RADIOSENSITIVITY OF AFRICAN SESAME CULTIVARS TO GAMMA-RAYS

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ABSTRACT

The effects of gamma irradiation on germination, seedling height and survival rate of two sesame (*Sesamum indicum* L.) cultivars from Senegal were investigated. Seeds of the extensively grown cultivars in Senegal, "32-15" and "38-1-7", were irradiated with 0, 100, 200, 300, 400, 500, 600, 700 and 800 Gy. The irradiated seeds (0, 300 and 400 Gy) of a Turkish cultivar "Birkan" were also used as a reference. Irradiated seeds were sown with their respective controls both in field and greenhouse conditions to assess germination rate, seedling height and survival rate as affected by the different doses of gamma rays. Germination, seedling height and survival rate significantly decreased with increasing irradiation dose. The depressive effect of radiation on germination was more pronounced in the field than in the greenhouse conditions. Cultivar "32-15" was more sensitive to gamma irradiation than cultivar "38-1-7". The effective dose which caused 50 % growth reduction was 645 Gy for variety "32-15" and 740 Gy for variety "38-1-7". The lethal dose (LD_{50}) determined at 50 days after sowing was 550 Gy and 740 Gy for "32-15" and "38-1-7", respectively. From the parameters studied, seedling height reduction or LD_{50} can equally be used in determining effective dose suitable for inducing artificial mutations.

Key words: Mutagenesis, *Sesamum indicum* L., primary damage, chimerism

INTRODUCTION

Sesame (*Sesamum indicum* L.) is a very ancient oilseed crop and one of the earliest domesticated oil crop in the world. It acquired importance as a source of cheap vegetable oil and proteins, good source of natural antioxidants (sesamin and sesamolin) which are unique for sesame and present in the oil (Ashri, 2007).

Ionizing radiation has been routinely used to generate genetic variability for breeding and genetic studies. Mutagens used may cause genetic changes in an organism, break linkages and produce many new promising traits for the improvement of crop plants (IAEA, 1977). Induced mutation is highly instrumental in plant biology to induce genetic variability in a great number of crops. The technology is simple, relatively cheap to perform and equally usable on a small and large scale (Siddiqui and Khan, 1999). By varying the mutagenic agent dose, the frequency and saturation of mutations can be regulated (Menda *et al*., 2004) and mutagenic agents can induce different extensions of genomic lesions, ranging from base mutations to larger fragment insertions or deletions (Kim *et al*., 2006). Mutants induced by gamma irradiation are often generated by deletion of large DNA fragments, up to 6 Mb (Naito *et al*., 2005).

There is a great variability between and within species with regard to sensitivity to gamma irradiation (Pathirana and Subasingbe, 1993). Sesame (*Sesamum indicum* L.) does exhibit such a great genotypic variability in its response to gamma rays and has seeds highly resistant to irradiation (IAEA, 1994). It is therefore advisable to conduct pilot dosage experiments on mutagen sensitivity of cultivars chosen for mutation induction. Gamma rays doses ranging from 150-800 Gy proved successful in inducing useful mutations (Van Zanten, 2001). Generally, seed germination, seedling growth and chromosomal aberration are the commonly used criteria for radiosensitivity in plants and in certain cases M1 sterility has been shown to be appropriate criterion (Kivi, 1964).

The present study was carried out to (1) obtain practical knowledge about the effectiveness of gamma irradiation (physical mutagen) in the M1 generation in two African sesame cultivars and (2) estimate the mutagen doses effective to reduce the growth and the survivors by a given proportion.

MATERIALS AND METHODS

Parent material

The seeds of two African sesame cultivars, "32-15" and "38-1-7", widely grown in Senegal and a new Turkish mutant cultivar, "Birkan" as reference of the experimental location, were selected for irradiation as parent material. The experiment was conducted in Antalya, Turkey. Characteristics of the cultivars are shown in Table 1.

Table 1: Characteristics of the cultivars used in the radiosensitivity study.

Cultivars	Branching	Physiological maturity (days)	Seed colour	1000-seed weight (g)
32-15	Semi	90	White	4.03
38-1-7	Semi	95	Cream	3.03
Birkan	Semi	90	Yellowish-light brown	4.07

Gamma irradiation treatment

Prior to the mutagenic treatment, African varieties were grown for one generation to ensure their homozygosity. Five grams of air-dried seeds of varieties "32-15" and "38-1-7" were exposed to different doses of gamma irradiation, i.e. 100, 200, 300, 400, 500, 600, 700 and 800 Gy (Gray) from a Cobalt ^{60} source. In another set, 50 g seeds of "Birkan" (as a reference) were irradiated only with 300 and 400 Gy doses which were found effective for inducing useful unique mutations in genetically related Turkish sesame genetic backgrounds in the previous studies (Cagirgan, 1996, 2001). Gamma irradiation to the seeds was applied at the FAO/IAEA Agriculture and Biotechnology

Laboratory in Seibersdorf, Austria in February 2008. Irradiated seeds were kept at 4 °C in a refrigerator and subsequently used for greenhouse and field studies.

Greenhouse study

The treated and untreated control seeds were sown in trays in greenhouse. The experiment was laid out as a Randomized Complete Block Design with five replicates. For each replicate, sixteen seeds per treatment were planted. Thereafter, the trays were placed in a germination room maintained at 24±1°C. Emergence started 4 days after sowing and the germination rate was recorded one week after sowing by counting the number of germinated seeds for each treatment. Three weeks after sowing, seedling height was recorded in 5 randomly selected plants from each treatment and 50 % growth reduction dose (GR_{50}) for each cultivar was determined by the ratio of irradiated over the control. One month later and before flowering, seedlings were transplanted in the field in 5 m rows, 70 cm apart with a distance of 12 cm between plants and sprinkler irrigated just after transplantation. Thereafter, irrigation was set when necessary based on soil condition. Survival rate was recorded 3 weeks after transplantation (50 days after sowing).

The data was subjected to analysis of variance and 50 % growth reduction dose for seedling height and the lethal dose (LD_{50}) were determined by using computer software "R". To perform statistical analysis, we have considered a 2 (varieties) x 9 (doses) factorial design.

Field studies

The seeds were directly sown in well prepared field in 2 m rows and 70 cm apart with closer spacing (1 cm between the plants within the row) to prevent multiple-branching in a Completely Randomised Blocks Design with four replicates. Before planting, the soil was ploughed to 20 cm depth and 80 kg ha^{-1} of complex N-P-K (15-15-15) fertilizer was applied before sowing. Just after planting, water was supplied by sprinkler irrigation and subsequently whenever soil water potential indicates water deficit. Seed emergency rate was recorded.

RESULTS AND DISCUSSION

Germination

In the controlled condition, "Birkan" exhibited the highest overall seed germination percentage of 94.58 % compared with 92.98 % and 89.67 % for "32-15" and "38-1-7" respectively for 0, 300 and 400 Gy (Table 2). Germination rate showed significant negative correlation with gamma rays ($r = -0.963$, $p<0.001$) (Table 3). All three varieties showed negligible effect of lower gamma irradiation doses on germination percentage. These results are similar to those of previous studies in sesame and in other crops reported negligible effect of gamma irradiation on germination percentage at lower doses (IAEA,1977).

In the field, percentage of emergence was significantly reduced in all varieties. Seeds irradiated with doses higher than 400 Gy did not germinate in the conditions of our study (data not shown).

Cheema and Atta (2003) examined varietal differences of three basmati rice cultivars with regard to sensitivity to gamma irradiation. They found a negative correlation between germination rate and radiation dose under field conditions in the M1 generation although the gamma ray treatments induced some stimulatory effects on total spikelets at maturity. Reduction in germination percentage with increasing dose of gamma irradiation has also been reported in many crops including cowpea (Uma & Salimath, 2001), chickpea (Toker & Cagirgan, 2004), lentil (Kumar & Sinha, 2003) and rice (Pons *et al.,* 2001).

Table 2: Mean of seed germination percentage and days to first flowering in three sesame cultivars after irradiation with gamma rays.

Treatment	Germination (%)			Days to first flowering		
Radiation (Gy)	32-15	38-1-7	Birkan	32-15	38-1-7	Birkan
Control	96.25	91.25	95	74	86	36
100	93.75	91.25	-	74	86	-
200	93.75	90	-	74	86	-
300	92.5	89.06	95	74	86	36
400	90.2	88.75	93.75	74	86	36
500	90.2	87.3	-	77	90	-
600	86.25	86.75	-	81	105	-
700	81.25	84	-	81	105	-
800	81	80	-	81	105	-

Seedling height

Seedling height is widely used as a criterion in determining the biological effects of various mutagens in M1 plants. The present study showed that seedlings height decreased in all varieties with increasing radiation dose (Figure 1). Significant differences ($p < 0.01$) among varieties in response to the various doses of radiation were observed for seedling height. This difference is more pronounced with the highest doses. Variety “38-1-7” appeared to be less radio-sensitive than variety “32-15” after 500 Gy. Varietal and genotypic variation in radiosensitivity has partly been attributed to difference amount of linoleic acid in seeds (Bowen and Thick; 1961). The rhythm of seed hydration during germination may also play a role in determining the degree of radiosensitivity (Gelin *et al.,* 1958).

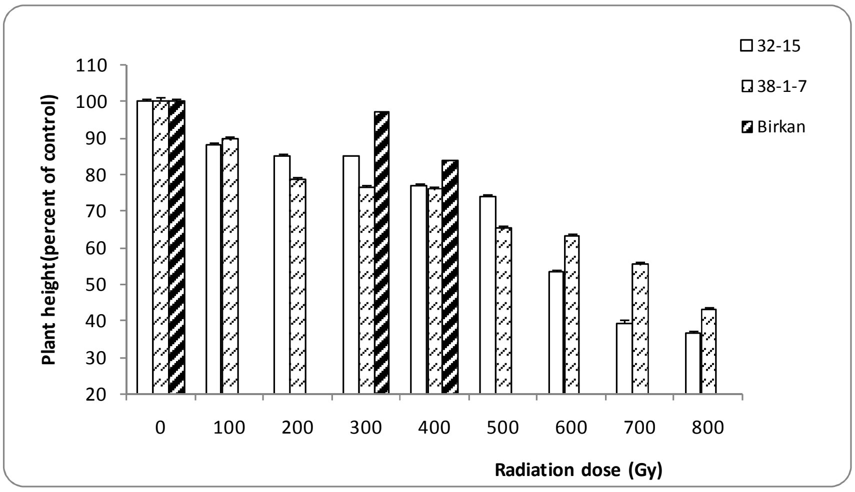

Figure 1: Seedling height reduction in percent of control

Seedling height showed significant negative correlation (r = - 0.985; p<0.001) with gamma rays (Table 3). Reduction in seedling height by increasing doses of gamma irradiation have also been reported in other species including basmati rice (Cheema & Atta, 2003), rice (Pons *et al.*, 2001). The reduction in seedling height may be attributed to damage induced during cell division and elongation following mutagenic treatment (Iqbal, 1969; Walther, 1969). Khanna & Maharchandani (1981) measuring peroxidases activity in chickpea seedlings obtained from irradiated seeds found that gamma irradiation caused damage to tissues by producing H_2O_2 and organic peroxy radicals and inhibited peroxidase activity at higher doses. They concluded that at higher doses the whole of the cell metabolism was impaired resulting in lower enzyme activity.

Table 3. Correlation of varieties with radiation dose and their response to gamma irradiation.

Plant character	Correlation	Control	Reduction (%)
Germination (%)	- 0.962***	94.17	14.13
Seedling height (cm)	- 0.985***	4.96	59.94
Survival rate (%)	- 0.959***	100	67.16

Reduced growth has been attributed to auxin destruction, changes in ascorbic acid content and physiological and biochemical disturbances (Gunckel and Sparrow, 1954; Usuf and Nair, 1974).

The dose of gamma rays which reduced the seedling height by a proportion of 50 % (GRD_{50}) expressed as percent of control was 645 Gy for variety “32-15” and 740 Gy for variety “38-1-7 (by interpolation). GRD_{50} was not estimated for the variety “Birkan” which showed a higher resistance to the two doses (300 and 400 Gy). The growth reduction of the seedling height is one of the first observable quantitative effects at irradiated seeds (IAEA, 1977). The correlation between morphological or physiological damage of seedlings and genetic damage over a wide range of dose with x-rays, gamma rays or neutrons is well established (Mikaelsen, 1968).

At doses higher than 600 Gy of gamma rays, seedling growth is generally inhibited. Moreover, several morphological and physiological injuries were observed. One visible chimeric case (thick white stripes on the leaves edges) was observed in the M1 population of “Birkan” irradiated with 400 Gy (Figure 2) and a high frequency of male sterile plants. Such chlorophyll mutation-like modifications were observed previously in M1 of other Turkish genetic backgrounds of sesame at high doses (600 and 750 Gy, Cagirgan, 1994, unpublished) and in the M1 of a durum wheat irradiated with fast neutrons (Cagirgan, 2009). In the M1, traits that encompass broader categories of biological processes, such as reduced apical dominance and dwarfism, were observed at higher rates in both mutagenized populations of the present study.

Figure 2: A visible chimeric case in one of the M1 plants of “Birkan” with thick white stripes on the leaves edges irradiated with 400 Gy of Gamma rays.

Growth reduction of 30 % in M1 plants caused by the physical damage of the mutagen should be enough to get a probability of obtaining high frequencies of useful mutations in the M2 generation but specific deletions causing loss of function (e.g. stay closed capsule mutants) may require higher physiologic damage or growth reduction (Cagirgan, 2001) .

Days to first flowering

Days to first flowering was delayed by higher doses of gamma rays starting from 500 Gy for the two cultivars “32-15” and “38-1-7”. For “Birkan” (300 and 400 Gy) there was no difference in days to first flowering between the treatments (Table 2). Ayiecho and Nyabundi (2001) studied the effect of gamma irradiation on three Kenyan sesame cultivars and they found that in M1 generation the effects of irradiation were expressed in reduced and delayed seedling emergence, delayed flowering, reduced plant height and sectorial deformed plants.

Survival rate

The number of survivors determined at 50 days after sowing (DAS) was steadily reduced in irradiated plants with increasing dose of gamma irradiation (Figure 3). The depressive effect of gamma rays on the survivors was not considerably drastic up to 500 Gy in both two varieties. The highest doses of mutagen decreased the number of survivors to 19.74 % for variety "32-15" and 45.95 % for "38-1-7".

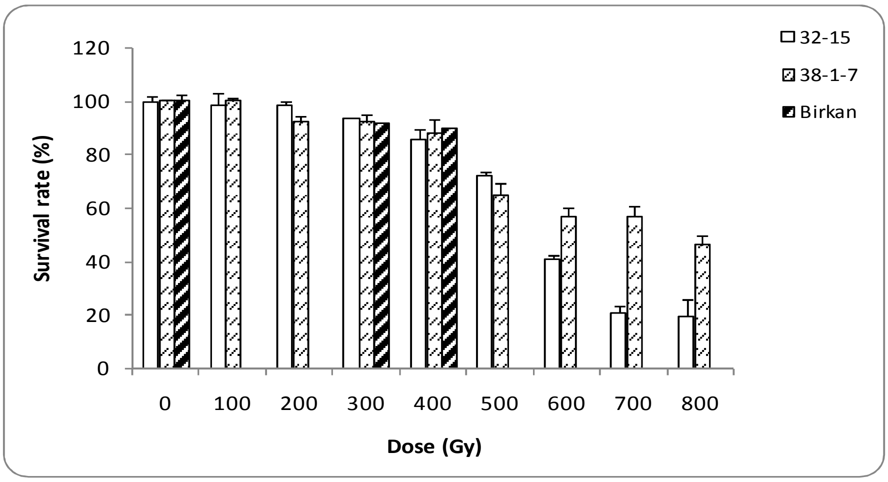

Figure 3: Survival rate at 50 days after sowing

The Analysis of variance for the effect of different doses of gamma irradiation on survival rate revealed significant genotypic differences ($p<0.01$). Genotype x dose interactions was also significant ($p<0.01$) indicating that differences exist within genotypes regarding sensitivity to different doses.

The mutagen dose which killed half of the tested population (LD_{50}) was 550 Gy for variety "32-15" and 740 Gy for variety "38-1-7" (by interpolation). The LD_{50} showed highly significant negative correlation ($r = - 0.959$; $p<0.001$) with gamma irradiation (Table 3). Kumar *et al.,* (2003) determined the effects of gamma irradiation (200, 400, 600, 800 and 1000 Gy) on the germination, growth and survivors of *Phaseolus lunatus* L.. They found that the germination and survival percentage decreased with increasing rates of gamma irradiation.

Our results indicate that out of the four parameters studied seedling height and survival rate can equally be used for estimating the effective dose of gamma irradiation for main treatment in a breeding program. Days to first flowering was not adequate in our study according to the photosensitivity of the varieties compared which took longer to flower in Antalya (three months instead of one month in Senegal).

It might be concluded that the range of gamma ray doses used fit well to the some African sesame cultivars under the study. From the GRD_{50} and LD_{50} doses it seemed appropriate to use gamma irradiation doses of 500 Gy, 650 Gy and 800 Gy for variety "32-15" and 600 Gy, 750 Gy and 900 Gy for "38-1-7" since IAEA recommended three doses including 20 % dose lower and higher than the effective dose. But in this study, conditions are optimal for germination and plant growth which is not the case in field conditions which may be harsh. So these doses are higher than reported by Cagirgan (1996) who recommended that 300-450 Gy should be enough for deletions to obtain closed capsules mutants which is our main goal in the furtherance of our project. Otherwise, higher doses are not advisable since lower doses produce the desired genetic changes with less primary physiological damages (Cagirgan, 2001). At higher doses drastic morphological abnormalities were found. According to these experimental results, it was concluded that seedling height and survivals were practically applicable to radiosensitivity studies.

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LITERATURE CITED

Ashri, A. 2007.Sesame (*Sesamum indicum* L.). In: R.J. Singh, Ed.,Genetics Resources, Chromosome Engineering, and Crop Improvement, Vol.4, Oilseed Crops, p.231-289, CRC Press, Boca Raton, FL, USA.

Ayiecho, P.O. and J.O. Nyabundi. 2001. Yield improvement of Kenyan sesame varieties. In: Sesame improvement by induced mutations. Final report of an FAO/IAEA co-odinated research project organized by the joint FAO/IAEA division of nuclear techniques in Food and Agriculture (1993-1998) pp.129-135.

Bowen H.J.M. and J. Thick. 1961. Effects of seed extracts on radiosensitivity. Symposium on the effects of ionizing radiation on seeds IAEA, 75-82.

Cagirgan, M.I., 1996. Radiosensitivity of Turkish sesame to Gamma Rays. Turk.J.of Field Crops, 1:39-43.

Cagirgan, M.I. 2001. Mutation techniques in sesame (*Sesamum indicum* L) for intensive management: confirmed mutants. In: Sesame improvement by induced mutations. Final report of an FAO/IAEA co-odinated research project organized by the joint FAO/IAEA division of nuclear techniques in Food and Agriculture (1993-1998) pp. 31- 40.

Cagirgan, M.I. 2007. Selection and modification of closed capsule mutants in sesame. In proceedings of Türkiye 7. Tarla Bitkleri Kongresi,25-27 Haziran 2007, Atatürk Üniversitesi, Erzurum, Türkiye s.408-411 (Abstract in English).

Cagirgan, M.I. 2009. Chlorophyl mutation-like chimeric cases induced by fast-neutrons in the M1 generation of a durum wheat. Turkish Journal of Field Crops, this issue.
Cheema, A.A. and B.M. Atta. 2003. Radiosensitivity studies in basmati rice. Pak. J. Bot. 35(2):197-207.
Gelin O., L. Ehrenberg and ST. Blixth. 1958. Genetically conditioned influences on radiation sensitivity in peas. Agr.Hort.genetica 16: 78-102.
Gunchel, J.E. and A.H. Sparrow. 1954. Aberrant growth in plants by ionizing radiations. Brookhaven Sym. Biol. 6: 252-279.
IAEA, 1977. Manual on mutation breeding. 2nd Ed., Technical Reports Series No. 119. IAEA, Vienna, 288p.
IAEA, 1994. Induced Mutations for Sesame Improvement. Report of the First Research Coordination Meeting for the FAO/IAEA Coordinated Research Program held in Vienna, Austria, 21-25 March 1994.
Iqbal, J. 1969. Radiation induced growth abnormalities in vegetative shoot apices of *Capsicum annum* L. in relation to cellular damage. Radiat. Bot. 9: 491-499.
Khanna, V.K. and N. Maherchandani. 1981. Gamma radiation induced changes in the peroxidase activity of chickpea seedlings. Curr. Sci. 50(16): 732-733.
Kim Y., K.S. Schumaker and J.K. Zhu. 2006. EMS mutagenesis of *Arabidopsis*. Meth. Mol. Biol. 323:101-103.
Kivi, E.I. 1964. Some aspects of sterility affects of irradiation on the basis of gamma and X-ray treated barley. In: *The Use of Induced Mutations in Plant, FAO/IAEA, Vienna*, 131-158.
Kumar, D.S., T. Nepolean and A. Gopalan. 2003. Effectiveness and efficiency of the mutagens gamma rays and ethyl methane sulfonate on limabean (*Phaseolus lunatus* L.). Indian J. Agric. Res. 37(2): 115-119.
Kumar, R. and R.P. Sinha. 2003. Mutagenic sensitivity of lentil genotypes. J. Appl. Bio. 13(1/2): 1-5.
Menda, N., Y. Semel, D. Peled, Y. Eshed and D. Zamir. 2004. *In silico* screening of a saturated mutation library of tomato. Plant J. 38:861-872.
Mikaelsen, K. 1968. Effects of fast neutrons on seedling growth and metabolism in barley. In: Neutron irradiation of seeds II. IAEA. Tech. Rep. Ser. No: 92. pp. 63-70.
Naito, K., M. Kusaba, N. Shikazono, T. Takano, A. Tanaka, T. Tanisaka and M. Nishimura .2005. Transmissible and nontransmissible mutations induced by irradiating *Arabidopsis thaliana* pollen with g-rays and carbonions. Genetics 169: 881-889.
Pathirana, R. and S. Subasingbe. 1993. Response of two sesame cultivars to seed irradiation with gamma rays. J. Natn. Sci. Coun. Sri Lanka 21(2):183-188.
Pons, L.M., W.A. Millan and G.M.C. Cepero. 2001. Radiosensitivity of rice variety Perla. Radiosensibilidad de la variedad de arroz Perla. Centro-Agricola 28(2): 5-8.

Siddiqui, B.A. And S. Khan. 1999. Breeding in Crop Plants: Mutations and *in Vitro M*utation Breeding. 1st ed. Kalyani Publishers, Ludhiana.
Toker, C. and Cagigran M.I. 2004. Spectrum and frequency of induced mutations in chickpea. *ICPN* 11: 20-21.
Uma, M.S. and P.M. Salimath. 2001. Effect of ionizing radiations on germination and emergence of cowpea seeds. Karnataka J. Agric. Sci. 14(4): 1063-1064.

Usuf, K.K. and P.M. Nair. 1974. Effect of gamma irradiation on the indole acetic acid synthesizing system and its significance in sprout inhibition of potatoes. *Rad.* Bot. 14: 251-256.
Van Zanten L., 2001.Sesame improvement by induced mutations: Results of the Coordinated research project and recommendation for future studies.In: Sesame improvement by induced mutations. Final report of an FAO/IAEA co-odinated research project organized by the joint FAO/IAEA division of nuclear techniques in Food and Agriculture (1993-1998) pp 1-12.
Walther, F. 1969. Effectiveness of mutagenic treatment with ionizing radiation in barley. In: induced mutation in Plants. IAEA, Vienna. pp. 261-270.