Evaluation of gamma rays-induced changes in oil yield and oleic acid content of niger, *Guizotia abyssinica* (L. f.) Cass.

Guizotia abyssinica is an annual herb belonging to the family Compositae/ Asteraceae, mainly grown for its edible oil and seeds. Its cultivation originated in the Ethiopian highlands and has spread to other parts of Ethiopia as well as outside Africa[1]. Common names for the plant are noog/nug, niger, nyjer, ramtil and blackseed. Niger is mainly cultivated for its edible oil that constitutes about 0.7% of the Indian oilseed production. The pale yellow oil of niger seed has a nutty taste and a pleasant odour. It is mainly used for cooking, lighting, anointing, painting and cleaning of machinery[2–4]. Niger oil is used as substitute for sesame oil for pharmaceutical purposes and is also used in soap-making.

Niger oil has a fatty acid composition typical of the Asteraceae oilseeds like safflower and sunflower with linoleic acid (polyunsaturated fatty acid) being the dominant fatty acid. Reduction in the levels of polyunsaturated fatty acids and their substitution for the monounsaturated oleic acid is an important goal for the production of higher quality oil. Polyploidy and mutations have played a significant role in the evolution of oilseed crops[5]. In recent years, induced mutations have been used extensively for genetic enhancement of the annual oilseed crops, especially to modify the fatty acid composition of mustard, sunflower, peanut, linseed and soyabean[6].

At present, gamma rays are the most favoured physical mutagenic agents because they are highly energetic and can penetrate deeply into tissues. The number of mutations induced depends on the radiation type, the applied dose and the dose rate. If gamma rays cause mutations in the plants that grow from the irradiated seeds, then such plants may possess altered genotypic properties. Among 13 varieties of niger, IGP-76 was concluded as the best for mutation breeding based on some promising features such as early maturity, high survival, high oil content, high mitotic index and fewer mitotic abnormalities[7].

Seeds of *G. abyssinica* were collected from the Zonal Agricultural Research Centre of Mahatma Phule Krishi Vidyapeeth, Igatpuri, Maharashtra. Healthy, dry seeds were irradiated with gamma rays (100, 200 and 300 Gy) at the Bhabha Atomic Research Centre (BARC), Mumbai. The source used for irradiation was cobalt 60. The experiment was carried out in three consecutive generations, mainly M1, M2 and M3, along with control. For the M1 generation, 300 irradiated seeds were sown in the field at Kelve Road, Thane, Maharashtra. Seeds were sown in three rows with 100 seeds in each row. Distances of 30 cm row-to-row and 10–15 cm seed-to-seed were maintained[8–10]. Proper irrigation conditions were maintained throughout the growth period.

M1 plants that have shown considerable mutation in their characters were selected and their seeds were used to grow M2 generation crop. Individual plants from every mutant line were harvested separately. Seeds from M2 crop each selected for a specific mutant were sown in the field to raise M3 plants. The performance of these mutants with respect to the characters was studied in the M3 generation to confirm their breeding nature. Different characters like oil yield, oleic acid content and oil yield and oleic acid content were studied for all three generations simultaneously with control.

Plants with oil content above 40% were identified and labelled as oil yield mutants (OY). Fatty acid composition of seeds shown the oleic acid percentage higher than linoleic acid were identified and labelled as oleic mutants (HO), and seeds showing high oil content as well as high oleic acid content were labelled as oil–oleic mutants (OO).

Oil estimation (%) was carried out using low-resolution pulsed nuclear magnetic resonance (PC 20) at the Nuclear Research Laboratory (NRL), Indian Agricultural Research Institute (IARI), New Delhi, according to the method suggested by Madsen[11].

The fatty acid composition of the seed oil was determined by simultaneous oil extraction and methyl esterification[12]. This was followed by gas–liquid chromatography of fatty acid methyl esters on a Nucon gas–liquid chromatograph (Nucon GC 5765) equipped with packed column (1/8 inch, packing S.S.).

The oil yield estimated from control niger seeds was 34.60%, whereas the oleic acid content was estimated to be 28.31%. In the M1 generation, few plants have shown oil content more than the control (Table 1). Plants with oil content above 40% were identified and labelled as OY. Mutants for characters like oil yield, high oleic acid and oil yield and oleic acid have been isolated at 100 Gy, but not for higher doses like 200 and 300 Gy. The mutants isolated have shown higher values for all the characters compared to the control. Similar results were obtained from the M2 and M3 generations.

From the above studies, four better-performing lines (OY-155, OY-325, HO-308 and OO-309) of niger were finally isolated for further agricultural use. In the present study, at 100 Gy both oil yield and oleic acid content improved. Oil yield improved remarkably and oleic acid content was almost double. In contrast, results for irradiation at 200 and 300 Gy did not show any improvement for these characters. This may be due to the fact that increased doses of irradiation often lead to the development of various lethal effects.

The stimulating effect of low doses of gamma ray irradiation on plant growth may be due to stimulation of cell division or cell elongation, alteration of metabolic processes that affect synthesis of phytohormones or nucleic acids[13].

Niger seeds are known for their hull thickness or thick seed coat, which is the main reason for less oil yield in different accessions[14]. There may be two possible reasons for increase in oil content in the present study. The first may be induction of mutations by which the proportion of seed hull decreased, thereby increasing the oil content of the seeds. The other reason may be induction of mutations by which oil content of the embryo tissue increased[15,16].

The fatty acid composition of niger seed is typical of the Asteraceae family, with linoleic acid being the major fatty acid, thereby showing the semi-drying nature of the oil. There is a negative correlation between linoleic and oleic acid. That is, with increase in oleic acid content the linoleic acid content decreases, and vice versa. This is expected since oleic acid is the precursor fatty acid for

Table 1. Different characters, percentage oil yield, oleic acid yield and oil yield, and oleic acid content of selected niger mutants (M1–M3 generations) and control

Character	Control (%)	M1	M2	M3
Oil yield (OY)	34.60	OY-153-43.50 ± 0.04%	OY-153-39.70 ± 0.06%	OY-153-39.60 ± 0.01%
		OY-155-47.60 ± 0.39%	OY-155-44.70 ± 0.55%	OY-155-48.40 ± 0.01%
		OY-325-47.20 ± 0.04%	OY-325-44.50 ± 0.03%	OY-325- 47.80 ± 0.01%
High oleic acid (HO)	28.31	HO-271-43.72 ± 0.01%	HO-271-43.10 ± 0.03%	HO-271-42.90 ± 0.01%
		HO-272-50.20 ± 0.03%	HO-272-49.90 ± 0.01%	HO-272-49.70 ± 0.01%
		HO-307-55.56 ± 0.02%	HO-307-53.30 ± 0.01%	HO-307-53.22 ± 0.02%
		HO-308-43.80 ± 0.01%	HO-308-43.00 ± 0.01%	HO-308-43.10 ± 0.02%
		HO-310-58.21 ± 0.01%	HO-310-53.34 ± 0.02%	HO-310-53.20 ± 0.03%
Oil yield + oleic acid (OO)	34.60 + 28.31	OO-259-(44.00 + 59.95) ± 0.02%	OO-259-(37.50 + 52.89) ± 0.01%	OO-259-(37.60 + 52.77) ± 0.03%
		OO-267-(46.50 + 47.71) ± 0.03%	OO-267-(45.10 + 47.62) ± 0.01%	OO-267-(45.00 + 47.10) ± 0.01%
		OO-309-(45.00 + 59.61) ± 0.01%	OO-309-(44.60 + 42.27) ± 0.01%	OO-309-(45.90 + 59.75) ± 0.02%

linoleic acid. An increase in oleic acid will, therefore, lead to a corresponding decrease in linoleic acid[17–19].

It is possible that the increased level of oleic acid observed in the present study is the result of an induced mutation or mutations in the gene or genes responsible for desaturation of oleic acid. This may have reduced the level of polyunsaturated fatty acids in the oil, resulting in an increased level of oleic acid. The results obtained in the present study clearly demonstrate the stimulative effect of low doses of gamma irradiation in oleic acid and oil production in niger seeds.

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