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GAMMA RAYS-INDUCED MUTANT SPECTRUM AND FREQUENCY IN SESAME

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ABSTRACT

A comparative study was made to determine the spectrum and frequency of different mutants induced by gamma rays in three genetic backgrounds of sesame and to test the hypothesis that "closed capsule mutants are inducible with efficient mutagenesis and screening large populations". Treatments concerned seeds of the two cultivars "32-15" and "38-1-7" extensively grown in Senegal and a recently released Turkish cultivar, "Birkan", irradiated by two doses (300 and 400 Gy) of gamma rays. Both irradiated and untreated seeds (control) were sown greenhouse to raise the M1 populations. The M2 populations produced from the bulked M1 plants at harvest were grown twice under irrigated conditions in two sets; first set in Antalya, Turkey, in 2008 and the second set in Bambey, Senegal, in 2009. The selected potential mutants in the 2-set of M2 populations were grown in the M3 stage in Bambey to confirm their true breeding behaviour. Results indicate that the mutants confirmed shared a wide and unique spectrum of the mutants such as closed capsules, branching habit, flowering types, capsule number and shape. Also at least one closed capsule mutant could be induced from each of the three genetic backgrounds of sesame tested. Among the cultivars tested "38-1-7" had the highest total mutant frequency (5.6 x 10^{-3}) followed by "Birkan" (4.2 x 10^{-3}). The lowest mutant frequency (2.7 x 10^{-3}) was recorded in "32-15". From these results, it appears that medium dose range of gamma rays was enough to induce viable and useful mutations in sesame.

Key words: Irradiation, *Sesamum indicum* L., mutation, unique mutants.

INTRODUCTION

Sesame (*Sesamum indicum* L.) is an important oilseed crop, highly tolerant to both heat and drought (Weiss, 2000). Its seeds are a good source of proteins (23-30 g/100 g) and very rich in oil (50 g /100 g), and other minerals and vitamins. Nowadays, there is a renewed interest of Senegalese farmers to integrate this crop in their production system. Sesame acreage in Senegal increased from 1,615 ha in 1994/95 to 52,420 ha in 2005/06 with a total seed production of 31,839 t in 2006 (Anonymous 2006).

However, average seed yield is low (350- 400 kg ha^{-1}) due to the improper cultivation with high planting density using photoperiod sensitive cultivars and with low amounts of fertilizer used, late planting as a secondary crop, and seed loss following capsule shattering. At maturity and in most commercial cultivars, capsules dehisce along their sutures and shatter their seeds on the ground (Ashri 1998; Çağırgan 1996b). This shattering is more accentuated during windy conditions (Langham 2007). Indehiscence, capsules remain closed at maturity, is therefore an important prerequisite for developing more intensive farming of sesame in Senegal. However, currently all available commercial sesame cultivars in Senegal and other countries are dehiscent. In order to increase sesame productivity, cultivars suited to high input levels under irrigated conditions should be developed since its yield can thus be increased two to three times in comparison to non-irrigated conditions (Çağırgan 1994). To our knowledge, the only available natural recessive gene for indehiscent capsules was found in Venezuela in 1942 by Langham (1946) but despite intensive efforts it could not be used successfully in the variety improvement because of many undesirable side characteristics, including reduced yields due to poor seed set and susceptibility to diseases, (Çağırgan 1996b, 2001 and Ashri 2007). Çağırgan (2001) obtained wide spectrum of the useful mutants including 8 independent induced closed capsule mutants for the first time in two sets of the Turkish genetic backgrounds of sesame. Further claimed Çağırgan (2007) that the unique mutants such as closed capsule are inducible in any background of sesame if large enough mutated populations are screened preferably derived from 5,000 M1 plants with efficient mutagenesis causing deletions. So, closed capsule mutants can probably only be obtained through induced or spontaneous mutations, or else through transgenic manipulations, since the trait is not repeatable/frequent in the current germplasm collections.

In general, mutagenesis has been successfully used to induce genetic variability in many crops, allowing to isolate mutants with desirable characters of economic importance such as increased seed yield, earliness (Wongyai et al., 2001), modified plant architecture, closed capsules, disease resistance (Çağırgan 1994, 2001; Ashri 1998), seed retention, larger seed size, desirable seed colour and high oil content (Hoballah 2001). Many new cultivars have been directly or indirectly released in the world through induced mutations. The number of mutant varieties officially released and recorded in the FAO/IAEA Mutant Variety Database by the beginning of the 21st century reached 2252 varieties for such crop as cereals, oilseeds, pulses, vegetables, fruits, fibres and ornamental plants (Kharkwal et al., 2004). With appropriate exposure doses of gamma rays which are known for their simple application, good penetration, reproducibility, and limited disposal problems (Chahal and Gosal 2002), sesame may undergo adequate mutations that could be of significant benefit to agriculture.

Therefore, the aims of the study were to determine the spectrum and frequency of the mutants to be induced in three genetic backgrounds of sesame irradiated by gamma rays and to test the hypothesis that "closed capsules mutants are inducible with efficient mutagenesis causing deletions and screening large enough populations".

MATERIALS AND METHODS

Plant material: Seeds of two African sesame (*Sesamum indicum* L.) cultivars, “32-15” and “38-1-7”, widely grown in Senegal and a new Turkish mutant cultivar, “Birkan” as a reference of the experimental location, were selected for irradiation material. Cultivars characteristics are shown in Table 1.

Gamma irradiation and growing M1: Fifty g of air-dried seeds of the three test cultivars, which all three have one capsule per leaf axil, were irradiated with 300 and 400 Gy of gamma rays from a ^{60}Co source at the Agricultural Laboratory of the International Atomic Energy Agency, Seibersdorf, Austria, in February 2008.

Table 1. Characteristics of the cultivars used as parent material

Cultivar	Branching	Physiological maturity (days)	Capsule type	Seed color
32-15	Semi-branched	90	Bicarpel	White
38-1-7	Semi-branched	95	Bicarpel	Yellowish-light
Birkan	Semi-branched	90	Bicarpel	Yellowish/light brown

These doses had been found to be effective in Turkish sesame cultivars in previous studies (Çağırgan 1996a, 1996b). Irradiated seeds were taken to Antalya, Turkey and kept in a refrigerator (±4 °C) until planting time. Both irradiated and untreated seeds (control) were sown in greenhouse to grow the M1 in off-season to speed up the programme. Sowing was done under close spacing (trays) in order to prevent multi-branching in green house in March 2008. At physiological maturity, plants were bulk-harvested per parent cultivar and per dose. Capsules were threshed in the laboratory after they had been air-dried for 3- 4 days.

Mutant selection and confirmation: The M2 generation of cultivar “Birkan”, a well-adapted Turkish cultivar, was grown outdoor during the regular season in June 2008 under irrigated conditions. To optimise germination and increase probability of finding closed capsules mutants, seeds were first sown in the controlled conditions to obtain plantlets and then the plantlets were transplanted to the field at three weeks after emergence. Closed capsule mutants were screened at the cotyledonary stage and confirmed at the harvest on the individual mutant plants. Prior to transplantation, the soil was ploughed at a 25 cm depth. Nitrogen (15 % N), phosphorus (15 % P_2O_5) and potassium (15 % K_2O) were applied at a rate of 60 kg ha^{-1} as a composite fertilizer. Populations were grown in rows 80 m long and 70 cm apart. Intra row spacing was 12 cm. Sprinkler irrigation was done before and after transplantation and thereafter when necessary based on soil and plant conditions. During the growth and development of plants, all potential mutants were tagged and selected. The M2 generation of cultivars “32-15” and “38-1-7” was grown in Bambey (Senegal) during the dry season of 2009 under irrigated conditions. This precaution was taken according to the photosensitivity of two cultivars which had needed more time prior to flowering in higher latitudes of Turkey (Boureima et al., 2009). The M1 plant progenies were grown in 30 m rows, 60 cm apart with plants at 20 cm within rows. After each ten rows, one parental control row (untreated) was sown. Agronomical practices were done as described above. Weed was controlled by hand. Potential mutants selected from the three cultivars in the 2008 and 2009 seasons were grown as the M3 generation in Bambey to confirm true breeding behaviour during the 2009 rainy season in progeny rows of 2 m long and 60 cm apart. Spacing between plants within rows was 20 cm. After emergence, plants were thinned to two plants per hole within each row. Mutant frequency was estimated according to Çağırgan (2006) by dividing total number of mutants confirmed by total number of the M2 plants in the bulk population.

RESULTS AND DISCUSSION

Mutant spectrum and frequency

Large M2 populations were grown in the summer season of 2008 in Antalya Turkey and in 2009 in Bambey Senegal under irrigated conditions. In total, 27,208 M2 plants were grown in bulk during 2008 and 2009 seasons for the three cultivars under consideration. M2 population sizes for each cultivar are given in Table 2 and Table 3. The so-called viable mutations induced gross morphological changes in branching, stem structure, phenology, growth habit, capsules shape and size, seed coat color and plant architecture. All mutants were confirmed to breed true in the M3 generation. A detailed analysis of the spectrum and mutants frequencies per cultivar and gamma rays doses is given in Table 2 and Table 3. Since populations were grown in bulk, the frequencies of viable mutations were determined as in Çağırgan (2006). It can be seen in Table 2 and Table 3 that there is no specific mutation type for any cultivar but that all

Table 2. Spectrum and frequencies of induced mutants in three sesame cultivars for the first group of characters

Cultivars	Dose (Gy)	M2 Plants	Spectrum and mutant frequency				
			Closed cap.	Hairy	Multi-cap./ leaf axil	Cap. Shape & size	Early flowering
32-15	300	8680	2.3×10^{-4}	1.2×10^{-4}	6.9×10^{-4}	1.2×10^{-4}	3.5×10^{-4}
	400	3472	–	2.8×10^{-4}	8.6×10^{-4}	5.7×10^{-4}	2.8×10^{-4}
	Overall	**12152**	$\mathbf{1.6 \times 10^{-4}}$	$\mathbf{1.6 \times 10^{-4}}$	$\mathbf{7.4 \times 10^{-4}}$	$\mathbf{2.4 \times 10^{-4}}$	$\mathbf{3.3 \times 10^{-4}}$
38-1-7	300	5952	1.7×10^{-4}	6.7×10^{-4}	1.5×10^{-3}	5×10^{-4}	3.4×10^{-4}
	400	829	–	–	–	–	2.4×10^{-3}
	Overall	**6781**	$\mathbf{1.5 \times 10^{-4}}$	$\mathbf{5.9 \times 10^{-4}}$	$\mathbf{1.3 \times 10^{-3}}$	$\mathbf{4.4 \times 10^{-4}}$	$\mathbf{5.9 \times 10^{-4}}$
Birkan	300	6429	7.8×10^{-4}	1.5×10^{-4}	1.2×10^{-3}	–	1.6×10^{-4}
	400	1846	5.4×10^{-4}	5.4×10^{-4}	2.7×10^{-3}	–	–
	Overall	**8275**	$\mathbf{7.3 \times 10^{-4}}$	$\mathbf{2.4 \times 10^{-4}}$	$\mathbf{1.6 \times 10^{-3}}$	**–**	$\mathbf{1.2 \times 10^{-4}}$
	Total	**27208**	$\mathbf{3.3 \times 10^{-4}}$	$\mathbf{2.9 \times 10^{-4}}$	$\mathbf{1.1 \times 10^{-3}}$	$\mathbf{2.2 \times 10^{-4}}$	$\mathbf{3.3 \times 10^{-4}}$

-- No mutant recorded

mutation types can take place in any of the 3 cultivars. Otherwise, some mutations such as multi- capsules per leaf axil and male sterility can be more easily induced than other types of mutations.

Differences were observed between cultivars for total mutation rate (Table 4). Among the cultivars tested, total viable mutation frequency indicated a maximum rate for "38-1-7" (5.6×10^{-3}), followed by "Birkan" (4.2×10^{-3}). Lowest mutation frequency (2.7×10^{-3}) was observed for cultivar "32-15" which was shown the more sensible cultivar to gamma rays (Boureima et al., 2009).

Individual Mutant Traits

Closed capsule mutants: Prevention of seed loss or its reduction at maturity and during harvest is the key to a successful cultivation of sesame for intensive production. During the two experiments, 9 mutants were found with closed capsules (Fig.1a). The highest mutation frequency for closed capsule mutants was observed in "Birkan" (7.3×10^{-4}) with 6 mutants (Table 2). From these six mutants, one was selected in the M3 generation from a mono stem mutant found in M2. Cultivar "32-15" yielded two similar looking mutants selected from the same population derived from an irradiation dose of 300 Gy. These two closed capsule mutants may derive from the same M1 plant. Only one closed capsule mutant was selected from "38-1-7", which was infertile. Cultivars "32-15" and "38-1-7" had similar mutant frequencies for closed capsules, 1.6×10^{-4} and 1.5×10^{-4} respectively. As reported earlier by Çağırgan (1996a; 2007), 300-400 Gy dose range of gamma rays is effective enough to induce closed capsule mutants from any sesame background. The present findings appear to support this conclusion.

Table 3. Spectrum and frequencies of induced mutants in three sesame cultivars for the second group of characters

Cultivars	Dose (Gy)	M2 Plants	Spectrum and mutant frequency			
			Multi-carpels	Split corolla	Uniculm	Male Sterile
32-15	300	8680	2.3×10^{-4}	1.2×10^{-4}	–	1×10^{-3}
	400	3472	–	–	2.9×10^{-4}	–
	Overall	**12152**	$\mathbf{1.6 \times 10^{-4}}$	$\mathbf{0.8 \times 10^{-4}}$	$\mathbf{0.8 \times 10^{-4}}$	$\mathbf{7.4 \times 10^{-4}}$
38-1-7	300	5952	1.7×10^{-4}	–	3.4×10^{-4}	1.7×10^{-3}
	400	829	–	–	–	6×10^{-3}
	Overall	**6781**	$\mathbf{1.5 \times 10^{-4}}$	**–**	$\mathbf{2.9 \times 10^{-4}}$	$\mathbf{2.2 \times 10^{-3}}$
Birkan	300	6429	–	1.6×10^{-4}	–	1.4×10^{-3}
	400	1846	5.4×10^{-4}	–	–	1.1×10^{-3}
	Overall	**8275**	$\mathbf{1.2 \times 10^{-4}}$	$\mathbf{1.2 \times 10^{-4}}$	**–**	$\mathbf{1.3 \times 10^{-3}}$
	Total	**27208**	$\mathbf{1.5 \times 10^{-4}}$	$\mathbf{3.7 \times 10^{-5}}$	$\mathbf{3.7 \times 10^{-5}}$	$\mathbf{1.3 \times 10^{-3}}$

-- No mutant recorded

Table 4. Total mutation rate pooled over cultivars

Cultivars	Dose (Gy)	Population Size	Total number of mutants	Frequency
32-15	300 + 400	12152	33	2.7×10^{-3}
38-1-7	300 + 400	6781	38	5.6×10^{-3}
Birkan	300 + 400	8275	35	4.2×10^{-3}

However, high mutation frequency of closed capsules in "Birkan" should be reasoned with care because of the further segregations in M3 which reminds outcrossing contribution to this high frequency. However, the seed of African cultivars were obtained from the plants grown in an area where no closed capsule mutants were grown previously. Indeed, we could finally select at least one closed capsule mutant in two of the three sesame genetic backgrounds of our study free from any suspicion. Çağırgan (2001) stated that when screening for closed capsules it is advisable to arrange M2 populations in the form of M1 plant progenies instead of in bulk although he selected the cc-1 in a bulk, the first induced closed capsule mutant isolated for the first time in the world. But, if bulk harvesting of M1 is preferred for labor and space efficiency, then the M2 populations must be grown in a very well-prepared seed beds with spaced planting to avoid loosing incompetitive mutant phenotypes.

Hairy mutants: Hairiness may be related to drought tolerance. Since the target environment is frequently subjected to drought, developing drought tolerant lines is another key requirement for cultivation of sesame under rainfed conditions. We selected 8 mutants in which only the capsules or both the capsules and other parts of the plant were covered by dense hairs. Hairy mutants are easy to obtain (Çağırgan 2001). Two mutants from "Birkan" background had dense hairs both on capsules and stems whereas 6 from "32-15" and "38-1-7" had hairs only on the capsules. Cultivar "38-1-7" had the highest frequency (5.9×10^{-4}) while the lowest frequency was observed in "32-15" (1.6×10^{-4}).

Multi-capsules per leaf axil mutants: In this category, 30 mutants with up to 3 capsules per leaf axil were selected (Fig. 1b) whereas the source parents have only one capsule per leaf axil. In general, these mutants have rarely multi-capsules at each node and present single capsules at the bottom and the top of the main stem. "Birkan" had the highest frequency for multi-capsules per leaf axil mutants (1.6×10^{-3}) while the lowest (7.4×10^{-4}) was observed in "32-15" (Table 2). Many authors Asthana and Raj (1970), Osman Khidir and Osman (1970), Ganesh and Sakila (1999) stated that number of capsules on main stem and number of capsules on branches showed a high positive direct effect on single plant seed yield in sesame. Baydar (2005), breeding for the ideal plant type in sesame, considered lines with multi-capsules per leaf axil as ideal plant type in breeding for high-yielding varieties. In the USA, Langham (2007) reported that farmers commented that varieties should be developed with triple capsules.

Capsule size or shape: Six mutants were confirmed under this category with "38-1-7" having the highest mutant frequency (4.4×10^{-4}). No confirmed mutant for capsule size or shape was scored for "Birkan" (Table 2). The contribution of this character in plant seed yield may be investigated.

Early flowering mutants: Earliness has been an important objective of breeding in several crops under different ecological conditions. Nine early mutants were confirmed. Frequencies were different from a cultivar to another with "38-1-7" having the highest frequency (5.9×10^{-4}) followed by "32-15" (3.3×10^{-4}) and "Birkan" having the lowest (1.2×10^{-4}). Precocity of one to two weeks was obtained comparative to their source parents. The seed coat color of all these mutants was also different from that of the source cultivars. Eight of them had brown seed coat while the source parents were whitish. The other one had whitish seed coat while the parent source (Birkan) seed coat was yellowish-light brown (data not shown). This trait is related with day-length sensitivity and Birkan is an insensitive genetic background in comparison to the African cultivars of this study, which are very sensitive to photoperiod and causes no capsule set in the first half of the plant. Consequently, it should be considered a useful drought escape mechanism.

Multi-carpel mutants: Four multi-carpel mutants with capsules having 8 locules were selected (Fig. 1c). In one mutant from "32-15" background, the stem is fasciated at the top. Another multi-carpel derived from "38-1-7" had split corolla and was grouped under "split corolla mutants". Cultivars "32-15" and "38-1-7" had the highest and similar mutant frequencies, 1.6×10^{-4} and 1.5×10^{-4} respectively while the lowest was observed in "Birkan" for this trait (Table 3). Langham (2007) reported that by converting lines from 2 carpels to 4 carpels, the seed weight per 4 carpel capsules was greater although it didn't double. Baydar (2005) screening for the ideal plant type in sesame found that lines with quadricarpels, tricapsules per leaf axil and unbranched had the highest oil content. Since it is a recessive character, it is obtained easily by mutagenesis (Çağırgan 2001)

Sterile mutants: For intensive management conditions, development of male sterility mechanism for hybrid seed production is a key factor. Although crossing is not difficult in sesame, genetic male sterility should be useful in population development and facilitating cyclic breeding to modify unwanted side effects of mutations (Çağırgan et al., 2009). Many authors (IAEA 1994a, 1994b; Murty 1994) described some interesting male sterility systems used in hybrid seed production. We confirmed 35 sterile mutants with different types of sterility: sterile without flowers and very bushy (Fig. 1d), sterile with split corolla derived from "Birkan", sterile with very small capsules. The highest frequency of sterile mutant was observed in "38-1-7" (2.2×10^{-3}) and the lowest (7.4×10^{-4}) in "32-15" (Table 3). A close relationship was observed between sterile mutation rate and total viable mutation. Cultivars with highest sterile mutant frequencies had the highest total viable mutation as revealed

Figure 1. General view of some mutation types. Closed capsule (a), multicapsules per leaf axil (b), multicarpels (c), sterile mutant (d), uniculm (e), split corolla (f).

in Tables 2, 3 and 4. However characterization of these sterile mutants remains as an important task.

Uniculm: Non-branched cultivars are suitable for dense stands under high input conditions. In this category, we selected 3 uniculm mutants (Fig. 1e). One mutant from "32-15" and 2 mutants from "38-1-7". "Birkan" did not yield any uniculm mutant.

Split corolla: This character is also useful for open pollination and constitutes another mechanism for hybrid seed production. Two mutants with split corolla were confirmed (Fig. 1f). High sterility was noticed in one of these mutants derived from "Birkan" due to rudimentary floral parts. Çağırgan (2001) reported a male sterile mutants linked with split corolla trait in a large mutant population in Turkish genetic backgrounds.

Other mutants: Some other mutants including dwarf type, early maturity and white seed coat color were also selected. In our target area of Sahel, wind should be taken into account in the breeding program due to the possibility of lodging. In the reproductive phase, high capsules bearing and the height of the plant may make plants more susceptible to lodging or break of the stem due to winds event. In these conditions dwarf mutants are more suitable. Clear seed coat color is more appreciated in the world market. Otherwise, it has been shown by many studies that seed color may be associated with oil content and that accessions with high oil content had white seeds (Namiki 1995; Were et al., 2001).

Based on these results it could be concluded that, although sesame seeds were known to be resistant to gamma radiation, it is not necessary to apply higher doses since medium doses (300-400 Gy) could be effective to generate viable mutations. Cultivars with moderate gamma rays resistance (Birkan and 38-1-7) can be expected to give higher rates of mutations than the sensitive ones (32-15) and is in agreement with the observations in other crops like chickpea (Kharkwal 1999). A close relationship between frequency of male sterility and viable mutations rates was also observed.

At least, one closed capsule mutant was yielded by each of three cultivars irradiated by 300 and 400 Gy doses of gamma rays. It was finally concluded that closed capsule mutants are easily obtainable also in African genetic backgrounds when grown big enough populations mutated with efficient mutagenesis of gamma rays causing deletions as stated and hypothesized by Çağırgan (2007).

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