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# Mutation breeding in niger (*Guizotia abyssinica* Cass): Review paper

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#### Abstract

Niger is a minor oilseed crop of India. It is cultivated in Ethiopia and India. The crop is cultivated in unproductive lands, marginal lands and hilly areas. It is a neglected crop and farmers are cultivating local varieties. It is a highly cross pollinated crop and is self incompatible. The capitula are very small and difficult to make hybridisation. So mutation breeding is the option to create variations. Physical and chemical mutagens are used to create mutations. An effort was made to review the mutation breeding in niger. A very limited work was carried out on mutation breeding in niger. Mutants were isolated for high oleic acid and high oil yield (Shabnum *et al.*, 2011), improved oil content and early flowering (Naik and Murthy, 2009) and improvement in seed weight per plant (Suvarna *et al.*, 2020). Other mutagenic effects of physical and chemical mutagens were also reported in niger (Maloo and Agrawal, 1995; Suvarna *et al.*, 2020, Premajyoti, 2006). Meiotic properties of C<sub>1</sub> plants was also studied (Dagne, 2001). Mutation breeding helps in isolating mutants with desirable traits. This crop needs more attention to improve the seed yield and oil content.

Keywords: Niger, guizotia, mutations

#### Introduction

Niger (Guizotia abyssinica Cass) is one of the important minor oilseed crop cultivated in Ethiopia and India. It constitutes about 50% of Ethiopian and 3% of Indian oilseed production (Getinet and Sharma, 1996)<sup>[3]</sup>. In Ethiopia, it is cultivated on waterlogged soils where most crops and all other oilseeds fail to grow and contributes a great deal to soil conservation and land rehabilitation. In India, it is cultivated on marginal lands, hilly areas and by tribal people. But this crop thrives best on well drained loamy soils of good depth and texture. It is known by various names such as Ramtil or Kalatil in India and Noog in Ethiopia. India, Ethiopia, East Africa, West Indies and Zimbabwe are the important niger growing countries in the world. In India, it is cultivated mainly in the states of Orissa, Maharashtra, Madhya Pradesh, Bihar, Karnataka and Andhra Pradesh and to some extent in hilly areas of Rajasthan, Uttar Pradesh, Gujrat, Tamilnadu, Assam, and also in some parts of North Eastern Hill states of the country. The productivity is very low and unpredictable due to its cultivation in marginal lands with negligible inputs and non-availability of suitable cultivars for diverse agro-climatic conditions. Niger is a cross pollinated crop with chromosome no. 2n = 30. The genus *Guizotia* belongs to the family Compositae, tribe Heliantheae, subtribe Coreopsidinae. The seed contains about 40% oil with fatty acid composition of 75-80% linoleic, 7-8% palmitic and stearic, and 5-8% oleic acids (Getinet and Teklewold, 1995)<sup>[4]</sup>.

Niger has been recognized as one of the 'neglected and underutilized' crop (Getinet and Sharma, 1996)<sup>[3]</sup> and thus, so far, it has received little attention from the scientific community. Despite its great potential, it suffers from a lack of improvement programme through modern breeding efforts. It is a self incompatible crop and there are no sources of male sterility. This creates practical problems in improvement of the crop. Increasing the seed yield, oil content and diversifying the oil quality and identification of self compatible lines and creation of male sterility are some of the major objectives of niger breeding. In order to overcome the practical problems mentioned above, there is a need to create variations through mutations breeding as it is difficult to make hybridisation in niger.

#### Mutations and mutation breeding

Genetic variation is a prerequisite for selection and crop improvement. The variations that are found in nature do not represent the original spectra of spontaneous mutations. Rather, they are the result of genotypes recombining within populations and their continuous interaction with environmental factors (Novak and Brunner, 1992)<sup>[10]</sup>. Mutations are the primary source of all genetic variations existing in any organism, including plants (Kharkwal, 2012)<sup>[5]</sup>.

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Assistant Professor, Department of Genetics and Plant Breeding, College of Agriculture, Raichur, UAS, Raichur, Karnataka, India The resulting variation provides the raw material for natural selection and is also a driving force in evolution. Spontaneous mutations are very rare and random in terms of time of occurrence, which makes them more difficult to use in plant breeding programmes (Lonnig, 2005)<sup>[6]</sup>. Primarily, simple selection of desirable offsprings was the first method of plant breeding and this utilized the occurrence of spontaneous mutations. Variations are created by hybridisation method and also through mutations artificially. If natural variations for the particular trait do not exist in the collected germplasm, the next option is creating the variation. Trait specific development of mutant varieties through inducing the mutations artificially is called mutation breeding. Thus mutation breeding involves the development of new varieties by generating and utilizing genetic variability through chemical and physical mutagenesis. It is now a pillar of modern plant breeding, along with recombinant breeding and transgenic breeding (Shu et al., 2012)<sup>[14]</sup>.

Mutagenesis is the process, whereby sudden heritable changes occur in the genetic information of an organism not caused by genetic segregation or genetic recombination, but induced by chemical, physical or biological agents (Roychowdhury and Tah, 2013) <sup>[12]</sup>. The induced mutagenesis, in which mutations occur as a result of irradiation (gamma rays, X-rays, ion beam, etc.) or treatment with chemical mutagens. Mutations as a mechanism of creating variability were first identified by Hugo de Vries in the late nineteenth century and coined the term 'mutation'. Radiation-induced mutagenic action of X-rays demonstrated in maize, barley and wheat by Stadler (Stadler, 1930)<sup>[15]</sup>.

**Mutagens:** Agents which induces artificial mutations in an organisms are called mutagens. They are generally grouped into two broad categories, namely chemical mutagens and physical mutagens (Acquaah, 2006 and Mba *et al.*, 2010)<sup>[1, 10]</sup>. To induce mutations in crops, planting materials are exposed to physical and chemical mutagenic agents. Mutagens can be treated to all types of planting materials, e.g. whole plants, usually seedlings, multiple forms of plant propagules, such as bulbs, tubers, corms and rhizomes in vegetatively propagated plants and *in vitro* cultured cells.

Physical mutagens are the radiations, which include alpha, beta, gamma, UV and X-rays, neutrons, protons and ion beams. The chemical mutagens includes the chemicals namely ethyl methane sulphonate (EMS), Methyl methane sulphonate, Alkylating agents, base analogues etc. Colchicine is also a chemical mutagen, which is used to induce polyploidy in plants will also create variations (mutations) in plants. This chemical is known to inhibit mitosis in a wide variety of plant and animal cells by interfering with the orientation and structure of the mitotic fibers and spindle fibers (Khan and Goyal, 2009). Since chromosome segregation is driven by microtubules, colchicine is therefore applied to interfere with mitosis to induce polyploidy and mutations in plant cells. The concentration of the mutagen, the length of treatment and the temperature at which the experiment is carried out affect the efficiency of mutagenesis. So that, the main advantage of mutational breeding is the possibility of improving one or two quantitative characters without changing the rest of the genotypes.

## Mutation breeding in niger

An effort was made to review the mutation breeding in niger. A very few efforts were made in niger and explained as below. Mutants were isolated for high oleic acid and high oil yield (Shabnam *et al.*, 2011)<sup>[13]</sup> and improved oil content and early flowering (Naik and Murthy, 2009)<sup>[9]</sup> in niger. Improvement in other characters was also reported in niger (Premajyoti, 2006)<sup>[11]</sup>.

Mutagenic effects of chemical mutagens (Ethyl Methane Sulfonate (EMS) and Methyl Methane Sulfonate (MMS) were studied by Maloo and Agrawal, (1995)<sup>[7]</sup>, EMS by Premajyoti, 2006<sup>[11]</sup>; Naik and Murthy, 2009<sup>[9]</sup>.

Physical mutagens (gamma rays) were used to study the mutagenic effects on niger (Maloo and Agrawal (1995), Premajyoti (2006); Naik and Murthy, 2009, Shabnam *et al.* 2011 and Suvarna *et al.*, 2020)<sup>[7, 11, 9, 13, 16]</sup>.

Maloo and Agrawal (1995)<sup>[7]</sup> treated the seeds of cultivar UN 4 with two doses of Ethyl Methane Sulfonate (EMS) and Methyl Methane Sulfonate (MMS) and also gamma rays. Variable response to the mutagens was observed for different characters in the M<sub>1</sub> generation. Plants with normal phenotype were selected from each treatment and advanced to the M<sub>2</sub> generation. These estimates of genotypic variation, heritability and genetic gain were high in the lines derived from treatment with EMS (0.25% dose, followed by 0.5%) and gamma rays (20 kR). EMS was most effective at lower doses in both the generations.

The variety N-71 was irradiated with five doses of gamma rays (24, 26, 28, 30 and 32 kR) and two varieties N-71 and IGP 76 were treated with three doses of EMS (0.3, 0.5 and 0.6%) (Premajyoti, 2006) <sup>[11]</sup>. The LD 50 dose for gamma irradiation was in between 30-33 kR. But LD 50 dose for EMS was in between 0.5-0.7 per cent. The mutagenic damage was more pronounced with EMS treatments compared to gamma irradiated populations. She studied chlorophyll mutants in M<sub>1</sub> populations. Maculate and striata mutants were more common in EMS treated and xantha and chlorine were more in gamma irradiated populations. Mutagenic damage in M<sub>1</sub> generation was more pronounced with EMS treatments compared to gamma irradiated populations. EMS at 0.3% was more effective in inducing varieties in both genotypes and 28 kR gamma irradiation was more effective in inducing variations in N 71. There was improvement in nine quantitative characters studied in mutated generations compared to normal. Correlation and path analysis revealed that plant height, number of branches, number of capitula per plant, number of seeds per capitula had significant positive association and direct effect on seed yield (Premajyoti, 2006) [11]

The variety N-71 was irradiated with different doses of gamma rays (0, 38, 76, 114 and 152 Gy) and EMS (0, 26, 52, 78 and 104 mM for 24 hrs). 200 seeds were used for each treatment (Naik and Murthy, 2009) [9]. They studied 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 M1 and M<sub>2</sub> generation. Observations were made on 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 for M<sub>1</sub> and M<sub>2</sub> generation. Variability was observed for the traits studied both in M<sub>1</sub> and M<sub>2</sub> generation. The plants showed decreased growth with increased dose and concentration of the mutagens. They observed reduction in traits such as germination and survival percentages, plant height, leaf length and width, number of primary branches and 1000 seed weight, but increases in number of capitula / plant, number of seeds / capitulum and seed yield / plant was observed in 52 and 78 mM treated M2

generated plants. Improved oil content was found in gamma rays 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.

Shabnam *et al.* (2011) <sup>[13]</sup> studied the effects of gamma irradiation on the oil yield and oleic acid content of niger. Seeds were irradiated with 100, 200 and 300 Gy doses of gamma rays. M1, M2 and M3 populations were studied. Plants were selected based on the expression of considerable variations and carried to next generations up to M<sub>3</sub>. Oil content and oil yield were studied in all three generations. Mutants with 40% were identified as oil yield mutants. Higher percentage of oleic acid containing mutants than linoleic acid was identified. Oil yield and oleic acid content was improved with 100 Gy irradiation. Four better performing mutants (OY 155, OY 325, HO 308 AND OO 309) were identified for further agricultural use.

Suvarna *et al.*,  $(2020)^{[17]}$  evaluated the gamma irradiated M<sub>1</sub> generation of RCR 18 and DNS 17 varieties. The seeds of these varieties were exposed to six doses of gamma rays viz., 150, 175, 200 225, 250 and 275 Gy. The characters plant height, number of primary branches and number of capitula at harvest and seed weight was studied for each plant. Mutants showed variations for all the characters. The LD<sub>50</sub> dose was found to be 250 Gy for DNS 17 and 275 Gy for RCR 18. Maximum seed weight / plant was found in the control (1.7360 g) followed by 150 Gy irradiated mutants in DNS 17, but in RCR 18 maximum seed weight / plant was found in mutants irradiated with 150 Gy (3.92280 g) followed by 250 Gy (3.1334 g). Improvement in the characters studied was observed in RCR 18 mutants. So isolation of mutants for high seed weight / plant helps in increasing the yield of niger and also contribute to more productivity.

The colchicine is generally used to induce the polyploidy in plants. So it also creates mutations in the treated plants. An effort was made to induce the polyploidy using colchicine and studied the meiotic properties in the induced  $C_1$  plants (Dagne, 2001) and its mutagenic effects on niger plants were studied (Suvarna, unpublished).

Five autopolyploid C1 plants realized from the seeds obtained on intercrossing the two normal plants (Dagne, 2001). Aneuploid C<sub>1</sub> plants with one plant of 2n=61, two plants of 2n=59, one plant of 2n=57 and one plant of 2n=56 chromosome number were reported based on Somatic and meiotic chromosome analysis. Univalents although in a low frequency of 1.15 to 7.39, were present in all pollen mother cells (PMCs), except in a few PMCs of the 2n=56 plant. Bivalents were the most frequent type of association present, with their mean ranging from 24.21 to 27.23. Only a total of four cases of trivalents were observed in two of the plants. Also quadrivalents occurred in low frequency with a mean range of 0.38 to 1.49, and were predominantly of the ring type. Four of the plants had pollen stainability of about 50-85%, and one had 6%. None of the  $C_1$  plants set seeds even though they were exposed to pollinators and flowers were rubbed together by hand.

Niger variety RCR 18 was treated with 0.05%, 0.1%, 0.15%, 0.2% colchine (Suvarna, unpublished). The soaked seeds were treated with colchicine solution with different concentrations for 6-8 hours. 0.1 % was found to be LD 50 dose. Among  $C_1$  plants, some showed abnormality, seedling mortality, chlorophyll deficiency. One plant exhibited three leaves in a single whorl and was healthy (Fig.1). But unfortunately, the plant was damaged and could not maintain the plant. Other

plants were selfed and harvested the seeds. The seeds harvested per plant were less in number compared to control.



Fig 1: C<sub>1</sub> plant which had three leaves per node.

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