



STUDIES ON INDUCED MUTATIONS IN NIGER [*Guizotia abyssinica* (L.f.) Cass.]

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Abstract:

Success of any breeding programme depends on the presence of significant genetic variability, which permits an effective selection. In last some years induced mutations have been used as an important supplementary tool, to other conventional methods of plant breeding for improvement of crops. This clearly indicates that mutation breeding has a significant momentum and still remained as the highly preferable tool for inducing the genetic variability in large number of plant species of interest. In present study seeds of niger were exposed to different doses of gamma rays (GR) ranging from 300, 400 and 500Gy, concentration of ethyl methane sulphonate (EMS) ranging from 0.3, 0.4 and 0.5%EMS and Sodium azide (SA) 0.03, 0.04 and 0.05%SA. Variations in the percentage of seed germination, root and shoot length, seedling injury, pollen sterility and survival of plants at maturity, were recorded in the M₁ generations.

Key words: Mutagens, niger, germination, seedling injury, pollen sterility.

INTRODUCTION

Niger [*Guizotia abyssinica* (L. f.) Cass.] is also called as khurasni or karala and belongs to family Asteraceae. It is a minor oilseed crop, grown predominantly under rainfed conditions in India for its edible oil. The plant is an annual herb and grows up to 5-6 feet. The tap root system is well developed, the stem is soft, hairy, hollow, branched, pale green, leaves are simple, opposite, alternate at the apices, leaf blade is lanceolate either entire or toothed, hairy, capitulum inflorescences with achenes seeds. (Bulcha, 2007).

Niger seeds yield oil and oil cake which is rich in protein and fibre, free from any toxic contents. Niger seed oil contains 75-80% linoleic acid, 7-8% palmitic and stearic acids, and 5-8% oleic acid (Getinet et al., 1996). Niger seeds are used as food in many dishes, condiments, snacks, chatnies and pickles. Niger oil cake contains protein 22%, lignin (12%) and fibre is the main supplement for livestock (Bulcha, 2007 and Getinet et al., 1996). It is a valuable cover crop between cereal crops and it can be turned into green manure. Seeds contain about 30-35% oil appearing yellow in colour, with a faint odour and nutty, slightly sweet taste. The oil is used for cooking, lighting, soap making, painting, the extraction of perfume from flowers and cleaning of machinery. In India, about 75% of the niger crop is used for oil extraction. The variation in minerals, protein, fatty acid and amino acid contents depend on location, climatic factors and crop varieties (Bhagya et al., 2003, Gebremedhin et al., 2009).

Niger is grown as intercrop with finger millets, maize and pulses or as pure stand. Seed is sown in July or August after the first heavy rains. It grows on almost any soil as long as it is not coarse-textured or extremely heavy. It is sown in areas with a rather poor soil or on heavy clay soil under poor cultural conditions. Niger tolerates waterlogged soils since it grows equally well on either drained soils or waterlogged clays (Naik and Hosakatte, 2009).

The productivity level of almost all oilseed is very low. The major constraints are- Lack of high yielding varieties adapting to diverse agroclimatic conditions. Biotic and abiotic stress conditions. Poor plant stand. Inadequate seed replacement rate and unavailability of quality seeds. The improvement in production of oilseed in India is the dire need of the country. Hence the improvement in oilseed like niger is the urgent need of the hour (Bolbhat and Dhumal, 2011). Success of any breeding programme depends on the presence of significant genetic variability, which permits an effective selection. In last few years induced mutations have been used as an important supplementary method, to other conventional methods of plant breeding for improvement of crops. Micke *et al.*, (1985) used induced mutations for obtaining desirable genetic changes like high yield, flower colour, disease resistance and early maturity in various crops, fruits and ornamental plants. Improvement in economic and quality characters can be achieved with the help of induced mutations within the shortest possible time as it is a powerful tool of creating new and useful variability (Maluszynski *et al.*, 1995, Senapati *et al.*, 2008).

MATERIALS AND METHODS

The seeds of Niger [*Guizotia abyssinica* (L. f.) Cass.] 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 employed in present work 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 niger with moisture content of 12 to 14 % were treated with 300, 400 and 500 Gy. Ethyl methane sulphonate ($\text{CH}_3\text{SO}_2\text{OC}_2\text{H}_5$) molecular weight 124.16, and 8% soluble in water, manufactured by Sigma chemical Co. Ltd. USA was used for the seed treatment of niger. Various concentrations of EMS (0.3, 0.4 and 0.5%) 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 (0.03, 0.04 and 0.05%) 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, 300, 400 and 500Gy, EMS 0.3, 0.4 and 0.5% while SA 0.03, 0.04 and 0.05% were finally selected for the seed treatment and the duration fixed was four hours. Selected seeds were soaked in distilled water for 8 hours and the wet seeds were treated with different concentrations of EMS (0.3, 0.4 and 0.5%) and SA 0.03, 0.04 and 0.05% for four hours. The untreated seeds served as control. For each treatment 480 seeds were used. The treated seeds washed thoroughly with tap water for two hours 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 7th day. The remaining lot of treated seeds (450) from each treatment was used for raising M_1 generation in field.

The field experiments were conducted on the experimental field at Department of Botany. The soil type of the experimental field was slightly deep, fine and calcareous with good drainage. The average minimum temperature was recorded as 17.63°C and maximum 32.73°C with average annual rainfall 641.03mm. All the experiments were carried out in triplicate following RBD design. Each plot had 150 plants. The distance between two rows and two plants was 45 X 30 cm.

Observations on M_1 generation : The number of seeds showing emergence of the radical and plumule was used to calculate percent seed germination. On 7th 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.

Pollen sterility was determined from 5 randomly selected plants/ treatment. The pollen grains from freshly dehisced anthers were stained with 1% aceto-carmin. Pollen grains stained as uniform deep red colour were counted as fertile and others as sterile. 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 ascertain 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

Results on seed germination, seedling injury, pollen fertility and survival of plant at maturity in M_1 generation of niger are recorded in Table-1. Seed germination in control and mutagen treatments clearly indicated that it was decreased in all the treatments as compared to control. The mutagens had exerted negative effects on seed germination. The percent seed germination decreased from 83.31% to 59.87% in GR, 85.03% to 56.19% in EMS and 90.36% to 68.39% in SA. The maximum (50%) decrease in percent seed germination was observed with GR treatment 500Gy (59.87%), EMS 0.5% (56.19%) and in SA 0.05% (68.39%). The results of present study have clearly shown that niger was sensitive to all the mutagens. EMS treatments were more effective in reducing seed germination percent, followed by GR and SA.

Reduction in seed germination in M_1 generation with increasing dose/ conc. of mutagens was reported in groundnut (Badigannavar and Murty (2007)), soybean (Kartika and Subba Laximi, 2006) and in sesame (Uttarde et al., 2020). GR and EMS are good mutagens, well known for their action causing point mutations, enzyme inhibitions and chromosomal aberrations (Auti, 2005). The recorded reduction in seed germination in niger as a result of treatments of these mutagens might be due to point mutations or the injuries caused to the genetic material.

Results indicates that doses of gamma radiation and concentrations of EMS and SA treatments showed inhibitory effect on seedling height. Maximum decrease in seedling height (6.96cm) was noted in 500Gy, 0.5%EMS (6.46cm) and 0.5%SA (7.36cm). Data on the effect of mutagens on seedling injury at M_1 revealed that all mutagenic treatments were highly injurious to the seedlings. EMS treatments had caused highest seedling injury, followed by the

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

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling height (cm)	Seedling injury (%)	Pollen sterility (%)	Plant survival (45 days) (%)
Control	96.27±13.48	4.71±0.66	5.32±0.74	10.03±1.40	0.00±0.00	7.92±1.11	88.19±12.35
300Gy	83.31±6.66	4.53±0.36	5.11±0.41	9.64±0.77	3.89±0.31	9.03±0.72	80.42±6.43
400	72.43±7.97	3.85±0.42	4.16±0.46	8.01±0.88	20.14±2.22	14.37±1.58	70.57±7.76
500	59.87±7.78	3.22±0.42	3.74±0.49	6.96±0.90	30.61±3.98	23.19±3.01	55.13±7.17
0.3 % EMS	85.03±11.90	4.12±0.58	5.19±0.73	9.31±1.30	7.18±1.01	10.44±1.46	83.10±11.63
0.4	70.93±4.97	3.29±0.23	4.02±0.28	7.31±0.51	27.12±1.90	18.78±1.31	67.21±4.70
0.5	56.19±4.50	3.07±0.25	3.39±0.27	6.46±0.52	35.59±2.85	27.61±2.21	55.33±4.43
0.03%SA	90.36±13.55	4.65±0.70	5.25±0.79	9.90±1.48	1.30±0.20	8.02±1.20	86.94±13.04
0.04	85.64±11.99	4.03±0.56	4.92±0.69	8.95±1.25	10.77±1.51	12.56±1.76	74.15±10.38
0.05	68.39±6.16	3.52±0.32	3.84±0.35	7.36±0.66	26.62±2.40	19.15±1.72	60.76±5.47
SEM±	7.75	0.39	0.45	0.84	1.67	1.40	7.26
F-value	5.86	4.92	5.28	4.98	12.60	47.21	5.90
P-value	0.01	0.01	0.01	0.01	0.01	0.01	0.01
LSD _{0.05}	16.89	0.85	0.98	1.83	3.64	3.05	15.82

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

gamma radiation and sodium azide. The seedling injury increased with the increase in doses/ concentrations of mutagenic treatments. Maximum seedling injury (35.59%) was observed in 0.5%EMS, followed by 500Gy and 0.5% SA. Badigannavar and Murty (2007) in groundnut and Senapati *et al.*, (2008) in blackgram were in agreement with our results in niger. Busey (1980) claimed that it was due to chromosomal and extra chromosomal damage of the cells in treated seedlings.

The data recorded on pollen sterility induced by mutagens treatments indicated that there was linear increase in pollen sterility with increasing dose/ conc. of mutagens. In gamma radiation, EMS and SA treatments, the range of pollen sterility % was 9.03 to 23.19%, 10.44 to 27.61% and 8.02 to 19.15%. The highest pollen sterility (27.61%) was recorded in 0.5% EMS. The rate of pollen sterility increased with increase in conc. or dose. Induction of pollen sterility with mutagens was reported earlier by Uttarde et al., (2020) in sesame, Bolbhat and Dhumal (2009) in horsegram. Different chromosomal abnormalities like anaphase bridges, translocation and laggarads were found in the progenies obtained from treated seeds.

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. The lowest survival % at the higher treatments was noted (55.13%) in 500Gy, 0.5% EMS (55.33%) and 0.05% SA (60.76%) as compared to control (88.19%). All mutagens reduced the rate of survival at maturity, Uttarde et al., (2020) in sesame and Kavithamni *et al.*, (2008) in soybean supported the above findings.

CONCLUSION

Percent seed germination and seedling growth was inhibited due to increasing doses/ concentrations of mutagens. All mutagens (GR, EMS and SA) were effective in inducing pollen sterility in M₁ generation. The rate of pollen sterility increased with increase in dose/ conc. of the mutagens and the survival rate was highly reduced with increasing dose/conc. of mutagens.

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