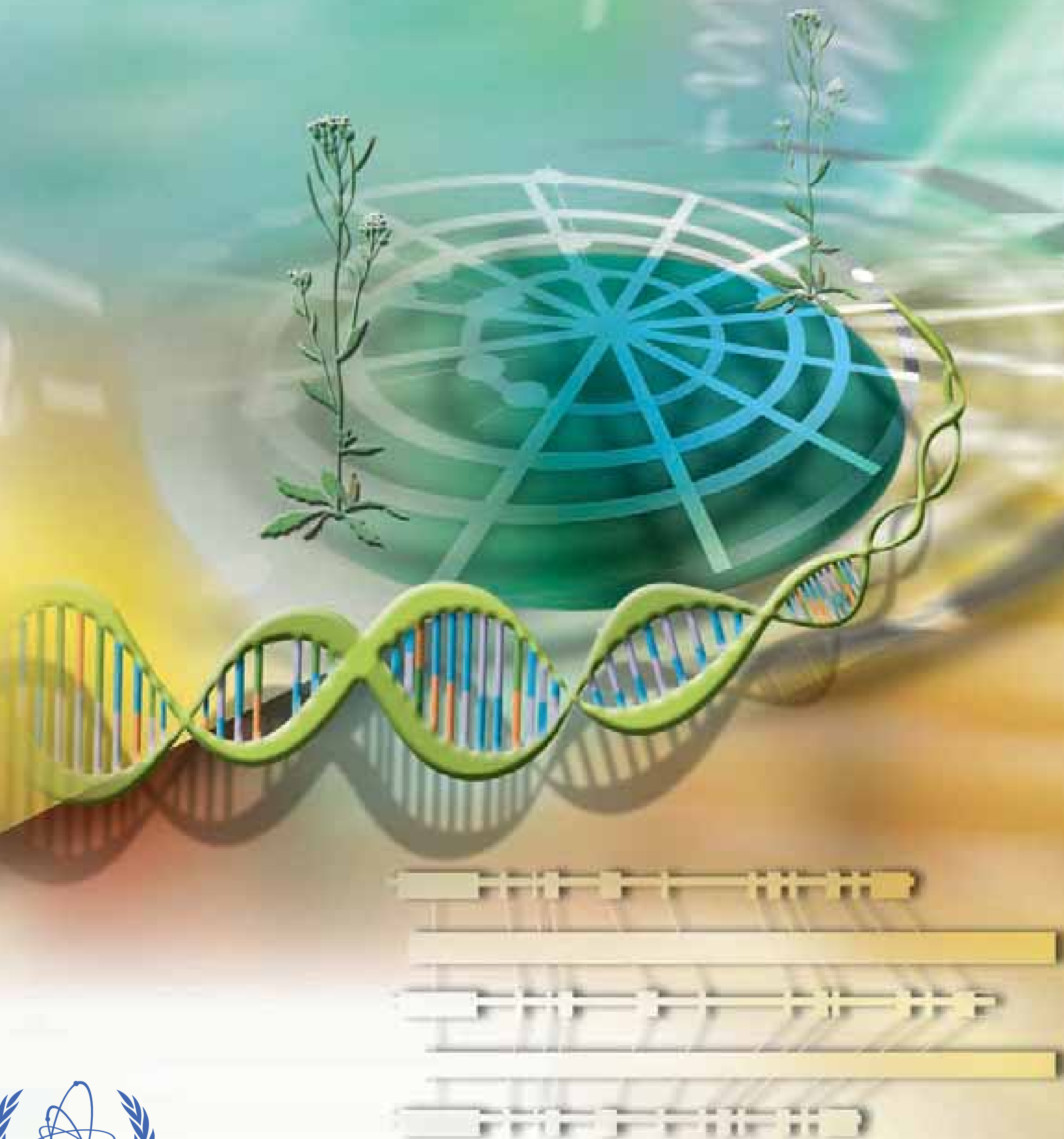
Induced Plant Mutations in the Genomics Era

Edited by Q. Y. Shu



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Plant Breeding Section,
Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture,
International Atomic Energy Agency,
Vienna, Austria

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Preface

The year 2008 marks the 80th anniversary of mutation induction in plants. The application of mutation techniques, i.e. Gamma-rays and other physical and chemical mutagens, has generated a vast amount of genetic variability and has played a significant role in plant breeding and genetic studies. The widespread use of induced mutants in plant breeding programmes throughout the world has led to the official release of more than 2,700 plant mutant varieties. A large number of these varieties (including cereals, pulses, oil, root and tuber crops, and ornamentals) have been released in developing countries, resulting in enormous positive economic impacts.

During the last decade, with the unfolding of new biological fields such as genomics and functional genomics, bioinformatics, and the development of new technologies based on these sciences, there has been an increased interest in induced mutations within the scientific community. Induced mutations are now widely used for developing improved crop varieties and for the discovery of genes, controlling important traits and understanding the functions and mechanisms of actions of these genes. Progress is also being made in deciphering the biological nature of DNA damage, repair and mutagenesis. To this end, the International Symposium on Induced Mutations in Plants was organized by the International Atomic Energy Agency (IAEA) and the Food and Agriculture Organization (FAO) of the United Nations through the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

The Symposium comprised an open session, two plenary sessions and ten concurrent sessions, covering topics from induced mutations in food and agriculture, plant mutagenesis, genetic diversity, biofortification, abiotic stress tolerance and adaptation to climate changes, crop quality and nutrition, seed and vegetatively propagated plants, gene discovery and functional genomics. A workshop on low phytate rice breeding was also organized. About 500 participants from 82 Member States of the IAEA and FAO, and nine international organizations/institutions attended the Symposium, with a good balance between the private and public sector, as well as developing and developed Member States. The Symposium received valuable assistance from the cooperating organizations and generous support from the private sector, for which the sponsoring organizations are most grateful.

This publication is a compilation of peer-reviewed full papers contributed by participants. They were either oral or poster presentations given in different sessions except Concurrent Session 3 (which will be compiled by the Human Health Division in a separate publication). These papers not only provide valuable information on the recent development in various fields related to induced mutations, but also on the social and economic impact of mutant varieties worldwide. Therefore, these Proceedings should be an excellent reference book for researchers, students and policy makers for understanding applications of induced mutations in crop improvement and biological research.

Qu Liang
Director
Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture
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Intervarietal Differences in Response of Sunflower (*Helianthus annuus* L.) to Different Mutagenic Treatments

S Gvozdenovic[1,*], S Bado[2], R Afza[2], S Jocic[1] & C Mba[2]

Abstract

For much of the past century, mutagenesis has gained popularity in plant genetics research as a means of inducing novel genetic variation. Induced mutations have been applied for the past 40 years to produce mutant cultivars in sunflower by changing plant characteristics that significantly increase plant yield and quality. The present study was focused on generating baseline data to elucidate the role of genotypic differences in the response of sunflower to induced mutagenesis with the aim of expanding the applicability of the use of induced mutant stocks in the genetic improvement of the crop and in its functional genomics. The strategy adopted was to estimate the optimal treatment conditions (doses of mutagens) through relating the extent of damage in seedling progeny to the exposure levels of the initiating propagules to mutagens. Seeds of 15 elite sunflower genotypes commonly used as breeding stocks and grown on commercial scales were treated with a range of mutagens: Gamma-rays (γ rays); fast neutrons and with ethyle-methane-sulphonate (EMS) at different treatment doses. The three mutagenic agents affected seedling height, reducing it with increasing dosage. Based on the mutagen damage on seedling height, the 50% and 30% damage indices (D_{50} and $D_{30,}$ respectively) were estimated for the 15 sunflower genotypes for the three mutagens. The D_{50} (D_{30}) values for the sunflower lines ranged from 120 to 325Gy (5 to 207Gy) for gamma irradiation; 9 to 21Gy (0.1 to 10Gy) for fast neutrons and 0.69 to 1.55% (0.01 to 0.68%) concentration of EMS.

Introduction

Sunflower (*Helianthus annuus* L.) is one of the world's most important oil crops, used for human consumption and industrial processes. It is also used as a confectionery, ornamental plant and flower, and as bird feed. It is currently cultivated on over 21 million hectares world-wide annually. The largest sunflower producers in the world are Russia, the United States, Argentina, China, and France [1].

The main objective of sunflower breeding is to develop productive sunflower hybrid cultivars that are stable, high yielding, and resistant to biotic and abiotic stresses. Yield is a complex trait, is controlled by multiple gene effects. Seed yield is variously estimated as: number of plants per hectare (55,000-60,000), number of seeds per plant (>1,500), hectoliter mass of the seed (45-50 kg/ha), thousand seed mass (>80 g), low hull percentage (20-24%) and high seed oil content (>50) [2].

Induced mutations have been applied for the past 40 years to produce mutant cultivars in sunflower by changing plant characteristics for significant increase in plant productivity [3], [7]. Mutagenic treatments, usually on seed, have induced high-oleics, semi-dwarfs and dwarfs, male-sterile plants and other interesting variants such as earliness and seeds with thin hull [4], [5], [6].

In 1976, Soldatov produced a mutant of significant practical importance for sunflower breeding by treating the seed of the cultivar VNIIMK 8931 with a solution of 0.5% dimethyl-sulphate (DMS); M_3 lines possessing a high content of oleic acid in oil were obtained. After further breeding, the high-oleic cultivar Pervenetz was developed [4]. The high oleic content of this cultivar has proved to be very stable under varying temperatures and the trait can be easily transferred into other genotypes by normal breeding procedures.

The main objectives of this research were to increase genetic variation in sunflower inbred lines and to assess the efficiency of different mutagenic treatments, since basic information on this is lacking. The first step was to estimate optimal treatment conditions (doses). Germination of the M_1 seed provides a good test of the sensitivity of the material to the mutagenic treatment.

Materials and Methods

Fifteen genetically different sunflower inbred lines chosen for their importance in commercial hybrid production (**Table 1**) were used for this study. Seed of these genotypes varied morphologically. The Institute of Field Vegetable Crops, Novi Sad, Serbia, supplied the seeds.

For gamma irradiation, 50 seeds of each genotype were irradiated at 100, 200, 300, 400 and 500Gy using a Cobalt-60 gamma source at the IAEA Laboratories in Seibersdorf, Austria. Prior to mutagenic treatment, the seeds were kept in a desiccator over a 60 % glycerol/water mixture for seven days at room temperature for seed moisture equilibration.

For fast neutron treatment, 50 seeds were treated with five different doses: 10, 20, 30, 40 and 50Gy at the Atomic Energy Research Institute, Budapest, Hungary. The samples were bombarded inside a cadmium (Cd) capsule with wall thickness of 2mm. Exposure temperature was less than 30°C, at normal air pressure and humidity was less than 70%. The samples were rotated at 16 revolutions per minute. Ten days after the treatment, 25 seeds of each genotype were sown and germinated to assess radiosensitivity.

For chemical treatment, seeds were pre-soaked in distilled water for 24 hours. Twenty-five seeds of each genotype were treated with five concentrations of ethyle-methane-sulphonate (EMS) solution, 0.5, 1.0, 1.5, 2.0 and 2.5%, for 3.5 hours; treatment concentrations were based on studies of other species [8]. After EMS treatment, the seeds were washed and sown. The control, non-mutagenized seeds were treated similarly, except for exposure to the mutagen.

The treated seeds and the controls were sown in boxes in three replications using the flat method [9] in a glasshouse under controlled environmental conditions (22-35°C, lighting of 12-hour photoperiod). The parameter used to assess the dose response was the seedling height. The measurements were taken when cotyledons emerged above the soil and had split up (12 days after sowing).

The mean seedling height of the control was used as an index of the normal growth of each inbred line. The mean seedling height of each treatment was expressed as a percentage of the corresponding control value. Based on these values, regression equations were obtained.

[1] Institute of Field and Vegetable Crops (IFVC), Oil Crops Department, Novi Sad, Serbia
[2] Plant Breeding Unit Joint FAO/IAEA Agriculture and Biotechnology Laboratory, International Atomic Energy Agency Laboratories, Seibersdorf, Austria

* Corresponding author. E-mail: sandra@ifvcns.ns.ac.yu

Radiobiological effects of mutagenesis were observed in the M_1 and calculated on the basis of the absorbed dose or EMS of the seedling height. According to [10] and [11] seedling height reduction of 30-50% is generally assumed to give high mutation yield. Seedling height is highly correlated to survival [12]. This is usually designated as D_{30} and D_{50}, respectively.

Table 1. List and characteristics of treated sunflower inbred lines

Inbred lines	Type of inbred line	Branching	Days to flowering	Plant height (cm)	Oil content (%)	Seed size ratio	Thousand seed mass (g)	Seed color	Seed coat type
HA-26	Standard female(B analogue)	no	62	126	44	0.39	46.15	black	thick
VL-A-8	Standard female(B analogue)	no	65	108	47	0.5	38.42	black	thick
HA-48	Standard female(B analogue)	no	72	150	48	0.49	44.30	black	thick
HA-19	Standard female(B analogue)	no	56	80	47	0.53	50.70	black	thick
OD-3369	Standard female(B analogue)	no	71	105	55	0.42	52.16	black	thick
V-8931-3-4-OL	High oleic	yes	73	95	54	0.47	47.47	black	thin
HA-26-OL	High oleic	no	65	119	47	0.40	51.96	black	thick
VK-66-tph_1	Altered tocopherol quality	yes	57	75	41	0.42	46.28	black	thick
VK-66-tph_1tph_2	Altered tocopherol quality	yes	58	64	37	0.47	52.46	black	thick
VK-66-OL-tph_2	High oleic and altered tocopherol quality	yes	60	68	28	0.44	50.96	black	thick
RUS-RF-168	Standard restorer	yes	74	134	40	0.49	38.31	black	medium
RHA-SELEUS	Standard restorer	yes	71	112	47	0.45	32.49	brown	medium
RHA-M-72	Standard restorer	yes	70	114	51	0.38	41.38	brown	thin
CMS-ANN-15	Standard restorer	yes	53	33	35	0.37	41.12	black	thin
RHA-S-OL-26	High oleic restorer	yes	69	88	55	0.38	28.43	cream	medium

Three mutagenic agents were used

Results and Discussion

All seeds, the control and the irradiated, germinated. The seedling height in all three treatments decreased with increasing dose. For gamma irradiation the D_{50} and D_{30} values for the 15 sunflower inbred line seeds ranged from 120Gy and 5Gy, respectively for inbred line HA-19 to 325Gy and 207Gy, respectively for genotype VK-66-tph_1. For fast neutron, the D_{50} and D_{30} for seeds of the 15 sunflower inbred lines seeds ranged from 9Gy and 0.1Gy, respectively (genotype HA-19) to 21Gy and 10Gy, respectively (genotype VK-66-tph_1tph_2). The trend was therefore similar to the responses to gamma irradiation by these genotypes. The D_{50} and D_{30} values for these 15 sunflower inbred line seeds treated with EMS ranged from 0.69% and 0.01%, respectively EMS concentration (genotype OD-3369) to 1.55% and 0.68%, respectively for the line HA-19 (**Table 2**).

The data indicated that all genotypes produced a wide range of responses. With respect to radiation damage by Gamma-rays, the genotype HA-19 showed the least radiation damage with VK-66-tph_1 displaying the highest damage. In the case of fast neutron, the genotype HA-19 was most affected while VK-66-tph_1 and VK-66-tph_1tph_2 had the least radiation damage. The study of EMS revealed OD-3369 to be least sensitive while VK-66-tph_1tph_2 again was highly susceptible. Reduction of seedling height was more pronounced in genotype HA-19 than any other genotype for both gamma and fast neutron irradiation and clearly demonstrated a genotypic response to mutagenic treatment. Interestingly, the same genotype showed the greatest resistance to high doses of EMS, inferring again a genotype - mutagen interaction. This line is very early maturing and it has round and large seed. Lines OD-3369 and V-8931-3-4-OL were generally more sensitive to all three mutagens than the others. These inbreds have very high oil contents in the seeds, normal sized seeds and high thousand seed mass. Inbred lines VK-66-tph_1, VK-66-tph_1tph_2 and VK-66-OL-tph_2 showed the greatest resistance to both physical and chemical mutagenic treatments. These genotypes are nearly isogenic lines, with different oil quality but low oil quantity. They have large, black seeds but a thick coat that is probably the reason for such high resistance to mutagenic treatments.

Table 2. D_{50} and D_{30} values for 15 inbreds for exposure to Gamma-rays, fast neutron bombardment and EMS solution

Genotypes	Gamma-rays (GY)			Fast neutrons (GY)			EMS (%)		
	D_{50}	D_{30}	S_e	D_{50}	D_{30}	S_e	D_{50}	D_{30}	S_e
HA-26	202	102	13.28	15	3.6	19.00	1.34	0.50	13.44
VL-A-8	218	100	12.54	12	0.6	22.95	1.41	0.55	12.03
HA-48	220	109	11.84	17	3.8	18.75	1.40	0.58	13.68
HA-19	120	5	22.76	9	0.1	25.67	1.55	0.68	9.82
OD-3369	151	18	20.34	11	0.08	24.56	0.69	0.01	22.39
V-8931-3-4-OL	155	44	15.96	13.5	1.5	21.21	0.82	0.07	22.95
HA-26-OL	181	76	13.39	12.5	1	22.27	1.16	0.43	14.16
VK-66-tph1	325	207	9.03	20	9	15.50	1.41	0.53	13.75
VK-66-tph1tph2	294	151	6.90	21	10	12.61	1.54	0.64	11.79
VK-66-OL-tph2	289	164	3.45	19	8	16.14	1.36	0.55	14.78
RUS-RF-168	201	101	14.33	20	7.3	20.86	1.09	0.30	14.88
RHA-SELEUS	206	95	13.43	15	2.6	21.80	1.15	0.39	12.40
RHA-M-72	188	93	19.03	13	1.7	22.34	1.46	0.62	16.91
CMS-ANN-15	237	146	14.89	13	0.4	20.52	0.94	0.25	13.51
RHA-S-OL-26	197	79	12.89	14.5	2	15.11	1.36	0.50	16.17

The three mutagenic agents affected seedling height, reducing it with increasing dosage. Based on the mutagen damage on seedling height, the D_{50} and D_{30} values for 15 sunflower genotypes were estimated for the three mutagens. Retardation of growth due to the mutagenic treatments has been used to determine the dose rate for mutation induction. It is the most functional parameter to be used in radiobiological investigations because it is generally considered to be a result of primary injury due to nuclear DNA damage. Sensitivity in seedlings height had been demonstrated in earlier dose response studies of bean [13], soybean [14], and other crops.

In this experiment, we established relationships between the D_{50} values due to gamma and fast neutron irradiation and EMS to the thousand seed mass (TSM), seed size ratio, oil content in the seed, plant height and days to flowering (**Table 3**). A significant negative correlation was found between the treatment and seed oil content, indicating that genotypes with relatively high seed oil content were more sensitive to gamma irradiation, fast neutrons and EMS. Also, larger seeds were generally more resistant to EMS treatment than to gamma and fast neutron irradiation. There was a negative correlation between early flowering, short stature plants and gamma irradiation. Mutagenic damage depended on the biological traits of the variety.

Table 3. Correlations between biological traits and response to mutagenic treatments

Biological traits	Gamma-rays	Fast neutrons	EMS
TSM	0.15	0.00	0.14
Seed size ratio	-0.17	-0.18	0.38*
Oil content	-0.69**	-0.37*	-0.39*
Plant height	-0.39*	-0.20	0.11
Days to flowering	-0.41*	-0.14	-0.24

r(0.05)=0.349 r(0.01)=0.449

The results obtained from this study indicated that the radiation damage due to mutagenic treatment was not similar amongst the genotypes. The same differential response to radiation among different genotypes in plant species was reported by many researchers. These intervarietal differences in radiation damage to seeds have been reported to be: a) under polygenic system in rice, tomato and barley [15], [16], [17], [18], [19], b) major gene control in einkorn wheat and soybean [20], [21], and c) influenced by heterozygosity in maize and peanut [22], [23], [24]. It is widely accepted that response to mutagens is species and genotype dependent, but the full explanation has not yet been provided.

The different D_{50} (D_{30}) values for sunflower inbreds were established: dose range of 120 to 325Gy (5 to 207Gy) for gamma irradiation, 9 to 21Gy (0.1 to 10Gy) for fast neutrons irradiation and 0.69 to 1.55% (0.01 to 0.68%) concentration of EMS. The radiation sensitivity studies indicated that all the genotypes treated exhibited a wide range of radiation damage to Gamma-rays and fast neutrons.

Based on the radiation damage, bulk irradiation with a dose giving rise to a 30% to 50% reduction in growth will be carried out and M_1 plants will be grown in the field. Different mutations will be observed in the field and promising mutants will be selected for further testing. Selection will be carried out in the M_2 generation for early flowering, short stature, deformations of leaves and heads, appearance of branches, head inclination, sterility and oil seed quantity and quality.

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The year 2008 will mark the 80th anniversary of mutation induction in crop plants. The widespread use of induced mutants has led to date to the official release of close to 3000 mutant plant varieties throughout the world. Many of these varieties have been widely grown by farmers, both in developed and developing countries, resulting in considerable positive economic impacts that are measured in billions of USD. In the past decade, induced mutations have become a means of choice for the discovery of genes that control important traits, and for understanding their functions and mechanisms of actions. The papers included in this book present some of the significant achievements, demonstrate the current development, and outline the perspectives in this dynamic field.

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