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STUDIES ON INDUCED MUTATIONS IN BARNYARD MILLET (ECHINOCHLOA FRUMENTACEA L.)

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ABSTRACT

In the present investigation barnyard millet seeds were mutagenized with different doses of gamma rays (GR) ranging from 100, 200 and 300Gy, varying concentration of ethyl methane sulphonate (EMS) 0.1, 0.2 and 0.3% and Sodium azide (SA) 0.01, 0.02 and 0.03%. Variations in the germination of seeds, seedling height, seedling injury and survival of plant at maturity of barnyard millet were recorded in M_1 generation. The effects of the mutagenic treatments on quantitative traits resulting in reduction in traits such as seed germination except 100Gy (91.02%), 0.1% EMS (90.87%) seedling height except 100Gy (7.02cm) and 0.01%SA (7.18cm) and survival of plant at maturity except 100Gy (88.09%) and 0.1%EMS (87.57%) but increases seedling injury except 100Gy (-00.86%) and 0.01%SA (-3.16%) was observed in treated M_1 generated plants.

Key words: Mutagens, barnyard millet, seed germination, seedling injury.

INTRODUCTION

Barnyard millet (*Echinocloa frumentacea* L.) is locally known as bhagar or varai. It is the fast growing millet, having high content of fibre and iron. It is cultivated on marginal lands as minor cereal where rice and other crops will not grow well. Barnyard millet is the second important small millet after finger millet (Ramesh et al., 2019). The genus *Echinochloa* belongs to family Poaceae, which includes 35 species that are widely distributed throughout the world (Arun, 2009). Indian barnyard millet species have good agricultural characteristics, including animal forage, easy cultivation, salt tolerance, drought resistance, cold tolerance, and assured crop harvest. Their usefulness has made them an essential crop (Nozawa, 2006 and Choi et al., 1991). The grain of barnyard millet is used as food and can be cooked similar to rice. The grains are cooked in water, like rice, or boiled with milk and sugar. Sometimes it is fermented to make beer. While also being part of staple diet for some communities in India. Its grains are highly nutritious, rich in proteins, lipids, and vitamin B1 and B2 compared to other cereals. Ugare et al., (2014) have been reported that, significant reduction of blood glucose levels and LDL cholesterol when eaten by diabetic patients.

The main objective of the plant breeder is to produce crops that perform better, usually in terms of yield and quality, than existing cultivars and this is dependent on the availability of genetic variation, preferably in the primary gene pool (Festus et al.,2016). If genetic diversity is insufficient, then new material needs variation, created through induced mutation. Induced mutagenesis has been successfully used to generate wider variability, portioning for isolating mutants with desirable characters of economic importance (Ganapathy et al., 2008 and Ramesh et al., 2019).

MATERIALS AND METHODS

The seeds of Barnyard millet (*Echinocloa frumentacea* L.) were obtained from local market of Manchar, Tal. Ambegaon, Dist.- Pune- 410503 (M.S.) India. Gamma rays (GR), ethyl methane sulphonate (EMS) and sodium azide (SA) were used as mutagens in present investigation for seed treatments. Gamma radiation from ^{60}Co source fixed in the gamma cell 200 installed at Department of Chemistry, Savitribai Phule, Pune University, Pune was used in the present work. Dry, healthy and uniform seeds of Barnyard millet with moisture content of 10 to 12 % were treated with 100, 200 and 300 Gy. Ethyl methane sulphonate ($CH_3SO_2OC_2H_5$) molecular weight 124.16, and 8% soluble in water, manufactured by Sigma chemical Co. Ltd. USA was used for the seed treatment of Barnyard millet. Different concentrations of EMS (0.1, 0.2 and 0.3%) were prepared in distilled water. Sodium Azide is inorganic compound. It is colour less salt, ionic compound, soluble in water and is highly toxic. Mol. Wt. is 65.0099g/mol. It is chemical mutagen and used for induction of mutations in the crop plants. Different concentrations of SA (0.01, 0.02 and 0.03%) was prepared in distilled water.

The experiments were conducted to determine the lethal dose (LD_{50}), suitable concentrations of EMS, SA and duration of seed treatment. The doses of gamma rays, 100, 200 and 300 Gy, EMS 0.1, 0.2 and 0.3% while SA 0.01, 0.02 and 0.03% were finally selected for the seed treatment and the duration fixed was four hours. Selected seeds were soaked in distilled water for 10 hours and the wet seeds were treated with different concentrations of EMS and SA for four hours. The untreated seeds served as control. For each treatment 180 seeds were used. The treated seeds washed thoroughly with tap water for one hour to leach out the residual chemicals. From each treatment 30 seeds was used for seed germination in laboratory. Three replications with 10 seeds per replication kept in petri dishes, containing seed germination paper, were used for recording seed germination, seedling height on seventh day. The remaining lot of treated seeds (150) from each treatment was used for raising M_1 generation in field. The field experiments were conducted on the research plot at Department of Botany. The soil type of the experimental field was slightly deep, fine and with good drainage. The average minimum temperature was recorded as 17.63^{0}C and maximum 32.73^{0}C with average annual rainfall 641.03mm. The experiments were carried out following RBD design. Each plot had 50 plants. The distance between two rows and two plants was 45 X 20cm.

Observations on M_1 generation : The number of seeds showing emergence of the radical and plumule was used to calculate percent seed germination. On seventh day of sowing, 5 seedlings from control and each treatment were randomly selected for measuring the root and shoot length and the average values were recorded in table. Reduction in the mean seedling length as compared to the control was regarded as seedling injury and expressed as percentage.

% seedling injury = (Control seedling height - Treatment seedling height) / Control seedling height X 100.

Survival percent was calculated by scoring the number of plants attaining maturity (45days).

STATISTICAL ANALYSIS

The data were summarized as the means of three replicates with standard deviation as the measures of variability. One-way ANOVA test was performed to determine significant differences due to various treatments. Fisher's LSD (Least significant difference) was used as post hoc test to as certain significant differences among treatments at p= 0.05. Statistical analysis and graphical data presentations were carried out by using Sigma stat (ver.25).

RESULTS AND DISCUSSION

The data on seed germination percent, seedling injury and survival of plant at maturity in M_1 generation of Barnyard millet were recorded in Table-1. Seed germination in control and mutagen treatments clearly indicated that it was decreased in all the treatments except in 100Gy, 0.1%EMS as compared to control. The mutagens had exerted negative effects on seed germination. The percent seed germination decreased from 77.13% to 62.59% in GR except 100Gy (91.02%), 75.31% to 59.84% in EMS and 87.05% to 60.95% in SA. The maximum (50%) decrease in percent seed germination was observed with GR treatment 300Gy (62.59%), EMS 0.3% (59.84%) and in SA 0.03% (60.95%). The results of present study have clearly shown that Barnyard millet was sensitive to all the mutagens except 100Gy and 0.1%EMS. 100Gy and 0.1%EMS showed increase in seed germination percent over control. Reduction in seed germination with increasing dose/ conc. of mutagens was reported in horsegram (Bolbhat and Dhumal, 2009, black turtle bean (Bolbhat et al., 2020) and in field pea (Kumar and Singh, 1996). GR, EMS and SA are good mutagenic agents, which causes point mutations, enzyme inhibitions as well as chromosomal aberrations.

Results indicates that doses / conc. of mutagen treatments showed negative effect on seedling height except in few treatments. Lowest seedling height (4.55cm) was noted in 0.3%EMS, 300Gy (4.72cm) and 0.03%SA (5.36cm). Treatments such as 0.01%SA (7.18cm) and 100Gy (7.02cm) showed increase in seedling height than control (6.96cm).

Table 1 : Effect of mutagens on growth parameters in M_1 generation of barnyard millet

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling height (cm)	Seedling injury (%)	Plant survival (45 days) (%)
Control	90.71±12.70	3.11±0.44	3.85±0.54	6.96±0.97	00.00±0.00	87.28±12.22
100Gy	91.02±7.28	3.05±0.24	3.97±0.32	7.02±0.56	-00.86±0.07	88.09±7.05
200	77.13±8.48	2.53±0.28	3.29±0.36	5.82±0.64	16.38±1.80	75.46±8.30
300	62.59±8.14	2.07±0.27	2.65±0.34	4.72±0.61	32.18±4.18	59.33±7.71
0.1 % EMS	90.87±12.72	2.85±0.40	3.18±0.45	6.03±0.84	13.36±1.87	87.57±12.26
0.2	75.31±5.27	2.49±0.17	2.91±0.20	5.40±0.38	22.41±1.57	71.91±5.03
0.3	59.84±4.79	2.31±0.18	2.24±0.21	4.55±0.40	34.63±2.31	57.15±4.57
0.01% SA	87.05±13.06	3.20±0.48	3.98±0.60	7.18±1.08	-3.16±0.47	85.28±12.79
0.02	71.22±9.97	2.75±0.39	3.57±0.50	6.32±0.88	9.20±1.29	67.02±9.38
0.03	60.95±5.49	2.24±0.20	3.12±0.28	5.36±0.48	22.99±2.07	56.44±5.08
SEM±	7.59	0.26	0.33	0.59	1.60	7.31
F-value	5.68	4.43	4.85	4.45	12.59	6.44
P-value	0.01	0.01	0.01	0.01	0.01	0.01
$LSD_{0.05}$	16.54	0.57	0.72	1.29	3.49	15.93

Data are means of three replicates ± standard deviation. Significant difference due to treatments was assessed by Fisher's LSD as a post-hoc test.

Data on the effect of mutagens on seedling injury at M_1 revealed that all mutagenic treatments except few were highly injurious to the seedlings. EMS treatments had caused highest seedling injury, followed by the gamma radiation and sodium azide. The seedling injury increased with the increase in doses/ concentrations of mutagenic treatments. Maximum seedling injury (34.63%) was observed in 0.3%EMS, followed by 300Gy and 0.03% SA. Lower treatments like 100Gy (-0.86%) and 0.01%SA (-3.16%) showed negative seedling injury. The seedling injury significantly increased with the increase in doses/ conc. of mutagenic treatments except few in barnyard millet. Parallel results has been reported by earlier researchers Bolbhat and Bhalerao (2020) in lentil, Senapati *et al*., (2008) in blackgram.

The results on the effects of gamma radiation, EMS and SA showed that in all the mutagenic treatments, survival % was decreased than the control. There was decrease in the survival % with increasing dose/ conc. of gamma radiation, EMS and SA except 100Gy and 0.1%EMS. The lowest survival % at the higher treatments was noted (59.33%) in 300Gy, 0.3%EMS (57.15%) and 0.03%SA (56.44%) as compared to control (87.28%). Results indicates that lower dose/ conc. showed increasing survival percentage. All mutagens except few reduced the rate of survival at maturity, Awate and Bolbhat (2020) in horsegram, Barshile et al., (2006) in chickpea supported the above findings.

CONCLUSION

Seed germination percent and seedling height was inhibited due to increasing doses/ concentrations of mutagens except few. All three mutagens (GR, EMS and SA) were effective in inducing seedling injury in M_1 generation. The rate of seedling injury percent increased while the rate of survival of plants at maturity was highly reduced with increasing dose/conc. of mutagens.

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