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STUDIES ON EFFECT OF INDUCED MUTAGENESIS ON FINGER MILLET [ELEUSINE CORACANA (L.) GAERTN.] IN M_1 GENERATION

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ABSTRACT

Induced mutations is the best method to improve genetic variation within short time. Creation of genetic variation by induced mutations proved best for strengthening crop improvement programmes and represents a more efficient source of genetic variability than the gene pool protects by nature. In the present investigation seeds of finger millet were treated with different doses of gamma rays (GR) ranging from 200, 300 and 400Gy, varying concentration of ethyl methane sulphonate (EMS) 0.2, 0.3 and 0.4% and Sodium azide (SA) 0.02, 0.03 and 0.04%. Variations in the germination percent of seeds, seedling height, seedling injury and survival of plant at maturity of finger millet were recorded in M_1 generation. The effects of the mutagenic treatments on quantitative traits resulting in reduction in traits such as percent seed germination except 200Gy (85.16%), 0.2% EMS (83.53%) and 0.02% SA (86.65%), seedling height except 200Gy (6.59cm), 0.2%EMS (6.54cm) and 0.02%SA (6.98cm) while survival of plant at maturity except 200Gy (79.26%), 0.2% EMS (78.41%) and 0.02%SA (81.54%) but increases seedling injury except 200Gy (-01.86%), 0.2% EMS (-01.08%) and 0.02%SA (-07.88%) was observed in treated M_1 generated plants.

Key words: Mutagens, EMS, SA, finger millet, seedling injury.

INTRODUCTION

Finger millet [*Eleusine coracana* (L.) Gaertn.] is locally known as *Nachani* or *Ragi*. It is annual herb and belongs to family Poaceae. Ragi is important crop in tropical regions and semi-arid regions with good nutraceutical properties and ensuring food security in harsh environment (Gupta et al., 2017). Nachani is a rain fed crop and cultivated under adverse climatic and soil conditions. These are important crops in tropical regions as they are resistance to pests and diseases, productivity, and short growing season under adverse and drought conditions. Its grains are highly nutritious and have an excellent seed storage quality. Anti-diabetic, anti-tumerogenic, antioxidant, atherosclerogenic effects, and antimicrobial properties are health beneficial effects of Nachni (Devi et al, 2014).

Nachani require very little amount of water so it can be cultivated under non-irrigated conditions with very low rainfall. They have various benefits like fiber, food, fodder, nutrition, health, environment and livelihood at minimal cost, making them the essential guardians of agricultural security. It has an ability to resist pathogens and tolerate to different abiotic stress (Gupta et al., 2017). The grain of millet have carbohydrates, dietary fibre, phytochemicals and essential amino acids; essential minerals. The nutritional composition of millet, is higher than other cereal grains. Grains are consumed by using processing methods such as soaking, malting, cooking, fermentation and popping. The processing improves the dietetic and sensory properties of seeds. Seed processing also controls anti-nutritional and inhibitory activities of indigestible proteins (Shonisani, 2019). The use of grain and its products helps to control cardiovascular diseases, diabetes, gastrointestinal cancers and some other disorders (McKeown, 2002).

The seeds of finger millet- contains protein 5–8%, ether extractives1–2%, carbohydrates65–75%, dietary fiber 15–20% and minerals 2.5–3.5% (Chethan and Malleshi, 2007). The seeds also contains Ca (344 mg/100 g), phytates (0.48%), polyphenols, tannins (0.61%), trypsin inhibitors and dietary fiber. Due to their enzyme inhibition activities are called as antinutrients (Thompson, 1993). But nowadays they are termed as neutraceuticals have an antioxidant activity which is beneficial for aging, health and metabolic diseases (Bravo, 1998).

MATERIALS AND METHODS

The seeds of finger millet [*Eleusine coracana* (L.) Gaertn.] were procured 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 finger millet with moisture content of 10 to 12 % were treated with 200, 300 and 400 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 finger millet. Different concentrations of EMS (0.2, 0.3 and 0.4%) 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 (0.02, 0.03 and 0.04%) 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, 200, 300 and 400 Gy, EMS 0.2, 0.3 and 0.4% while SA 0.02, 0.03 and 0.04% 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 30cm.

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 IBM SPSS (ver.25).

RESULTS AND DISCUSSION

The data on seed germination percent, seedling injury and survival of plant at maturity in M_1 generation of finger 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 200Gy, 0.2%EMS and 0.02%SA as compared to control. The mutagens had exerted adverse effects on seed germination. The percent seed germination decreased from 69.32% to 56.41% in GR except 200Gy (85.16%); 71.04% to 59.13% in EMS and 73.37% to 62.89% in SA. The maximum (50%) decrease in percent seed germination was observed with GR treatment 400Gy (56.41%), EMS 0.4% (59.13%) and in SA 0.04% (62.89%). The results of present study have clearly shown that finger millet was sensitive to all the mutagens except 200Gy, 0.2%EMS and 0.02SA. 200Gy, 0.2%EMS and 0.02SA showed increase in seed germination percent over control. Reduction in seed germination with increasing dose/ conc. of mutagens was reported in horsegram (Awate and Bolbhat, 2014, in black turtle bean (Bolbhat et al., 2020) and in barnyard millet (Bolbhat and Bhalekar, 2020). Results clearly indicates that lower doses/conc. of mutagens like GR, EMS and SA showed increase in seed germination.

Data recorded in table 1 indicates that doses / conc. of mutagen treatments showed negative effect on seedling height except in lower treatments. Minimum seedling height (4.39cm) was noted in 0.4%EMS, 400Gy (4.43cm) and 0.04%SA (4.98cm). Treatments such as and 200Gy (6.59cm), 0.2%EMS (6.54cm) and 0.02%SA (6.98cm) showed increase in seedling height over control (6.47cm).

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

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling height (cm)	Seedling injury (%)	Plant survival (45 days) (%)
Control	82.29±11.52	3.05±0.43	3.42±0.48	6.47±0.91	00.00±0.00	77.33±10.83
200Gy	85.16±6.81	2.91±0.23	3.68±0.29	6.59±0.53	-01.86±0.15	79.26±6.34
300	69.32±7.63	2.12±0.23	3.09±0.34	5.21±0.57	19.47±2.14	65.33±7.19
400	56.41±7.33	1.80±0.23	2.53±0.33	4.43±0.58	31.53±4.10	52.19±6.78
0.2 % EMS	83.53±11.69	3.08±0.43	3.46±0.48	6.54±0.92	-01.08±0.15	78.41±10.98
0.3	71.04±4.97	2.37±0.17	3.15±0.22	5.52±0.39	14.68±1.03	66.39±4.65
0.4	59.13±4.73	1.72±0.14	2.67±0.21	4.39±0.35	32.15±2.57	49.91±3.99
0.02% SA	86.65±13.00	3.24±0.49	3.74±0.56	6.98±1.05	-07.88±1.18	81.54±12.23
0.03	73.37±10.27	2.60±0.36	3.07±0.43	5.67±0.79	12.36±1.73	65.77±9.21
0.04	62.89±5.66	2.12±0.19	2.86±0.26	4.98±0.45	23.03±2.07	48.59±4.37
SEM±	7.22	0.26	0.31	0.57	1.59	6.67
F-value	4.47	9.39	3.50	5.45	16.53	7.26
P-value	0.01	0.01	0.01	0.01	0.01	0.01
$LSD_{0.05}$	15.73	0.57	0.68	1.24	3.47	14.53

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 GR, EMS and SA on seedling injury at M_1 generation showed that all mutagenic treatments except lower treatments 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 (32.15%) was observed in 0.4%EMS, followed by 400Gy and 0.04% SA. Lower treatments such as 200Gy (-01.86%), 0.2%EMS (-01.08%) and 0.02%SA (-07.88%) showed negative seedling injury. The seedling injury significantly increased with the increase in doses/ conc. of mutagenic treatments except lower treatments in finger millet. Similar results has been reported by earlier researchers Bolbhat and Bhalekar, (2020) in barnyard millet, Senapati *et al*., (2008) in blackgram.

The results on the effects of mutagens showed that in all the mutagenic treatments, survival % was decreased but increased in lower treatments as compared to the control. There was decrease in the survival % with increasing dose/ conc. of gamma radiation, EMS and SA except 200Gy (79.26%), 0.2%EMS (78.41%) and 0.02%SA (81.54%). The lowest survival % at the higher treatments was noted (48.59%) in 0.04%SA, (49.91%) in 0.4%EMS and (52.19%) in 400Gy as compared to control (77.33%). Results indicates that lower dose/ conc. of mutagens showed increasing survival percentage while all other showed reduced the rate of survival at maturity. Awate and Bolbhat (2020) in horsegram, Bolbhat and Bhalekar, (2020) and Ramesh et al., (2019) in barnyard millet supported the above findings.

CONCLUSION

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

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