African Journal of Biotechnology Vol. 8 (18), pp. 4459-4464, 15 September, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

The effects of gamma and ethylmethanesulphonate treatments on agronomical traits of niger (*Guizotia abyssinica* Cass.)

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Accepted 24 June, 2009

Seeds of niger (*Guizotia abyssinica* Cass.) cultivar N-71 were exposed to different doses of gamma rays ranging from 0 -152 Gy and varying concentration of ethyl methanesulphonate ranging from 0 - 104 mM. Variations in the percentage of germination and survival, plant height, number of leaves/plant, leaf length, leaf width, number of primary branches, capitulum size, head size, number of ray florets/capitulum, number of disc florets/capitulum, number of capitula/plant, number of seeds/capitulum, seed yield/plant, 1000 seed weight were recorded in the M_1 and M_2 generations. Plants showed decreased growth with increased dose and concentration of the mutagens. There were dose and concentration related effects of the mutagenic treatments on quantitative traits resulting in reduction in traits such as germination and survival percentages, plant height, leaf length and width, number of primary branches and 1000 seeds weight, but increases in number of capitula/plant, number of seeds/capitulum and seed yield/plant was observed in 52 and 78 mM treated M_2 generated plants. Improved oil content was found in gamma ray treated (38, 114, and 152 Gy) M_2 plants and also in 78 mM EMS treated M_2 plants. Early flowering was found in gamma (76 Gy) treated M_2 plants.

Key words: Gamma ray, ethyl methanesulphonate, niger, agronomical trait, M_1 generation, M_2 generation.

INTRODUCTION

Mutations are known to enhance the genetic variability of crop plants as the variability at species level has reached the ceiling due to high breeding intensity and rapid erosion of genetic resources. Since spontaneous mutations occur at very low frequency, induced mutations facilitate the development of improved varieties at a swifter rate (Maluszynski, 1990). Besides the vital role in plant breeding programs, a new role of induced mutations in releasing of gene silencing in transgenic plants has been reported (Bhatia, 1999).

Induced mutations have been used to generate genetic variability and have been successfully utilized to improve yield components of various crops like *Oryza sativa* (Awan et al., 1980; Singh et al., 1998), *Hordeum vulgare* (Ramesh et al., 2001), *Triticum durum* (Sakin and Yildirim, 2004), *Cicer arietinum* (Wani and Anis, 2001), *Vigna mungo* (Misra et al., 2001), *Cajanus cajan* (Srivastava and Singh, 1996; Ravikesavan et al., 2001), *Arachis hypogaea* (Venkatachalam et al., 1999), *Sesame indicum* (Mensah et al., 2007), *Helianthus annuus* (Elangovan, 2001), *Guizotia abyssinica* (Misra, 2001). These reports show that mutagenesis is a potential tool to be employed for crop improvement.

The biological effect of ionizing radiation like gamma rays depends primarily on the amount of energy that will be absorbed by the biological system of which of course, the chromosomes are the most important target (Van Harten, 1998). Ethyl methanesulphonate (EMS) is a chemical mutagen of the alkylating group and has been commonly used in plant breeding because it can cause high frequency of gene mutations and low frequency of chromosome aberrations (Van Harten, 1998).

Niger (*G. abyssinica* Cass. Asteraceae) is an important oil seed crop cultivated predominantly in Ethiopia and India. Seeds contain about 30-50% of yellow edible, semi-drying oil with little odour and pleasant nut-like taste which is very valuable dietically (Getinet and Sharma,

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1996). Although this crop is one of the important sources of edible and industrial oil in the tropics, it has not received much attention for yield improvement. This situation has changed in both industrialized and developing countries due to recent initiatives. Among the different breeding methods used, mutation induction has been used as an important tool to supplement existing variability and to create additional variability for qualitative as well as quantitatively inherited traits in niger. Especially various degrees of resistance to biotic and abiotic stress, seed yield and oil content. The present study deal with the effects of mutagens like gamma rays and EMS on some of the agronomical characters of niger.

MATERIALS AND METHODS

Seed material

Seeds of niger (*G. abyssinica* Cass.) cultivar N-71 were obtained from the Project coordinator, All India Coordinated Research Project (AICRP on sesame and niger), Jawaharlal Nehru Agricultural University campus, Jabalpur, India.

Gamma radiation treatment

Seeds were irradiated with gamma radiation at Bhabha Atomic Research Centre, Mumbai, India. The gamma radiation was derived from a Cobalt-60 (^{60}Co) source with a measured dose rate of 4.07 Gy/min. A completely randomized design was used. LD_{50} was determined at maturity of plants by preliminary experiments. There were 5 treatments with 200 seeds per treatment. The seeds were treated with gamma radiation at 0, 38, 76, 114 and 152 Gy.

Ethyl methanesulphonate treatment

The alkylating substances ethyl methanesulphonate (Himedia, India) was used as a chemical mutagen. LC_{50} was determined at maturity of plants by preliminary experiments. Seeds were initially soaked in double distilled water for 6 h at 4°C. Then the seed surface was blotted free of water with filter paper. There were 5 treatments with 200 seeds per treatment. The seeds were treated with 0, 26, 52, 78 and 104 mM solutions of EMS. The EMS solutions in phosphate buffer (0.1 M, pH 7), were prepared just before use. Seeds were left in the mutagen solution for 24 h at 4°C.Then seeds were washed for 4 h with tap water to completely remove EMS and seeds were surface blotted. Both gamma irradiated and EMS treated seeds were sown in experimental field at the University of Agricultural Science, Dharwad, in complete randomized-block design to raise the M_1 generation. The M_1 plants were harvested individually and M_2 generation as plant-to-row progeny were produced.

Field experiment

Field experiments were undertaken to determine the effects of the physical and chemical mutagen on germination, survival, plant height, number of leaves/plant, leaf length, leaf width and number of primary branches at maturity for M_1 and M_2 generation. Germination data were taken after 20 days of sowing, and all other data were taken after 90 days of sowing. The effects of the mutagens on the capitulum size, number of ray florets/head, number of disc florets/head, number of capitula/plant, number of seeds/capitulum, seed yield/plant and 1000 seed weight were also investigated for M_1 and M_2 generation. The oil content was scored for M_2 generation.

Determination of oil content

10 g seeds of each samples were first milled to a fine paste, homogenized and then extracted in a soxhlet apparatus using petroleum ether (40 - 60°C) as a solvent. The extract was refluxed for 6 h and the solvent was evaporated under reduced pressure. After evaporating the solvent the recovered oil was measured.

RESULTS AND DISCUSSION

M_1 generation

All the data of M_1 generation were represented in Table 1. Germination data were taken after 20 days of sowing in the field, and other agronomical characters were taken after 90 days of sowing. Gamma rays treated seeds showed 100% germination and survival with all the treatments, however, EMS treated seeds showed decreased seed germination and survival rate of the plants as the concentration of mutagen increased, except in 104 mM. Similar results observed in chemical mutagen treated *S. indicum* (Mensah et al., 2007). Plant heights were decreased in both gamma rays and EMS treated plants at higher dose and concentration respectively (152 Gy -74.8 cm; 104 mM - 88.5 cm respectively) when compared to the control (110.2 and 113.0 cm respectively). Total number of leaves/plant including withered leaves was also decreased in both mutagens as dose and concentration increased. Gamma ray (114 Gy) treated plants showed 12.9 cm leaf length which was slightly higher than the control (12.7 cm), but EMS treated plants showed decreased leaf length at higher concentration. Plants treated with higher dosages of mutagens (gamma ray/EMS) showed decreased leaf width. In the similar way number of primary branches also decreased.

Capitulum size decreased in both mutagens (gamma ray/EMS) treated plants at higher dose and concentration. Gamma ray treated plants showed decreased head size at higher dose (152 Gy - 0.8 cm) when compared to control (1.2 cm), but EMS treated plants showed at 52 mM concentration head size was equal to that of control. In gamma ray treated plants number of ray florets/capitulum showed in decreased manner, but in EMS treated plants showed higher number of ray florets/capitulum in all concentrations except in 26 mM when compared to control. The number of disc florets/capitulum was higher at 114 Gy gamma ray (46.8) and 52 mM EMS (50.0) treated plants when compared to control (43.6 and 48.7 number of disc florets/capitulum respectively). The number of capitula/plant was decreased in gamma and EMS treated plants at higher dose and concentration respectively.

The number of seeds/capitulum decreased in gamma

Table 1. Effects of gamma ray and ethyl methanesulphonate (EMS) on some agronomic traits of niger in the M_1 generation.

Parameters	Gamma rays dosages (Gy)					EMS concentration (mM) for 24 h				
	0	38	76	114	152	0	26	52	78	104
Germination (%)*	100.0	100.0	100.0	100.0	100.0	100.0	97.8	95.6	76.5	91.4
Survival (%)	100.0	100.0	100.0	100.0	100.0	100.0	97.8	93.6	73.9	78.2
Plant height (cm)	110.2	80.7	77.7	95.3	74.8	113.0	108.8	108.8	109.0	88.5
No of leaves/plant (number of withered leaves)	11.6 (12.2)	11.6 (10.6)	11.1 (10.0)	12.0 (10.8)	11.1 (11.2)	12.0 (13.1)	12.2 (11.4)	12.5 (10.8)	11.5 (11.0)	11.4 (10.8)
Leaf length (cm)	12.7	12.3	11.2	12.9	11.9	12.8	12.2	9.9	10.8	9.2
Leaf width (cm)	3.6	3.3	3.5	3.5	3.2	3.3	2.6	2.0	2.4	2.5
No. of primary branches (pair) at flowering stage	5.7	4.9	4.8	5.5	4.9	5.3	3.0	3.2	3.2	2.4
Capitulum size (cm)	4.3	3.6	3.3	3.7	3.3	4.4	4.0	4.3	3.7	3.9
Head size (cm)	1.2	0.9	0.8	1.0	0.8	1.2	1.0	1.2	1.1	0.9
Number of ray florets/capitulum	8.0	7.9	7.6	7.6	7.6	8.0	7.9	8.5	9.2	8.5
Number of disc florets/capitulum	43.6	43.9	41.5	46.8	40.6	48.7	39.2	50.0	45.8	40.9
Number of capitula/plant	43.9	39.9	31.5	36.4	11.8	41.1	34.1	24.5	28.1	22.1
Number of seeds/capitulum	15.9	11.2	10.9	8.0	11.8	16.4	14.3	14.2	16.4	10.1
Seed yield /plant	698.0	449.4	345.4	293.7	399.8	674.0	425.1	352.1	421.5	241.3
1000 seed wt (g)	4.2	4.1	3.7	4.0	3.8	3.5	3.2	3.5	3.2	3.4

*Germination (%) results were taken 20 days after sowing.
All other data were taken after 90 days of sowing.

ray treated plants at higher dose and similar way in EMS treated plants except at 78 mM (16.4) in which number of seeds/capitulum was equal to that of control (16.4). Gamma ray treated plants were showed decreased seed yield/plant and 1000 seed weight at higher dose. In EMS treated plants seed yield/plant decreased at higher concentration but 1000 seed weight also decreased except at 52 mM (4.3 g) when compared to the control (4.1 g). Increased 1000 seed weight (4.2 g) is observed in EMS treated *G. abyssinica* (Misra, 2001).

M_2 generation

All the data of M_2 generation were represented in Table 2 and Figures 1-4. In M_2 generation 100% germination and survival rate was found in all the dose of gamma treated plants. In EMS treated plants showed drastic decreased in germination and survival rate at 78 mM concentration (4.5 and 4.1% respectively). Plant height (118.4, 110.7, 98.5 and 85.7 cm; 125.4, 115.7, 110.7, 101.4 and 98.7 cm) was decreased as the dose and concentration of the gamma ray (0, 38, 114 and 152 Gy) and EMS (0, 26, 52, 78 and 104 mM) was increased respectively except in 76 Gy gamma treated plants (116.7 cm). Total number of leaves/ plant including withered leaves was decreased in gamma and EMS treated plants. Leaf length and width was decreased in gamma treated plants as dose increased. EMS induced plants showed decreased leaf length (15.5, 13.9, 12.5, 12.6 and 12.3 cm) and leaf width (3.9, 3.3, 3.2, 2.5 and 2.2 cm) with increased mutagen concentrations. The number of primary branches are decreased at higher dose and concentration of mutagens used.

Gamma rays and EMS treated plants showed early flowering and late flowering mutants. The number of days to flowering was 6, 3, and 1 day early at 76, 38, and 152 Gy gamma ray treated plants respectively when compared to the control (Figure 1). Early flowering is observed in gamma rays treated *C. cajan* and *C arietinum* (Ravikesavan et al., 2001; Wani and Anis, 2001). Late flowering was observed in the EMS treated plants. Increase in the concentration of EMS lead to increase in number of days to flowering (Figure 2). Capitulum

Table 2. Effects of gamma ray and ethyl methanesulphonate (EMS) on some agronomic traits of niger in the M_2 generation.

Parameters	Gamma rays dosages (Gy)					EMS concentration (mM) for 24 h				
	0	38	76	114	152	0	26	52	78	104
Germination (%)*	100.0	100.0	100.0	100.0	100.0	100.0	52.0	15.8	4.5	6.8
Survival (%)	100.0	100.0	100.0	100.0	100.0	100.0	51.9	14.9	4.1	6.4
Plant height (cm)	118.4	110.7	116.7	98.5	85.7	125.4	115.7	110.7	101.4	98.7
No of leaves/plant (number of withered leaves)	15.4 (11.3)	14.7 (12.1)	16.2 (8.4)	15.1 (9.4)	14.7 (10.7)	19.7 (7.8)	19.5 (7.5)	16.7 (8.4)	14.8 (8.2)	16.0 (6.0)
Leaf length (cm)	15.9	15.5	14.7	14.2	13.8	15.5	13.9	12.5	12.6	12.3
Leaf width (cm)	4.4	4.3	4.1	3.9	3.8	3.9	3.3	3.2	2.5	2.2
No. of primary branches (pair) at flowering stage	7.5	7.2	6.5	6.6	5.9	6.7	6.2	5.4	4.7	4.0
Capitulum size (cm)	3.8	3.7	3.8	4.0	3.5	4.0	3.7	4.0	4.0	3.7
Head size (cm)	0.9	0.8	0.8	0.9	0.8	1.0	0.9	0.9	1.0	0.8
Number of ray florets/capitulum	8.0	8.0	7.6	7.9	7.4	8.0	8.0	8.1	8.4	8.0
Number of disc florets/capitulum	44.6	36.6	37.0	43.1	36.5	46.2	39.5	45.8	45.1	40.1
Number of capitula/plant	41.1	35.4	15.2	21.5	11.1	40.4	23.6	67.1	148.2	42.0
Number of seeds/capitulum	6.7	7.0	5.2	7.4	8.9	7.7	7.2	11.3	15.0	7.3
Seed yield /plant	279.4	248.6	79.2	159.5	99.6	308.5	172.4	765.4	2233.0	308.0
1000 seed wt (g)	3.7	3.5	3.4	3.4	3.6	3.5	3.2	3.5	3.2	3.4

*Germination (%) results were taken 20 days after sowing.
All other data were taken after 90 days of sowing.

size was 4.0 cm at 114 Gy gamma ray treated plants which was higher than that of control (3.8 cm). In EMS treated plants showed 4.0 cm capitulum size in both 52 and 78 mM which were equal to control. Lower and higher concentration showed decreased capitulum size. Head size was more or less equal in both mutagens treated plants when compared to control. The number of ray florets/capitulum was decreased at higher dose of gamma treated plants. But in EMS treated plants showed higher number of ray florets/capitulum, 8.1 and 8.4 at 52 and 78 mM respectively when compared to the control (8.0 ray florets/capitulum). The number of disc florets/capitulum was decreased at higher dose and concentration of mutagens used. The number of capitula/plant (41.1, 35.4, 15.2, 21.5 and 11.1) was decreased at higher dose (0, 38, 76, 114 and 152 Gy respectively) of gamma ray. In EMS treated plants number of capitula/plant were found higher at 52 mM (1.66 times) and 78 mM (3.66 times) when compared to the control. More number of capitula/plant (26.2) is found in EMS treated *G. abyssinica* (Misra, 2001) and it is very less when compared to 78 mM EMS treated plants in which 148.2 capitula/ plant was observed (Table 2).

Slightly higher number of seeds/capitulum was found in gamma ray treated plants. Higher number of seeds/capitulum was found at 52 mM (11.3 seeds/capitulum) and 78 mM (15.0 seeds/ capitulum) concentration of EMS treated plants when compared to the control (7.7 seeds/ capitulum). Increased number of seeds/pod and seeds/capitulum was observed in EMS treated *V. mungo* and *G. abyssinica* respectively (Misra et al., 2001; Misra, 2001). Gamma ray treated plants showed decreased in seed yield/plant and 1000 seed weight at higher dose. 52 mM and 78 mM concentration of EMS treated plants showed 2.48 and 7.23 times higher seed yield/plant when compared to the control respectively. High yield was observed in EMS treatment of *A. hypogaea* (Venkatachalam et al., 1999). 1000 seed weight was decreased in the plants at higher concentration of EMS. Gamma ray treated plants showed improved oil content at 38, 114 and 152 Gy (38.70, 36.24 and 37.21% of oil content respectively), and EMS treated plants, they showed increased oil

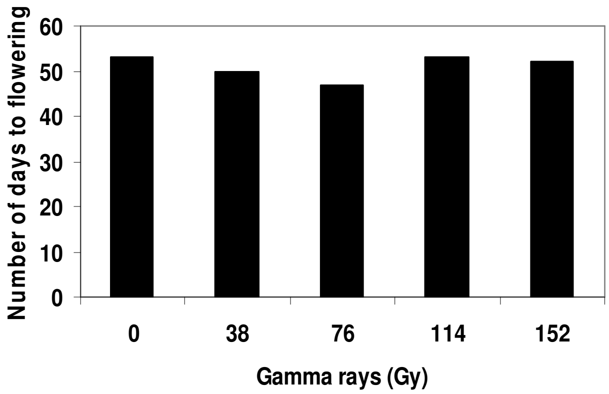

Figure 1. Effect of gamma rays on flowering of niger in the M_2 generation.

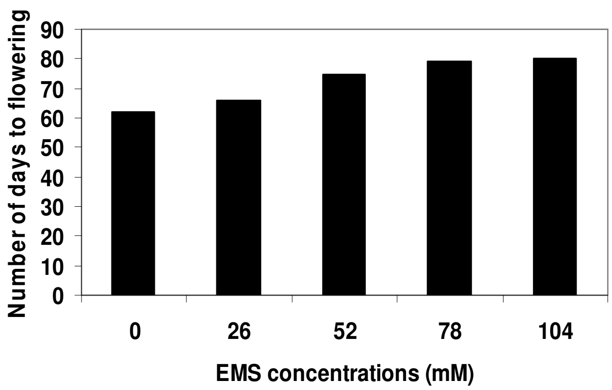

Figure 2. Effect of EMS on flowering of niger in the M_2 generation.

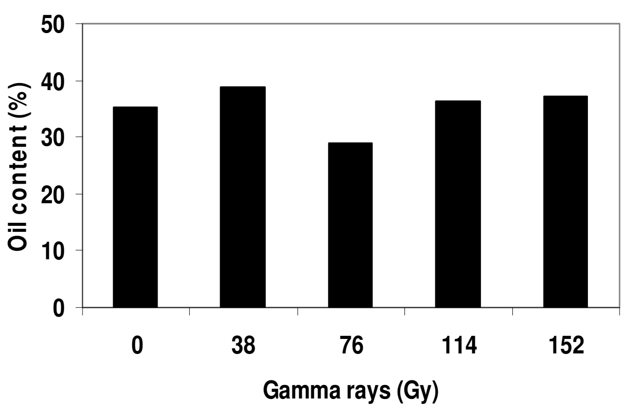

Figure 3. Effect of gamma rays on oil content of niger in the M_2 generation.

content of 36.49% at 78 mM, but in control it was 35.61% (Figures 3 and 4). Similarly, high oil content was observed in both gamma and EMS treated *A. hypogaea* (Venkatachalam et al., 1999).

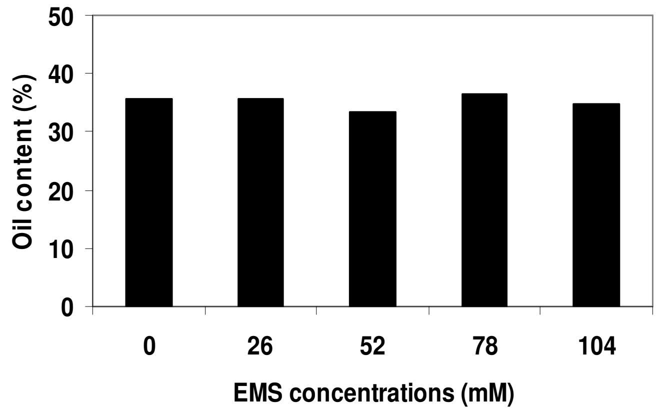

Figure 4. Effect of EMS on oil content of niger in the M_2 generation.

Conclusion

Generally, reduced plant height, primary branches and seed weight were recorded. However, gamma ray treated M_2 generated plants showed improved oil content at 38, 114, and 152 Gy and early flowering plants at 76 Gy. The high seed yield and improved oil content were observed at 78 mM EMS treated M_2 generated plants. The useful mutants isolated through the present study need to test further on a wider scale to establish any changes in chromosome or allele frequency and also to assess its performance in later generations.

ACKNOWLEDGMENT

This work was partially financed by Council of Scientific and Industrial Research (Scheme number: Number 38 (1098) /04/EMR-II), New Delhi, India.

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