
	50kR	52.45±4.75	149.54±8.11	5.75±0.47	6.30±0.53	22.54±2.44	5.67±0.35	8.30±0.46	9.42±0.27
	60kR	55.05±0.52	147.27±7.80	5.20±0.28	5.90±0.39	20.95±1.34	5.54±0.40	7.25±0.37	8.89±0.36
EMS	10mM	48.45±0.45	190.37±9.95	7.65±0.54	8.05±1.00	28.40±1.68	6.56±0.38	10.31±0.57	10.52±0.26
	20mM	50.60±0.43	166.30±7.07	6.70±0.55	7.45±0.70	26.88±1.87	6.20±0.41	9.97±0.42	10.01±0.30
	30mM	51.65±0.39	154.34±9.01	6.15±0.38	7.40±0.34	26.34±1.43	5.93±0.51	9.70±0.46	9.83±0.35
	40mM	52.55±0.29	147.39±7.33	5.25±0.40	6.40±0.37	23.03±1.49	5.70±0.42	8.39±0.56	9.83±0.33
	50mM	55.50±0.27	133.33±8.79	4.85±0.38	5.45±0.44	21.61±1.47	5.29±0.38	7.15±0.41	9.11±0.30

Length of earhead, Breadth of earhead, 1000 grains weight, Yield per plant. The data's were analyzed by using NPRC software.

RESULTS

Effect of Gamma rays and EMS mutagenesis on Germination

The increase in concentration of EMS and Gamma rays the decrease in germination was observed in M_1 generation as well as the increase above the non- treatment control.

Effect of Gamma rays and EMS on Root length and seedling height

Seedling height and Root length decreased with the increase in Gamma rays and EMS. According to results obtained seedlings height and root length decreased in the proportion with increase in applied Gamma rays and EMS concentrations. In this research, root length decreased after increasing concentrations of Gamma rays and EMS as compared to non-treatment control. In 7th and 15th day root length was maximum (4.9 to 8.0) and minimum (5.9 to 9.0) when induced with 60kR and 50mM concentration of Gamma rays and EMS as compared to non- treatment control in 7th day (11.02 to 16.0) and 15th day (16.7 to 24.5) maximum reduction in shoot length was observed after mutagenesis(Table- 1) when induced with (60kR and 50mM) concentration of Gamma rays and EMS.

Days to first flowering

Days to first flowering showed different effect with different doses of Gamma rays and EMS as compared to the non-treatment control.

This character ranged from control 40 to 48days. Minimum reduction ranged from (41 to 50) in 10kR and maximum reduction ranged (45 to 60) in 60kR gamma rays and maximum reduction ranged (42 to 57) in 10mM and minimum reduction ranged from (46 to 64) in 50mM EMS (Table- 2).

Delayed flower was observed in higher doses/ concentrations of both EMS and Gamma rays. These findings showed that the mutagens can change the flowering time of the plants.

Plant height (cm)

The plant height was observed higher in control when compared to the treated plants. The plant height was ranged from 176.2 to 224.4 cm in control(Table- 2). In gamma treated plant, the maximum height was observed in 10kR (161.6 to 220.3) and minimum was observed in 60kR (109.0 to 167.1). In EMS the plant height was observed between (156.3 to 102.0). In higher doses/ concentrations of both Gamma rays and EMS plant height was decreased as compared to the non-treatment control. In EMS, at highest concentrations the plant shows stunted growth.

Number of leaves

The number of leaves was observed higher in control when compared to the treated plants. The number of leaves was ranged between 7 to 11 numbers in control. In gamma treated plant, the maximum number of leaves was observed in 10kR (7 to 10) and minimum was observed in 60kR (4 to6).

In EMS the number of leaves observed in 10mM (6 to 10) and minimum was observed in 50mM (4 to6). In higher doses/ concentrations of both gamma rays and EMS number of leaves is decreased as compared to the non-treatment control.

Number of nodes

The number of nodes was ranged between (9 to 13) number in control. In gamma treated plant, the number of nodes observed between (8 to 12) and minimum (5 to 7). In EMS treated plant, the number of nodes observed between 8 to 11 and 4 to 8 (Table- 2).

Length of earhead (cm)

The highest length of earhead 27.8 to 38.0 has been observed among the control group. Length of earhead reduced during EMS- induced mutagenesis (40 and 50mM) and gamma rays 60kR. The medium length of earhead was observed (22.0 to 35.6) in 20kR gamma rays and 20.0 to 33.9 was observed in 30mM EMS (Table-2).

Breadth of earhead (cm)

Breadth of earhead has been similar range in M_1 generation with increasing radiation dosage of 30, 40, 50 and 60kR when compared to control (Table- 2). In EMS, 10mM treated populations, the breadth of earhead was observed maximum between 5.3 to 8.1. The minimum breadth of earhead was observed in 50mM (3.7 to 6.6).

1000 grains weight (gm)

The range of 1000 grains weight was observed 7.500 to 12.662 gm in control. In gamma rays maximum grains was observed in 10kR (7.440 to 11.729) and minimum was observed in 60kR (5.945 to 9.040). 20mM and 30mM of EMS treated plants showed statistically similar results through the values were different. In higher doses/ concentrations of both gamma rays and EMS, grains weight was decreased as compared to the non- treatment control.

Yield per plant (gm)

Mean yield per plant in M_1 generation has shown shift towards negative direction in treated population. In higher doses / concentration of both gamma rays and EMS yield per plant was decreased as compared to the non- treatment control. The mean value observed 11.34±0.45 in control and the higher mean value was observed in 10kR (10.64±0.45). The lowest mean value was observed in 60kR (8.89±0.36) gamma rays. In EMS yield per plant, highest mean value was observed in 10mM (10.52±0.26) and lowest (9.11±0.30) was observed in 50mM EMS (Table- 2).

DISCUSSION

The results showed that there was a significant difference among seedling height and root length in gamma rays in EMS treated plants. In the germination test, significant differences were not observed. But, there was a significant decrease in the level of germination, seedling height, root length and plant growth under field condition with the increased concentration of EMS and gamma rays comparing to non- treatment control. In this survey, the results of gamma irradiation was confirmed with the finding of germination test has done by Borzuei *et a l*., 2010.

The reduction in germination percentage might have been due to the effect of mutagens on meristematic tissue of the seed. The mutagenic treatments also delayed the germination process (Kdeinhofs *et al*., 1978).

20kR of gamma rays revealed 50 percent of germination. Same result has also been reported in Pearl millet by gamma rays (Vijendra das, 1978). 30mM of EMS revealed 50 percent of germination result has also been reported in pearl millet (Burton and Powell, 1966).

Reduction of plant height was increasing level of concentration was observed in rice by Ali Benjavad Talebi *et al*., 2012. Days to first flowering increased with increasing doses/ concentrations of gamma rays and EMS was observed by Constatin *et al*., 1976 and found linear relationship between dose and reduction survival of yield growth of soybean. However, number of leaves, Number of nodes, Length of earhead, Breadth of earhead, 1000 grains weight, Yield per plant decreased mean performance value with increasing dosage. The mutagenic effect was found, decrease in quantitative characters in Soybean (Pepo, 1989; Pavadai and Dhanavel ., 2004.

Length of earhead, grains weight, Yield per plant were decreased as compared to the non- treatment control. The same result has also been reported by Larik *et al*., 2009 in gamma rays treated *sorghum bicolor*.

M1 generation morphological and yield characters were decreased increasing dose/ concentrations of gamma rays and EMS by Velu *et al*., 2007 and 2008 ; Sanjai Gandhi *et al*.,2014 . The decrease quantitative and yield characters have been attributed to the physiological disturbance or chromosomal damage caused to the cells of the plant by the mutagen (Thilagavathi and Mullainathan., 2011).

Mean yield per plant in M_1 generation has shown shift towards negative direction which is an agreement with findings of earlier workers (Ashraf *et al* ., 2003, Majeed, 1997., Potdukhe 2004, Shah *et al* ., 2008). Present results suggest that lower dose (10kR) of gamma radiation can be useful for breeding point of view for selecting higher yielding plant in M_1 generation Larik *et al*, 2009; Gregory, 1965. The success of selection will, however, be greater in subsequent generations when there will be increased recombination and elimination of cytological variants (Larik *et al* ., 1981; Laric *et al* ., 1982). From breeding point of view increased variation assumes greater significance. (Frey 1969) reported that mutagen derived variability for quantitative characters in cereal plants are heritable and response to selection is good. Use of relative value of this source of variability in crop improvement, therefore, depends almost entirely upon nature of phenotypic expression caused by mutation induced at polygenic loci. It is only necessary to know if such deviation from the mean is identical and unidirectional for all yield components. The results indicated that change in means is always unidirectional for all yield components. The results indicated that change in means is always unidirectional and is effective for all traits, supporting conclusions of Bateman 1959 and Oka *et al* ., 1958 that induced genetic changes are unidirectional and negative and highly selected or adapted characters bring a greater shift in mean and a greater asymmetry in distribution (Brock, 1965).

CONCLUSION

In this research, Lethal dose was determined by measuring the seed germination, seedling height, Root length, Plant height, Days to first bloom, Number of leaves, Number of nodes,

Length of earhead, Breadth of earhead, 1000 grains weight , yield per plant and emergence under the field condition of the M_1 generation. M_1 generation was decreased in increasing doses/ concentrations of treatments. Mean performance of different quantitative traits were better in control when compared with treated plants. Induced mutagenesis is the best method to enlarge genetic variability within short time. Creation of genetic variability by induced mutagenesis proved best for strengthening crop improvement programmers and represents a more efficient source of genetic variability than the gene pool protect by nature.

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