IAEA-TECDOC-781

Mutation breeding of oil seed crops

Proceedings of a final Research Co-ordination Meeting of an FAO/IAEA Co-ordinated Research Programme organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and held in Vienna, 11–15 January 1993

INTERNATIONAL ATOMIC ENERGY AGENCY

December 1994

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The originating Section of this document in the IAEA was:

Plant Breeding and Genetics Section
Joint FAO/IAEA Division
International Atomic Energy Agency
Wagramerstrasse 5
P.O. Box 100
A-1400 Vienna, Austria

MUTATION BREEDING OF OIL SEED CROPS
IAEA, VIENNA, 1994
IAEA-TECDOC-781
ISSN 1011–4289

Printed by the IAEA in Austria
December 1994

Foreword

Oils and fats have been used in cooking already in ancient cultures, thousands of years ago They also had other uses such as lighting, soap making and religious and ceremonial ones Several plants were domesticated long ago and have been serving as sources of edible oil for several millennia, e g olive, rapeseed and sesame As the demand for vegetable oils for edible and industrial purposes grew additional plant species were domesticated or modified and brought into cultivation as oil crops, e g soy beans, oil palm, sunflower and safflower The oils obtained from these species differ in their acid composition and physical properties and hence in their usages Many of these oil crops serve also as sources of protein-rich meal for food and animal feed As with the oils, there is variation between the proteins of the different species in their amino acid composition and presence of undesirable substances, which affect their suitability and value as food or feed for various farm animals

Many of the breeding objectives of the various oil crops are identical to those of other crops, e g higher yields, disease resistance, modified plant architecture, and tolerance to abiotic stresses In addition, however, there are in the crops unique aspects of genetic manipulation of the oil and/or protein quality and of breeding varieties with "tailor made" properties Natural and induced mutations have been used successfully in the genetic modification of the metabolic pathways and the improvement of the products, e g erucic acid-free rapeseed oil, high oleic acid sunflower and glucosinolate free rapeseed meal (Canola quality)

The Co ordinated Research Programme (CRP) on "Mutation Breeding of Oil Seed Crops" was implemented in view of the above This publication reppresents the proceedings of the third and final Research Coordination Meeting, held in Vienna, Austria on January 11 - 15, 1993

Most of the projects dealt with *in vivo* application of mutation breeding approaches Some *in vitro* research was also conducted The investigations dealt mainly with *Brassica* species (rape) and sesame but also with sunflower and more minor oil plants, namely castor and poppy The objectives included higher yield, modification of plant architecture, modified oil and protein qualities, pest and disease resistance, earliness etc The CRP included also the use of induced mutations in the domestication of potential new oil producing species of *Cuphea*

It is hoped that this publication will be of help to researchers interested in the use of induced mutations in breeding oil crops as well as in breeding seed propagated crops in general

This document was jointly edited by the Scientific Secretary, E Amano, and by A Ashri, Israel, whose valuable contribution is acknowledged

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INTRODUCTION

OIL CROPS : STATUS AND OUTLOOK

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The worldwide expansion in the production of oil crops in the past 50 years has been remarkable [1]. Overall, from 1935 to 1985/86 the production of oil seeds grew nearly four-fold (Table I). During the same period the production of edible vegetable oils increased nine-fold (Table II), and that of oil cake or meal increased about ten-fold (Table III). On the other hand, the production of industrial oils remained nearly the same (Table II). It should be noted that edible oil imports have increased markedly from 1962/63 to 1985/86; the increase was most marked in developing countries, where the imports grew eight-fold (Table IV).

The vegetable oils are obtained from several perennial trees and from annual crops, where induced mutations were employed. The major annual oil crops, together with their chromosome numbers (2n) and their fatty acid compositions are listed in Table V.

The growth in the production of three annual oil crops from the 1930's to the 1950's was explosive. Thus, the increment in soybean production was 653 %, in sunflower 640 % and in rapeseed 367 % (Table I). Plant breeding efforts have been the key to this tremendous increase in these three crops. They also contributed to the increase in the other oil crops, as in peanuts and oil palm.

Table I. AVERAGE ANNUAL PRODUCTION OF WORLD OILSEEDS FOR 1935/39, THREE FIVE YEAR INTERVALS, THE 1984/85-1985/86 PERIOD, THE PERCENT CHANGE FROM 1935/39 TO 1984/86 AND THE ANNUAL GROWN RATE FROM 1957/61 TO 1984/86 IN MILLIONS OF TONNES

Plant	1935/39	1957/58 -1961/62	1972/73 -1976/77	1984/85 -1985/86	% change 1935:1986*	% ann. growth 1957/61-1984/86
Soybean	12.62	25.67	58.26	94.98	+ 653	+ 5.2
Cottonseed	13.87	18.03	23.59	32.34	+ 133	+ 2.3
Groundnut(shelled)	6.08	9.04	11.24	13.70	+ 125	+ 1.6
Sunflower seed	2.53	5.86	10.52	18.74	+ 640	+ 4.6
Rapeseed	3.82	3.72	7.18	17.83	+ 367	+ 6.2
Sesame	1.62	1.39	1.78	2.07	+ 24	+ 1.5
Copra/palm kernel	3.83	4.05	5.44	6.82	+ 78	+ 2.0
Linseed	3.42	3.26	2.46	2.48	- 27	- 1.0
Castor+tung nut	1.28	1.26	1.56	1.21	- 6	--
World production	49.07	72.28	122.03	190.17	+ 288	+ 3.8

*:Increment.
Source: Hatje [1].

Table II. AVERAGE WORLD PRODUCTION OF VEGETABLE OILS FOR SELECTED FIVE YEAR INTERVALS AND FOR 1985/86, IN MILLIONS OF TONNES (VISIBLE OILS ONLY)

Plant	1909/1913	1935/1939	1958/1962	1973/1977	1985/1986
Edible oils:					
Olive oil	0.59	0.88	1.32	1.59	1.57
Soybean	0.30	0.93	3.28	8.50	14.16
Cotton	0.98	1.23	2.29	2.87	3.57
Groundnut	0.65	1.42	2.45	2.67	3.50
Sunflower	0.12	0.57	1.90	3.71	6.88
Rapeseed	1.08	1.21	1.18	2.50	6.36
Sesame + corn	0.55	0.75	0.57	0.92	1.67
Coconut	0.75	1.94	1.85	2.55	3.25
Palm kernel	0.15	0.36	0.43	0.48	1.04
Palm	0.28	0.99	1.30	2.83	7.72
Sub-total	5.45	10.28	16.57	28.62	49.72
Industrial oils:					
Linseed	0.86	1.04	0.90	0.67	0.68
Castor	0.13	0.18	0.22	0.34	0.39
Tung	0.10	0.14	0.11	0.11	0.10
Sub-total	1.09	1.36	1.23	1.12	1.17
Total	6.54	11.64	17.80	29.74	50.89

Source: modified from Hatje [1].

Table III. AVERAGE WORLD PRODUCTION OF OILCAKE AND MEAL FOR SELECTED FIVE YEAR INTERVALS AND FOR 1985/86 IN MILLIONS OF TONNES*

Plant	1909/1913	1935/1939	1958/1962	1973/1977	1985/1986
Soybean	1.32	4.19	14.47	37.13	61.95
Cotton	3.13	4.02	7.31	9.44	13.23
Rape	1.77	1.98	1.94	3.94	10.30
Sunflower	0.13	0.64	2.12	3.98	8.06
Groundnut	0.88	2.04	3.31	3.70	4.60
Copra/palm kernel	0.60	1.51	1.54	2.05	3.15
Linseed	1.22	1.48	1.72	1.28	1.28
Sesame	0.61	0.79	0.50	0.50	0.74
Total	9.66	16.65	32.91	62.12	103.31

*:No distinction has been made in the production statistics between oilcake and meal.

Source: modified from Hatje [1].

Table IV. WORLD IMPORTS OF EDIBLE OILS AND OILSEEDS (ON AN OIL EQUIVALENT BASIS) BY REGION IN 1962/63 AND 1985/86 IN MILLIONS OF TONNES

Region	Year	
	1962/63	1985/86
Western Europe	3.50	5.68
Other developed countries*	1.11	3.51
USSR/East Europe/China	0.34	1.74
Developing countries	1.11	8.94
Total	6.06	19.87

*:USA, Canada, South Africa, Japan, Australia, New Zealand.
Source: modified from Hatje [1].

Table V. ANNUAL OIL CROPS AND THEIR FATTY ACID COMPOSITION (%)

Plant	Chr.No. 2n	Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)	Erucic (22:1)	Ricinoleic (18:1)	Other
Edible oils:									
Canola[1] (*Brassica napus*)	38	5	2	63	19	9	-	-	2
Cotton (*Gossypium hirsutum*)[2]	52	23	3	18	54	-	-	-	2
Linola (*Linum usitatissimum*)	30	6	4	17	72	1	-	-	-
Maize (*Zea mays*)	20	11	2	24	58	1	-	-	5
Peanut (*Arachis hypogaea*)	40	9	3	57	23	-	-	-	8
Safflower[3] (*Carthamus tinctorius*)	24	7	2	12	79	-	-	-	-
Sesame (*Sesamum indicum*)	26	8	4	47	39	-	-	-	2
Soybean (*Glycine max*)	40	12	4	25	51	8	-	-	-
Sunflower[3] (*Helianthus annuus*)	34	7	4	16	73	-	-	-	-
Industrial oils:									
Castor (*Ricinus communis*)	20	1	1	3	4	Trace	-	90	1
Linseed (*Linum usitatissimum*)	30	7	4	20	17	44	-	-	8
Rape[4] (*Brassica napus*)	38	4	-	11	14	8	52	-	11[5]
Turnip rape[4] (*Brassica campestris*)	20	3	-	27	18	9	31	-	12[5]

1:Rape, low (zero) erucic acid and low (zero) glucosinolate.
2:Major species. *G. barbadense* (Egyptian cotton) second cultivated species.
3:Standard type.
4:High erucic acid.
5:Mainly eicosenoic fatty acid (20:1)

In the earlier years, natural genetic variability was utilized [2]. However, already in the 1950's and more so since, researchers turned also to mutagenic treatments to generate genetic variation. They produced through induced mutations 110 cultivars in ten crop species by 1990 (Table VI). In general, mutation breeding of oil crops shares many improvement objectives with crops of other types, e.g. modified plant architecture, higher yields, disease resistance, tolerance to abiotic stresses, adaptation to new environments, earliness, male sterility etc. However, for oil crops, there are additional unique objectives dealing with the aspects of the oils and/or the proteins [3]. It should be noted that some of the oil crops, such as soybean, can be labelled protein crops [4].

Table VI. NUMBERS OF RELEASED VARIETIES DEVELOPED THROUGH MUTATION BREEDING IN ANNUAL OIL CROPS[1]

Crop	Direct	Cross	Total
Cotton	13	3	16
Peanut	19	14	33
Rape (B. napus)	6	1	7
Rape (B. campestris)	1	-	1
Safflower[2]	-	-	-
Sesame	2	-	2
Soybean	38	3	41
Sunflower	1	-	1
Castor	2	1	3
Linseed	3	3	6
Total	85	25	110

1:Source: Mutation Breeding Newsletter (= MBNL) No. 38: 16-21, 1991, IAEA, Vienna. Includes only officially released varieties reported in Mutation Breeding Newsletters, up to No. 37, 1991. For further details see MBNL 38: 22-49, 1991.
2:Source: Micke et al. [9].

Oil crops are the source of edible oils and industrial oils, which have a variety of uses, some are listed in Table VII. Recent economic developments and present and potential plant breeding achievements which will yield oils of new qualities or chemical purities may make the oil crops also renewable sources of raw materials for the chemical industries [5,6] and renewable sources of fuel [7]. Pryde and Rothfus [5] note that the future will see a shift from "petrochemistry" to "botanochemistry". With new developments in molecular genetics, induced mutations and plant breeding, the shift may well go further to "genochemistry". Thus, it can be anticipated that within the next few years it will be possible to develop genotypes that will provide "tailor made" vegetable oils to meet the needs of the edible and industrial markets [6, 8]. It will also be possible to modify the quality of the proteins in the meal and to reduce the content of anti-nutritional substances, such as glucosinolate in rape or lupinin in *Lupinus* spp., or to eliminate them.

At present though, the major efforts are directed to a handful of annual oil crops: rape, soybean, sunflower and maize. This will lead to greater breeding advancement in these crops which may in turn harbour some dangers. One danger is that of genetic vulnerability. If larger and larger areas are planted to fewer crops and within each to fewer genotypes, the damage from outbreaks of epidemics caused by new pathogenic races or from environmental changes such as drought over large areas, could be very severe. The second danger is that

Table VII. USES OF VEGETABLE OILS

Edible oil uses	Technical oil uses
Salad oils	Pharmaceutical products
Margarine	Soaps
Vanaspati	Paints and resins, coatings
Shortenings	Linoleum
Cooking oils	Cosmetics
Fats for baking,	Lubrication
confectionery industry	Chemicals
and mayonnaise manufacturing	Candles
Oil for the fish and canning industry	Technical products
Feed fats	Plastic coatings

Source: Hatje [1].

crops of developing countries could be made redundant by genetic engineering of temperate crops. For instance, if rape is engineered to produce ricinoleic acid, the market for castor oil will be greatly reduced.

Thus, there is a vital need to devote more research and development efforts to the wide range of oil crops, those of cool weather and those adapted to warmer climates. There is also a clear need to search, domesticate and develop species which can serve as new sources for existing or new requirements. These efforts will give more viable marketable production options to the farmers. They will fit in with the need to diversify the crops grown in both the developing countries (to supply local demand and to produce exportable commodities) and in the developed ones (to reduce surpluses).

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EVOLUTION OF IMPROVED VARIETIES OF RAPESEED/MUSTARD THROUGH INDUCED MUTATION

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Abstract

Mutation breeding using gamma rays led to the development and release of two high yielding yellow sarson mustard mutant varieties, 'Safal' and 'Agrani', which are now commercially cultivated by the farmers of Bangladesh. Further studies with *Brassica napus* created a lot of variabilities in M_2 with the selection of eight early maturing mutants which took 82 - 85 days to mature compared to 105 days by the original cultivar. The mutants bred true in M_3 for earliness. A few indehiscent and semi-indehiscent mutants were also developed. Seed yields of the early mutants were about 2 000 kg per hectare which was comparable to the original cultivar. The mutants have 44 - 45 % oil content in the seed compared to previously released varieties with 42 - 43 % oil content. Mutants are moderately resistant to *Alternaria* blight in field condition. Other mutants segregated in M_3 in different ratios and further selection was made. Evaluation of yellow sarson mutant BINA 2 along with other high yielding varieties in three saline zones of Bangladesh revealed that BINA 2 is more tolerant to salinity compared to existing high yielding varieties with an average yield of 1 888 kg followed by 1 758 kg by 'Agrani' and 1 699 kg by 'Safal', per ha.

1. INTRODUCTION

Work on mutation breeding on oil seed rape started in the Plant Breeding Division of Bangladesh Institute of Nuclear Agriculture (BINA) since 1981 in collaboration with the International Atomic Energy Agency through a project, 2991/RB. Success has been achieved in this regard with the release of two high yielding mutant varieties 'Agrani' and 'Safal'. The present work on induced mutations was initiated in 1988 to fulfill the need for the development of a high yielding mustard variety that matures by 80 days. The planners and the scientists of Bangladesh felt that it would fit well into the existing cropping pattern.

2. MATERIALS AND METHODS

2.1. Collection and evaluation of germplasm

About 150 germplasm of *Brassica campestris*, *B. juncea* and *B. napus* were collected from the various parts of Bangladesh. These materials were field evaluated in a randomized complete block design with three replications in a unit plot size of 2 x 5 m^2 for selection of materials for irradiation.

2.2. Induction of genetic variability

Dry seeds of the sarson variety, Sonali Sarisha of *B.campestris* and Nap-3 and Oro of *B. napus* were exposed to different doses of gamma rays from a ^{60}Co source and the seeds were sown directly in the field with three replications with a plot size of 2 x 5 m^2 for M_1 studies. Records on various agronomic traits were taken from seedling to maturity and M_1 plants were harvested individually to grow M_2.

2.3. Studies on segregation and selection of desirable mutants in M_2 and M_3 generations

M_2 plant progeny rows of mustard were raised under different doses along with original cultivar. The materials were very carefully studied from seedling to maturity.

Selection on the basis of earliness, bold siliqua type, resistance to *Alternaria* blight with high yielding attributes were made in mustard under different doses. The selected M_2 materials were grown in M_3 in plant progeny rows along with the original cultivar for screening, and further selection was made.

2.4. Progeny tests of selections of rape in more advanced generations

The experiments were conducted at three locations of Bangladesh with mutants BINA 1, BINA 2, BINA 3 and check varieties YS 52 (original cultivar), and two recommended varieties, Sampad and Sonali Sarisha. Randomized complete block design was followed at all the locations with four replications. The mutants were also evaluated under low and high input conditions in the farmers' field at 7 agro-ecological zones of Bangladesh in split plot design (dispersed) along with the check varieties.

2.5. Evaluation of the mutant varieties/mutants in saline zones

Mutant BINA 2 and the two mutant varieties Agrani and Safal were evaluated in three saline zones of Bangladesh to see their agronomic performances and yield compared to the existing high yielding varieties of mustard. The experiments were conducted in randomized complete block design with four replications with unit plot size of 5 x 5 m^2.

Soil samples from all the experimental plots were collected regularly after 15 days interval and were analyzed by the Soil Science Division of BINA. Soil pH of the experimental plots ranged between 7.6 - 8.0. E.C. (dS/m) of the soil during experiment ranged between 0.4 - 0.9.

3. RESULTS AND DISCUSSION

3.1. Collection and evaluation

Variation was observed in different agronomic characters of the varieties of mustard evaluated. Varieties Sonali Sarisha, Nap-3 and Oro of mustard were selected for irradiation.

3.2. M_1 studies

Records on germination, seedling height, number of leaves and leaf area after 21 days of germination, plant height at maturity, number of siliqua/capsule per plant, days to maturity and oil content were taken from a random sample of 10 plants under each dose of mustard. With increasing doses most of these characters showed a linear decrease (Table I). Statistical analysis showed a significant differences among all the characters excepting days to maturity and oil content in mustard. Abidi and Haq [1] reported a positive response in two mustard varieties, S-2 and S-4 of *B. campestris* to gamma rays treatment for seed index which could be exploited for the evolution of better varieties.

3.3. Selection of desirable mutants in M_2 generation

A large number of variants was observed in M_2 with respect to earliness, bolder siliqua, resistance to *Alternaria* blight, longer siliqua with a higher number of seeds per siliqua, wax deficient mutants, coloured siliqua in *B. napus*. Only two mutants with two chambered siliqua were selected from Sonali Sarisha where the original cultivar was four chambered. About 45 mutants with morphological variations were selected in the field from Oro and Nap 3 which were screened in the laboratory by the NMR for oil content and finally 35 mutants were kept. Gupta [2] reported morphological changes like flower colour, seed characters in M_2 generation of diploid and autotetraploid mustard after treatment with X-rays.

Table I. EFFECT OF GAMMA RAYS ON M_1 POPULATION OF RAPESEED AND MUSTARD

Variety	Dose (Gy)	21 Days Old Seedling			Plant height (cm)*	Siliqua /plant (No.)	Days to maturity	Oil content
		height (cm)	Leaves					
			No.	Area (cm^2)				
Sonali	0	14a	6.4a	139a	122a	45a	96	42
Sarisha	700	11b	5.2b	69b	114b	44a	96	42
	800	10b	5.0bc	65b	112b	40ab	95	41
	900	9c	4.8bc	49c	102c	41ab	96	41
	1 000	7d	4.5c	47c	98c	38d	97	41
Oro	0	11a	6.0a	112a	110a	121a	130	44
	700	8b	4.0b	77b	105a	105b	130	44
	900	8b	4.0b	41c	98b	101bc	131	45
	1 000	6c	3.0c	38d	98b	96c	132	43
	1 100	5d	3.0c	35d	91c	85d	131	45
Nap 3	0	15a	6.0a	121a	107a	99a	105	45
	700	12b	5.0b	118a	96b	82bc	105	44
	900	9c	4.0c	70b	89c	80bc	106	44
	1 000	8d	4.0c	62c	83d	77cd	107	44
	1 100	7c	3.0d	50d	85d	72d	107	44

* : At maturity.

3.4. Growing of M_3 plants of variety Nap-3 and Oro (*B.napus*) for selection

Thirty five M_2 mutants of Nap-3 and five mutants of Oro induced by different doses of gamma rays were grown in plant progeny rows along with control at BAU farm, Mymensingh, during 1991 - 92 winter season to study their agronomic characters in M_3.

All the eight early mutants of Nap-3 were confirmed to mature earlier by 82-85 days whereas the original cultivar took 105 days to mature. Seed yield of the early maturing mutants were comparable to the yield of the original cultivar which was around 2 000 kg/ha (converted yield). The indehiscent type of mutants segregated further and were grouped as indehiscent and semi-indehiscent while the dehiscent types of mutants were discarded. Mutants with longer and bolder pods segregated further for maturity and those mutants which had 90 days maturity were only kept and the rest were discarded.

The dwarf mutants bred true with indehiscent pods characteristics but their yield was remarkably low compared to the original cultivar. The pink coloured pod mutants segregated further as pink to normal colour. Wax deficient mutant also segregated further as normal and wax deficient. Mutants with medium sized pod also segregated further as medium, long and short types pod compared to the pod of the original cultivar. Based on the morphological variation in the field further selections in M_3 were made which were again screened in the laboratory for oil content through NMR techniques and only the mutants having 44% oil and above were kept for further studies in M_4 generation. Gupta [2] reported further segregation and selection in M_3 generation of mustard.

Most of the mutants were moderately resistant to *Alternaria* blight in field condition including the original cultivar. Only the coloured pod mutants were resistant to the disease.

Table II. PERFORMANCE OF DIFFERENT AGRONOMIC CHARACTERS OF THE SELECTED MUTANTS OF MUSTARD AND ORIGINAL VARIETIES IN M_3 GENERATION

Variety/ mutant & dose	Days to maturity	Plant height (cm)	Pod height (cm)	Branch/ plant (No.)	Pods/ plant (No.)	Seeds/ pod (No.)	Characteristics of mutants
Var. Nap-3							
Nap-3	105	93	34	6.0	70	19.5	Branched, Dehiscent, medium pod
Mutant lines							
700 Gy							
7-75-90	85	79	31	2.9	65	16.3	Long pod
8-88-90	84	80	32	2.0	58	21.6	Medium pod
7-25-90	98	81	29	2.4	36	24.8	Indehiscent pod
7-25-90-1	98	74	28	2.0	85	21.4	Semi-indehiscent pod
7-76-90	98	74	29	2.2	67	22.2	Bolder pod
7-10-90	96	75	28	2.8	108	20.8	Medium pod
22-71-90	98	69	29	2.6	46	19.0	Dwarf,long pod
7-9-90	98	67	26	2.4	44	16.4	Dwarf
8-38-90	95	74	34	2.3	107	21.0	Coloured pod
800 Gy							
43-66-90	82	82	42	2.4	56	24.8	Coloured bolder pod
4-8-90	82	73	39	2.2	50	22.5	Medium pod.
36-41-90	92	79	40	2.0	82	19.9	Coloured pod
5-37-90	82	83	43	1.2	47	23.2	Early maturing
16-45-90	84	84	35	2.0	66	20.1	Early and bolder pod
17-7-90	100	84	40	2.1	70	20.2	Medium pod
18-55-90	99	76	34	1.9	61	22.3	Long pod
18-55-90-	100	94	39	1.8	67	16.8	Long pod
3-23-90	100	82	33	1.8	66	20.7	Coloured pod
17-21-90	100	85	37	2.0	67	25.8	Long pod
19-33-90	100	84	36	2.4	66	21.7	Wax deficient
1 000 Gy							
5-55-90	98	87	36	3.4	58	29.7	Early and long pod
8-58-90	98	98	55	2.8	101	18.8	Coloured pod
5-100-90	98	74	38	1.8	60	21.1	Erect plant
7-31-90	98	81	38	1.9	67	25.0	Long,bolder,color pod
19-48-90	101	83	35	2.3	79	24.5	Long,bolder pod
5-58-90	101	88	40	2.0	81	25.9	Coloured pod
6-93-90	101	90	28	1.4	65	23.2	Indehiscent pod
6-93-90-1	101	90	33	2.0	80	23.6	Semi-indehiscent pod
1 100 Gy							
19-51-90	83	81	42	2.0	77	22.0	Early maturing
19-19-90	83	84	34	1.9	70	18.3	Coloured pod
Var. Oro							
Oro	110	85	39	7.5	69	19.0	Branched med.pod
1 000 Gy							
16-92-90	90	78	34	0.8	43	22.2	Early maturing

Rahman *et al.* [3] reported selection of resistant mutants against *Alternaria* blight in their earlier work with *B. campestris* variety yellow sarson. Out of five mutants selected from Oro, four bred true for earliness and of the rest one segregated for different characters and was discarded in the field. Three more mutants were discarded after oil analysis since they had oil content below 44 % (Table II).

Evaluation of the mutants BINA 1, BINA 2 and BINA 3 for yield compared to the existing high yielding varieties Sampad and Sonali Sarisha at different agro-ecological zones of Bangladesh in research stations and in farmers field proved the superiority of the mutants BINA 1 and BINA 3 in respect to seed yield, biomass yield and tolerant to *Alternaria* blight over the checks. The mutants have potentiality of producing 2,500 kg seed yield with an average of 1,700-1,750 kg compared to the national average of 700 kg per hectare. Both the mutants were released in 1991 by the National Seed Board of Bangladesh with the popular names, Safal (BINA 1) and Agrani (BINA 3) for commercial cultivation by the farmers.

The promising mutants/varieties were further tested for their performance in saline zones. Data on seed yield, biomass yield and other important agronomic characters were recorded from 20 randomly chosen plants from each replicate of the mutants and the checks (Table III). The highest seed yield (1 888 kg) was obtained by BINA 2 followed by Agrani (1 758 kg) and Safal (1 699 kg). Sonali Sarisha gave 1 538 kg while Sampad and Tori 7 have produced 1 218 kg and 869 kg seed/ha, respectively. The performance of BINA 2 was found to be better in two places. In these two places E. C. (dS/m) was recorded higher than the 3rd location.

The highest biomass yield was produced, on an average by Safal (5 122 kg) followed by BINA 2 (4 959 kg) and Agrani (4 378 kg). Samapd and Sonali Sarisha gave 3 802 kg and 3 650 kg biomass yield per hectare. Tori 7 gave only 1,910 kg.

BINA 2 had the tallest plant height of 128 cm followed by Safal (124 cm), Agrani (120 cm), Sonali (111 cm), Sampad (109 cm) and Tori 7 (77 cm).

On the other hand, Tori 7 had the highest number of pods/plant (73 pods) followed by BINA 2 (50 pods) and Safal (41 pods), Agrani (37 pods), Sampad (35 pods) and Sonali Sarisha (35 pods).

Seeds/pod was highest in Sonali Sarisha (25 seeds) followed by BINA 2 and Safal (22 seeds), Agrani (21 seeds) and Sampad (20 seeds). Tori 7 produced lowest number of seeds/pod (15 seeds).

Thousand seed weight of Agrani was higher (3.65 g) compared to other varieties and was closely followed by Sonali Sarisha (3.30 g). Seed weight of Safal, BINA 2 and Sampad was comparable (2.63 g, 2.64 g and 2.50 g, respectively). The lowest seed weight was recorded in Tori 7 (2.05 g). Safal and BINA 2 were found more tolerant to *Alternaria* blight and to aphid compared to other varieties in field conditions.

The results of the experiment was encouraging. Farmers of these places showed interest to grow mustard in their areas where most of the land remains fallow from December to May. The experiment needs to be repeated.

Acknowledgement

The authors acknowledge with gratitude the financial assistance of the International Atomic Enery Agency through the RC. Nos. 2991/RB and 5432/RB.

Table III. MEANS OF SEED AND BIOMASS YIELDS AND OTHER AGRONOMIC CHARACTERS OF MUTANT/VARIETIES OF MUSTARD GROWN IN THREE COASTAL AREAS OF BANGLADESH

Variety/ Location	Days to maturity	Plants/ m^2	Plant height (cm)	Pods/ plant (No.)	Seeds/ pod (No.)	1000-seed wt.(g)	Yield (kg/ha)	
							Seed	Biomass
BINA 2								
L-1	93	--	131	50	19	2.75	1 409a	6 457b
L-2	93	87	132	44	20	2.60	2 105a	4 220a
L-3	92	60	122	55	27	2.58	2 150a	4 400a
MEAN	93	74	128	50	22	2.64	1 888	4 959
SAFAL								
L-1	93	--	126	47	18	2.75	1 618a	7 797a
L-2	92	86	123	38	19	2.53	1 560b	3 368b
L-3	91	68	123	38	29	2.60	1 918bc	4 200a
MEAN	92	77	124	41	22	2.63	1 699	5 122
AGRANI								
L-1	88	--	121	44	17	3.73	1 330ab	5 622c
L-2	84	87	129	35	18	3.63	1 921a	3 369b
L-3	88	62	111	32	29	3.60	2 023ab	4 144a
MEAN	87	75	120	37	21	3.65	1 758	4 378
SAMPAD								
L-1	85	--	114	46	15	2.56	1 030bc	5 417c
L-2	86	92	118	30	17	2.45	1 129c	2 538c
L-3	87	75	95	30	27	2.48	2 494d	3 450b
MEAN	86	84	109	35	20	2.50	1 218	3 802
SONALI								
L-1	93	--	116	39	23	3.50	1 308abc	5 288c
L-2	92	83	121	36	21	3.08	1 481b	2 750c
L-3	92	62	97	30	30	3.33	1 825c	2 913c
MEAN	92	73	111	35	25	3.30	1 538	3 650
TORI 7								
L-1	81	--	75	115	13	2.13	985c	2 869b
L-2	75	52	88	54	13	1.98	759d	1 330d
L-3	73	49	67	49	18	2.05	864e	1 531d
MEAN	76	51	77	73	15	2.05	869	1 990

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STUDIES ON BREEDING FOR QUALITY IN *Brassica napus*

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Abstract

Sichuan province is the largest rape producing area in China. The current popular rape varieties in China contain high percentages of erucic acid and glucosinolates which have limited the utilization of the oil and cake. Therefore it is necessary to develop good varieties of *Brassica napus* for the autumn sown rape production areas in China. One new *Brassica napus* variety with high yield, high oil content, strong lodging resistance and low erucic acid has been developed by the combination of irradiation with crossbreeding in the present programme. At the same time some new mutant lines, that is, two with high protein content, two with "double low", three with low linolenic acid, two with high oil content and low erucic acid, one with high oleic acid and low erucic acid and one with high linoleic acid and low erucic acid, have also been developed.

1. INTRODUCTION

China is a chief producer of rapeseed, ranking first in both the sown area and the total production of rapeseed in the world [1], and Sichuan province is the largest rape producing area in China [2]. Rape oil is one of the main edible oils of the Chinese people [3]. *Brassica napus* L. is the main cultivated rape species in China [4]. However, the current rape varieties grown in China contain high percentages of erucic acid and glucosinolates which have limited the qualities and utilization of the rape oil and rape cake. Low erucic acid or "double low" (low erucic acid and low glucosinolates) exotic varieties have been introduced, but they can not be used directly in the field production because they are not fully adapted to the local environments [5]. Therefore, it is urgent to develop well adapted double low rape varieties of *Brassica napus* for the autumn sowing in China [3]. The rape varieties developed by conventional methods so far are undesirable in one or several characters such as yields, resistances and maturing dates. In the present research programme, it is planned to develop high yield, high quality and multi-stress resistant rape varieties of *Brassica napus* suitable for growing in Sichuan province by means of the radiation induced mutation or the combination of mutation induction with crossbreeding.

So far, one new *Brassica napus* variety with high yield, high oil content, low erucic acid, strong lodging resistance and *Sclerotinia* resistance has been developed. At the same time, some new strains, that is, two with high protein content, two with "double low", three with low linolenic acid, two with high oil content and low erucic acid, one with high oleic acid and low erucic acid, and one with high linoleic acid and low erucic acid, have also been developed.

2. MATERIALS AND METHODS

2.1. Materials

Three groups of *Brassica napus* varieties have been irradiated:

Varieties with some shortcomings including "single low" (low in either of erucic acid or glucosinolates) and "double low" varieties were exotic varieties. Their defects in agronomic characters are expected to be improved by the irradiation treatment.

Varieties with high yield but with high erucic acid or with both high erucic acid and high glucosinolates were mainly local high yielding varieties. Their quality was expected to be improved.

The third group was a hybrid progeny, especially F_1 seeds crossed between the "single low" or "double low" varieties and the popular high yielding varieties. The variation range and the frequency of induced mutation were the objectives to be increased.

2.2. Irradiation treatments

2.2.1. Irradiation of seeds

Fifty grams of seeds (moisture content 8-9%) for each dose were treated by 695, 869 and 1 043 Gy of ^{60}Co gamma rays at the dose rate of 2.44 Gy/min, respectively. The seeds were sown seven days after the irradiation treatment.

2.2.2. Irradiation of pollen grains

Flowers blooming in the morning were irradiated by 26.08 Gy ^{60}Co gamma rays at the dose rate of 0.94 Gy/min. Pollen grains were collected immediately from the treated flowers to pollinate.

2.3. Identification and selection of the progeny

The identification and selection of the agronomic characters were the main work conducted in the M_2 generation. For the lines selected for favorable agronomic characters, examinations of quality characters were made and promising lines were selected in the M_3 generation.

2.4. Determination of chemical compositions

Oil content was determined by the use of the YG-2 fat extractor (improved Soxhlet Method). The composition of the fatty acid and glucosinolates contents was determined by the use of the gas chromatography, and the protein content was calculated from the total Kjeldahl nitrogen, multiplying with the conversion factor, 6.25.

3. RESULTS

3.1. Breeding of the variety 26-1 with low erucic acid

The variety 26-1 was selected from the irradiated B-26 seeds, which were treated with 869 Gy ^{60}Co gamma rays and whose erucic acid content was about 50 %. The breeding process of the variety 26-1 is shown in Fig.1.

The oil content of the variety 26-1 is 42.3 %. The composition of fatty acids is shown in Table I.

TABLE I. THE COMPOSITION (%) OF FATTY ACIDS OF VARIETY 26-1

Variety	Fatty acids (%)						
	C16:0	C18:0	C18:1	C18:2	C18:3	C20:1	C22:1
B26(CK)	3.15	0.21	16.10	11.25	7.78	11.20	50.31
26-1	5.10	0.20	62.00	16.45	9.00	6.30	0.95

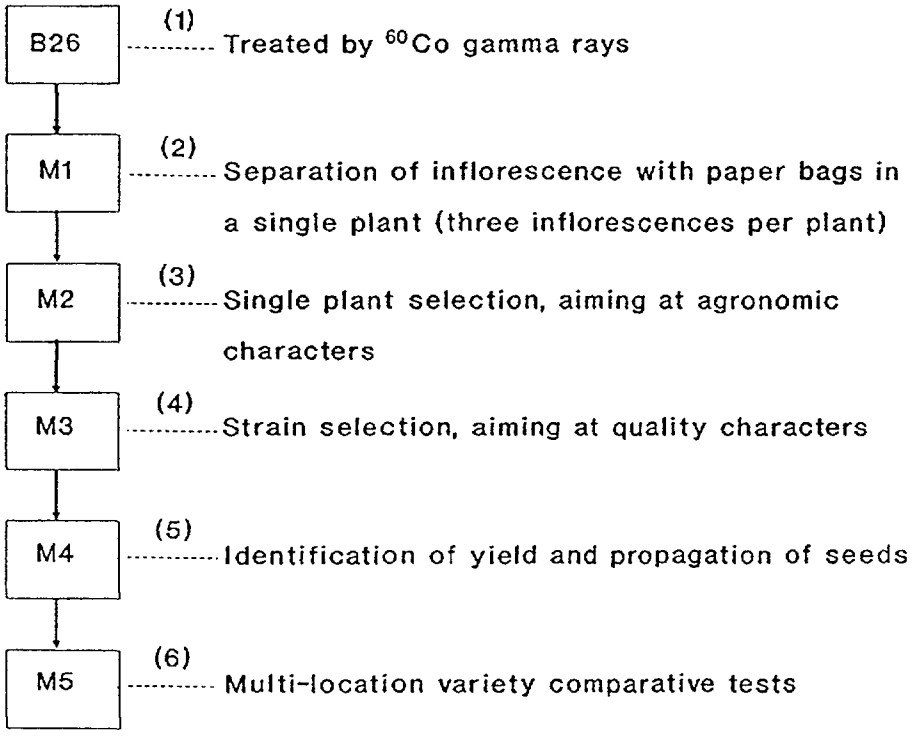

Figure 1. The breeding process of variety 26-1 with low erucic acid.

During the period from 1991 to 1992, the multi-location variety comparative tests for the variety including 26-1 were conducted in five sites where the area of each plot was 66.7 m^2 with triplicates. The mean yield of the variety 26-1 was 16.04 % higher than that of the original strain B-26, and 10.53 % higher than that of the popular variety Chuan-You 11 (Table II), significantly so (<0.01 and <0.05, respectively).

TABLE II. YIELD COMPARISON OF THE VARIETY 26-1, THE ORIGINAL STRAIN B-26 AND THE POPULAR VARIETY CHUAN-YOU 11

Variety	Yield of rapeseeds		Increases (%) of 26-1
	kg/66.7m^2	kg/ha	compared with others
B-26 (CK 1)	13.84	2076.0	16.04
Chuan-You 11 (CK 2)	14.53	2179.5	10.53
26-1	16.06	2409.0	-----

The variety 26-1 has strong lodging resistance and quite strong *Sclerotinia* resistance (Table III).

The variety 26-1 has been well accepted by farmers because of its low erucic acid, strong lodging resistance, quite strong *Sclerotinia* resistance, high oil content of seeds and at least 10 % higher yield than that of the local popular varieties.

3.2. Other promising strains

Some strains with better agronomic and quality characters have also been developed.

TABLE III. COMPARISON OF DISEASE AND LODGING RESISTANCE OF 26-1, B-26 AND CHUAN-YOU 11

Variety	Disease incidence of *Sclerotinia* (%))	Lodging resistance
26-1	8.9	Strong
B-26	14.7	Medium
Chuan-You 11	29.6	Weak

3.2.1. New mutant lines with high protein content

In the crossbreeding in which the variety Zhong-You 821 was used as the female parent and the variety Jin 6 as the male parent, the pollen grains of the male parent were treated by 26.08 Gy ^{60}Co gamma rays. Two new strains, 924-24 and 924-46, with high protein content have been developed after the directional selection for four years in their crossbreeding progenies, and the strain 924-46 is also a strain with low erucic acid (Table IV). The agronomic characters and yield performance of the two strains with high protein content are quite good, and the variety comparative tests will be conducted in 1993. The rapeseed cake is an important protein resource [6], and those with high protein content are good livestock feed. Therefore, the demand for creating the high protein germplasm has been suggested in China.

TABLE IV. THE CAKE PROTEIN CONTENTS AND THE FATTY ACID COMPOSITION OF OILS OF TWO NEW STRAINS WITH HIGH PROTEIN CONTENT

Strain	Protein content (%)	Fatty acids (%)					
		C16:0	C18:1	C18:2	C18:3	C20:1	C22:1
924-24	41.32	3.48	15.61	15.47	6.48	9.21	49.75
924-46	40.81	9.92	54.02	23.18	9.12	2.19	1.57

3.2.2. New strains with "double low"

F_1 seeds of the crossbreeding between Xi-Nan 302 and Marnoo were treated with 695 Gy ^{60}Co gamma rays, and then two new strains, 926-7-4 and 926-7-1, with "double low" have been selected from their progenies (Table V). The oleic acid content of the strain 926-7-4 is up to 64.31%. The yield test will be conducted in 1993.

TABLE V. FATTY ACID COMPOSITION (%) AND GLUCOSINOLATE CONTENTS (MOL/G) OF THE TWO NEW STRAINS WITH "DOUBLE LOW"

Strain	Fatty acids (%)						
	C16:0	C18:1	C18:2	C18:3	C20:1	C22:1	Glucosinolates
220 (CK)	4.15	15.45	16.45	9.25	11.10	43.60	30.10
926-7-4	4.63	64.31	18.70	9.35	1.56	1.45	25.00
926-7-1	5.10	61.65	19.90	8.90	2.10	2.35	27.50

TABLE VI. THE FATTY ACID COMPOSITION (%) OF THREE NEW STRAINS WITH LOW LINOLENIC ACID

Strain	Fatty acids (%)					
	C16:0	C18:1	C18:2	C18:3	C20:1	C22:1
925-1-5	2.44	61.88	8.03	2.77	1.95	22.93
925-4-1	4.46	58.50	29.89	3.64	2.10	1.41
925-6-1	4.33	32.19	17.96	4.99	21.55	18.98
57-1	5.56	23.17	14.43	9.91	17.40	29.53

3.2.3. New strains with low linolenic acid

The pollen grains of the strain 57-1 were treated by 26.08 Gy of ^{60}Co gamma rays and used to pollinate the same plant. One new strain 925-1-5 with less than 3% of linolenic acid and two, 925-4-1 and 925-6-1, with less than 5% of the same have been selected from their progenies (Table VI). Among them, the strain 925-4-1 has low linolenic acid and low erucic acid, and the strain 925-1-5 is a strain with low linolenic acid and high oleic acid.

3.2.4. New strains with high oil content and low erucic acid

Two new strains with high oil content and low erucic acid have been selected from the progenies of the treated seeds of the variety B-26 by 869 Gy ^{60}Co gamma rays (Table VII).

TABLE VII. THE OIL CONTENT AND FATTY ACID COMPOSITION OF TWO NEW STRAINS WITH HIGH OIL CONTENT AND LOW ERUCIC ACID

Strain	Oil content (%)	Fatty acids (%)					
		C16:0	C18:1	C18:2	C18:3	C20:1	C22:1
925-100	44.7	9.17	41.93	24.18	18.35	4.65	1.72
925-37	41.5	7.65	54.23	27.08	7.30	2.30	1.44
B-26	38.8	3.15	16.31	11.25	7.78	11.20	50.31

3.2.5. New line with high oleic acid and low erucic acid

The new line 925-40 with high oleic acid and low erucic acid has been selected from the progenies of the treated seeds of the variety B-26 by 869 Gy gamma rays, and its fatty acid compositions are shown in Table VIII.

TABLE VIII. FATTY ACID COMPOSITION OF THE NEW STRAIN 925-40

Fatty acids	C16:0	C18:1	C18:2	C18:3	C20:1	C22:1
Content (%)	6.91	64.33	18.99	6.31	1.61	1.85

3.2.6. New line with high linoleic acid and low erucic acid

The pollen grains of the introduced variety Savaxia were treated by 26.08 Gy ^{60}Co gamma rays, and used to pollinate the same plant. The new line 925-10-2 with high linoleic acid and low erucic acid has been selected from their progenies.

TABLE IX. THE FATTY ACID COMPOSITION OF THE NEW STRAIN 925-10-2

Fatty acids	C16:0	C18:1	C18:2	C18:3	C20:1	C22:1
Content	6.44	51.99	30.04	9.78	0.80	0.95

4. DISCUSSION

Significant achievements in breeding have been attained by the combination of irradiation treatments with the crossbreeding in many countries [7, 8, 9]. If an appropriate breeding materials could be selected to irradiate, and the combination of the radiation mutagenesis with the crossbreeding is used, it is very likely to breed new varieties of *Brassica napus* with high yield and improved quality characters suitable for local ecological conditions and cultivated systems. This idea has been well approved by these promising varieties or lines which have been obtained in the present programme.

During these researches, pollen grains of some materials have been irradiated by ^{60}Co gamma rays and all the plants pollinated by these treated pollen grains were fertile, producing seeds. Some new mutant lines have been obtained. Therefore, it is suggested that 26.08 Gy be a suitable dose for this method as one of the techniques applicable to rape.

The contradiction between high yield and high quality may be resolved easily if agronomic characters are selected in M_2 generation and quality characters are screened in the later generations. Crossbreeding may be used to combine desired mutant gene with other favorable genotype.

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IMPROVEMENT OF RAPESEED (*Brassica napus* L.) FOR AGRONOMIC AND QUALITY CHARACTERS THROUGH INDUCED MUTATIONS AND HYBRIDIZATION

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Abstract

Mutation breeding research of oilseed rape (*Brassica napus* L.) with the objective to develop improved varieties characterized by having high yield potential, high oil content of better quality, and resistance/tolerance to different stresses was initiated during 1988 - 89. Based on GR_{50} dose, bold and uniform seeds with 10 - 12 % moisture of different rapeseed varieties/lines were subjected to 800, 1 000 and 1 200 Gy ^{60}Co gamma radiation and treated with 1.0, 1.5 or 2 % ethyl methanesulphonate (EMS) and $1x10^{-4}$, $1x10^{-3}$, $1x10^{-2}$ M sodium azide (NaN_3) solutions for four hours. The treated seeds were directly sown in the field in isolation as M_1. Selection of desirable mutant plants was made in M_2 - M_3. The stable progenies were evaluated in microplot tests in M_5 and M_6, for yield potential and other agronomic and quality characters in comparison with respective parent and a local commercial cultivar as checks. Results of yield trials indicated significant variability with regards to grain yield. A number of mutants expressed high yield potential and many had significantly outyielded both the checks. Plant height was reduced in certain mutants but reduction to an ideal level could not be induced. Variability in physiological maturity was also noted and four mutants matured significantly earlier than the checks by a margin of three weeks. Yield tests under drought conditions revealed that 16 out of 28 mutants of cv. Pak-Cheen and Tower significantly outyielded the checks. As a result of significant increase in seed size, total oil content was also enhanced in many mutants and the highest oil content (50 %) was observed in mutant line RM-13-1. Quality analysis indicated that 15 mutants produced oil of Canola quality. Cross breeding of promising mutant lines with exotic Canola quality lines was started during 1990 - 91. So far, the F_2 generation has been raised and 102 recombinants with desired plant attributes have been selected. Further studies are being conducted to confirm the previous results and to ascertain the genetic stability and adaptability of promising rapeseed mutants.

1. INTRODUCTION

Pakistan is extremely deficient in edible oil and this deficit is continuously being enlarged with the increase in annual consumption. The domestic production is sufficient to meet only 30 % of the requirement, the remaining demand is met through heavy imports. Pakistan has spent more than four hundred million U.S. dollars on the import of edible oil during 1991 - 92, out of which 1.4 million was spent on the import of rapeseed oil [1, 2]. The import bill can, however, be reduced only by increasing the domestic oil production.

Rapeseed and mustard, collectively referred to as Oilseed Brassica, are the only established traditional oilseed crops of the country, as these are grown for centuries and are well entrenched in the present cropping system. The farmers are also well familiar with their production technology. Brassica oilseeds are presently grown over an area of 0.3 million hectares with an average national yield of 752 kg/ha [3]. The reasons for the low yields are many but lack of high yielding cultivars with better oil quality is the most important one. With the development of improved varieties, brassica oilseeds would help narrow the gap between production and consumption of edible oil in Pakistan, and attaining self-sufficiency in brassica oilseeds alone would result in reducing the import bill of the country by 1.4 million US dollars annually.

It is with these facts in view that a research project on the "Improvement of rapeseed for agronomic and quality characters through induced mutations and hybridization" was initiated during 1988-89. The thrust of the project is to make oilseed rape more competitive with other field crops by increasing its productivity, improving the product quality (oil and meal) and

making it resistant/tolerant to various biotic and abiotic stresses, through the development of improved cultivars using both conventional and nuclear techniques. This paper describes the results of various experiments undertaken during 1991 - 92 and recapitulates the previous results reported in the first and second RCM.

2. MATERIALS AND METHODS

The procedure outlined by Konzak and Mikaelsen [4] was adopted for raising M_1 - M_3 generations of rapeseed mutants. The methods used for conducting various experiments are briefly mentioned below.

2.1. Seed treatment with gamma rays

About 10 000 dry and uniform seeds having 10 - 12 % moisture from each of four varieties of rapeseed viz. Pak-Cheen, Westar, Tower and Salam were subjected to various doses of gamma rays and based on GR_{50} dose, three doses i.e. 800, 1 000 and 1 200 Gy were used for mutation breeding of these varieties. The treated seeds along with untreated controls were sown directly in the field in isolation, during 1988 - 89. At maturity four pods from every primary branch of each plant were harvested and seeds were bulked dose and variety wise. Parent varieties were also harvested at maturity and their seed were then used as control in M_2.

2.2. Seed treatment with chemical mutagens (EMS and NaN_3)

Based on GR_{50} dose, presoaked uniform seeds of four rapeseed varieties viz. Westar, Pak-Cheen, Sweden line 1517 and Tower were treated with 1.0, 1.5 and 2 % solution of EMS for 4 h. After 4 h post washing under running tap water, the seeds were dried on filter paper under room temperature and then planted in the field. An equal number of untreated seeds as control (but subjected to pre and post washing) were also sown. Similarly seeds of these varieties were treated with $1x10^{-4}$, $1x10^{-3}$ and $1x10^{-2}$ M concentrations of NaN_3. The procedure for the NaN_3 treatment was the same as mentioned above for EMS treatment. Seeds after drying on filter paper were planted in the field dose/variety wise, along with the controls. At maturity four pods from each primary branch of every plant were harvested, seed threshed and bulked dose and variety wise.

2.3. Selection procedure and stability tests

M_2 generation of each variety was raised dose wise in separate blocks. Parent variety was planted at the start and after every 20 rows of treated seeds for easy comparison. Selection of desirable mutants was carried out at appropriate growth stages. The mutants were tested in M_3/M_4 progeny rows for ascertaining stability of the selected traits. Seed treatment, selection and stability tests are routinely carried out every year for keeping continuity in the mutation breeding research programme.

Two breeding generations were raised every year in winter at NIFA and in the hilly area in summer (at Kaghan). This practice resulted in advancement of breeding generations of rapeseed mutants and the rapid progress of this research project.

2.4. Agronomic evaluation

The stable M_4 mutants were evaluated for yield potential and other agronomic traits in yield trials in M_5, during 1990 - 91. Each trial was laid out according to randomized complete block (RCB) design with two replications. Each entry was planted on a plot of 5.4 m^2, having six rows, 3 m long and 30 cm apart. Data of plant height was recorded at maturity on 5 randomly selected plants in each entry, by measuring from ground level to terminal inflorescence. Days to maturity were estimated from date of sowing to physiological maturity (i.e. when 30 % of the pods on main stem changed colour from green to straw). At maturity four central rows were harvested for recording grain yield data per plot (size 3.6 m^2).

Chemical analysis for total oil content, fatty acid composition and total glucosinolate contents were carried out with the help of Gas Liquid Chromatography (GLC).

The yield tests were repeated during 1991 - 92, with same experimental design but every entry was planted on comparatively larger plot size (9.0 m^2) with four replications. Remaining procedure was same as outlined above. The results were statistically analyzed applying the Least Significant Difference (LSD) test at 5% level of significance [5].

3. RESULTS AND DISCUSSION

3.1. Selection of desirable mutants

Selection for desirable mutant plants was carried out in a large M_2 populations of different rapeseed varieties during 1989 - 1992, for earliness, short stature, heavy bearing i.e. more primary branches and pods/plants, long effective pods, bold seeds and resistance/tolerance to stress conditions. Details of the plants selected for particular characteristics are given in Table I. A total of 425 desirable mutants were selected out of which 44 expressed short stature, 77 were early maturing, 133 exhibited heavy bearing, 269 produced long pods, 160 possessed more grains/pod, 186 had bold seeds and 99 plants showed stress tolerance. It is also clear from the results that rapeseed mutants exhibited very useful genetic variability regarding different traits, and some mutants possess more than one desirable characteristic.

3.2. Stability tests

Genetic stability of the desired traits of mutants selected in M_2/M_3 was ascertained in M_3/M_4 progeny rows. Results (Table II) indicate that maximum number of mutants were isolated from mutagenised population of variety Westar followed by Tower, however, high number of stable mutant progenies regarding different plant traits were observed in case of cv. Pak-Cheen. Most of the mutants were segregating and further selection was made.

3.3. Agronomic evaluation

3.3.1. Preliminary Yield Trials (PYT)

The results indicated that 42 out of 72 mutants tested in yield trials produced higher grain yields than their respective parents and local commercial cultivar. The results also revealed that mutants of ideal plant height (100 - 120 cm) for irrigated areas could not be isolated, though reduction in plant height was noted in some mutants. It seems that genes controlling plant height in *Brassica* are not easily mutated by gamma ray irradiation and the mutation frequency for this trait appears to be very low. Four mutants matured significantly earlier than the checks, by a margin of three weeks, whereas some were late maturing. Seed size was significantly increased in many mutants due to increase in length of the siliquae, which provided more space for growing seeds. Positive correlation is also reported [6] between pod length and seed weight. Increase in seed size would also result in high yield and high oil content with reduced level of fibre content [7].

3.3.2. Micro plot tests

Mutants showing good performance in preliminary yield trials were further evaluated in micro plot tests in M_6 during 1991 - 92 to study their genetic stability. Results of some promising mutants of cv. Pak-Cheen, Westar and Tower are presented in Table III and IV.

It can be observed from the results (Table III) that plant height of most of the mutants remained unchanged, indicating good genetic stability. Mutant RM-9-7 produced significantly shorter plants than the checks. Mutants RM-1-2 and RM-1-4 exhibited high degree of stability regarding maturity, both mutants matured significantly earlier than all the

TABLE I. TYPES OF DESIRABLE PUTATIVE MUTANTS SELECTED IN M_2 GENERATION DURING 1989-92

Variety	Dose (Gy)	Total plants selected	Short stature	Early maturity	Heavy bearing	Long pods	More grains /pod	Bold seed	Stress tole-rance
Westar	800	42	6	2	16	36	22	28	12
	1 000	55	1	6	14	42	20	22	18
	1 200	30	2	8	8	30	6	20	8
Sub total		127	9	16	38	108	48	70	38
Tower	800	38	2	2	15	12	10	15	7
	1 000	56	3	4	20	21	17	19	10
	1 200	12	1	2	9	7	3	4	5
Sub total		106	6	8	44	40	30	38	22
Pak-Cheen	800	30	5	12	2	17	8	6	3
	1 000	18	4	2	2	12	4	4	2
	1 200	23	10	5	-	12	14	12	4
Sub total		71	19	19	4	41	26	22	9
Marnoo	800	30	-	3	4	20	9	18	5
	1 000	13	1	5	6	7	6	7	2
	1 200	4	-	1	1	3	2	3	4
Sub total		47	1	9	11	30	17	28	11
Salam	800	9	1	3	4	5	2	3	1
	1 000	15	1	4	9	7	4	5	3
	1 200	5	1	1	2	3	2	1	1
Sub total		29	3	8	15	15	8	9	5
Altex	800	5	-	1	2	2	2	2	1
	1 000	14	-	2	7	10	8	8	5
	1 200	5	1	-	2	2	1	1	1
Sub total		24	1	3	11	14	11	11	7
Sweden-3	800	7	1	4	2	4	6	2	2
	1 000	10	3	7	6	15	12	5	4
	1 200	4	1	3	2	2	2	1	1
Sub total		21	5	14	10	21	20	8	7
Grand total		425	44	77	133	269	160	186	99

TABLE II. PROMISING SELECTION OF RAPESEED MUTANTS AND STABLE M_3/M_4 PROGENIES, DURING 1989-92

S.No.	Variety	No. of M_2/M_3 selections	Stable M_3/M_4 progenies
1.	Westar	127	20
2.	Tower	106	17
3.	Pak-Cheen	71	46
4.	Marnoo	47	17
5.	Salam	29	4
6.	Altex	24	4
7.	Sweden-3	21	12

entries. Mutant RM-1-4 not only matured 3 weeks earlier than the parent and check, but also expressed comparable yield potential. Most of the mutants produced bold seeds, however, significantly ($P < 0.05$) higher seed weight was expressed by ten mutants. Mutant RM-16-1 followed by RM-10-1, RM-11-2 and RM-13-1 significantly outyielded the checks and the rest of the mutant lines. Eight other mutants also yielded higher than the checks.

TABLE III. MEAN VALUES OF DIFFERENT PLANT CHARACTERS OF M_6 MUTANTS IN MICRO PLOT TESTS, DURING 1991-92

Mutant/variety	Plant ht. (cm)	Days to maturity	1 000 seed wt. (g)	Yield (kg/ha)
Pak-Cheen (P)	190.3	187.0	3.6	1 452.1
PR-7 (L.C.)	202.4	186.0	3.6	1 409.5
RM-1-2	175.7	167.5	4.4	1 040.3
" 1-4	174.4	165.7	4.0	1 440.0
" 6-1	185.7	184.8	4.8	1 593.0
" 9-2	172.0	184.3	4.8	1 616.7
" 9-7	169.4	184.5	4.4	1 581.9
" 10-1	186.1	185.0	4.4	1 994.1
" 11-2	184.0	185.0	4.4	1 984.1
" 13-1	172.0	188.7	4.8	1 947.7
" 15-3	197.3	186.0	4.8	1 859.3
" 16-1	198.0	186.0	4.8	2 011.9
" 19	199.6	183.5	4.4	1 732.0
" 21	191.6	183.8	3.6	1 623.6
" 35	191.4	183.5	4.0	1 693.0
" 39	184.4	183.3	3.6	1 651.4
LSD P<0.05	22.5	5.7	0.7	446.3

P = Parent L.C = Local commercial variety

Results (Table IV) indicate that plant height and maturity period of most of the mutants of cultivars Tower and Westar remained unchanged. No marked deviation in the magnitude of results of these traits was noted when compared with the results of the previous year. Significantly higher seed weight than checks was noted only in case of five mutant lines. Mutant RM-156-2 followed by RM-159-2 and RM-161-2 significantly outyielded the checks. Grain yield, however, is dependant on various yield components such as number of branches and pods/plant, number of seeds/pod and seed weight. A good combination of these traits results in high yielding and stable lines as significant positive correlation is reported amongst these yield components [6, 8].

Data with regards to grain yield ha^{-1} of some promising mutants of cultivars, Pak-Cheen and Tower, tested for two consecutive years were further evaluated for ascertaining their stability over years. It is clear from the results (Table V) that all the entries except three produced higher grain yield in the first year compared to the second, however, mutant line RM-156-2 exhibited higher grain yield in both the years. The next highest mean yields were given by RM-11-2, RM-10-1, RM-16-1 and RM-13-1. Although all the mutant lines shown in Table V exhibited higher mean yields in two growing seasons over the check, yet most of the entries, including parent, were not stable in their performance over the years which might be due to drastic fluctuation in the weather during second year (1992). Three mutants viz. RM-16-1, RM-159-1 and RM-15-5, however, expressed fair degree of stability with regards to this trait.

TABLE IV. MEAN VALUES OF DIFFERENT PLANT CHARACTERS OF M_6 MUTANTS IN MICROPLOT TESTS, DURING 1991-92

Mutant/variety	Plant Ht. (cm)	Days to maturity	1 000 seed wt. (g)	Yield (kg/ha)
Tower (P)	187.4	186.5	3.6	1 477.8
PR-7 (L.C)	204.9	185.3	3.6	1 526.4
RM-152-2	192.5	184.3	4.4	1 929.2
" 155-1	181.2	184.3	4.0	1 820.1
" 156-2	188.6	183.8	4.4	2 022.0
" 158-1	198.1	186.0	3.6	1 838.9
" 158-2	188.9	185.8	4.0	1 790.3
" 159-1	173.8	184.3	4.0	1 651.4
" 159-2	201.5	185.3	3.2	1 957.0
" 161-2	184.4	184.5	3.6	1 957.0
" 167	187.1	182.3	4.0	1 519.5
" 169	178.4	183.0	4.4	1 575.0
" 182	173.6	182.5	4.4	1 575.0
" 184	171.9	184.3	4.0	1 533.3
" 319	171.7	181.3	3.6	1 470.8
" 325	181.5	182.8	4.0	1 547.2
LSD P<0.05	23.5	2.5	0.6	420.2

P = Parent L.C = Local commercial variety

TABLE V. MEAN GRAIN YIELD OF RAPESEED MUTANTS AND PARENTS TESTED FOR TWO CONSECUTIVE YEARS (1990 - 91) AND (1991 - 92)

Mutants/variety	Mean yield (kg/ha) (1990-91)*	(1991-92)**	Difference (kg/ha)	Average (kg/ha)
Pak-Cheen (P_1)	1 670.1	1 452.1	-218.0	1 561.7
Tower (P_2)	1 530.6	1 277.5	-253.1	1 404.5
RM-9-2	2 047.2	1 616.7	-430.5	1 831.9
" 9-5	1 907.7	1 640.3	-267.4	1 774.0
" 10-1	2 080.6	1 994.1	- 86.5	2 037.3
" 11-2	2 205.6	1 984.1	-221.5	2 094.8
" 13-1	2 061.1	1 947.7	-113.3	2 004.4
" 15-5	1 862.5	1 829.6	- 32.9	1 846.0
" 15-6	1 926.4	1 672.2	-254.2	1 799.3
" 16-1	2 032.0	2 011.9	- 20.1	2 021.9
" 152-1	1 958.3	1 727.8	-230.5	1 843.0
" 152-2	1 994.5	1 929.2	- 56.3	1 962.1
" 156-2	2 234.7	2 022.0	-212.7	2 128.3
" 158-1	1 677.8	1 838.9	+166.1	1 758.3
" 159-1	1 983.3	1 651.4	- 31.9	1 667.3
" 159-2	1 415.3	1 957.0	+541.7	1 686.1
" 161-1	1 683.3	1 827.0	+143.7	1 755.1

* : 289.3mm ** : 409.3mm [Rainfall (Oct. to Apr.)]

P_1, P_2 : Parents

3.3.3. Performance of mutants under drought conditions at D.I.Khan during 1991 - 92

Rapeseed is successfully grown under rainfed conditions through out the country and about 30 % of the planted rapeseed area is dependent on rainfall, resulting in poor yields. The development of high yielding and drought resistant/tolerant varieties of brassica oilseeds is very essential for enhancing their productivity and increasing domestic production, as no suitable varieties are available for cultivation in rainfed areas. Research activities were, therefore, initiated also in this direction, to develop high yielding and drought tolerant/resistant varieties of rapeseed. Mutants of cultivar Pak-Cheen and Tower showing good performance under moderate rainfall conditions, were evaluated for yield and other agronomic traits in two yield trials under arid condition (rainfall less than 250 mm), during 1991 - 92, the results of which are presented as below.

All mutant lines except two i.e. RM-9-7 and RM-9-2 of cultivar Pak-Cheen were similar in maturity as compared with the parent and local commercial cultivar (Table VI). Mutant RM-9-7 which matured significantly earlier than the checks, also produced significantly shorter plants. The plant height of rest of the mutants except RM-6-3 was non-significantly different from parent and check. Mutant RM-9-7 significantly outyielded all the entries. Besides it, seven other mutants also produced significantly ($P < 0.05$) higher grain yield than the checks and only five mutants were poor yielders. The results of this trial indicate that mutant RM-9-7 possess valuable agronomic characters like high yield, reduced plant height, early maturity, and holds promise for further extensive testing in arid zone or low rainfall areas.

TABLE VI. AGRONOMIC PERFORMANCE OF RAPESEED MUTANTS OF CV.PAK-CHEEN UNDER DROUGHT CONDITIONS AT D.I.KHAN, DURING 1991-92*

Mutants/variety	Days to 50% flowering	Days to maturity	Plant Ht. (cm)	Yield (kg/ha)
Pak-Cheen (P)	94	167.0	184.0	753.5
PR-7 (L.C)	94	166.5	186.5	579.9
RM-6-3	92	168.0	190.0	1 069.4
" 9-2	94	165.0	185.5	1 072.9
" 9-5	95	166.0	186.0	1 454.9
" 9-6	94	167.5	179.5	1 274.3
" 9-7	92	164.0	178.0	1 479.2
" 10-1	94	166.0	183.0	1 361.1
" 11-1	97	166.5	184.5	1 368.1
" 11-2	92	167.0	185.0	958.3
" 12-1	94	166.0	185.5	201.4
" 13-1	92	168.5	186.5	600.7
" 15-4	96	165.5	185.0	83.3
" 15-5	95	167.5	185.5	270.8
" 16-1	94	167.5	184.0	691.0
" 36	95	165.5	184.5	941.0
LSD P<0.05	3.4	1.5	4.6	191.9

* : Rainfall=138mm, P : Parent, L.C : Local commercial variety

The results of another trial comprising 14 mutants of cultivar Tower, parent and local check are shown in Table VII. Results regarding flowering and maturity were almost similar. Only one mutant (RM-152-1) matured significantly late ($P < 0.05$) than the rest of the mutants and parent but was at par with the commercial cultivar. No significant differences were observed in the mean values of mutants and checks regarding plant height except RM-161-2,

which produced significantly shorter plants. Eight mutants significantly outyielded the checks, and mutant RM-152-2 was on top of all. Only five mutants showed poor performance regarding this trait.

The results of these trials revealed that some rapeseed mutants performed very well under drought conditions. The yield potential of some of the mutants viz. RM-9-5, RM-9-7, RM-152-2 and RM-182 was very high under low rainfall. However, these mutants also need to be tested extensively on farmers fields in future, to ascertain their genetic stability and adaptability in the arid zone farming conditions.

TABLE VII. AGRONOMIC PERFORMANCE OF RAPESEED MUTANTS OF CV.TOWER UNDER DROUGHT CONDITIONS AT D.I.KHAN, DURING 1991-92 *

Mutants/variety	Days to 50% flowering	Days to maturity	Plant Ht. (cm)	Yield (kg/ha)
Tower (P)	94	164.0	185.0	711.8
PR-7 (L.C)	95	168.0	185.0	684.9
RM-152-1	93	168.0	186.5	906.3
" 152-2	92	166.0	185.0	1 673.6
" 156-2	95	166.0	187.0	416.7
" 158-1	94	164.0	185.0	444.4
" 159-1	94	165.0	187.5	1 239.7
" 161-2	93	164.0	182.0	909.7
" 167	95	166.0	184.0	583.3
" 178	92	165.0	182.5	812.5
" 179	93	163.0	184.0	927.1
" 182	94	164.0	185.0	1 607.6
" 184	95	163.0	186.0	583.3
" 185	93	163.0	185.5	1 048.6
" 187	91	166.0	185.5	1 279.2
" 190	96	165.0	183.0	236.1
LSD P<0.05	3.6	3.0	2.6	167.7

* : 138mm rain fall was recorded at the experimental site.
P : Parent, L.C : Local commercial variety

3.4. Oil quality assay of rapeseed mutants

Chemical analysis for total oil content and fatty acid composition of some promising mutants was done, courtesy oilseed analytical lab, National Agric. Res. Centre, Islamabad. The results are presented in Table VIII. The mutants RM-13-1 and RM-161-1 possessed higher oil content, thus out-classing the parent by a margin of 20 %. Besides, 26 other mutants also exhibited higher oil content than both the checks. The total oil content in rapeseed (*Brassica napus* L.) is controlled by multiple genes and gene action appears to be additive [11, 12]. From the results of chemical analysis of rapeseed mutants it appears that gamma rays irradiation has induced sufficient genetic variability and increased oil content of the seed might have resulted from partitioning of increase amount of photosynthates into the embryo i.e. into the oil and decreased amount into the hull or seed coats [12]. Fifteen mutants also contained less than 5 % erucic acid in oil and hence their oil is suitable for human consumption [13]. High proportion of this fatty acid in brassica oil is reported to cause lipidosis and heart lesions in animals [11] and it is feared that erucic acid may also cause similar problems in human beings. The oleic acid (C 18:1) content has been increased to a desirable level in four mutant lines, while in some it has been reduced markedly than the parent cultivars (P_2). Mutants RM-9-1 and 9-4 possessed high amount of erucic acid and their

oil may be most suitable for industrial uses. The results also revealed that some mutants of cv. Tower and Westar are characterized by possessing less than 5 % erucic acid, over 60 % oleic acid, about 26 % linoleic acid and around 9 % linolenic acid in their oil and as such are within the prescribed Pakistan standards of Canola quality rapeseed oil [13].

TABLE VIII. CHEMICAL COMPOSITION OF OIL OF SOME PROMISING RAPESEED MUTANTS, M_6 GENERATION DURING 1992

Mutants/ variety	Total oil(%)	Oleic (C 18:1)	Linoleic (C 18:2)	Linolenic (C 18:3)	Erucic (C 22:1)
Pak-Cheen (P_1)	41.9	16.3	16.4	10.7	40.7
Tower (P_2)	42.2	59.0	19.6	10.0	4.6
RM-9-1	46.5	18.2	15.2	10.2	40.2
" 9-4	45.0	20.2	14.0	9.7	40.8
" 12-1	45.6	16.3	15.8	12.9	39.5
" 13-1	50.0	16.2	16.4	13.0	35.4
" 15-1	46.8	30.0	19.1	11.3	21.2
" 152-1	44.5	59.5	17.2	9.3	4.2
" 153-1	46.9	64.1	17.2	8.9	3.1
" 154-1	46.5	63.0	19.2	9.6	3.7
" 157-1	47.7	27.3	19.5	9.2	22.6
" 157-2	42.5	48.5	17.8	8.2	5.1
" 158-1	48.8	30.5	16.8	11.0	25.5
" 158-3	46.7	62.7	15.3	9.2	4.0
" 159-1	48.9	53.1	18.4	10.8	4.9
" 159-2	48.2	60.5	17.0	9.0	3.7
" 159-3	47.2	59.6	17.0	9.9	4.1
" 160-1	45.3	30.4	13.8	9.8	21.4
" 160-2	46.1	48.8	16.3	10.0	9.0
" 161-1	49.9	59.5	17.1	9.3	4.3
" 161-2	46.5	58.5	18.9	10.8	4.3
" 162-1	46.9	35.2	19.0	11.7	20.9
" 163-1	47.1	58.4	19.1	10.1	4.8
" 164-1	47.1	48.7	18.1	11.0	7.1
" 19	49.6	21.6	14.3	9.1	38.3
" 172	41.1	49.6	24.7	12.0	4.0
" 181	44.4	58.8	18.7	9.6	4.2
" 182	42.0	39.8	22.3	12.7	10.8
" 195	45.0	56.4	19.3	9.7	4.4
" 312	46.8	43.6	19.8	10.1	7.6
" 313	45.4	49.8	26.3	9.0	7.6
" 319	44.2	54.6	20.8	9.5	4.8

P_1 and P_2 = Parents

3.5. Recombination breeding

Hybridization of rapeseed mutants with exotic Canola quality cultivars has been initiated during 1991. Certain mutants with improved attributes like high yield potential, high oil content etc. but suffering from some genetic defects such as lodging (due to tall and weak stem), late maturity, poor oil quality, etc. were cross bred in reciprocal combinations with promising Canola quality exotic cultivars (having short stature and early maturity but are poor yielders under local conditions), pursuing the classic intraspecific hybridization scheme. So far, the F_2 generation has been raised and 102 desirable recombinants, characterised by superior plant attributes, have been selected.

4. CONCLUSION

It can be concluded from the results of various experiments conducted since 1988 that gamma ray irradiation created very useful genetic variability in certain characters of economic importance in different rapeseed varieties. Oilseed rape cultivars showed lot of phenotypic and genotypic plasticity when subjected to physical and chemical mutagens and hence it supports the previous findings [14]. The improvement made in some polygenically inherited traits through irradiation such as total oil content of seed and grain yield, might be due to genetic changes induced in certain other related, simply inherited characters like plant architecture, photoperiodic response and seed coat, which could have dramatic positive effect on seed yield and oil content [12, 15, 16]. The findings of this research project are also in full agreement with the results reported by other researchers [17, 18, 19]. Some of the mutants with desired traits such as high yield potential, high oil content, drought tolerance, early maturity, better oil quality etc. are expected to lead directly or through recombination breeding to the development of improved rapeseed varieties for irrigated and rainfed areas of Pakistan. However, some of the characters like oil content, and grain yield are influenced markedly by different environments [11, 12], genotype-environment interaction studies over locations and years would therefore, be needed to confirm the present findings and to ascertain adaptability and genetic stability of promising mutant lines, which would help confirm their value for Pakistan.

Acknowledgement

The authors gratefully acknowledge the financial help extended by the International Atomic Energy Agency (IAEA) for the partial funding of this research under Research Contract No. PAK 5438/RB.

The authors are grateful to Director, NIFA for providing all necessary facilities for conducting this research. The authors are also thankful to Mr. Saeed Gul and Mr. Bismillah Jan for the statistical analysis and typing of the manuscript on computer.

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THE IMPROVEMENT OF OguCMS AND HETEROSIS BREEDING SYSTEM IN *Brassica napus* L. BY MUTATION BREEDING

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Abstract

To develop a successful hybrid seed system in rape, insufficient development of chlorophyll in the OguCMS line had been a problem. Difficulty in obtaining a good fertility restorer was another very important problem. To improve these shortcoming, mutation breeding was applied to the OguCMS materials. BC_7, BC_8 seeds of OguCMS back-crossed with three maintainer lines, Kuo077, 2432 and H23 were treated by ^{60}Co-gamma rays in 1990 and 1991. BC_8 ovaries of OguCMS maintained with the line 2432 were irradiated with ^{60}Co-gamma rays in 1990. The results indicated that LD_{50} of OguCMS maintained with H23 and Kuo077 was about 800 Gy, while LD_{50} of OguCMS maintained with 2432 was about 1000 Gy. There were three tentative mutant plants without chlorophyll deficiency found in M_1 of OguCMS maintained with Kuo077 and treated by ^{60}Co-gamma ray 1200 Gy. One of them was male fertile and the other two were male sterile when they reached to flowering stage. There were two male fertile plants with chlorophyll deficiency in M_1 of OguCMS maintained with H23, irradiated by ^{60}Co-gamma ray 1200 Gy. Their progenies were investigated. The results were discussed in this report. There were 19 male sterile plants without chlorophyll deficiency in M_2 of OguCMS maintained with Kuo077 and irradiated by ^{60}Co-gamma ray 1000 Gy. The morphology of their flowers was very similar to that of OguCMS flowers.

1. INTRODUCTION

Ogura [1] found a system of cytoplasmic male sterility in *Raphanus sativus*. By successive back-crossing Bannerot *et al.* [2] introduced the nucleus of *Brassica napus* into the S- *Raphanus* cytoplasm and bred a cytoplasmic male sterile line of *B. napus* L. with *Raphanus sativus* cytoplasm (OguCMS). OguCMS has been one of the main research materials of rapeseed heterosis utilization. The male sterility of OguCMS is very stable in many climatic conditions. However, there are no fertility restorer genes for OguCMS reported in rapeseed group (*B. napus*, *B. campestris*, *B. juncea*) and the current OguCMS line suffers by insufficient chlorophyll development at seedling stage under low temperature. F. W. Heyn [3] reported that there were restorer genes for OguCMS in *Raphanus sativus*. But it is very difficult to transfer restorer genes from *Raphanus sativus* to rapeseed. In addition, the transferring of restorer genes from *Raphanus sativus* to rapeseed is often accompanied with many undesirable characters. Effort was made to improve OguCMS line by protoplast fusion and the problem of chlorophyll deficiency was partly solved [4, 5 and 6]. However, the effort to introduce fertility restorer gene for OguCMS through crossing a *Raphanobrassica* which maintain restorer genes with OguCMS line has not succeeded yet.

Sasaki *et al* [7] treated wheat male sterile lines in *T. timopheevii* cytoplasm (TCMS) by X-ray and obtained fertile restorer mutation. Peiru He *et al* [8] irradiated TCMS with ^{60}Co-gamma rays and were also successful in obtaining fertile plants perhaps by restoring mutation and developed fertility restorer lines with excellent agronomic characteristics with high restorability in wheat.

In the present research, seeds of OguCMS were treated by ^{60}Co-gamma ray in 1990 and 1991, intending to induce fertility restorer gene. Part of the research has been reported elsewhere [9].

2. MATERIALS AND METHODS

2.1 Seed irradiation

BC_7, BC_8 seeds of OguCMS backcrossed with three maintainer lines Kuo077, 2432 and H23 were treated by ^{60}Co-gamma rays with four doses: 0 (control), 800 Gy, 1000 Gy and 1200 Gy in 1990 and 1991. In each year, 100 seeds in each dose/cross class were irradiated amounting to 1 200 seeds in total. Among the maintainer lines used, H23 and 2432 had high erucic acid and high glucosinolate and Kuo077 was a low erucic acid variety.

To prevent outcrossing, only a few buds of OguCMS maintained with 2432 were retained in each inflorescence, which were carefully bagged. Pollinations were carried out with maintenance line 2432 in the following morning. The flowers were then tagged with label of cross information.

2.2 In vitro culture

Forty BC_8 ovaries of OguCMS pollinated with the maintainer line 2432 were collected 8 days after pollinations and irradiated by ^{60}Co-gamma rays with four doses: O (control), 3, 6 and 9 Gy. Ten ovaries were irradiated in each dose. The surface was sterilized with chlorine water for 7-10 minutes, rinsed twice in sterile distilled water and cultured on B5 medium.

3. RESULTS AND DISCUSSION

3.1. Influence of different dosages to three materials

Numbers of survived seedlings and plants of OguCMS seeds irradiated by ^{60}Co-gamma rays with four dose classes were shown in Table I. It may be seen that LD_{50} of OguCMS seeds pollinated with the maintainer line 2432 was about 1000 Gy. LD_{50}s of OguCMS seeds pollinated with other maintainer lines H23 and Kuo077 were both about 800 Gy.

Table I. AVERAGE NUMBER OF SURVIVING SEEDLINGS AND PLANTS OF OguCMS IRRADIATED BY ^{60}Co GAMMA RAYS IN 1990 AND 1991 EXPERIMENTS

Dosage	Pollen source					
	Kuo077		H23		2432	
(Gy)	S	P	S	P	S	P
0	82	79	83	79	80	77
800	60	48	62	50	63	58
1000	32	10	44	12	52	45
1200	14	4	20	8	25	10

S:Seedlings (three leaves stage), P:Plants

3.2. Effect of mutagen treatment on chlorophyll content

Five plants survived in the M_1 generation of OguCMS pollinated with maintainer line Kuo077 and irradiated by ^{60}Co-gamma ray 1200 Gy in 1990 - 1991. Three of them showed no chlorophyll deficiency at the seedling stage. One plant was male fertile and the other two were male sterile when they reached to the flowering stage.

The male fertile plant was self-pollinated. This male fertile plant was used to pollinate the other two male sterile sister plants. It was also used to pollinate other OguCMS plants maintained by pollination with H23, 2432 or Kuo077 maintainer lines.

The two male sterile plants were also test-crossed with other varieties Shan 2B, Shan 2C and restorer line 1. The phenotypes expressed in the progenies of their sib-crossing and test crossing are shown in Table II.

Table II. PHENOTYPIC EXPRESSION IN PROGENY OF THE TWO MALE STERILE PLANTS WITHOUT CHLOROPHYLL DEFICIENCY

	Male fertile[1]			Shan 2B[2]			Shan 2C[3]			Restorer line 1[3]		
	T	F	S	T	F	S	T	F	S	T	F	S
Sterile-1	17	4	13	21	18	3	34	30	4	32	27	5
Sterile-2	11	2	9	26	25	1	28	25	3	29	26	3

T: Total number of plants, F: No. fertile, S: No. sterile,
1: Without chlorophyll deficiency but sister plant to the male sterile female plants.
2: Maintainer line of Shan 2ACMS,
3: Restorer lines of Shan 2ACMS.

Segregation ratio between male sterile plants and male fertile plants in progenies of sib-crossing were about 3 sterile :1 fertile. Most of the progenies of the male sterile plants test-crossed with Shan 2B, Shan 2C and restorer line 1 were male fertile. But there were a few male sterile plants in every progeny population (Table II). There were 31 M_2 plants in self-pollinated progenies of the male fertile plant. Among them 23 were male fertile plants and 8 were male sterile. However, there was no male fertile plant in the test-crossed progenies of OguCMS pollinated with this male fertile plant as pollen donor.

These results might exclude the idea that genes of the cytoplasm were modified in the OguCMS line maintained with Kuo077 and irradiated by ^{60}Co-gamma ray 1200 Gy, even if three plants were found normal in terms of the chlorophyll. As for chlorophyll deficiency of OguCMS, many scientists thought that it was the result of S-cytoplasm of *Raphanus sativus*. However, the male sterile plants were different from Shan 2ACMS. Progenies of test-cross with Shan 2B showed restoring of male fertility in most of the cases. At the same time, progenies of OguCMS test-crossed with the male fertile sister plant without chlorophyll deficiency did not show restoration of male fertility when used as pollen donor. It might be possible that modification of the cytoplasmic gene(s) was not sufficient to restore male sterility of the OguCMS.

3.3 Analysis of two fertile mutants and their progeny

There were seven plants with chlorophyll deficiency at the seedling stage in OguCMS line maintained by pollination from H23 and irradiated by ^{60}Co-gamma ray 1200 Gy in 1990 - 1991. Two of them were male fertile at the flowering stage. In addition to the self-pollination, they were also used to pollinate OguCMS plants which have been maintained by H23, 2432 or Kuo077 lines.

Before harvesting, one male fertile plant died of disease. The other could yield some seeds. There were seven seeds by self-pollination and 42 seeds by open pollination. The morphology of pods of the fertile plant was very similar to those of radish. Unfortunately, the self-pollinated seeds did not grow well in 1991 - 1992 season. There were 106 plants derived

from the cross with these two male fertile plants as pollen donors. All the plants were male sterile.

From these results, it could be deduced that the male fertilities in the two plants which retained their chlorophyll deficiency, might be due to only minor changes of nuclear genes and such minor change of nucleus genes were in sufficient to restore the male fertility of OguCMS line.

3.4. Result of ovary culture on B5 medium

The ovaries irradiated with four dose classes and cultured on B5 medium produced variable results as shown in Table III. There were 15, 21 and 13 seeds in the dose class of 3, 6 and 9 Gy, respectively. Seven, 13 and eight plants for each dose class survived in the 1991 - 1992 season. All plants were with chlorophyll deficiency and male sterile, typical character of OguCMS.

Table III. FORMATION OF SEED IN CULTURED OVARIES

Radiation dose (Gy)	Initial size (cm)		Size at maturity (cm)		Number of ovaries having seed	(%)
	Length	Width	Length	Width		
0	1.80	0.158	3.10	0.29	2	20
3	1.81	0.161	3.22	0.30	3	30
6	1.80	0.160	3.43	0.31	4	40
9	1.79	0.158	3.34	0.30	3	30

3.5. Characters observed in M_2

Apart from tentative variant plants found in M_1 generation of OguCMS irradiated by ^{60}Co gamma rays in 1990 - 1991, all other plants were of chlorophyll deficiency type and male sterile. These plants were individually planted and the seeds were harvested. Three thousand M_2 seeds of every experimental classes were sown. The range of morphological variation in M_2 generation was very large. There were 19 plants with normal leaf colour but male sterile in the M_2 population of OguCMS experimental class of "maintained with Kuo077 and irradiated by ^{60}Co gamma rays 1000 Gy". The morphology of their flower was the same as that of OguCMS. All other M_2 plants had chlorophyll deficiency and were male sterile, typical for OguCMS. No variation was found in M_1 in 1991 - 1992 season. In the control treatment classes in both 1990 - 1991 and 1991 - 1992 seasons, no such variation could be observed.

Larik [10] pointed out that radiation could promote the production of chlorophyll in wheat by induced mutation. Peiru He *et al.* [8] treated TCMS of wheat with ^{60}Co gamma rays and obtained fertility restorer mutation. They reported that those restorer mutation seemed to be a kind of dominant mutation involving one or more loci of nuclear genes. Chengzhang Zhao *et al.* [11] treated rice callus of Basmati 370 with ^{137}Cs gamma rays and obtained cytoplasmic male sterile line, although the research was a preliminary work.

We tried to modify the interaction of nucleus and cytoplasm of OguCMS line, to develop new CMS system and fertility restorer mutant gene necessary for the system. In our work on the irradiation of OguCMS lines, male sterility and chlorophyll deficiency reacted independently. There were both male fertile and male sterile plants in the normal green seedling variants. There were male fertile plants of chlorophyll deficiency type in the present experiments. The results were not sufficient to prove the induction of a good fertility restorer. Further studies of nucleus/cytoplasm interaction are necessary.

Acknowledgements

Authors wanted to express their thanks to IAEA for the support of this research. Thanks are also to Mr. B. Sigurbjörnsson, Ms. T. Benson-Wiltschegg and Mr. M. Maluszynski for the support of the programme, and Mr. E. Amano for discussion of many problems related to the programme.

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PROGRESS IN THE IMPROVEMENT OF INDIAN MUSTARD (*Brassica juncea* (Linn.) Czern & Coss.) WITH YELLOW SEED COAT COLOUR

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Abstract

The main objectives in the mustard breeding programme at BARC are to evolve high yielding strains with yellow seed coat colour. Two of the Trombay mustard (TM) selections, TM-2 and TM-4 have been released for commercial cultivation in Assam State. In 1991 - 92 crop season, these two cultures were also evaluated in 60 and 200 minikit trials respectively at the farmers fields in Rajasthan State. The mean yield of TM-2 and TM-4 was however lower than the improved cultivar grown as the check. In the trials conducted at 3 - 4 locations by the Punjabrao Agricultural University for the past four years, TM-9 gave the highest mean yield of 840 kg/ha. TM-9 and TM-21 were the top yielders in the non-traditional mustard growing areas in Maharashtra State. TM-18 is presently the earliest maturing mustard available in the country. Such early cultures have the potential to replace toria (*Brassica campestris* var. toria) in the multiple cropping system. Simple and reliable methods for screening mustard germplasm for aphid tolerance at three different stages of plant growth have been developed. Using these methodologies, selections showing tolerance against crucifer aphid (*Lipaphis erysimi* Kalt.) have been identified. Powdery mildew (*Erysiphe cruciferarum* Opiz ex Junell) resistant selections have been isolated from a cross between TM-18-8 and *Brassica carinata*.

1. INTRODUCTION

The main emphasis of the mustard breeding programme at this Centre, as stated previously [1], is on developing new strains with yellow seed coat colour. This paper reports the progress made since then.

2. EXPERIMENTS AND RESULTS

2.1. TM-2 and TM-4

2.1.1. Release for commercial cultivation in Assam State

Cultivars TM-2 (Black seed coat) and TM-4 (Yellow seed coat) were earlier approved for release in the central and lower Brahmaputra valley of Assam State by the Assam Agricultural University, Jorhat [2]. Both these varieties mature in 105 - 115 days and the seed yield ranges between 1200 - 1600 kg/ha [3]. These varieties are to replace *B. campestris* var. *toria* grown in the area. This state has also recommended to replace areas under irrigated wheat with TM-2 and TM-4. In 1990-91 crop season, twenty demonstration trials were conducted with TM-2 and TM-4 at farmers fields in Assam State [4].

2.1.2. Farmers field trials in Rajasthan

Both these varieties have also shown higher yield in the trials conducted by the Department of Agriculture, in the State of Rajasthan. Joint Director (Oilseeds), Jaipur conducted 200 and 60 minikit trials each with TM-4 and TM-2 respectively during 1991 - 92 crop season in farmers fields [5]. The mean yield of TM-2 and TM-4 was however lower than the improved cultivar grown as check in these trials.

2.2. TM-9 and TM-21 in non-traditional mustard growing areas

In view of the edible oil shortage in the country, efforts are being made at the national level to introduce mustard cultivation in some of the traditional wheat growing areas. TM-cultures were tested in the non-traditional mustard growing areas in the States of Maharashtra and Andhra Pradesh. Among the ten promising cultures tested by the Punjabrao Agricultural University (PAU), for four years at four locations in Maharashtra, TM-9 and TM-21 were the top yielders both for seed and oil yield (Table I).

Table I. SEED YIELD (q/ha) AND OIL YIELD (kg/ha) OF PROMISING TM-CULTURES IN MAHARASHTRA

	Mean seed yield*				Overall mean of 4 years (q/ha)	Increase over Varuna (%)	Oil content overall mean of 4years (%)	Oil yield kg/ha mean of 4 years (kg/ha)	Increase over varuna (%)
	1988-89 (4 locations) (q/ha)	1989-90 (3 locations) (q/ha)	1990-91 (4 locations) (q/ha)	1991-92 (5 locations) (q/ha)					
Varuna	11.4	7.1	5.9	5.2	7.4		28.4	213	
Pusa bold	12.3	8.7	6.0	5.4	8.1		28.7	207	
Pusa barani	12.8	7.6	5.9	5.4	7.9		25.6	203	
Seetha	10.0	9.6	5.6	5.0	7.6		30.8	232	
TM-9	12.5	9.2	6.2	5.7	8.4	13.5	29.8	250	17.4
TM-17	10.0	8.6	5.1	4.6	7.1		31.5	223	
TM-21	12.5	8.6	5.6	5.1	8.0		29.8	236	

* : q = 100 kg

Source: Agricultural Research Station (PAU), Washim, Maharashtra.

2.3. Early culture, TM-18

Early maturing cultures have the advantage of escaping from pests, diseases and moisture stress. TM-18 is presently the earliest maturing mustard available requiring 24 - 28 days for flowering and 65 - 70 days for harvest at Trombay compared to 35 - 40 and 95 - 100 days respectively for the national check cultivar Varuna. Three years data on seed yield, oil percent and oil yield/ha are given in Table II. Though the yield *per se* of TM-18 was lower than Varuna, the productivity of TM-18 on per day basis was higher. Such early cultures have the potential to replace early maturing *B. campestris* var. toria cultures in the multiple cropping systems [6].

Table II. YIELD AND YIELD COMPONENTS OF TM-18 COMPARED TO CV. VARUNA AT TROMBAY

Line	Days for first flowering	Crop duration	Seed yield q/ha*			Productivity on per day basis (kg/ha mean)	Oil** content (%)	Harvest index (%)
			87-88	88-89	89-90			
Varuna	40	100	9.3	13.1	9.1	10.5	33.8	15.6
TM-18	25	70	9.5	8.1	7.8	12.1	35.2	21.0
L.S.D. (at 5% level)	-	-	1.78	2.50	3.89	-	-	-

* : q = 100 kg

** : Mean of three years

When grown in the northern latitudes the crop duration increased but TM-18 maintained its earliness over Varuna. During 1990 - 91 crop season it was evaluated in the All India Coordinated Research Project on Oilseeds (AICORPO), with 49 entries at 24 locations all over the country. TM-18 was earliest maturing culture among the selections tested (Table III).

Table III. CROP DURATION (DAYS) OF TM-18 COMPARED TO OTHER EARLY CULTURES AND NATIONAL CHECKS IN AICORPO TRIAL, 1990 - 91*

Cultivar	(**Zone-2) 9 locations	(Zone-3) 6 locations	(Zone-4) 6 locations	(Zone-5) 3 locations
Varuna	138	122	113	140
Kranti	139	123	114	140
DLM-23	140	122	111	141
JGM-9062	139	123	114	142
NDR-190	142	122	114	142
RJ-10	134	127	110	135
RW-8718	132	113	107	136
RW-873	134	114	108	141
RW-8716	131	120	107	140
TM-18	133	114	104	134

* : Source : AICORPO Proceedings 1990-91.
Total of 49 entries in AICORPO trial.
** : Zones : Zone 1-North hill region
Zone 2-North plain region
Zone 3-North central region
Zone 4-North West region
Zone 5-North East region

2.3.1. Growth analysis of TM-18 compared to Varuna

Growth of TM-18 was compared with cultivar Varuna at Trombay (Fig. 1, A-F). The parameters studied from 20 - 95 days after sowing (DAS) at 15 - 20 day intervals were plant height, fresh leaf weight, fresh and dry plant weight, leaf area and moisture percent. At all stages of growth, fresh and dry phytomass of cultivar Varuna was higher in comparison to TM-18. Fresh leaf weight of TM-18 was highest at 35 DAS while for Varuna it reached the maximum at 50 DAS. The fresh leaf weight of Varuna was about three times more than TM-18 (Fig. 1-B). The leaf area of TM-18 was also maximum at 35 DAS. At all stages of growth, moisture content of TM-18 plants was markedly lower as compared to Varuna (Fig. 1-C). TM-18 plants showed less succulence compared to Varuna.

2.3.2. Days required for first flowering in TM-18 and Varuna

In order to study the number of days required for first flowering, seeds were sown on the first day of every month for 12 months and the duration for first flowering was recorded based on five plants (Fig. 1-D). In general, in July-October sowing both Varuna and TM-18 required longer duration for flowering and this was minimum during December-January sowing. The range of variation for flowering in TM-18 was 22 - 34 days compared to 32 - 52 days required for Varuna. At all sowing dates, TM-18 was early to flower (Fig. 1-D).

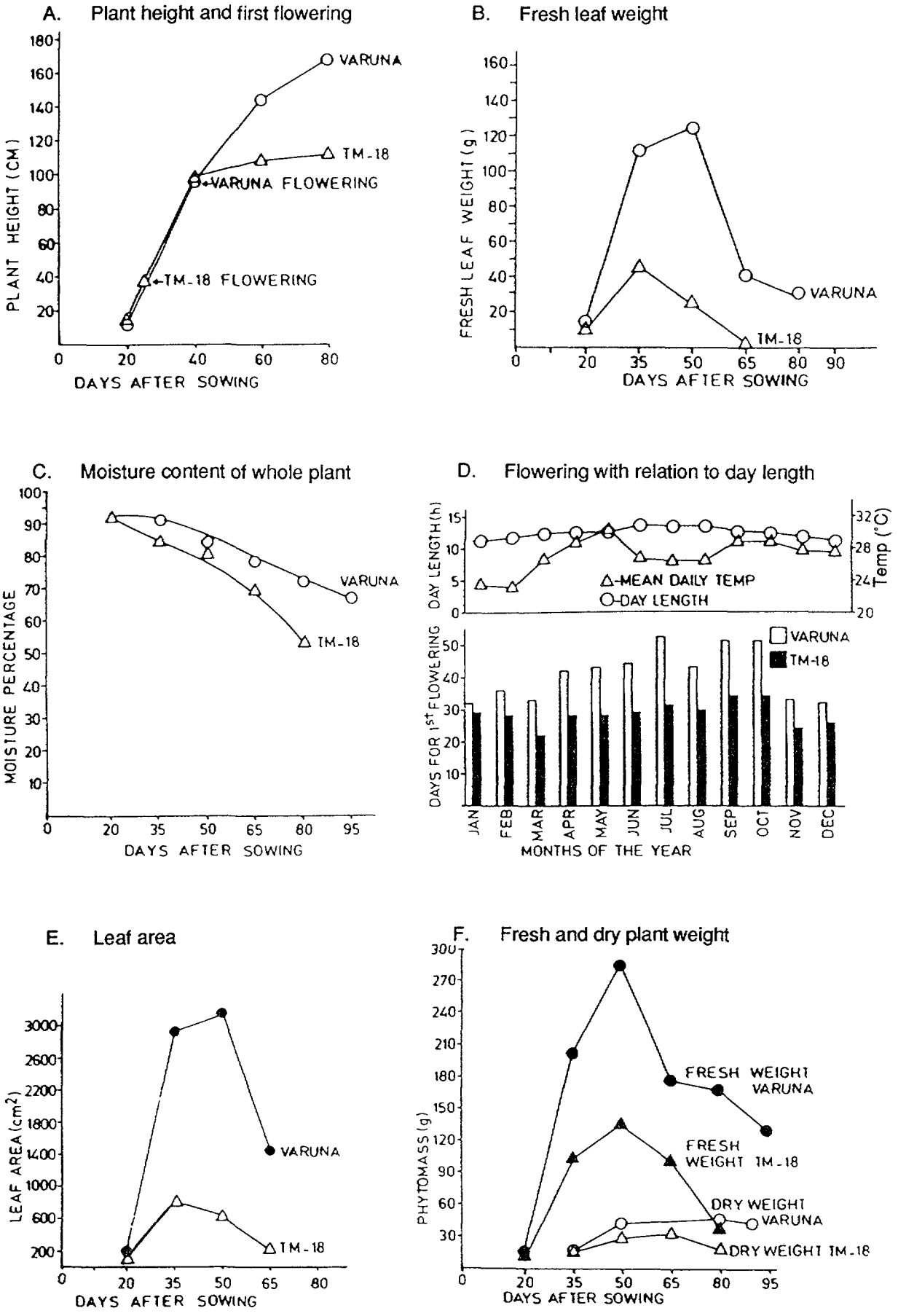

Figure 1. A-F Growth analysis of TM-18 compared to Varuna.

2.3.3. Further selections in TM-18 for seed yield improvement

Twenty single plant selections based on seed yield were made in 1988 - 89. These selections were evaluated at Trombay during 1989 - 90 and 1990 - 91 (Table IV). One of the selections, TM-18-8 was entered in the AICORPO trials at 28 locations in the country in the 1991 - 92 crop season (Table V). TM-18-8 was identified as least susceptible to downy mildew, white rust and *Alternaria* blight during 1991 - 92 crop season (Table VI) and four centres reported TM-18-8 free of white rust or with least attack [7]. This probably is due to early maturity of TM-18-8.

Table IV. SEED YIELD OF SINGLE PLANT SELECTIONS FROM TM-18 (g/m^2)

Culture	1989-90	1990-91	Oil content (%) 1989-90
Varuna	9.1	11.0	33.8
TM 18	7.8	7.8	34.5*
TM-18-1	8.4	5.4	36.0*
" 2	8.6	8.4	37.1*
" 3	9.6	6.6	37.8*
" 4	9.3	9.2	-
" 5	9.9	7.2	34.5*
" 8	9.2	8.8	36.9*
" 16	9.1	6.0	36.2*
" 18	10.8	7.8	37.6*
L.S.D. (at 5% level)	1.82	2.45	0.90

Table V. SEED YIELD (kg/ha) AND CROP DURATION (DAYS) OF TM-18-8 IN AICORPO TRIALS

	IRRIGATED								RAINFED			
	Zone-2 (8 locations)		Zone-3 (8 locations)		Zone-4 (5 locations)		Zone-5 (3 locations)		Zone-2 (2 locations)		Zone-5 (2 locations)	
Cultivar	Y	D	Y	D	Y	D	Y	D	Y	D	Y	D
Varuna	1275	151	1184	125	1464	107	1039	111	1629	133	743	116
Kranti	1611	148	1536	125	1789	105	1011	112	1568	129	701	113
DIR-489	1529	147	1355	124	2097	105	1586	110	1208	129	793	118
TM-18-8	1000	144	988	119	1159	101	916	101	1627	127	512	106

Source: AICORPO Report, 1991-92
Combined statistical analysis are not reported.
Y = Seed yield in kg/ha
D = Crop duration in days

4. APHID TOLERANCE

Institutional, national and international collaboration is called for developing an environmentally safe aphid control strategy and antibiosis test [8]. All the screening efforts till date have failed to identify source of aphid resistance.

Table VI. REACTION TO DOWNY MILDEW, WHITE RUST AND *Alternaria* BLIGHT UNDER NATURAL AND ARTIFICIAL INOCULATION IN AICORPO DISEASE NURSERY

Cultivar	Kangra	Sriganga-nagar+	Nav-gaon	Morena	Faiza-bad+	Kangra*	Morena*	Kanpur*	Faiza-bad
Downy mildew									
Varuna	0.2	31.2	2.0	2.1	5.0	0.0	0.0	0.0	4.0
Kranti	0.2	91.3	2.0	2.7	5.0	3.0	3.0	0.0	2.0
TM-18-8	0.2	0.0	1.0	0.5	0.0	2.0	2.0	2.0	0.0

Cultivar	Kangra	Sriganga-nagar	Nov-gaon*	Bathinda	Hisar	Kanpur*	New Delhi	Faiza-bad	Pant Nagar*	Berham-pur
White rust										
Varuna	3.0	3.0	3	2	4	0	2.5	2	5.0	2
Kranti	3.0	3.0	3	2	3	0	2.5	4	5.0	4
TM-18-8	3.0	1.0	2	1	0	0	0.5	0	4.0	0

Cultivar	Kangra	Sriganga-nagar*	Nav-gaon*	Bathinda	Hisar*	Morena*	Kanpur*	New Delhi	Faiza-bad	Pant Nagar*	Ber-hampur	Dholi
Alternaria blight												
Varuna	2	3	3	4	4	3	4	2.5	5	5	4	3
Kranti	3	3	3	4	3	3	4	2.5	4	4	3	2
TM-18-8	3	2	4	4	3	3	3	1.0	-	5	3	3

Source = AICORPO Report 1991-92, Total 76 entries were included

\+ Stag head %

* Under artificial inoculation

Score of disease : 0-5 scale : 0 : No disease, 1 : 1 to 10%, 2 : 11 to 25%, 3 : 26 to 50%, 4 : 51 to 75%, 5 : 76 to 100%.

Simple and reliable methods have been developed to screen mustard germplasm for aphid tolerance at three different stages of plant growth [9, 10]. Seedlings grown in wooden trays were screened in the glass house by keeping aphid infested trays on either side. The progeny of plants surviving this test were evaluated in field trials without insecticidal sprays. The detached leaves of the tolerant lines were rooted in petri dishes and aphid nymphs were introduced. Their life span, reproductive ability and survival were scored. This method was useful as a simple and reliable antibiosis test. Using these methodologies, tolerant selections against crucifer aphid have been identified (Table VII, VIII).

5. POWDERY MILDEW RESISTANCE

B. carinata was found to remain free from powdery mildew (*Erysiphe cruciferarum*) in field experiments. It was further tested under controlled environment by inoculating the detached leaves. The two *B. carinata* accessions tested remained free from the powdery mildew disease. Hence TM-18-8 culture of *B. juncea* (2n = 36, AABB genome) was crossed with the two resistant accession of *B. carinata* (2n = 34, BBCC genome). The F_1 plants remained free from the disease in the field [12]. The F_2 generation showed segregation for resistance and susceptibility (Table IX). Resistance was dominant and showed monogenic segregation. Inoculation of the detached leaves from the F_2 plants under controlled environment confirmed the field observations. Back crossing of the resistant plants to *B. juncea* has been initiated to transfer the powdery mildew resistance gene(s) from *B. carinata.*

Table VII. SURVIVAL OF SEVEN AND 14 DAY OLD SEEDLINGS OF TOLERANT SELECTIONS

Culture	Number survived upto 50 days*	
	Seven day old seedlings	14 day old seedlings
Eruca sativa	10.0	10.0
Pusa bold	0.0	0.0
RH-30	0.0	0.0
TM-4	0.0	0.0
Varuna	0.0	0.0
S-34	3.7	5.3
S-37	3.0	4.8
S-41	5.3	6.3
S-42	5.0	6.3
S-44	8.7	9.0
S-45	5.7	6.0
S-46	6.0	6.0
S-48	3.7	1.7
L.S.D. (at 5% level)	2.89	1.69

* Mean of ten seedlings in four replications.

Table VIII. FIELD EVALUATION OF MUSTARD SELECTIONS FOR APHID PREFERENCE*

Selections	Leaves without aphid on 40th day	Healthy plants at harvest	Seed yield** (q/ha)	Oil content (%)
Eruca sativa	2.0	34.3	1.3	30.2
Varuna	1.4	5.5	0.5	31.5
S 34-1	9.0*	6.0	1.4*	33.3
S 34-2	3.8*	5.3	2.0*	34.8
S 34-4	1.6	4.7	1.1*	30.8
S 35-1	3.8*	7.3	1.5*	35.8
S 35-2	3.4	9.3	2.1*	33.6
S 35-3	4.4*	6.6	1.4*	35.7
S 41-5	5.6*	7.3	1.0	-
S 42-3	4.4*	13.6	1.3*	32.2
S 44-2	1.2	16.6*	1.3*	31.8
S 44-3	2.6*	10.0	1.3*	31.2
L.S.D. (at 5% level)	2.50	8.33	0.57	

* : Mean of 40 plants in three replications.
** : q = 100 kg/ha

6. SELECTIONS FOR INCREASED OIL PERCENTAGE

Selections made for increased oil percent (OP) were used in crossing programme and the F_2 to F_6 progenies were screened for oil percent. In the progeny of a cross between a high oil line and Pusa Bold with large seed size, a selection with consistently higher OP and 1 000 seed weight has been obtained (Table X).

Table IX. F_2 SEGREGATION FOR POWDERY MILDEW RESISTANCE IN TM-18-8 x *BRASSICA CARINATA*

F_2 families		Resistant	Susceptible	Chi. square (3:1)
TM-18-8xBC 11-9	1	19	6	0.013
	2	11	3	0.095
	3	6	2	0.000
	4	11	3	0.095
	5	6	2	0.000
	6	8	6	2.381
	7	14	2	1.333
	8	12	2	0.857
	9	7	5	1.777
	10	32	15	1.198
	11	7	4	0.757
TM-18-8xBC 10-9	1	23	3	2.513
	2	8	4	0.444
	3	18	2	2.40
	4	7	1	0.667
	5	17	2	2.123

Table X. OIL CONTENT OF PARENTS AND SELECTIONS FROM F_2 TO F_8 GENERATIONS

Parents	1984-85	1985-86	1986-87	1987-88	1988-89	1990-91	1000 Seed wt.(g)	Days to maturity
Varuna	33.9	33.5	33.2	32.4	32.5	34.6	3.56	95
Pusa bold	33.5	36.9	33.3	32.7	32.5	35.5	4.7	93
TM-4	38.6	36.8	34.5	35.8	33.5	38.1	2.99	90
Primary derivatives (TM-4 x Lethbridge)								
S 2-1	42.8**	38.3**	36.6**	34.6**	34.9*	39.8	1.98	83
S 22-1A	40.6**	32.3	35.4**	31.6	33.0	38.2	2.50	83
S 25-1	40.0*	36.0*	36.8	33.6*	34.3*	38.2	2.50	83
Secondary derivatives (Pusa bold x S 2-1)								
	F_2	F_3	F_4	F_5	F_6	F_8		
S 3 pl-3	43.3**	38.4	34.3	36.3	35.4	39.3	2.30	90
S 5 pl-13	41.9*	38.2*	35.5**	34.6*	34.1*	38.5	4.30	86
L.S.D. (at 5% level)	1.83	1.44	0.70	1.06	0.57	2.51	0.26	

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DEVELOPMENT OF HIGH YIELDING WINTER RAPESEED WITH IMPROVED OIL QUALITY

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Abstract

In order to secure and increase the consumption of rapeseed oil in the food industry, lower linolenic acid contents are required. Varieties reaching this quality standard must also produce high oil yields for such production to become economic. In a pertinent research programme, first mutation experiments were started in 1968 resulting in an improved fatty acid (f.a.) composition of the seed oil in spring rapeseed genotypes. In the following years selected alleles controlling the expression of low linolenic acid content were transferred from spring type mutants to winter rapeseed via backcrossing. In a further mutation experiment with winter forms, genotypes were selected exhibiting less than 3 % linolenic acid content in seed triacylglycerols during tests run over four years. Thus, very low linolenic acid levels were realized also in winter rapeseed varieties. By simultaneous selection for low glucosinolate (gsl) content together with the desired oil quality selection gain for seed yield was reduced considerably. Further backcrossing and continuous selection resulted in high yielding 0_l0 lines. On the other hand S_1, recurrent selection proved to be another effective method to accumulate favorable alleles for seed yield in a 0_l+ base population without changing its favorably low linolenic acid content. Therefore, the transferred mutant alleles do not seem to restrict yield performance.

1. INTRODUCTION

Rapeseed oil is commonly used in human consumptions for cooking, baking and frying purposes as well as in products such as margarine or salad oils. To secure or even increase oilseed production, special oil qualities are required.

In industrialized countries oil stability is a major concern of the food distributors. It is well known that high contents of unsaturated fatty acids are directly related to low stability and quality of the oils. Fresh Canola oil is light colored and odorless, but during storage rancidity tends to develop, causing an unpleasant odor and flavour. Reasons for this deterioration are auto-, photo- and thermal oxidations of the unsaturated fatty acids. During normal cooking processes, such as deep-fat frying, an oil may be heated up to temperatures of 180 °C - 190 °C. Under such conditions and in the presence of air oxygen and moisture, oxidation, hydrolysis, and thermal degradation of the oil occur relatively rapidly. The by-products of these processes change not only the odor and flavour of the oil, but they also contribute to an undesirable flavour of the fried foods. Moreover, the formation of lipid peroxides is suspected to have carcinogenic effects *in vivo* [1]. Thus quality demands in rapeseed oils are definitely directed to low levels of linolenic acid.

Canola oil obtained from low erucic acid, low glucosinolate (gsl) rapeseed (i.e. so-called 00-varieties) exhibits a fatty acid composition of about 60 % oleic acid (C18:1), 20 % linoleic acid (C18:2) and about 10 % linolenic acid (C18:3). The aims of breeding are to reduce the C18:3 content to less than 3 % of the total fatty acid composition and increase the proportion of C18:2 or C18:1. At this level of the trienoic acid the stability and quality of the oil is remarkably improved [2].

Since in the available germplasm of rapeseed, variation in polyunsaturated fatty acids is limited, a mutation experiment was initiated at the Plant Breeding Institute of the University of Göttingen in 1968 using the Canadian spring type variety 'Oro' [3]. Selection and further mutagenesis [4] resulted in several mutants deviating in fatty acid composition. These genotypes were the first verified sources of alleles for an improved polyenoic fatty acid (f.a.) composition. Moreover, the low C18:3 levels of the first commercially available Canadian variety 'Stellar' [5] was derived from these mutants.

For rapeseed production under European conditions high performing winter type varieties exhibiting Canola quality are required. Our work, therefore, was directed to further improve the f.a. composition of winter types and to establish high yields of these winter Canola varieties.

2. MATERIAL AND METHODS

The first mutants were developed as described above from the Canadian low erucic acid spring variety 'Oro' applying to 12 h soaked seeds 80 Gy X-rays and 0.2 % or 0.5 % EMS (ethyl methanesulfonate), respectively. The M_2 was subjected to gaschromatographical (GLC) half-seed analysis [6]. Table I represents the C18 f.a. composition and agronomic traits of the starting material, i.e. the two initial mutants (M6 and M11), and their recombination product (M48) in comparison to 'Oro'. The alleles desired for an improved oil quality as present in M48 were then transferred by recurrent crossing into winter breeding lines using different pollinators. For each cross, plants exhibiting low linolenic acid contents (0_1+ quality*) were reselected from a broad F_2 population by GLC. After two backcrosses, lines were selected in this way exhibiting sufficient winter hardiness. These BC_2-lines were used to improve oil quality on the one hand and seed yield on the other hand.

Table I. STARTING SPRING RAPESEED MATERIAL FOR THE IMPROVEMENT OF OIL QUALITY IN WINTER TYPES

Mutant/Variety	C18:1	C18:2	C18:3	Height	Fertility
M 6	64.2	25.3	4.0	80 cm	low
M11	50.5	35.4	7.7	110 cm	medium
M48	63.8	27.7	3.0	75 cm	medium
Oro	59.5	21.8	8.4	140 cm	high

2.1. Improvement of oil quality

A new mutation experiment was initiated in 1986 with winter rapeseed lines which were already improved in their f.a. composition. Each 2,500 seeds were soaked for 10 h and treated with 0.2 % and 0.5 % EMS, respectively. After prescreening 12 000 M_2 plants by means of a TBA (thiobarbituric acid), test samples with low TBA values were checked by GLC. Selected mutants were evaluated for performance under field conditions for several generations.

2.2. Yield improvement

After the transfer of alleles controlling an improved f.a. composition into winter material, two different strategies were applied to increase the seed yield.

2.2.1. Backcross selection

Four backcrosses were executed so far. For the first time the parents of the third backcross exhibited Canola quality; therefore, this segregating BC_3F_2 population was also selected for low glucosinolate (gsl) contents: Open pollinated plants were first screened by Palladium test [7] for low glucosinolate contents. The selected plants then were analysed for their f.a. composition resulting in 0_10 quality. In three successive years from 1989/90 to 1991/92 0_1+- and 0_10-lines were field tested together with 00-cultivars for seed yield in a randomized complete block design with three replications at Göttingen.

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* The designation refers in its first position to the given oil quality, i.e. in this case "zero erucic" (= 0) and "low linolenic" (= 0_1), and in its second position to the meal quality, here "high glucosinolate" (= +)

2.2.2. Recurrent S_1 selection

In addition to backcrossing, seed yield was selected for using a recurrent selection system based on the performance of progenies of selfed plants (S_1). In May 1987, 10 BC_2 lines each represented by two plants were selected and crossed in a diallel manner. Hybrid plants were self pollinated in the next spring and 98 S_1 populations were tested together with two Canola varieties for seed yield. The experimental design was a 10 x 10 lattice with two replications in Göttingen and Thüle. For starting the second cycle, the 10 highest yielding S_1-populations were selected. Remnant seed was used for establishing a new cycle of recombination. Each selected S_1 was represented by 10 single plants which were crossed in a diallelic manner resulting in 450 combinations. In September 1990, from each cross three F_1 plants were planted in the field. During the following spring season in each S_1 combination 4 plants were selfed to produce the progenies for yield testing. The field performance test of the second cycle S_1-populations was conducted in Göttingen as previously and in Hohenlieth in three 6x6 lattice designs with two replications.

Oil (%), protein (%) and gsl content (µmol/g seed) were determined by NIR and f.a. composition (% of total f.a. in the seedoil) by GLC.

3. RESULTS

3.1. Quality improvement

M_3-families were planted as individual plants (3-9) in the field and in the greenhouse. After GLC analysis the M_4 generation was cultivated in field observation plots (3.5 m^2) during the 1989/90 season in Göttingen. Table II shows the mean and range of agronomic traits and of oil quality of the M_4-lines in comparison with the winter rapeseed variety "Diadem". Generally, the mutants were well developed before winter. In the spring, they remained in the rosette stage for a longer period and correspondingly flowered later. At flowering, the mutants were weaker in vigour than the standard variety, which is reflected in a mean height of 120 cm and a stronger sensitivity to lodging. Moreover, the mutants were more heavily infested by *Cylindrosporium*, a fungal pathogen, which became widespread in Germany only since 1988. On the other hand, the yield component "pod length" of the mutants was scored with 6 as compared to 7.2 for "Diadem". However, in the polyenoic f.a.

Table II. MEAN AND RANGE OF DIFFERENT AGRONOMIC AND SEED OIL TRAITS OF 106 M_4 LINES IN COMPARISON WITH THE WINTER CANOLA VARIETY "DIADEM"

Trait	Mean	Min.	Max.	CV	Diadem
Growth before winter[1]	6.7	4.0	8.0	13.8	7.0
Growth after winter[1]	3.9	2.0	7.0	30.7	6.0
Growth at anthesis[1]	5.5	3.0	8.0	27.2	7.0
Lodging resistance[2]	5.2	1.0	8.0	34.6	7.0
Pod length[3]	6.0	2.0	8.0	23.3	7.2
Cylindrosporium resistance[1]	3.7	1.0	7.0	35.1	7.7
Plant height (cm)	120	80	160	13.4	150
C16:0 (%)	5.0	3.4	8.0	16.4	4.3
C18:1 (%)	54.8	42.8	68.5	9.8	58.8
C18:2 (%)	32.4	13.8	42.0	15.4	22.1
C18:3 (%)	3.6	1.8	9.5	47.2	10.4

1 = Visual scores: 1 = very weak, 5 = medium, 9 = very strong
2 = " " : 1 = lodging flat on the ground, 9 = upright
3 = " " : 1 = very short, 5 = medium, 9 = very long

Table III. C18 FATTY ACID COMPOSITION OF FOUR WINTER RAPESEED MUTANTS WITH A LOW LINOLENIC ACID CONTENT TESTED OVER FOUR YEARS IN GÖTTINGEN

Genotype	Year	C18:1	C18:2	C18:3
M 19	1989	63.4	26.7	1.8
	1990	59.5	30.0	2.0
	1991	65.2	23.9	1.7
	1992	55.5	32.8	2.3
M 22	1989	65.8	23.5	1.8
	1990	53.8	35.0	2.2
	1991	55.5	32.0	2.1
	1992	60.5	29.5	1.9
M 87	1989	62.4	27.4	2.0
	1990	58.0	31.5	2.2
	1991	62.4	27.3	1.8
	1992	54.5	32.9	1.8
M 89	1989	63.5	26.0	2.4
	1990	62.1	27.1	2.2
	1991	62.0	26.3	1.8
	1992	60.9	28.6	1.9

composition, the mutants were strongly improved. On the average, they exhibited 32.4 % C18:2 and 3.6 % C18:3. In particular, the mutants covered a broad range of variation in linolenic acid contents. By further selection, lines exhibiting less than 3% C18:3 were isolated.

Four genotypes with very low linolenic acid contents are presented in Table III regarding their C18 fatty acid pattern over the last four years. All mutants exhibited no more than about 2 % linolenic acid throughout the years under conditions in Göttingen; this means that the low linolenic acid content of these mutants is surprisingly stable. For the genotype M19, C18:3 varied between 1.7 % in 1991 and 2.3 % in 1992. Compared with this, the linoleic acid content of this mutant ranged from 23.9 % in 1991 to 32.8 % in 1992. This confirms earlier observations of stronger environmental sensitivity of the oleic acid desaturation in comparison with the linoleic acid desaturation. The same holds true for the genotypes M22, M87 and M89 exhibiting stably low linolenic acid contents and varying shares of linoleic acid on the total fatty acid composition. In order to utilize such mutants in a breeding programme they have to be backcrossed to the available best Canola varieties. In a first approach we have started to transfer in this way alleles for low linolenic acid content.

3.2. Yield improvement

3.2.1. Backcross selection

In order to determine, in the backcrossing programme, the effect of simultaneous selection for f.a. composition and gsl content compared to selection for oil quality only, 0_l0 lines together with 0_l+ lines and two high yielding (00) Canola varieties were tested in three successive years in Göttingen. Table IV demonstrates that the yield of 0_l+ material compared to the cultivars was increased within three years by nearly 26 %, and one line was found which outyielded the cultivars by 10 % in 1992. On the average, the yield of 0_l0 lines was also constantly improved from 67.2 % of the standard varieties in 1990 to 79.4 % in 1992. An unexpectedly great progress was made in 1992. Whereas in 1990 and 1991 the best performing lines still yielded about 10 % less than the cultivars, the best line in 1992 had a yield advantage of about 14 % compared to the cultivars.

Regarding selection of the main quality traits i.e. gsl and linolenic acid content, the 0_10 lines were strongly improved over the three years (Table V). The mean gsl content declined by nearly 42 %. Since the glucosinolate content is strongly influenced by the environment further evaluations are necessary for confirmation. In 1992 the share of C18:3 reached 3 % only. The 0_1+ lines exhibited nearly the same level of polyenoic fatty acid, except in 1991 where it raised to 4.9 %. However, compared to 00-cultivars the oil quality in both groups of material was considerably improved. Moreover, the oil and protein content in both quality types did not differ from 00-cultivars.

Table IV. RELATIVE YIELD (00-CULTIVARS = 100) IN 3 SUCCESSIVE YEARS OF 0_1+- AND 0_10-LINES IN COMPARISON TO 00-CULTIVARS

	Year	0_10	0_1+	00-cultivars (absolut)
Mean	1990	67.2	84.3	54.0
n		30	4	2
Min.		46.6	77.8	53.6
Max.		89.6	92.5	54.4
Mean	1991	75.2	88.4	50.3
n		19	4	2
Min.		57.7	84.0	49.0
Max.		91.4	91.3	51.6
Mean	1992	79.4	110.2	48.5
n		22	1	2
Min.		50.7	110.2	43.1
Max.		114.4	110.2	53.8

Table V. MEAN QUALITY CHARACTERISTICS (GSL, C18:3) IN 3 SUCCESSIVE YEARS OF 0_1+-, 0_10-LINES AND 00-CULTIVARS

Material	Year	gsl (µmol/g seed)	C18:3 (%)
0_10	1990	30.5	3.6
0_1+		107.4	3.6
00-cultivars		20.3	11.8
0_10	1991	29.7	3.3
0_1+		55.1	4.9
00-cultivars		25.5	-
0_10	1992	17.7	3.0
0_1+		81.4	3.2
00-cultivars		16.8	10.4

3.2.2. Recurrent S_1 selection

The final step of the first cycle, i.e. the selection based on the performance of S_1 lines at two locations was completed in 1989. Unexpectedly, several S_1 lines reached and even exceeded the yield level of the standards already after this first cycle. Table VI shows the 10 selected S_1 lines. All lines showed only moderate lodging and disease resistance. The

Table VI. RELATIVE SEED YIELD (00 = 100 %), SCORES OF LODGING RESISTANCE (LODG) AND DISEASE RESISTANCE (RES: 1=LOW, 9=HIGH) AND F.A. COMPOSITION (%) OF THE SELECTED S_1 LINES COMPARED TO THE MEAN OF THE STANDARD VARIETIES "CERES" AND "LIRABON"

S_1 line	Yield (rel.)	Lodg (score)	Res (score)	C18:1 (%)	C18:2 (%)	C18:3 (%)
16	**111.0**	4.0	**4.3**	57.6	27.9	**2.8**
45	103.2	3.2	5.3	61.1	25.6	3.6
59	101.5	4.3	5.3	61.6	28.2	3.7
62	100.5	5.7	6.2	62.3	29.2	3.0
34	100.0	4.5	5.3	59.2	**25.0**	**5.1**
90	100.0	**3.7**	4.8	61.1	26.9	2.9
84	99.0	5.8	**6.0**	58.4	27.5	2.8
17	98.0	5.2	5.5	57.8	26.7	4.1
53	97.8	**6.7**	5.7	56.8	**29.4**	4.1
58	**95.6**	5.3	5.8	63.5	27.5	3.7
00-standard	4.08 t/ha	7.3	7.7	54.3	24.5	11.8

polyenoic f.a. composition of the S_1's varied between 25.0 % to 29.4 % for C18:2 and for C18:3 between 2.8 % and 5.1 %. Line 16 outyielded the varieties by 11 % and contained no more than 2.8 % linolenic acid. The average seed yield of the selected lines was 4.09 t/ha, whereas the population mean reached 3.47 t/ha. This results in a selection differential (D) of 0.62 t/ha (4.09 t/ha - 3.47 t/ha).

Since after the second cycle the new population is not synthesized yet, the S_1's of both cycles are compared in Figure 1 on the basis of relative performance to the standard varieties. The S_1's of the second cycle yielded an average 8.6 % less than the standard varieties. The seed yield ranged between 73 % and 109 % indicating still a broad variability in alleles controlling yield. Moreover, 9 S_1's exhibited at least 3 % higher yields than the standard varieties compared to only 2 S_1's in the first cycle. Comparing the mean values of both cycles

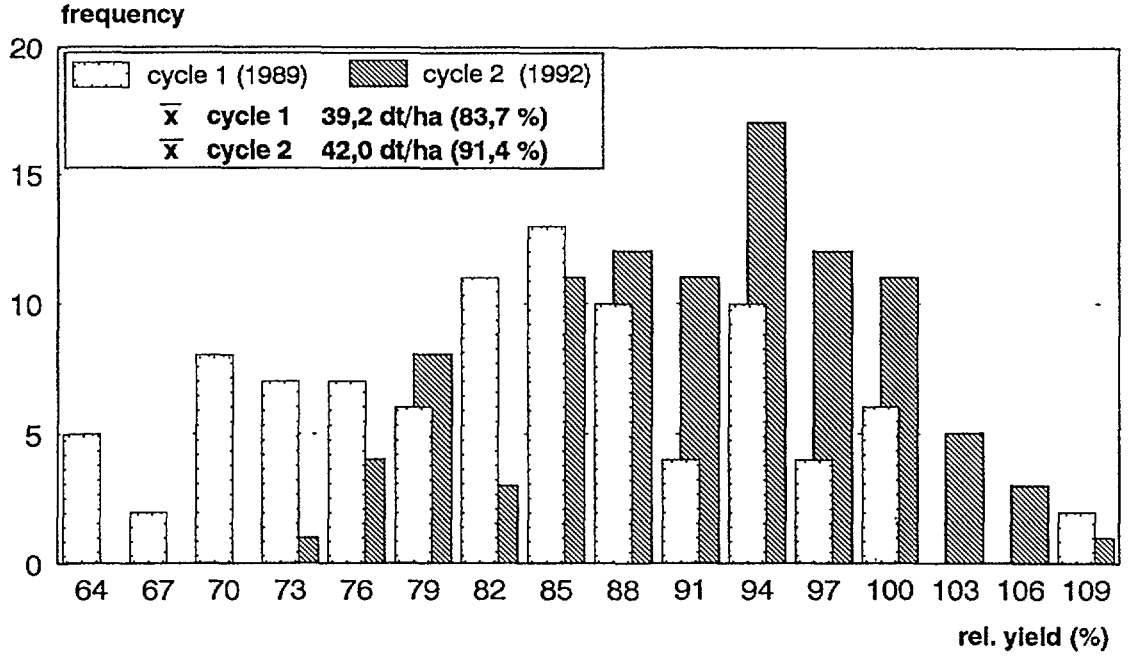

Fig.1. Relative yield of 95 S1's in cycle 1 and 2 compared to varieies (=100%)

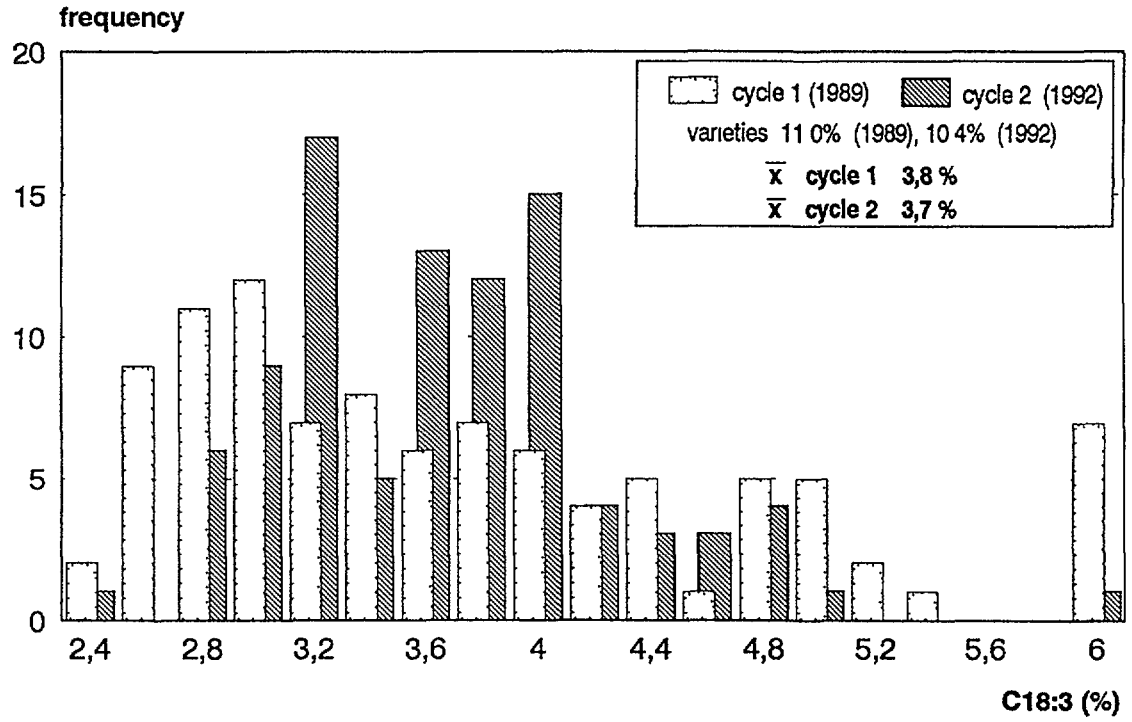

Fig.2. Linolenic acid content of 95 S1's in cycle 1 and 2 (=100%)

the realized selection gain was 6.4 % in relation to the standard varieties over three years or calculated on a per year base of 2.1 % per year. However, this calculation may be misleading since both populations were tested in different environments. Therefore, representative populations of both and further cycles have to be tested under the same conditions to obtain the real selection gain.

Comparing the linolenic acid content of the two cycles the average values were about the same (Fig. 2). Therefore, alleles controlling low C18:3 content seem to be fixed in the population. Small deviations in the trienoic f.a. content may result from genetic background effects.

4. DISCUSSION

After chemical mutagenic treatment of winter rapeseed, lines were selected containing less than 3 % linolenic acid expressed over four successive seasons. This confirms that EMS is a powerful tool to improve the oil quality of rapeseed [8, 9]. However, until now only spring type lines are reported to exhibit very low C18:3 contents [10]. Therefore, the expression of an altered f.a. composition in the seedoil seems not to depend on spring or winter habit. In general, the altered lipid composition of some of the mutants might reflect secondary effects of alleles at loci affecting other cellular functions, such as deficiencies in hormone biosynthesis or transport [11], while others may be directly involved in lipid biosynthesis. Only the latter mutants can be used to identify and clone genes participating in the triacylgycerol biosynthesis by molecular techniques. Moreover, our rapeseed mutants may be useful to study gene expression in an amphidiploid organism as compared with a diploid one like *Arabidopsis*, where similar mutants have been selected [12].

Lines with an improved fatty acid composition and fairly high seed yields appeared after two backcrosses already (see also Kräling 1989 [13]). An additional selection for low glucosinolate content reduced yield again as demonstrated with 0_I0 lines tested in 1990 and 1991. In comparing winter rapeseed lines selected exclusively for seed yield and lines selected simultaneously for low gsl and erucic acid content, Sauermann *et al.* [14] demonstrated the lower yielding capacity at the quality selection path. The limited number of selected 0_I0 plants in the segregating generation reduced selection gains for seed yield.

However, using developed 0_l0 lines in further crosses with Canola varieties, the genetic progress for seed yield increased resulting in one 0_l0 line which outyielded the standard varieties by about 14 % in 1992. Therefore, a reduced linolenic acid content in the seedoil does not necessarily cause physiological disturbances as assumed by Thies [15]. However, after the first backcross to winter rapeseed, lines were selected exhibiting low linolenic acid contents and chlorosis in young leaves under cool temperatures [16]. In *Arabidopsis* Hugly and Somerville [17] described a similar phenomenon. They found that chloroplast desaturation was reduced contributing to lower fitness under 5 °C cultivation. However, our selection of high yielding lines exhibiting low linolenic acid contents in seed triacylglycerols demonstrates that it is possible to eliminate undesirable side mutations. Similar to mutants in flax [18] and sunflower [19] we assume our mutants to result from a change in the desaturation system of the endoplasmic reticulum which is suggested to be active only during embryo development. Nevertheless, it is still open how much plastidical desaturation contributes to storage lipids in rapeseed.

Recurrent selection methods are cyclical, i.e. conducted in a repetitive manner to gradually increase the frequency of favorable alleles of quantitatively inherited traits in plant populations. Our starting point was a BC_2-population with a broad genetic base deriving from high yielding backcross cultivars. From this population S_1 progenies were developed. In the second phase, these progenies were tested with two replications at two locations. Decisions were based on the results of the performance trials to determine which genotypes were to recombine for starting the second cycle. The S_1 populations of the second cycle outyielded those of the first cycle by 0.73 t/ha or 6.4 % in relation to the tested varieties. In maize intrapopulation recurrent selection via S_1 selection resulted in a grain yield improvement of 5.9 % per cycle [20]. On a yearly basis this gain is reduced to 3.0 % whereas in our rapeseed populations a per year gain of 2.1 % was attained. Therefore, S_1 recurrent selection seems to be a useful method to improve the grain yield of rapeseed populations. However, since only low gsl varieties are commercially accepted, 0_l0 populations have to be built up which then may again be improved by S_1 recurrent selection. In general, population improvement strategies are of essential importance not only for populations characterized by a fixed quality trait but also for hybrid breeding systems which meanwhile are under worldwide development.

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BREEDING PROGRAMME IN SESAME (*Sesamum indicum* L.) TO DEVELOP WILT DISEASE TOLERANT LINES UNDER EPIDEMIC FIELD CONDITIONS

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Abstract

The objectives of the sesame mutation breeding programme were to improve agronomic characters, seed yield components, quality traits and tolerance to sesame wilt pathogens. To achieve these goals, air dried seeds of the local cultivars Giza 24 and Giza 25 were irradiated with gamma ray doses of 100, 200, 300 and 400 Gy at the Egyptian Atomic Energy Authority. Fifteen promising mutant lines were selected from M_3 bulk populations of Giza 24. Selection was practiced for white seeds, increased length of fruiting zone, high number of capsules per leaf axil, 1 000 seed weight, seed yield/plant, and seed oil + protein content (%). In Egypt, local cultivars were highly susceptible to wilt pathogens. To develop sesame varieties that would withstand wilt disease, more than 90 exotic lines, induced mutant lines and local cultivars were screened at seedling stage under artificial inoculation with *Sclerotium bataticola* spores. The best tolerant exotic lines were crossed with local varieties as well as mutant lines with good seed yield potential. Meanwhile, progeny families of mutant 48 and local variety Giza 32 were screened under highly infected field condition for wilt pathogens at EL-Saff location, Giza Governorate where selection was practiced for three successive seasons. In all characters, marked differences among sesame genotypes were observed under infected field conditions in both two seasons. Lines which proved to be highly tolerant to wilt pathogens in the field over two seasons were selected and included in yield trials.

1. INTRODUCTION

Disease resistant varieties are key factors in increasing yield and keeping crop production safe from epiphytotic attack. The development of resistant varieties as a mean of controlling disease infestation has become a common object in plant breeding programmes.

As noted by Simons [1] breeding for generalized resistance is more difficult than breeding for specific resistance. First, there is the general problem of selecting for a polygenic trait which is a slower and less precise procedure than selecting for single gene traits. Johanson [2] suggested one specific method for breeding for general resistance, a selection method among the progeny of crosses between susceptible genotypes. It would in fact yield genotypes which might have epistatically determined gene-for-gene resistance and it might be difficult to distinguish between tolerance and general resistance.

In Egypt, local cultivars are highly susceptible to wilt pathogens particularly under excess use of irrigation water. Wilt pathogens caused more than 40 % of seed production losses yearly, therefore it was very important to develop sesame varieties tolerant to wilt disease. The objective of this study was to screen and evaluate the promising tolerant sesame crosses under natural epiphytotic field conditions of wilt disease pathogens.

2. MATERIALS AND METHODS

To develop tolerant sesame varieties against wilt disease pathogens, more than 90 exotic lines were screened under artificial infection with wilt disease pathogens, especially

Sclerotium bataticola. The best tolerant lines were selected and crossed to some of the induced mutant lines and local varieties. Some progenies derived from induced mutant 48 and local variety Giza 32 were selected under highly infested field conditions with wilt pathogens at EL-Saff, Giza governorate, Egypt.

In 1990 season, seeds of 14 F_2 hybrids as well as progeny families of mutant 48 and local variety Giza 32 were sown in the infected field in the new reclaimed area at EL-Saff, Giza using a randomized complete block design with three replications. Each plot contained four rows, 3.5 m long and 60 cm apart. The plants were thinned out to two plants per hill with spacing 20 cm apart within rows. Normal field practices for this crop were applied.

Four pathogens were isolated from the soil of the epidemic field at EL-Saff, Giza. According to their spread and seriousness, these pathogens were as following : *Sclerotium bataticola, Rhizoctonia solani, Fusarium* spp and *Phytophthora parasitica.*

The precedures used to test the reaction of different germplasm of sesame to infection with the aforementioned pathogens are described below.

2.1. Pot experiments

Inoculation preparation and soil infestation : Barley medium consisting of barley, sand and water was prepared in glass bottles 500 - 1,000 ml capacity. Bottles containing medium were sterilized in the autoclave according to our laboratory standard. When cooled they were inoculated with the test fungi in separate treatment under aseptic conditions and were incubated at 25 to 30 °C for 2-3 weeks. Inocula of the fungi were mixed with semi-sterilized soil with 5 % formalin in clay pots at the rate of 5 - 20 % of soil weight*, before or after seed germination. In all treatments, five replicates were usually used for each entry. In case of inoculation of pathogens into soil before sowing, percentages of pre- and post-emergence damping off were scored after 15 and 30 days, respectively. When the pathogens were inoculated after germination, only post-emergence damping off was scored.

* : Experiment on inoculum potential was carried out before inoculation test.

Interaction between the tested fungi was studied also in different treatments i.e. each two fungi or three fungi together. This experiment was carried out to investigate possible existence of antagonistic or synergistic effects between fungi in the soil around the roots of the different sesame lines.

The same method mentioned before could be used to inoculate plants, via their roots, at various growth stages. The purpose of this experiment was to evaluate the reaction of different germplasm at their older growth stages (adult plant stage).

In all experiments, the survived plants were continuously observed until harvest for disease development. At harvest, roots of the surviving plants were examined for infection of the pathogen according to the following scale:

0 = No root infection, no wilt
1 = Few secondary roots infected + no wilt
2 = Few secondary roots infected + the main root + no wilt
3 = Root infection + discoloration of the lower part of the stem and no wilt
4 = Root infection + discoloration of most of the stem + wilt (seedling infection + adult infection).

2.2. Field experiment

The same methods were used, except that the plants were grown in plots under field conditions and for two successive seasons. Data obtained from these experiments were compared and used for final selection of the best tolerant germplasm of sesame. The best

tolerant plants from each line in F_2 were selected under the epidemic conditions and bulked seeds were used to raise F_3 generation in the same field. In 1991 season, the best tolerant crosses (seven crosses out of 14 crosses) of F_3 generation, best tolerant progenies of mutant 48 and local cultivar Giza 32, as well as recurrent irradiation of the induced mutant 48 with gamma ray dose 200 Gy (Mut.48 R) were sown in a randomized complete block design with three replications under the epidemic field conditions at EL-Saff, Giza. Each plot contained four rows, 3.5 m long and 60 cm apart. The plants were thinned out to two plants per hill with spacing 20 cm apart within rows. Normal field practices for this crop were applied. Screening for disease tolerant plants continued in the F_4 and subsequent generations.

3. RESULTS AND DISCUSSION

Results in Table I and II showed significant differences among sesame genotypes for all the examined characters in both two seasons in 1990 and 1991, except for seed yield/plant in 1991 season. Concerning length of the fruiting zone in 1990 season, means ranged from 64.3 cm for H.4 to 94.0 cm for H.20, with highest values for H.20, H.7, H.18 and Mut.48. However, in 1991 season, means ranged from 43.0 cm for Giza 32 to 113.7 cm for H.17, with highest values for H.17, H.7, H.12, H.18 and Mut.48. Over two seasons, H.17, H.7, H.12, H.18 and Mut.48 showed higher values in length of fruiting zone than that of other genotypes. Similar results were obtained by Ibrahim [3]. He noticed that fruiting zone length in all non-branching mutants exihibited an obvious increase over check varieties in both seasons.

Regarding number of capsules/plant in 1990 season, means ranged from 51.0 for H.15 to 104.0 for H.12, with highest values for H.12, H.21, H.18, H.5 and Mut.48. However, in 1991 season, means ranged from 82.3 for H.3 to 148.3 for H.17, with highest values for H.17, H.21, H.18 and Mut.48. Over two seasons, H.17, Mut.48, H.21, H.18 and H.12 exhibited higher number of capsules/plant than that of other genotypes. These results were in harmony with those obtained by Pathirana [4].

TABLE I. SCREENING AND EVALUATION OF SOME SESAME CROSSSES IN AN INFECTED FIELD WITH WILT PATHOGENS IN 1990 SEASON

Lines	First branch height (cm)	Plant height (cm)	Length of fruiting zone (cm)	No.of branch per plant	No.of capsule per plant	Seed yield/ plant (g)	Oil content (%)	Tole-rance degree (%)
H.3	74.0	164.0	90.0	2.8	89.0	11.0	60.0	76.0
H.4	60.7	125.0	64.3	2.2	60.7	8.9	58.8	31.0
H.5	67.0	136.3	69.3	3.2	95.3	11.4	58.4	45.0
H.7	61.0	153.0	92.0	2.3	85.0	13.2	58.1	82.0
H.12	58.0	148.0	90.0	3.2	104.0	13.4	59.6	61.0
H.13	71.4	155.7	84.3	2.5	61.3	9.7	60.3	25.0
H.14	66.0	144.0	78.0	1.9	53.0	10.4	58.3	32.0
H.15	60.7	128.0	67.3	2.5	51.0	7.7	58.2	20.0
H.16	71.3	146.7	75.3	2.3	70.8	10.1	59.5	39.0
H.17	63.0	140.3	77.3	2.5	80.0	14.2	58.6	65.0
H.18	65.0	157.0	92.0	2.6	97.0	13.0	59.2	71.0
H.19	70.3	146.0	75.7	1.9	69.8	9.8	58.9	22.0
H.20	70.0	164.0	94.0	2.3	64.7	12.5	59.7	50.0
H.21	81.0	157.0	76.0	3.0	100.0	13.3	57.8	61.3
Giza 32	66.7	150.0	83.3	2.8	55.0	9.3	59.2	60.0
Mut.48	66.0	156.3	90.3	2.3	94.0	12.8	60.1	74.0
	*	**	**	*	**	**	**	**
LSD 5%	9.65	12.63	13.30	0.16	14.40	1.35	0.98	5.53
1%	10.80	14.13	14.90	0.18	16.10	1.51	1.10	6.19

Table II. SCREENING AND EVALUATION OF SOME SESAME CROSSES IN AN INFECTED FIELD WITH WILT DISEASE PATHOGENS IN 1991 SEASON

Lines	First capsule height (cm)	Plant height (cm)	Length of fruiting zone (cm)	No.of branch per plant	No.of capsule per plant	Seed yield/ plant (g)	Oil content (%)	Tole-rance degree (%)
H.3	60.0	151.0	91.0	1.3	82.3	13.2	59.5	78.0
H.7	58.3	161.3	103.0	2.3	95.0	15.1	57.4	84.3
H.12	52.7	153.3	100.7	2.7	112.3	14.8	59.2	67.0
H.17	59.3	173.0	113.7	3.3	148.3	16.8	58.0	68.7
H.18	88.3	186.3	98.0	4.3	125.0	16.0	58.7	68.0
H.20	69.7	158.0	88.3	2.3	110.0	14.0	57.3	52.0
H.21	78.0	164.7	86.7	3.0	125.0	16.3	56.9	61.3
Giza 32	85.0	128.0	43.0	2.7	87.3	10.6	59.9	79.0
Mut.48	56.7	153.3	96.7	2.7	139.3	15.7	60.7	81.7
Mut.48R	46.7	121.3	71.3	3.7	108.7	13.5	60.8	83.0
	**	**	**	*	**	NS	**	*
LSD 5%	5.60	12.10	12.99	1.10	14.20	4.02	0.77	3.30
1%	7.70	16.60	17.80	1.50	19.40	5.51	1.05	4.50

With respect to seed yield per plant in 1990 season, means ranged from 7.4 g for H.15 to 14.2 g for H.17, with highest values for H.17, H.12, H.21, H.18 and Mut.48. However, in 1991 season, means ranged from 10.6 g for Giza 32 to 16.8 g for H.17, with highest values for H.17, H.21, H.18 and Mut.48. Over two seasons, H.17, H.21, H.18, Mut.48, H.7 and H.12 showed higher seed yield/plant than that of other genotypes. Similar results were obtained by Pathirana [4]. He observed that at least 13 selections which out-yielded MI3 indicated clear mutant character of *Phytophthora* field tolerance.

Concerning oil content, means ranged from 57.8 % for H.21 to 60.1% for Mut.48, with highest values for H.13, Mut.48, H.3, H.20 and H.12 in 1990 season. In 1991 season, means ranged from 56.9 % for H.21 to 60.8 % for Mut.48 R, with highest values for Mut.48 R, Mut.48, Giza 32, H.3 and H.12. Over two seasons, Mut.48, H.3, H.12 and Giza 32 showed higher oil content than that of other genotypes. Similar results were obtained by Ibrahim [3]. He mentioned that oil yield/ha for all mutant lines showed an increase of more than 0.5 t/ha oil , particularly in mutants Nos. 8, 9, 12, 14 and 15.

Regarding the tolerance degree to the wilt pathogens, means ranged from 20 % for H.15 to 82.0 % for H.7, with highest values for H.7, H.3, Mut.48, H.4 and H.12 in 1990 season. In 1991 season, means ranged between 52 % for H.20 and 84.3 % for H.7, with highest values for H.7, Mut.48 R, Mut.48, H.3 and Giza 32. Over two seasons, H.7, Mut.48, H.3, H.18 and Giza 32 were more tolerant to wilt pathogens under infected field conditions. Similar results were obtained by Kang [5] and Ibrahim and Kararah [6]. They expected that all the selected promising lines raised from the mutation programme or from the crossing block, would pass the pathological test and prove to be highly tolerant to sesame fungal disease. To support the expectation, Pathirana [4] also obtained eleven mutants with field tolerance to phytophthora disease.

From the previous results, it could be concluded that selection among progeniesy of crosses between susceptible genotypes may result in high yielding genotypes which have epistatically determined gene-for-gene resistance, i.e., H.7, Mut.48, Giza 32 and H.3 were more tolerant to wilt pathogens in the second cycle of selection than the first one under the epidemic field conditions. Also results showed that tolerance to wilt pathogens could be increased through recurrent irradiation of induced mutant 48 (Mut.48 R).

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MUTATION BREEDING FOR DISEASE RESISTANCE AND HIGH YIELD OF SESAME (*Sesamum indicum* L.) IN THE REPUBLIC OF KOREA

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Abstract

Two out of 14 sesame varieties released to farmers since 1955 in Korea were developed by mutation breeding. 'Ahnsankkae' was developed in 1984 by 20 krad X-ray irradiation and has been planted as major sesame variety in Korea. It occupied 31 % of total sesame area because of its high yield, uniformity in different regions and disease resistance. 'Suwonkkae' was developed in 1991 from a cross of an X-ray induced mutant and Korean local variety, to have superior quality with higher essential amino acids and linoleic acid (Vitamin F) content. 2 625 - mutant pedigree lines and 89 200 plants of all generations were planted from 1989 to 1992. By this operation, 1 540 promising mutant lines were selected. Sodium azide (NaN_3)-treatment was also used and a dwarf mutant line, 'Suwon 128', which was unique in its dwarf shape with strong lodging resistance, was obtained. This line was expected to give higher yield when planted densely. However, some susceptibility to *Phytophthora* blight was noticed in the yield trials, although it gives almost similar yield to the check cultivars. The only determinate gene so far known, 'dt 45', selected by Dr. A. Ashri after 50 krad of gamma-ray was first introduced to the cross breeding programme in Korea in 1985, and 'Suwon 129' was bred from the cross to 'Danbaeckkae'. It was put to the regional yield trial from 1990. Other determinate lines Suwon 131, 133, 134, and 135 followed. But almost all the determinate lines resulted in lower yield due to their few capsule setting nodes (3-5), although they showed strong resistance to diseases and lodging. Thousands of crosses have been made and evaluated since 1989. It should be noted that the dwarf mutant 'Suwon 128' and determinate varieties have been involved in all of the major crosses in 1992. Determinate, semi-dwarf, disease and lodging resistant, larger seed size, few or non branching along with higher maturity rate and yield have been the Ideal Plant Type of sesame breeding in Korea. 'SI 90033-2B' (Suwon 129//Suwon 9/IS 103) might be the first promising mutant cultivar to fulfil most of the requirements for Korea.

1. INTRODUCTION

Sesame has been cultivated and favored by the people of the Korean peninsula since 1 000 BC. Sesame has been used for many kinds of food as vegetable oil, seasonings for side dishes with steamed rice, rice cakes, sesame pastes especially for the sick, cookies, sesame crackers, etc. Sesame cultivation area had increased from 25.8 thousand hectares in 1970 to 94.3 thousand hectares in 1987. However, it has decreased since then due to shortage of farm labourers and also low market price of cheaper imported sesame. The breeding objectives of sesame in Korea have been focused on the reduction of labor and production costs.

Diseases resistant 'Ahnsankkae', dwarf and lodging resistant 'Suwon 128', and determinate 'dt 45' were successfully bred by induced mutation with X-rays, sodium azide (NaN_3) or gamma-rays in Korea and Israel. Mutation breeding in Korea has been performed to create new genes for disease resistance, higher and uniform yield performance, and unique architecture of plant types in sesame. All the usable genes induced by mutation have been directly adopted to traditional cross breeding programme in Korea. Mutants and mutant derived varieties have been fully used in crossing block in 1992.

'SI 90033-2B', determinate with longer capsule setting nodes, more capsules and higher maturity was selected from F_2 pedigree nursery in 1992. It is evaluated as the first fulfilment of the ideal plant type for Korea illustrated in the 1991 RCM Report.

2. MATERIALS AND METHODS

2.1. Experiment in 1992

Sodium azide (NaN_3), ethyl methanesulfonate (EMS) and gamma-rays were used to treat the seeds of 11 varieties. They were planted in the mutation breeding nursery together with 160 mutant pedigree lines and 25 000 plants, having whole generations of M_1 to M_4 in 1992 (Table I).

TABLE I. VARIETIES, PEDIGREE MUTANT LINES AND PLANTS, AND MUTAGENS ON THE MUTATION BREEDING PROGRAMME IN 1992

Generations	No.varieties used	No.of plants* or lines examined	Mutagens used
M_1	2	(5 000)	NaN_3
M_2	3	(30 000)	NaN_3, gamma-ray
M_3	4	129	EMS
M_4	2	31	EMS
Total	11	(35 000)+160	3 mutagens

* : Figure in ()

2.2. Experiments in 1989 - 1992

43 cultivars of sesame were employed in mutation induction experiments using three different mutagens, NaN_3, EMS and gamma-ray. Pedigree lines amounting to 2 625 lines, 89 200 plants, were planted in the M_1 to M_4 pedigree nursery during the season of 1989 to 1992 (Table II).

TABLE II. NUMBER OF VARIETIES USED, POPULATION SIZE, AND AGENTS USED DURING THE SEASON OF 1989 TO 1992

Gene-ration	No. varieties					Population size*					Mutagens used**			
	'89	'90	'91	'92	Sum	'89	'90	'91	'92	Sum	'89	'90	'91	'92
M_1	2	4	3	2	11	1 800	2 400	10 000	5 000	19 200	G-ray EMS	NaN_3 EMS	G-ray NaN_3	NaN_3
M_2	2	2	3	3	10	10 000	20 000	20 000	20 000	70 000	G-ray EMS	G-ray EMS	EMS NaN_3	G-ray NaN_3
M_3	2	4	3	3	12	843	458	537	127	1965	EMS	G-ray EMS	G-ray EMS	EMS
M_4	2	1	4	3	10	355	152	120	33	660	G-ray EMS	EMS	G-ray EMS	EMS
Total	8	11	13	11	43	1 198 11 800	610 22 400	657 30 000	160 25 000	2 625 89 200	2	3	3	3

* : Number of plants in M_1 and M_2 and number of lines in M_3 and M_4.

** : G-ray = gamma rays

3. RESULTS AND DISCUSSION

3.1. EXPERIMENT IN 1992

Sodium azide was used to treat the dry seeds of Suwon 137 and Mokpo 9 with the concentrations of 2, 4, 6 mM and 2, 3 or 4 hours duration in the Sörensen buffer solution.

Germination tests in the petri dishes were carried out to check the effect of mutagenic treatments with NaN_3, and the results are shown in Table III. Germination rate seemed to be generally higher in NaN_3 treatment than control. It might be due to a kind of sterilization effect of NaN_3 on microbes on the sesame seed. No significant differences in germination rate were observed between treatments except the 6 mM/4 hr, the highest dose treatment.

Seedling height appeared not to be affected much after the treatment. It was even increased by NaN_3 treatments except 4mM/2hr, 6mM/3hr and 6mM/4hr.

Seedling growth characteristics were examined to determine the optimum NaN_3 mutagen treatments. After termination of the NaN_3 treatment, the seeds were planted in sand filled plastic boxes under greenhouse condition.

TABLE III. GERMINATION RATE AND PLANT HEIGHT BY DIFFERENT NaN_3 TREATMENTS ON DIFFERENT SESAME VARIETIES IN KOREA 1992

NaN_3 treatment	Germination rate(%)				Plant height(cm)			
(mM/hr)	Suwon 137	Mokpo 9	Mean	Index	Suwon 137	Mokpo 9	Mean	Index
Control	90BC**	87C	89D	100	8.0AC	8.2AB	8.1AC	100
2/2	99A	99A	99A	111	7.4BC	8.9AB	8.2AC	101
2/3	96A	100A	98A	110	8.7AB	8.8AB	8.8AB	109
2/4	98A	99A	99A	111	8.5AB	9.2AB	8.8AB	109
4/2	97A	97AB	97AB	109	7.6AC	7.9B	7.8BC	96
4/3	97A	99A	98A	110	8.1AC	8.4AB	8.3AC	102
4/4	99A	100A	100A	112	8.5AB	9.2AB	8.9AB	110
6/2	95AB	99A	97AB	109	8.8A	9.4A	9.1A	112
6/3	89C	98A	94BC	106	7.0C	8.3AB	7.7C	95
6/4	89C	93B	91CD	102	7.1C	9.1AB	8.1AC	100
Mean	95	97	96		8.0	8.7	8.4	
CV(%)	3.1	2.4	3.2		9.0	8.3	10.0	
LSD(5%)	5.0	4.0	3.6		1.2	1.2	1.0	

* Pre-soaking : 6 hour 4 °C Washing : 6 hour in flowing tap water
Soaking : 20 hour 20 °C Control : Sörenson buffer solution
** DMRT (Duncan's Multiple Range Test)

Plant height, leaf length and width were examined at the full development stage of the second true leaves. Leaf length and width of the first true leaves were measured and the results were shown in Table IV. Plant height did not show much difference between NaN_3 treatments and was generally increased by NaN_3 treatments over that of control except 4mM/2h and 4mM/3h. It showed similar tendency as the results of germination rate and plant height on petri dish test shown in Table III. Leaf length and width showed some decreases, perhaps due to damages by NaN_3 treatment, in the treated materials. Consequently, the dose of the optimum mutagen treatment seemed to be more reasonably determined by the leaf length and width at full developed stage of second foliage leaves than germination rate or plant height in sesame seedling test.

TABLE IV. PLANT HEIGHT, LEAF LENGTH AND WIDTH OF DIFFERENT SESAME VARIETIES BY SODIUM AZIDE (NaN_3) TREATMENT IN 1992 (Unit:cm)

NaN_3 treatment (mM/hr)	Plant height				Leaf width				Leaf length			
	Suwon 137	Mokpo 9	Mean	Index	Suwon 137	Mokpo 9	Mean	Index	Suwon 137	Mokpo 9	Mean	Index
Control	7.9AC*	6.6A	7.2AB	100	1.05AB	0.92A	0.98A	100	1.99A	1.84A	1.92A	100
2/2	8.3AB	6.7A	7.5AB	104	0.94AB	0.87AB	0.90AB	92	1.90A	1.57AC	1.73AB	90
2/3	7.7AC	6.8A	7.2AB	100	0.92AB	0.91A	0.92AB	94	1.74A	1.64AC	1.69AB	88
2/4	8.8A	7.5A	8.2A	114	1.10A	0.91A	1.00A	102	2.04A	1.79A	1.91A	99
4/2	7.2BC	6.8A	7.0B	97	0.93AB	0.86AB	0.89AB	91	1.71A	1.75AB	1.73AB	90
4/3	6.8C	6.2A	6.5B	90	0.86B	0.78AB	0.82B	84	1.68A	1.46BC	1.57B	82
4/4	7.7AC	7.2A	7.5AB	104	1.00AB	0.84AB	0.92AB	94	1.72A	1.69C	1.71AB	89
6/2	8.4AB	6.2A	7.3AB	101	1.06AB	0.73B	0.89AB	91	2.00A	1.44C	1.72AB	90
6/3	8.1AC	6.8A	7.5AB	104	1.06AB	0.89A	0.97A	99	1.85A	1.64AC	1.75AB	91
6/4	8.0AC	6.8A	7.4AB	103	1.03AB	0.88A	0.95AB	97	1.89A	1.74AB	1.82AB	95
Mean	7.9	6.8	7.3		1.00	0.86	0.92		1.85	1.66	1.75	
CV(%)	10.8	14.9	9.2		14.0	9.9	9.0		16.9	10.9	10.5	
LSD(5%)	1.2	1.5	1.0		0.20	0.12	0.12		0.45	0.26	0.27	

* DMRT(Duncan's Multiple Range Test)

TABLE V. NUMBER OF SELECTED LINES AND AMOUNT OF SEEDS FROM MUTATION BREEDING NURSERY UNDER BLACK POLYETHYLENE FILM MULCHING CONDITION WITH NO CHEMICAL CONTROL IN 1992

Generation	Numbers examined		No. of selected lines	Selection rate(%)
	Varieties	(Plants) lines		
M1	2	(5 000)	75(g)	-
M2	3	(30 000)	360	1
M3	4	129	76	59
M4	2	31	15	48
Total	11	160(35 000)	451(75g)	--

The whole examined and selected lines and amount of seeds are shown in Table V. In 1992, 160 pedigree mutant lines and 35 000 plants were planted in the mutation breeding nursery and 427 lines and 75 g of M_1 seeds were selected from the nursery.

Selected lines and their main characteristics are summarized in Table VI. In M_2 generation 360 mutants were selected with unique characteristics of quadricarpels octo-loculi, dwarf, determinate, diseases tolerant, higher yield ability.

Selected lines and their main characteristics among M_3 and M_4 generations are summarized as Table VII and VIII. Semi-dwarf and quadri-carpels, octo-loculi, lines were selected in M_3 sodium azide treated Hansumkkae. Erect branched and higher yielding mutant were selected in M_4 gamma-ray treated Hansumkkae.

TABLE VI. SELECTED LINES AND THEIR MAIN CHARACTERISTICS OF SESAME IN M_2 GENERATION IN 1992

Planted lines	Original variety	Mutagen treatment*	No. of lines selected	Main character
SIM91128 r30-2B and 2 others	Suwon 128	Gamma ray 30, 40, 50 kR	30	Dwarf, No and few Branches
SIM91129 r30-2B and 2 others	Suwon 129	"	12	Determinate, Diseases Tolerance
SIM91JB r30-2B and 2 others	Jinbaeckkae	"	13	Many, few, no Branches
SIM91128 S2/2-2B and 8 others	Suwon 128	NaN_3 2mM 2hr and 8 others	108	Semi-Dwarf, High Yield, Diseases Tolerance
SIM91129 S2/2-2B and 8 others	Suwon 129	"	107	Determinate, Dwarf, High Yield
SIM91JBS 2/2-2B and 8 others	Jinbaeckkae	"	90	High Yield, Quadricarpels
Total	3	2(11)	360	

* : Number of treatment classes of different mutagens and doses in ( ).

TABLE VII. SELECTED LINES AND THEIR MAIN CHARACTERISTICS ON SESAME M_3 GENERATION IN 1992

Planted lines	Original varieties	Mutagen treatments*	No. selected lines	Main characteristics
SIM90128E-2B-1 and 34 others	Suwon 128	EMS 0.5% 24 hours	21	Dwarf, Diseases resistant
SIM90129E-2B-1 and 15 others	Suwon 129	"	8	Dwarf, Quadricarpels
SIM90HS2/2-2B-1 and 39 others	Hansumkkae	NaN_3 2mM 2hrs, 2mM 3hrs	24	Branch, Semi-dwarf, Quadricarpels
SIM90JBS2/2-2B-1 and 34 others	Jinbaeckkae	NaN3 2mM 2hrs, 2mM 3hrs	23	High Yield
Total	4	2(3)	76	

*:( ) are number of treatment levels of different kind of mutagens

TABLE VIII. SELECTED LINES AND THEIR MAIN CHARACTERISTICS IN M_4 GENERATION IN 1992

Planted lines	Original varieties	Mutagen treatments*	No.of line selected	Main characters
SIM89H r20-2B-81-1 and 22 others	Hansumkkae	Gamma ray 20, 30, 40KR	8	Erect Branchs, High Yield
SIM89JBE-2B-3-1 and 6 others	Jinbaeckkae	EMS 0.5% 12hrs	7	High Yield Quadri carpels
Total	2	2(4)	15	

*: ( ) are number of treatment classes of different doses and mutagens

3.3. Experiment from 1989 to 1992

Selected lines, plants and amount of seeds resulted from 1989 to 1992 in the mutation breeding programme are summarized in Table IX. From M_1 through M_4 generations planted in the field during this period, 1 540 pedigree mutant lines and 2 137g of seeds have been selected from the total populations of 2 625 lines and 89 200 plants.

TABLE IX. NUMBERS OF SELECTED LINES AND AMOUNT OF SEED FROM 1989 TO 1992 IN SESAME MUTATION BREEDING PROGRAMME IN KOREA

Generation	Number of plants or lines planted*					No. selected lines**					Selection percent
	'89	'90	'91	'92	Total	'89	'90	'91	'92	Total	
M_1	1 800	2 400	10 000	5 000	19 200	300g	360g	150g	1 327g	2 137g	- %
M_2	10 000	20 000	20 000	20 000	70 000	388	512	94	197	1 191	2
M_3	843	458	537	127	1 965	43	104	66	57	270	14
M_4	355	152	120	33	660	12	37	14	16	79	12
Total	1 198	610	657	160	2 625	443	653	174	270	1 540	2
	11 800	22 400	30 000	25 000	89 200	300g	360g	150g	1 327g	2 137g	

* : In M_1 and M_2, number of plants.
** : In M_1, amount of seed (g).

In the entire breeding programmes, mutants and hybrid progenies involving mutant line have amounted to 1 229, or 58 %, among whole cross breeding pedigree combinations (F_1 to F_5) of 2 122 from 1989 to 1992 as summarized in Table X. Percentages of mutants and mutant cross progenies in the whole generations of tested cross breeding combinations have been dramatically increased year by year. It finally reached to 100 percent on crossing blocks of pedigree nursery in 1992. Major parental mutant lines used in the crossing blocks were the dwarf 'Suwon 128', diseases resistant, semi-dwarf, higher maturity determinate lines and diseases tolerant high yielding 'Ahnsankkae'.

TABLE X. USE OF MUTANTS AND MUTANT-CROSS COMBINATIONS AMONG ALL THE SESAME PEDIGREE GENERATIONS FROM 1989 TO 1992 IN KOREA

Generation	No.released combinations(A)					No. mutants & mutant crossed combinations(B)					Proportion of B/A(%)				
	'89	'90	'91	'92	Total	'89	'90	'91	'92	Total	'89	'90	'91	'92	Mean
Crossing block	116	80	147	72	415	50	50	123	72	295	43	63	84	100	71
F_1	117	118	154	76	465	38	53	117	68	276	32	45	76	89	59
F_2	139	105	103	182	529	55	34	42	158	289	40	32	41	87	55
F_3	88	129	80	85	382	66	57	25	38	186	75	44	31	45	49
F_4	80	83	89	56	308	28	65	46	26	165	35	78	52	46	54
F_5	-	-	23	-	23	-	-	18	-	18	-	-	78	-	78
Total	540	515	596	471	2 122	237	259	371	362	1 229	44	50	55	77	58

Mutants and mutant cross progenies among all cross breeding pedigree lines have reached 5 015, or 57 %, among 8 751 planted pedigree lines of whole generations (F_3 - F_5) from 1989 to 1992. Those are shown in Table XI.

TABLE XI. CHANGES OF MUTANTS AND MUTANT CROSS PEDIGREE LINES AMONG ALL THE SESAME PEDIGREE LINES FROM 1989 TO 1992 IN KOREA

Gene-ration	No.planted lines (A)					No. mutants & mutant crossed lines(B)					Proportion of B/A(%)				
	'89	'90	'91	'92	Total	'89	'90	'91	'92	Total	'89	'90	'91	'92	Mean
F_3	1 398	1 835	1 177	816	5 226	1 183	1 020	302	447	2 952	85	56	26	55	56
F_4	559	975	1 564	286	3 384	239	788	733	179	1 939	43	81	47	63	57
F_5	-	-	141	-	141	-	-	124	-	124	-	-	88	-	88
Total	1 957	2 810	2 882	1 102	8 751	1 422	1 808	1 159	626	5 015	73	64	37	57	57

Mutants and mutant crossed combinations reached 276, or 54 %, among whole yield trial combinations of 509 from 1989 to 1992. It is summarized in Table XII.

TABLE XII. CHANGES OF MUTANTS AND MUTANT CROSSED COMBINATIONS AMONG ALL THE PLANTED COMBINATIONS ON YIELD TRIALS FROM 1989 TO 1992 IN KOREA

Yield* trials	No. of tested combination(A)					No. of mutants & mutant crossed combinations(B)					Proportion of B/A(%)				
	'89	'90	'91	'92	Total	'89	'90	'91	'92	Total	'89	'90	'91	'92	Mean
OYT	93	47	36	76	252	38	18	27	52	135	41	38	75	68	54
PYT	49	32	41	27	149	36	12	19	20	87	73	38	46	74	58
AYT	12	14	15	17	58	5	9	11	12	37	42	64	73	71	64
RYT	14	13	12	11	50	2	3	7	5	15	14	23	58	45	34
Total	168	106	104	131	509	81	42	64	89	276	48	40	62	68	54

* OYT : Observational Yield Trial AYT : Advanced Yield Trial
PYT : Preliminary Yield Trial RYT : Regional Yield Trial

Mutants and mutant crossed lines have reached 1 266, or 60 %, among 2 096 whole tested lines of yield trials since 1989 to 1992, as summarized in Table XIII.

TABLE XIII. CHANGES OF MUTANTS AND MUTANT CROSSED LINES AMONG ALL THE RELEASED LINES ON YIELD TRIALS SINCE 1989 TO 1992 IN KOREA

Trials*	No. of tested lines (A)					No.of mutants & mutant crossed lines(B)					Proportion of B/A(%)				
	'89	'90	'91	'92	Total	'89	'90	'91	'92	Total	'89	'90	'91	'92	Mean
OYT	343	184	348	767	1 642	127	121	307	414	969	37	66	88	54	59
PYT	139	60	61	59	319	109	26	36	50	221	78	43	59	85	69
AYT	26	20	19	19	84	18	13	13	14	58	69	65	68	74	69
RYT	14	13	12	13	52	2	3	7	6	18	14	23	58	50	35
Total	522	277	440	858	2 097	256	63	363	484	1 266	49	59	83	56	60

* OYT : Observational Yield Trial AYT : Advanced Yield Trial
PYT : Preliminary Yield Trial RYT : Regional Yield Trial

Six lines of mutant and mutant crossed progenies being bred in Crop Experiment Station were included in regional yield trials in 1992. Six mutants bred in Crop Experiment Station, Suwon 128, Suwon 131, Suwon 133, Suwon 134, Suwon 135, Suwon 136 (second cropping) and Suwon 137 amounted to 86 % among 7 all CES lines submitted to regional yield trial. Results were summarized in Table XIV. All those tested lines except Suwon 136 were mutants and mutant crossed lines of dwarf, determinate, diseases resistant and high yielding.

TABLE XIV. YIELD AND GROWTH CHARACTERISTICS OF MUTANTS AND MUTANT CROSSED LINES ON REGIONAL YIELD TRIAL OF MONO CROPPING IN KOREA IN 1992

No.	Tested varieties & lines	Plant type	Plant height (cm)	Length capsule setting nodes (cm)	No. branches	No. capsules/ plant	No. grains/ capsule	Maturity (%)
1	Jinbaeckkae	Indeterminate	133	102	0.1	98	63.7	77.9
2	Ahnsankkae	Indeterminate Mutant	122	83	2.7	83	62.8	86.3
3	Suwon 128	Dwarf Mutant	77	61	1.8	140	52.4	66.3
4	Suwon 131	Determinate M.	77	25	4.9	111	61.1	86.7
5	Suwon 133	"	69	18	4.6	72	61.0	81.3
6	Suwon 134	"	70	17	4.6	63	66.9	85.3
7	Suwon 135	" (Black)	54	16	3.9	91	58.7	96.0
8	Suwon 137	Indeterminate M.	125	98	0.9	118	55.7	80.0
9	Mokpo 9	Indeterminate	143	105	0.9	108	58.2	68.0
10	Mokpo 10	"	140	103	0.4	108	63.8	71.7
11	Iri 3	"	119	90	0.6	103	60.5	84.3
12	Iri 4	"	132	90	0.5	90	61.8	78.7
13	Iri 5	"	130	90	2.3	115	58.1	71.7

(Table XIV continued)

No.	Tested varieties & lines	1 000 grain weight (g)	1 Litre weight (g)	Diseases (%)	Lodging (%)	Yield (kg/ha)	Index
1	Jinbaeckkae	2.77	615	7	38	1 009	100
2	Ahnsankkae	2.64	619	12	30	1 033	102
3	Suwon 128	2.71	602	5	2	1 050	104
4	Suwon 131	2.64	593	3	2	1 034	103
5	Suwon 133	2.57	619	5	0	973	96
6	Suwon 134	2.77	621	5	2	795	79
7	Suwon 135	2.74	479	5	10	923	92
8	Suwon 137	2.57	611	8	35	1 014	101
9	Mokpo 9	2.58	599	10	42	970	96
10	Mokpo 10	2.35	609	5	20	1 021	101
11	Iri 3	2.53	611	13	30	967	96
12	Iri 4	2.88	605	8	33	983	97
13	Iri 5	2.79	615	12	67	962	95
						L.S.D (5%)	16.27
						(1%)	- 9.85

Regional yield and yield index of tested varieties and lines are described in Table XV. Suwon 128, the dwarf and lodging resistant mutant induced by sodium azide treatment showed almost same yield ability with Ahnsankkae but lesser than Jinbaeckkae in mid-northern area. Suwon 137, indeterminate, tolerant to diseases and high yielding mutant which was tested for the first time in RYT 1992 showed 9 percent yield increase in the whole area. Determinate mutants of Suwon 131, Suwon 133, Suwon 134 and Suwon 135 (black seed) showed very strong disease resistance but a little lesser yield ability compared to Jinbaeckkae.

Table XV. REGIONAL YIELD AND YIELD INDEX OF VARIETIES AND LINES ON REGIONAL YIELD TRIAL OF MONO CROPPING IN KOREA IN 1992

Mid-northern area (Unit.kg/ha)

Tested Varieties & Lines	Suwon	Hwasung	Chunchon	Chongju	Taejun	Average
	Yield (%)	Yield (%)	Yield (%)	Yield (%)	Yield (%)	Yield (%)
Jinbaeckkae	1 010(100)	1 350(100)	500(100)	750(100)	1 170(100)	960(100)
Ahnsankkae	1 030(102)	1 420(105)	470(93)	820(109)	860(74)	920(96)
Suwon 128	1 050(104)	1 060(79)	540(107)	800(107)	1 130(97)	920(96)
Suwon 131	1 030(103)	1 020(76)	450(90)	790(105)	980(84)	860(90)
Suwon 133	970(96)	- -	- -	740(99)	- -	860(97)
Suwon 134	800(79)	- -	- -	720(96)	- -	760(86)
Suwon 135	920(92)	- -	- -	- -	- -	920(92)
Suwon 137	1 010(101)	1 470(109)	590(118)	820(109)	1 310(117)	1 050(110)
Mokpo 9	970(96)	- -	- -	- -	- -	970(96)
Mokpo 10	1 020(101)	- -	- -	- -	- -	1 020(101)
Iri 3	970(96)	1 210(90)	320(63)	700(93)	1 120(96)	860(90)
Iri 4	980(97)	1 330(99)	550(109)	760(99)	1 080(92)	940(98)
Iri 5	960(95)	1 160(87)	460(92)	690(92)	1 160(99)	890(93)

Southern area (Unit.kg/ha)

Tested varieties and lines	Iri	Iri*	Naju	Muan	Taegu	Average	National Mean
	Yield (%)	Yield (%)	Yield (%)	Yield (%)	Yield (%)	Yield (%)	Yield (%)
Jinbaeckkae	830(100)	810(100)	820(100)	970(100)	680(100)	820(100)	890(100)
Ahnsankkae	740(90)	900(111)	800(98)	900(93)	670(99)	800(98)	860(97)
Suwon 128	650(80)	680(83)	560(69)	910(84)	510(74)	640(78)	780(88)
Suwon 131	600(74)	740(91)	550(68)	540(56)	540(79)	600(72)	730(82)
Suwon 133	- -	640(74)	530(65)	530(55)	370(53)	520(63)	690(77)
Suwon 134	- -	680(84)	560(68)	690(71)	480(71)	600(73)	680(77)
Suwon 135	- -	710(88)	- -	620(64)	- -	670(76)	750(81)
Suwon 137	940(115)	930(115)	850(104)	980(101)	740(108)	890(108)	970(109)
Mokpo 9	990(121)	830(102)	780(96)	930(96)	780(114)	860(105)	920(103)
Mokpo 10	830(102)	820(101)	820(100)	920(95)	750(109)	830(101)	920(104)
Iri 3	1 110(136)	860(105)	780(96)	940(87)	580(85)	830(101)	850(96)
Iri 4	1 090(133)	850(105)	870(106)	920(95)	670(99)	880(107)	910(102)
Iri 5	980(120)	910(112)	680(84)	880(91)	850(124)	860(104)	870(98)

* Chun-Buk PRDA

Three years ('90 - '92) tests of regional yield and yield index of dwarf mutant 'Suwon 128' were summarized in Table XVI. The yield of Suwon 128 appeared almost the same with Ahnsankkae in mid-northern area but values decreased in southern area. Suwon 128 will be

used as material for crossing block; it was decided not to release it as new variety, by the National Seed Committee of the Ministry of Agriculture, Forestry and Fishery.

Seed quality of the tested varieties and lines in regional yield trial are described in Table XVII. Besides their morphological characteristics, determinate type Suwon 131 and indeterminate mutant Suwon 137 showed highest content of linoleic fatty acid (Vitamin F). The highest oil content was found in Suwon 137, and the highest protein content in determinate Suwon 133 and Suwon 134.

TABLE XVI. THREE YEARS ('90 - '92) REGIONAL YIELD AND YIELD INDEX OF LINES AND VARIETIES AMONG REGIONAL YIELD TRIAL OF MONO CROPPING IN KOREA

Mid-northern area (Unit kg/ha)

Varieties and lines	Suwon		Hwasung		Chunchon		Chongju		Taejun		Average	
	Yield	Index	Yield	Index	Yield	Index	Yield	Index	Yield	Index	Yield	Index
Ahnsankkae	800	100	890	100	590	100	780	100	1 020	100	820	100
Suwon 128	910	113	770	87	620	106	710	91	980	96	800	98
Mokpo 9	780	97	-	-	-	-	-	-	-	-	780	97
Iri 3	630	78	830	94	470	81	700	91	960	94	720	88

Southern area (Unit kg/ha)

Varieties and lines	Iri		*Iri		Naju		Muan		Taegu		Average		Nation Mean	
	Yield	In-dex	Yield	In-dex	Yield	In-dex	Yield	In-dex	Yield	In-dex	Yield	In-dex	Yield	In-dex
Ahnsankkae	760	100	900	100	740	100	810	100	910	100	820	100	820	100
Suwon 128	570	75	770	86	590	80	800	98	700	76	690	83	740	90
Mokpo 9	870	114	860	93	790	107	960	118	1 020	113	900	109	880	108
Iri 3	910	119	910	101	780	106	830	102	830	91	850	103	790	96

* Chun-buk PRDA

The history of the recommended sesame varieties by Korean RDA organization were summarized in Table XVIII. Early Russian had been introduced and released to the farmers in Korea 1955. Early Russian was used in crosses to eight varieties and 14 lines derived were released as varieties. It was valued as the most useful and efficient gene source in Korea.

Ahnsankkae, a disease tolerant and stable high yielding mutant induced by X-ray irradiation of 20 krad, was developed in 1984. It became soon a major cultivated variety in Korea (Table XIX). 31 percent of whole sesame cultivating area in Korea has been still occupied with Ahnsankkae in 1992. Suwonkkae, another good quality high yielding, X-ray induced mutant of ME-93-4 crossed to Kyum, Korean local variety was newly developed in 1991 and being accepted by farmers. It will spread in farmer's field soon.

TABLE XVII. FATTY ACID COMPOSITION, OIL AND PROTEIN CONTENT OF VARIETIES AND LINES ON REGIONAL YIELD TRIAL IN 1992
(Unit: %)

Varieties and lines	Fatty acid composition						Oil content	Protein content
	Pal.	Ste.	P+S	Ole.	Lin.	O+L		
Chinbaeckkae	8.43	2.70	11.1	42.21	46.66	88.9	51.29	31.3
Ahnsankkae	8.01	2.93	10.9	40.30	48.75	89.1	42.35	30.4
Suwon 128	9.03	3.46	12.5	39.47	48.04	87.5	46.72	31.3
Suwon 131	7.64	2.84	10.4	38.95	50.57	89.5	50.49	30.2
Suwon 133	11.34	3.96	15.3	47.56	37.14	84.7	42.22	31.4
Suwon 134	7.58	2.55	10.1	41.88	47.99	89.9	46.57	31.5
Suwon 135	9.44	1.91	11.4	39.66	48.99	88.7	46.39	28.6
Suwon 137	8.57	3.27	11.8	36.25	51.91	88.2	53.44	30.8
Mokpo 9	7.74	2.19	9.9	41.42	48.65	90.1	42.25	29.7
Mokpo 10	10.48	3.54	14.0	40.77	45.21	86.0	50.85	31.2
Iri 3	7.46	2.62	10.1	39.77	50.13	89.9	47.01	30.9
Iri 4	8.01	2.71	10.7	41.05	48.23	89.3	52.93	31.4
Iri 5	8.47	2.95	11.4	40.91	47.68	88.6	53.78	29.6

* Pal. : Palmitic fatty acid Ste. : Stearic fatty acid
Ole. : Oleic fatty acid Lin. : Linoleic fatty acid

TABLE XVIII. YIELDS AND CROSS COMBINATIONS OF RECOMMENDED VARIETIES SINCE 1955 TO 1992 IN KOREA

No.	Recommended varieties	Released year	Cross combination and origins	Yield	Breeding techniques
1	Early Russian (90 Days Chamkkae)	1955	Russian local	470	Introduced from USA
2	Suwon 5	1971	Haenam/K10	490	Cross breeding
3	Suwon 9	1974	Anthalya/Early Russian	490	Cross breeding
4	Suwon 21	1978	Hongchun local collection	510	Pure line selection
5	Pungnyeonkkae	1980	Shirogoma/Suwon 5//Suwon 5	720	Cross breeding
6	Kwangsankkae	1981	Suwon 11/Early Russian	760	Cross breeding
7	Danbaeckkae	1982	Suwon 9/Early Russian// PI 195123	870	Cross breeding
8	Ahnsankkae	1984	X-ray 20KR irradiated to Early Russian	920	Mutation breeding
9	Yusungkkae	1984	Yusung local collection	830	Pure line selection (Black sesame)
10	Hansumkkae	1986	Suwon 9/Early Russian// PI 195123	930	Cross breeding
11	Samdakkae	1986	Namwon local collection	690	Pure line selection (Cheju island)
12	Jinjukkae	1988	Suwon 9/Suwon 11//Suwon 9	670	Cross breeding (Adapted to second cropping)
13	Chinbaeckkae	1989	Baeckchamkkae/Margo	920	Cross breeding
14	Suwonkkae	1991	Kyum/ME-93-4	874	Mutation & cross breeding

TABLE XIX CULTIVATION AREA OF SESAME CULTIVARS IN KOREA IN 1992

Varieties	Ahnsan-kkae	Danbaec-kkae	Hansum-kkae	Kwangsan-kkae	Samda-kkae	Jinju-kkae	Jinbaec-kkae	Others
Cultivation area(%)	31 0	24 5	18 0	4 6	7 1	1 7	0 3	12 4

* Others Yusungkkae, Korean local, etc

Ideal sesame plant type was created by Korean sesame breeding group and their efforts have been concentrated to achieve the new ideal plant type in the breeding nursery during the seasons of two years in Korea. The characteristics of ideal plant type are described in Fig. 1 and 2. Ideal Plant Type II was focused on the growth and yield component characteristics of semi-dwarf, determinate, only a few branches, lodging resistant, higher maturity, disease-resistant and higher yielding. Ideal Plant Type I was added with the characteristics of non branching and larger seed size to Ideal Plant Type II.

As the first step of creating Ideal Plant Types, genes controlling determinate plant type, numbers and longer-capsule setting nodes than in any other determinate mutant (3 - 5 nodes) were used so far. 'SI 90033-2B' with higher maturity rate and many capsules appeared promising in F_2 cross breeding nursery in 1992. Cross combinations and other growth characteristics were described in Table XX.

The new determinate plant type mutant line 'SI90033-2B' will be placed to yield performance trial in 1993. It will be used as parental line for crossing block because it has been demonstrated to have the characteristics of the Ideal Sesame Plant Type in Korea.

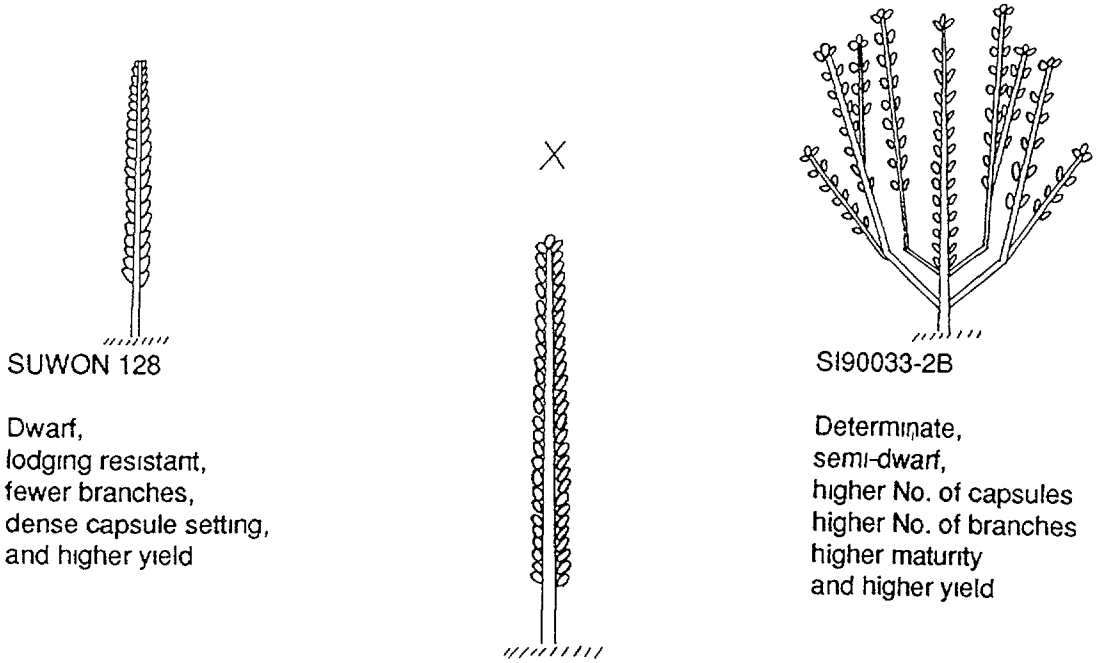

Fig.1. Ideal planbt type I.

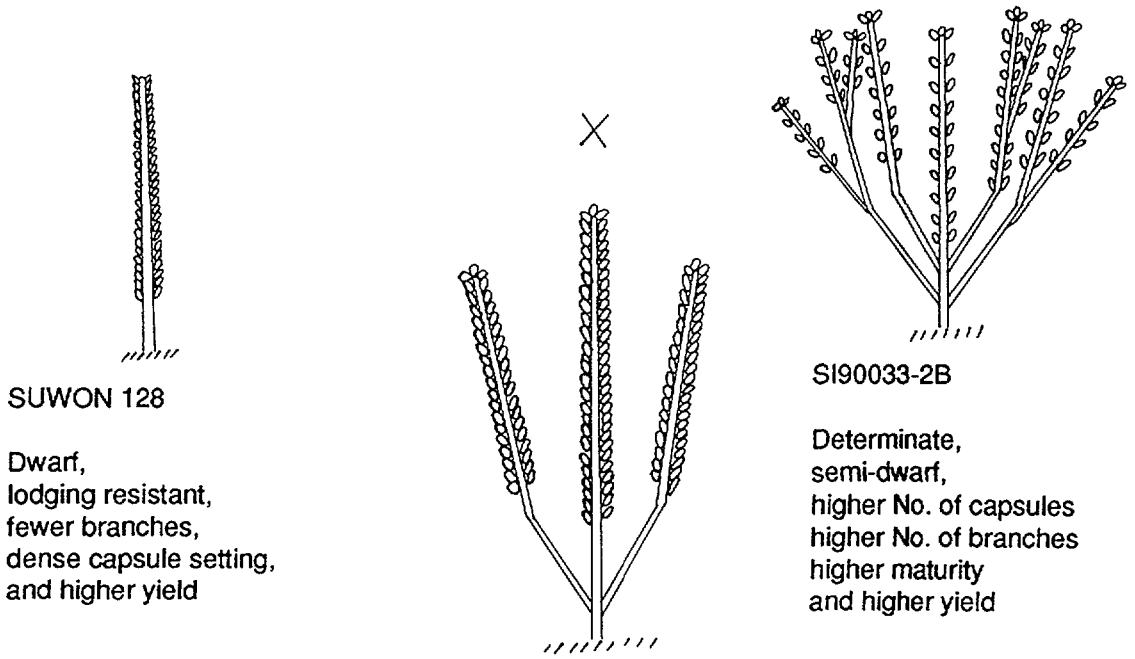

Fig.2. Ideal plant type II.

TABLE XX. YIELD COMPONENTS AND GROWTH CHARACTERISTICS OF NEWLY DEVELOPED PROMISING DETERMINATE MUTANT 'SI90033-2B' IN SUWON IN 1992

Lines & varieties	Cross combinations	Plant type	Plant height (cm)	No. of capsule setting nodes	Length of capsule setting nodes (cm)	No.of branches	No.of capsules	Maturity rate (%)
SI90033-2B	Suwon 129(Danbaeck-kkae/dt45)/Nongki S-1(Suwon 9/IS 103)	Determinate mutant	84	18	69	9	265	98
Suwon 131	dt45//MB-112-2-13-2/ Danbaeckkae	Determinate mutant	77	3	25	5	111	87
Jinbaec-kkae(check)	Baeckchamkkae/Margo	Indeterminate	133	24	102	0.1	98	78

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IMPROVEMENT OF SESAME BY INDUCED MUTATION IN THAILAND

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Abstract

Sesame is grown in Thailand on a small scale with minimal inputs. It has drought tolerance potential and can be grown in the rice-based cropping system or on upland as rotation crops to utilize residual soil moisture. Only cultivar introduction and cross breeding had been the procedures used in sesame breeding in Thailand. However, the desirable genotypes were rarely found in such a breeding program. Mutation breeding was expected to fulfill these demands. The use of gamma irradiation to induce desirable agronomic characters was investigated intending to evolve genetic variability of sesame germplasm, especially resistance to diseases and insects, drought tolerance, earliness, non-shattering and plant architecture suitable for cropping systems. M_2 seeds were harvested in bulk, but in the later generations the pedigree method was used for the promising lines. The plants in the experimental field often suffered from diseases, making harvest of the check cultivar difficult. But the promising mutant lines maintained the desirable characters of disease resistance, larger seed size, higher seed yield per plant etc. Yield trials will be continued to confirm the value of these improved lines.

1. INTRODUCTION

Sesame (*Sesamum indicum* L.) is a traditional and popular catch crop to obtain additional income in Thailand. It occupied an area of 58 000 hectares and yielded 29 200 metric tons with the average of 500 kg/ha in 1990/1991. Erratic rainfall during the growing seasons affected the output considerably.

Sesame is grown over the country on a small scale with minimal management. It has drought tolerance potential and can be grown in the rice-based cropping system both before and after rice and also after the harvest of upland crops to exploit residual soil moisture. At present, only cultivar introduction, plant hybridization and selection are the procedures used in sesame varietal improvement in Thailand.

The variability for desirable genotypes is rarely found in such a breeding program. Mutation breeding therefore could be successful especially for resistance to diseases and pests.

The objectives of this project were as follows:

1. To investigate the potential of radiation to induce desirable agronomic characters.
2. To evolve genetic variability of sesame germplasm especially for those desirable characters of high values in crop improvement such as resistance to diseases and insects, drought tolerance, earliness, non-shattering and plant architecture suitable for cropping systems.
3. To evaluate the sesame populations derived from induced mutation for utilization in the sesame crop improvement program through hybridization.

2. MATERIALS AND METHODS.

2.1. Experiment plan

There were two sets of experiments in this project.

In the Set I trial, two recommended cultivars, Roi-et 1 (RE) and Mahasarakam 60 (MK 60); two local cultivars, Nakornsawan 1 (NW) and Chaibadan (LW-local white) and one elite line, MKS-I-83042-1 (MKS) were treated by gamma rays with 0, 200, 400 and 600 Gy respectively.

In the Set II trial, three cultivars namely Mahasarakam 60, Nakornsawan 1 and MKS-I-83042-1 were treated by gamma rays with 0, 400, 600, 800 and 1 000 Gy respectively.

The experiments were conducted twice a year starting in 1989 at Ubon Rachathani Field Crops Research Center (Ubon FCRC). The first trial (Set I) was done in the early rainy season (Apr. - Aug.) while the second trial (Set II) was made in the late (Sept. - Dec.) rainy season in 1989.

2.2. Experimental procedure

The M_1 were harvested in bulk for advanced generation. In the M_2 - M_5 visual selections were made and followed for desirable plant type and agronomic characters in relation to earliness, disease and/or insect resistances, non shattering pod type, desirable plant architecture suitable for cropping systems, seed size, seed colour and yield.

In the M_3 - M_5 generations of the selected lines the pedigree method was used. The criteria for selection were the same as in the M_2.

In this programme, lines with promising mutant characters were put to preliminary yield trials. Hybridizations among these lines and further selections are also included in the future work plan.

3. RESULTS AND DISCUSSION (1991-1992)

Two experiments were conducted, firstly an advanced generation trial of M_5 and M_6 (SET II), and secondly a preliminary yield trial of M_6 and M_7 (SET I) in the late rainy season 1991 and in the early rainy season 1992 respectively at Ubon Rachathani FCRC.

3.1. EXPERIMENT 1 (M_5 AND M_6)

Advanced generation trial of 96 elite lines of M_5 and six check cultivars namely MK 60, RE, LW, MKS, local red (LR) and Hnanni was laid out in a randomized complete block (RCB) design with 2 replications in the late rainy season 1991. Most plants suffered from major diseases caused by *Macrophomina phaseolina*, *Pseudomonas solanacearum* etc. Only 36 lines and four checks could be harvested.

Twenty-one elite lines of healthy plants with good capsule arrangement and good seed set were selected and were tested in early rainy season 1992. Seeds of 21 lines were sent to the Division of Plant Pathology for *M. phaseolina* resistance screening in the laboratory. The work is now in progress.

Agronomic traits and seed yields are shown in Table I. The data showed that among 21 selected lines, only 9 lines gave higher seed yields than the check cultivars (Hnanni = 2.89 g/plant). The line MK 60/80/7/3-2 (MK 60 treated with 800 Gy of gamma rays) gave the highest yield of 7.18 g/plant. Average plant height ranged from 62.7 to 110.6 cm. while in the check cultivars it ranged from 55.7 to 79.9 cm. Average number of capsules and branches

Table I. AGRONOMIC TRAITS AND SEED YIELD OF SESAME CULTIVARS IN ADVANCED GENERATION (M_5) TRIAL IN LATE RAINY SEASON OF 1991 AT UBON FCRC

Lines	Capsule height		Number/plant		Seed Yield per plant
	top (cm)	first (cm)	capsule	branch	(g)
1.MK60/60/7/1-2	62.7	29.7	17.0	2.90	1.75
2.MK60/80/4/2	72.4	27.9	14.9	0.20	2.19
3.MK60/100/1/3	82.0	20.4	34.9	1.00	1.89
4.MK60/100/2/2	81.1	21.7	25.9	0.20	3.05
5.MK60/100/3/3-3	90.2	27.0	21.4	0.10	4.03
6.MKS/40/3/2	65.8	27.3	26.2	1.80	3.59
7.MKS/80/1/2-4	110.6	56.9	29.0	2.10	2.14
8.MKS/40/4/2-2	76.1	39.6	19.4	1.30	2.29
9.MK60/10/4/1	92.3	31.7	28.0	2.00	4.58
10.MK60/80/1/4-2	77.4	23.6	22.1	0.10	3.74
11.MK60/80/1/4-3	76.7	32.5	17.8	0.10	1.48
12.MK60/80/1/2-2	66.8	29.3	15.0	0.10	4.01
13.MK60/80/1/4-2	69.9	33.6	17.0	1.00	1.56
14.MKS/80/5/3	76.6	31.3	18.7	1.20	1.70
15.MKS/40/2/1-1	73.3	24.5	19.9	1.60	1.88
16.MKS/80/4/1	76.8	18.0	16.7	0.20	2.12
17.MK60/80/7/3-2	103.3	35.0	33.3	0.40	7.18
18.MK60/80/7/3-4	82.9	33.6	17.6	0.00	1.82
19.MK60/40/3-1	102.5	30.5	19.7	0.30	3.08
20.MK60/60/5/2-1	89.5	37.0	18.5	0.00	2.90
21.MK60/40/B/3	92.7	29.3	29.8	0.20	2.50
22.LR (check)	70.5	35.6	17.2	3.05	0.78
23.RE (check)	55.7	26.6	13.6	0.95	0.72
24.MK60 (check)	68.9	24.8	23.5	0.30	0.82
25.Hnanni(check)	79.9	31.5	31.0	2.90	2.89
Mean	79.9	30.4	21.9	0.96	2.59
F-test	NS	**	NS	**	*
C.V.(%)	18.90	21.78	33.73	67.61	5.09
LSD. 05	-	13.37	-	1.35	0.24
LSD. 01	-	17.79	-	1.79	0.32

per plant ranged from 14.9 to 34.9 and 0 to 2.9, respectively. Those of the check cultivars were from 13.6 to 31.0 and 0.30 to 3.05, respectively.

The 24 selected lines of M_6 (selected from M_5) and five check cultivars were tested in the early rainy season 1992 as Preliminary yield trial and laid out in RCB with three replications. The four check cultivars in the replication 1 and 3 were severely infected by *Macrophomina* and could not be harvested.

Agronomic traits and yield are shown in Table II. The data indicated that seed yields of 25 cultivars were not significantly different due to high value of coefficient of variance (41.2 %). It is assumed that the number of harvested plants in each treatment was different from plot to plot due to *Macrophomina* sp. infection. It was also observed that the mutant lines gave larger seed size than normal check cultivars, e.g. 1 000 seeds weight of MK 60 was 2.8 - 3.00 gram. Seed yield and agronomic traits being taken into consideration, 13 mutant lines were selected for further test in the next season. They were Nos. 1, 2, 6, 7, 9, 10, 13, 15, 16, 17, 18, 22, and 23.

Table II. SEED YIELD AND AGRONOMIC TRAITS OF SESAME (M_6) IN PRELIMINARY YIELD TRIAL IN EARLY RAINY SEASON IN 1992 AT UBON FCRC

Lines	Seed yield (kg/ha)	1,000 seed weight (g)	Capsule height		Number/plant	
			first (cm)	top (cm)	node	capsule
1.MK60/100/3/3-3	820	3.53	76	187	34	44
2.MKS/40/3/2	768	3.72	70	176	29	46
3.MKS/80/1/2-4	492	3.76	76	182	23	47
4.MKS/40/4/2-2	524	3.03	90	192	27	37
5.MK60/80/1/4-2	629	3.03	54	191	36	61
6.MK60/80/1/4-1	813	3.36	76	182	39	44
7.MK60/80/7/3-4	623	3.69	67	187	42	49
8.MK60/40/3/1	490	3.23	52	168	27	54
9.MK60/60/5/2-1	808	3.37	82	202	45	44
10.MK60/40/B/3	646	3.36	83	199	41	52
11.MKS/80/4/1	158	3.63	60	155	29	44
12.MKS/40/2/1-1	207	3.21	64	182	39	55
13.MK60/80/7/3-2	681	3.20	79	168	42	52
14.MK60/100/4/1	558	3.13	67	179	29	50
15.MK60/100/1/3	707	3.31	73	186	30	46
16.MK60/80/1/2-2	833	3.25	67	181	32	50
17.MK60/80/1/4-3	745	3.11	64	178	29	46
18.MK60/100/2/2	648	3.19	64	180	34	51
19.MK60/60/7/1-2	455	2.76	61	176	37	68
20.MKS/80/5/3	497	2.63	79	190	40	49
21.K60/80/4/2	578	3.45	79	182	29	40
22.MK60/80/7/4-2	609	4.06	61	174	40	53
23.MK60/80/3/6-2	605	3.58	67	173	32	52
24.MK60/80/7/3-2	429	3.62	75	182	38	43
25.Hnanni (C)	557	3.47	92	184	27	56
Mean	595	3.35	71	181	34	49
C.V. (%)	41.2(24.0)	4.23	16.88	8.65	15.20	16.22
L.S.D. (5%)	NS	0.23	19.66	NS	8.49	13.13
(1%)		0.31	26.23		11.32	17.52

3.2. EXPERIMENT 2 (M_6 AND M_7)

Advanced generation trial of 24 elite lines and six check cultivars were arranged in RCB with three replications and carried out in late rainy season 1991. Again, major diseases were very common in the field and most plants were infected by the diseases, resulting in high variation of seed yield and numbers of harvested plant. The four check cultivars RE, LW, MKS and NW were seriously damaged by the diseases and could not be harvested. Seeds of 24 lines were sent to the pathologist for *M. phaseolina* resistance screening in the laboratory.

Agronomic traits and seed yield were shown in Table III. The data revealed that the seed yield on a plant basis ranged from 1.03 to 2.98 while the check cultivar, MK 60 gave the lowest yield of 0.97 gram. Line MK 60/40/2 produced the highest seed yield of 2.98 g/plant.

Table III. AGRONOMIC TRAITS AND SEED YIELD OF SESAME CULTIVARS, ADVANCED GENERATION (M_6) TRIAL IN LATE RAINY SEASON IN 1991 AT UBON FCRC

Lines	Capsule height		Number/plant		Seed yield per plant
	top (cm)	first (cm)	capsule	branch	(g)
1. LW/40/2	66.3	30.3	31.5	0.0	2.55
2. LW/40/3	75.0	41.7	19.7	2.3	1.41
3. LW/40/4	67.1	20.9	15.7	2.3	1.59
4. MK60/20/1	79.8	31.3	17.7	0.0	2.85
5. MK60/20/2	78.6	31.2	22.9	1.4	1.57
6. MK60/20/3	72.0	35.0	25.1	3.1	1.55
7. MK60/40/2	74.7	29.6	27.5	0.0	2.98
8. MK60/40/3	79.5	39.9	48.0	0.0	1.45
9. MK60/40/4	83.8	27.4	20.4	0.0	1.65
10.MK60/40/5	69.3	32.8	15.0	0.4	1.28
11.MK60/40/6	64.3	22.7	14.3	0.7	1.19
12.MK60/40/7	89.6	30.2	28.1	0.0	1.55
13.MK60/40/8	96.4	27.7	29.5	0.0	1.95
14.MK60/40/9	78.1	35.1	24.5	2.1	1.37
15.MK60/40/11	62.3	20.1	14.5	0.4	1.03
16.NW/20/6	110.1	43.5	22.5	0.0	2.45
17.NW/40/4	99.9	41.6	26.3	0.0	2.72
18.NW/40/5	78.5	31.9	13.9	0.8	1.51
19.MKS/60/2	75.0	24.6	13.3	0.7	1.15
20.MKS/60/3	80.1	32.3	13.5	0.9	1.19
21.LW/40/B	90.0	37.8	26.3	0.0	1.85
22.MK60/20/B	80.4	36.8	34.8	0.0	2.11
23.MK60/40/B	76.9	24.8	12.2	1.6	1.13
24.MKS/40/B	72.7	26.4	18.8	2.3	1.09
25.LR (check)	54.1	27.2	21.4	1.7	1.65
26.MK60(check)	74.6	28.3	30.0	2.8	0.97
27.Hnanni(check)	59.3	21.8	10.1	0.2	1.45
Mean	77.3	30.8	22.1	0.88	1.68
F-test	**	*	**	**	**
C.V. (%)	14.1	25.98	38.41	67.62	12.22
LSD.05	17.9	13.29	13.93	0.97	0.34
LSD.01	23.9	17.7	18.56	1.29	0.45

Average plant height and number of capsules and of branches per plant ranged from 62.3 to 110.1 cm and 12.2 to 48.0, and 0 to 3.1, respectively, and that of the check cultivars from 54.1 to 74.6 cm and 10.1 to 30.0 and 0.2 to 2.8, respectively.

The Preliminary yield trial of 11 selected lines of M_7 (selected from M_6) and four check cultivars were laid out in RCB with three replications in the early rainy season 1992. One mutant line and one check cultivar were severely infected by *Macrophomina* sp. and could not be harvested.

Agronomic traits and yields were shown in Table IV. The data in Table IV showed that seed yields were not significantly different due to unequal number of plants in the plots caused by disease infection and consequently high variation of seed yields.

Table IV. SEED YIELD AND AGRONOMIC TRAITS OF SESAME (M_7) IN PRELIMINARY YIELD TRIAL IN EARLY RAINY SEASON IN 1992 AT UBON FCRC

Lines	Seed yield		1 000 seed weight	Capsule height		Number/plant	
	(kg/ha)	(g/plt)	(g)	first (cm)	top (cm)	node	capsule
1.LW40/3	600	5.2	2.89	88	187	23	78
2.MK60/20/2	367	2.5	3.04	65	185	31	55
3.MK60/40/3	363	3.4	3.34	58	167	32	56
4.MK60/40/4	613	3.1	3.29	63	181	33	64
5.MK60/40/5	551	3.7	3.19	54	166	29	51
6.MK60/40/8	285	1.6	3.31	65	176	29	52
7.MK60/40/9	427	2.4	3.37	51	168	29	55
8.NW/40/4	550	3.3	2.89	75	186	24	51
9.NW40/5	445	2.9	2.78	73	181	26	44
10.MK60/20/B	679	3.6	2.96	92	174	18	69
11.MK60 (C)	253	2.7	3.15	57	154	22	40
12.NW (C)	379	3.8	2.95	83	171	17	35
13.Hnnani(C)	544	2.5	3.45	73	161	23	47
Mean	456	3.1	3.12	69	174	26	54
CV. (%)	45.5	(28.2) 14.07	2.87	7.15	6.02	14.07	18.97
L.S.D.(5%)	NS	0.74	0.15	8.31	17.60	6.16	17.11
(1%)		1.01	0.20	11.26	23.86	8.35	23.19

Seed yields and agronomic traits being taken into consideration, mutant lines were selected to be tested in the next season. They were LW40/3, MK60/40/3, MK60/40/4, MK60/40/5, MK60/40/9, NW40/4 and MK60/20/B.

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GENETIC IMPROVEMENT OF SESAME FOR PLANT ARCHITECTURE AND GRAIN YIELD THROUGH NUCLEAR TECHNIQUES

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Abstract

To develop high yielding, widely adapted varieties of sesame a mutation breeding project was initiated with the partial support of FAO/IAEA. Genetic variation was observed for a number of yield components. Mutants with earliness in flowering, short stature, monostem, heavy bearing and high yield have been selected. True breeding lines developed from the present project have exhibited impressive yield potential in preliminary yield evaluation. This report discusses the results of these investigations.

1. INTRODUCTION

Pakistan is chronically deficient in the production of edible oils. Domestic production is hardly sufficient to meet 20 % of the demand of 115 million people. Thus the country is constrained to import edible oil in large quantity involving huge expenditure in hard earned foreign exchange (Table I). Under such a situtation it is imperative to adapt all appropriate measures to improve the domestic production and achieve self sufficiency in edible oils. Realizing the importance of induced mutations in improving the crop plants [1] to supplement our conventional breeding programme of sesame, a mutation breeding project was initiated entitled "Genetic improvement of *Sesamum indicum* through induced mutations".

TABLE I. EDIBLE OIL IMPORTS

Year	Rupees (million)
1980-81	2 610
1981-82	3 670
1982-83	6 440
1994-95	15 000

The main objectives of this project were to induce and manipulate the genetic variability in order to confer specific improvements, such as earliness, short stature, suitable plant type, heavy bearing, high seed yield and high oil content in agronomically important sesame varieties of the southern region of Pakistan. The present paper reports and discusses the results of mutation breeding of sesame in Pakistan.

2. MATERIALS AND METHODS

The homogeneous seeds of three varieties 'Pr.19-9', 'Pr.14-2', and 'S-17' were exposed to different doses of gamma radiation, i.e. 0, 100, 200, 300, 400, 600 and 800 Gy and fast neutron (Nf) i.e. 0, 10, 20, 30, 40, 60 and 80 Gy. Nf treatment of seeds was done at Seibersdorf Lab. through the courtesy of IAEA Vienna. The irradiated seeds were divided into two parts for laboratory and field studies.

2.1. Laboratory studies

For laboratory studies 100 seeds from gamma rays and Nf treated material along with respective controls were planted into sterilized soil in plastic trays and placed in germinator with temperature adjusted at 30 °C. Germination counts were recorded from 4th day of sowing till complete emergence. Two weeks after sowing the seedlings were uprooted, rinsed with water and placed in moistened paper towels. The measurements were recorded on 10 longest seedlings from all the treatments for seedling height and 50 % lethal dose (LD_{50}) values for various varieties were determined according to the procedure described earlier [2].

2.2 Field studies

To grow the M_1 generation, the second lot of irradiated seeds was planted in the field. Various observations were recorded on 10 randomly tagged plants per treatment. At maturity 5 seeds were collected from each M_1 plant and bulked by treatments to form the M_2 seeds population.

The M_2 populations were thoroughly screened at all the stages of crop growth in the field and desirable selections for various agronomic characters were made.

To confirm thebreeding behaviour, offspring putative mutants were grown in plant progeny rows. One row each of the respective mother variety was grown after every ten rows of mutant lines for comparison. On the basis of agronomic data and visual comparison, true breeding lines were selected. True breeding lines were further screened and evaluated in preliminary yield trials (PYT). Mutant lines showing better performance than the respective parent were identified for further evaluation in microyield trials (MYT) later during Kharif 1993.

3. RESULTS AND DISCUSSION

3.1. Radiosensitivity studies

Dose response studies were conducted to determine the optimum doses of gamma radiation for various varieties of sesame. The seedling emergence started on the 4th day after sowing in non-irradiated material, while in all the treated populations it was delayed. The frequency of germination in the irradiated material at different intervals was correspondingly delayed with increasing dose of radiation (Table II). The depressive effect of radiation on the germination percentage was not considerably greater upto 400 Gy. However, at 600 Gy and 800 Gy germination percentage was significantly reduced in all the varieties both under laboratory and field conditions.

Seedling height data indicated that with the increase of dose rate the seedling growth decreased and inhibition was more in variety Pr.19-9 followed by S-17 and Pr.14-2 respectively. However, 50 % reduction dose (RD_{50}) for seedling height lies between 400 Gy and 500 Gy, except for variety S-17 (600 Gy) which seems fairly radio-resistant (Fig. 1).

3.2. M_1 generation

The treated seeds were planted in the field to grow the M_1 generation. At higher doses i.e. 600 Gy and 800 Gy gamma rays and 60 Gy Nf the plant growth was generally stunted. Various observations were recorded on 10 randomly tagged plants per treatment. Results are depicted in Figures 2 and 3 for plant height. From M_1 studies the following conclusions were drawn:

(1) Plant height (Figs.2-3), numbers of mature capsules per plant and seed yield per plant decreased in treated populations [3].
(2) Branches per plant and number of immature capsules per plant slightly increased in irradiated populations.
(3) Flowering and maturity was delayed.

TABLE II. SEED GERMINATION AT 24 HOURS INTERVALS AS AFFECTED BY SEED IRRADIATION WITH GAMMA-RAYS

Varieties	Treatments	Days after sowing												Total No.of seeds germinated	Germination%	
		4	5	6	7	8	9	10	11	12	13	14	15		Lab.	Field
Pr.19-9	Control	5	22	18	20	15	7	5	1	-	-	-	-	93	93	80
	100 Gy	-	16	15	10	13	10	12	10	6	3	-	-	95	95	80
	200 Gy	-	9	13	14	13	14	10	9	6	3	-	-	91	91	79
	300 Gy	-	6	16	23	19	9	8	4	3	2	-	-	90	90	65
	400 Gy	-	6	14	20	22	8	6	3	4	1	-	-	84	84	60
	600 Gy	-	2	3	5	8	6	6	8	2	3	-	-	43	43	43
	800 Gy	-	-	2	2	3	3	5	4	4	3	-	-	26	26	15
Pr.14-2	Control	4	20	21	18	15	11	5	2	-	-	-	-	96	96	81
	100 Gy	-	12	20	19	16	10	8	5	3	-	-	-	93	93	79
	200 Gy	-	10	22	15	14	11	10	8	3	3	-	-	96	96	71
	300 Gy	-	7	13	22	10	10	9	8	3	2	-	-	84	84	62
	400 Gy	-	5	8	20	20	15	5	4	3	2	-	-	82	82	82
	600 Gy	-	3	5	8	10	6	6	4	2	1	-	-	45	45	45
	800 Gy	-	1	4	4	5	6	6	4	4	2	-	-	36	36	21
S-17	Control	8	18	20	19	11	10	4	2	-	-	-	-	92	92	78
	100 Gy	-	10	16	23	18	10	8	5	2	-	-	-	82	82	73
	200 Gy	-	8	12	20	15	12	10	7	2	1	-	-	87	87	62
	300 Gy	-	6	10	16	15	14	12	8	2	2	-	-	85	85	53
	400 Gy	-	5	9	10	19	10	14	10	4	2	-	-	83	83	41
	600 Gy	-	3	3	5	7	8	10	10	5	1	-	-	52	52	32
	800 GY	-	1	3	2	3	5	6	5	6	5	-	-	36	36	17

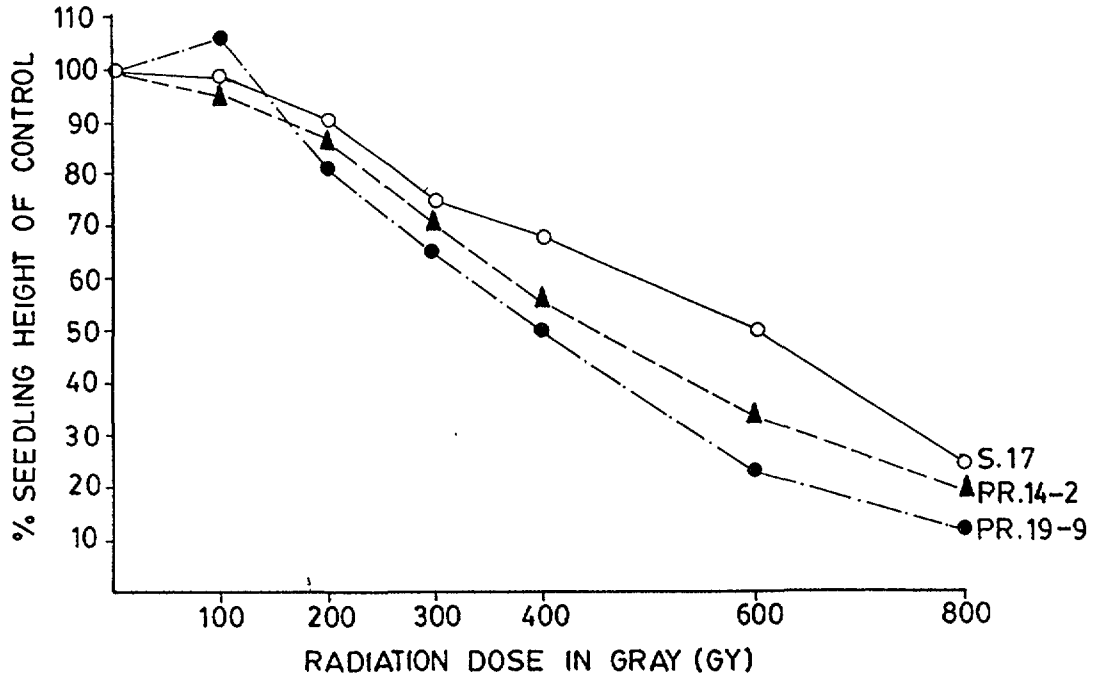

Fig.1. Effect of gamma rays on seedling height in sesame.

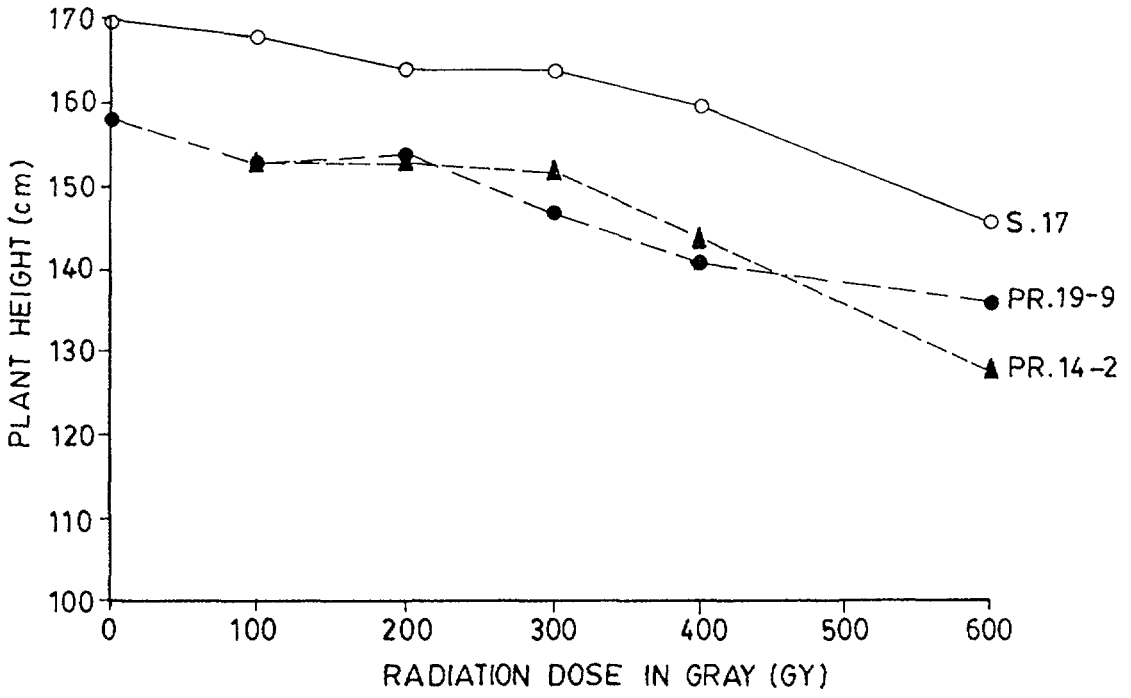

Fig 2. Effect of gamma rays on plant height in sesame.

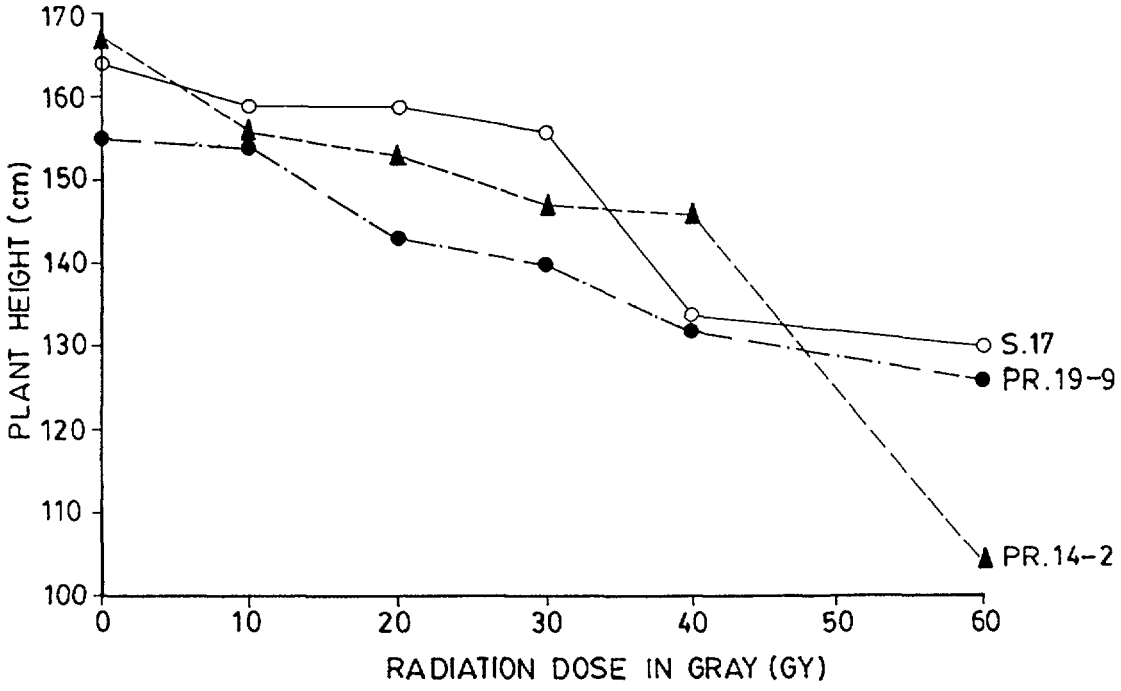

Fig.3. Effect of fast neutrons on plant height in sesame.

3.3. Selection of desirable mutants in M_2

In the treated populations 122 putative mutants were isolated for different desirable attributes (Table III). The characteristics of desirable segregants were, early flowering, short stature, mono-stem, semi-indehiscent type, compact plant type, heavy bearing and higher yield. Most selections on character basis were for monostem followed by early flowering, heavy bearing, short stature and semi-indehiscent. Mutation frequency when observed on varietal basis was maximum for Pr.14-2 followed by Pr.19-9 and S-17.

TABLE III. PUTATIVE MUTANTS SELECTED IN THE M_2 POPULATIONS OF SESAME.

Variety	Early flowering	Semi indehiscent	Short internode	Long capsule	Mono stem	Compact	Heavy bearing	Total
Pr.14-2	12	5	7	3	15	4	6	52
Pr.19-9	5	2	4	4	10	3	8	36
S-17	6	4	6	2	6	2	8	34
Total	23	11	17	9	31	9	22	122

3.4. Confirmation of true breeding lines (M_3)

On the basis of data and visual comparison 29 true breeding lines (seven from variety S-17, eight from variety Pr.19-9 and fourteen from Pr. 14-2) were selected for further screening and evaluation. Most of the M_2 selections (Table III) segregated and did not breed true in M_3. Only 23.8 % of the M_2 selections bred true for selected attributes. Segregation to such a large extent could be due to polygenic nature of the characters under study [4]. The agronomic data of these true breeding lines, along with their identifying characters are presented in Tables IV - VI. Two mutants with substantial improvement in fruiting were observed (Pr.14-2 HB-2 and Pr.14-2 PB-1). Induced mutations also improved seed yield, which is a character governed by polygenes. Mutant Pr.14-2 MS-1, Pr.14-2 MS-2, Pr.14-2 EF-2 and Pr.19-9 MS-1 showed much superior seed yield potential than their respective mother varieties.

TABLE IV. AGRONOMIC DATA OF TRUE BREEDING M_3 LINES DERIVED FROM VARIETY PR.14-2 AND THEIR IDENTIFYING CHARACTERS.

Sr. No.	Mutant lines	Days to flower	Plant height (cm)	Branches per plant	Capsules per plant	Seed yield/ plant (gm)	Identifying characters
1	Pr 14-2(cont.)	46	146	2	51	6.05	--
2	Pr 14-2 T-1	50	190	3	80	11 04	Very tall
3	Pr 14-2 T-2	47	196	-	136	11 53	Very tall, Mono-stem
4	Pr.14-2 HB-1	42	155	4	200	14.69	Heavy bearing, compact plant, profuse branching
5	Pr.14-2 HB-2	53	160	4	230	15 40	Heavy bearing, tall, high yield
6	Pr.14-2 D-1	48	87	1	143	13.30	Dwarf, short internodes, high yield
7	Pr.14-2 PB-1	55	173	5	232	19.94	Profuse branching, tall, high yield
8	Pr 14-2 PB-2	46	145	5	208	18.49	Profuse compact branching, high yield
9	Pr.14-2 PB-3	44	155	4	162	15.07	Profuse branching, high yield
10	Pr 14-2 PB-4	48	130	4	122	13.00	Profuse branching,short internodes, high yield
11	Pr.14-2 EF-1	38	153	1	144	16.90	Early flowering, high yield
12	Pr.14-2 EF-2	39	102	4	139	22.23	Early flowering, profuse branching, short internodes, high yield
13	Pr.14-2 MS-1	48	108	-	228	31.40	Mono-stem, short culm, heavy bearing, high yield
14	Pr.14-2 MS-2	42	116	-	220	24.00	Mono-stem, heavy bearing, high yield
15	Pr.14-2 MS-3	43	145	-	134	19.00	Mono-stem, high yield

TABLE V. AGRONOMIC DATA OF TRUE BREEDING M_3 LINES DERIVED FROM VARIETY PR.19-9 AND THEIR IDENTIFYING CHARTACTERS

Sr. No	Mutant lines	Days to flower	Plant height (cm)	Branches per plant	Capsules per plant	Seed yield/ plant (gm)	Identifying characters
1	Pr 19-9(cont.)	48	152	2	64	7.5	--
2	Pr.19-9 HB-1	46	148	4	139	15.5	Heavy bearing, profuse branching, high yield
3	Pr.19-9 T-1	55	185	4	195	19.5	Tall, profuse branching, heavy bearing, high yield
4	Pr 19-9 PB-1	48	142	5	200	14.5	Profuse branching, heavy bearing, compact plant type
5	Pr 19-9 EF-1	35	110	-	94	10 7	Early flowering, monostem, short stature
6	Pr.19-9 D-1	44	74	3	150	13.5	Dwarf
7	Pr.19-9 D-2	49	92	4	75	11.5	Dwarf, profuse branching
8	Pr.19-9 MS-1	50	160	-	210	20.0	Monostem, heavy bearing, high yield
9	Pr.19-9 MS-2	45	150	-	180	16.5	Monostem, heavy bearing, high yield

TABLE VI. AGRONOMIC DATA OF TRUE BREEDING M_3 LINES DERIVED FROM VARIETY S-17 AND THEIR IDENTIFYING CHARACTERS.

Sr No	Mutant lines	Days to flower	Plant height (cm)	Branches per plant	Capsules per plant	Seed yield/ plant (gm)	Identifying characters
1	S-17(cont)	53	144	2	79	8 5	--
2	S-17 EF-1	41	147	4	135	11 5	Early flowering, heavy bearing, profuse branching
3	S-17 EF-2	39	138	3	110	11.3	Early flowering
4	S-17 St-1	48	110	2	106	14 6	Short stature
5	S-17 MS-1	55	152	-	156	16 4	Monostem, heavy bearing, high yield
6	S-17 MS-2	46	147	-	172	17 2	Monostem, heavy bearing, high yield
7	S-17 D-1	52	72	4	130	10 5	Dwarf, profuse branching
8	S-17 SI-1	49	136	2	145	16 2	Semi indehiscient

3.5. Preliminary yield trial of true breeding mutants (M_4)

Twenty nine true breeding lines, selected for earliness, mono-stem, short internodes, compact branching, heavy bearing and high yield along with mother varieties (Pr.14-2, Pr.19-2 and S-17) were evaluated for seed yield performance in two separate trials.

3.5.1. Preliminary yield trial-1

Fifteen mutant lines developed from varieties Pr.19-9 and S-17 were evaluated in this trial. Mutant line Pr.19-9 MS-1 produced significantly highest seed yield (1533 kg/ha) followed by mutant line Pr.19-9 T-1 (1506 kg/ha). The mother variety (Pr.19-9) gave the lowest seed yield (551 kg/ha). Non-significant differences in oil content were observed among the mutant lines and parent varieties (Table VII).

TABLE VII. PRELIMINARY YIELD TRIAL-I OF SESAME MUTANTS (M_4)

Mutants/varieties	Days to 50% flowering	Seed yield (kg/ha)	Oil content (%)
S-17 (Cont.)	55	600	53.15
S-17 EF-1	43	807	52.95
S-17 EF-2	40	802	53.00
S-17 St-1	49	1109	53.10
S-17 MS-1	57	1201	52.95
S-17 MS-2	51	1298	53.00
S-17 D-1	53	906	53.15
S-17 S-1	52	1201	53.10
Pr.19-9 (cont.)	51	551	52.85
Pr.19-9 HB-1	48	1010	52.70
Pr.19-9 T-1	54	1506	52.60
Pr.19-9 PB-1	52	1018	52.60
Pr.19-9 EF-1	37	707	52.60
Pr.19-9 D-1	44	878	52.55
Pr.19-9 D-2	48	733	52.90
Pr.19-9 MS-1	54	1533	51.95
Pr.19-9 MS-2	47	1073	52.50
LSD 5 %		43.05	
1 %		56.58	

3.5.2. Preliminary yield trial-2

Fourteen mutant lines derived from variety Pr.14-2 were evaluated in this trial. Mutant line Pr.14-2 MS-1 produced significantly highest seed yield (1840 kg/ha) followed by Pr.14-2 MS-2 (1640 kg/ha) and Pr.14-2 EF-2 (1539 kg/ha). The parent variety Pr.14-2 produced the lowest seed yield (571 kg/ha). The oil content differences among mutant lines and parent variety were non-significant (Table VIII).

TABLE VIII. PRELIMINARY YIELD TRIAL-II OF SESAME MUTANTS (M_4)

Mutants/varieties	Days to 50% flowering	Seed yield (kg/ha)	Oil content (%)
Pr.14-2(cont.)	14	571	51.95
Pr.14-2 T-1	53	703	51.75
Pr.14-2 T-2	50	710	50.90
Pr.14-2 HB-1	45	1000	52.00
Pr.14-2 HB-2	56	1043	51.95
Pr.14-2 D-1	51	975	50.90
Pr.14-2 PB-1	58	1476	51.90
Pr.14-2 PB-2	49	1336	52.00
Pr.14-2 PB-3	47	1000	50.95
Pr.14-2 PB-4	51	965	51.90
Pr.14-2 EF-1	41	1100	51.97
Pr.14-2 EF-2	42	1539	52.00
Pr.14-2 MS-1	51	1840	51.90
Pr.14-2 MS-2	45	1648	51.75
Pr.14-2 MS-3	46	1330	51.80
LSD- 5 %		53.62	
1 %		72.27	

On the basis of field performance, from PYT I and II, ten high yielding mutants were selected to further evaluate in micro yield trial during Kharif 1993.

Sesame appears to be very suitable material for inducing useful mutations as in soybean [4 - 5], wheat [6] and sesame [7]. Our studies have clearly demonstrated that induced mutations can be successfully utilized also for the improvement of various quantitative characters of sesame. Further, extensive evaluation of our high yielding mutant lines of sesame will be useful in direct release of improved varieties.

New germplasm has also been generated for useful agronomic traits. This germplasm will be utilized for breeding new cultivars of sesame through synergistic approaches [8] including recurrent irradiation and recombination breeding.

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INDUCED MUTATIONS AND ANTHER CULTURE FOR SESAME IMPROVEMENT

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Abstract

Seeds of two Sri Lankan sesame (*Sesamum indicum* L.) cultivars MI 2 and MI 3 and of UCR82-203 NS which has indehiscent capsules were treated with gamma rays, ethyl methanesulphonate and diethyl sulphate. Selections were made in segregating populations for improved yield components and morphological characters. Radiation sensitivity studies indicated that the seeds of MI 3 variety were more resistant to irradiation than MI 2. The M_1 plants also showed varietal differences in growth reduction. Fifty percent growth reduction for plant height in MI 2 was in the range of 500 - 600 Gy and that for MI 3 was 750 - 1000 Gy. Promising mutants after screening in preliminary trials were tested for yield and adaptability in multilocational trials in different agro-climatic zones. A wide variation and increased mean values of yield and components were recorded in selected mutants in the preliminary trials. Two mutants, MB 29 and MB 33, consistently outyielded the recommended cultivar MI 3 in regional trials. The mutant MB 13 was also promising. MB 29 and MB 29w have a brittle seed coat easy for decortication. A number of mutants with improved characters have been isolated, characterized and included in the cross- breeding programmes. Mutants MB 29 and MB 33 recorded better germination than MI 2 under high osmotic pressure and should be tested for tolerance to drought at early growth phases. The shoot/root ratio was least in MB 29, MB1 and MB 1-1. The shortest and more synchronous flowering period recorded in MB 29 and C 10 is important in reducing shattering losses and escaping from drought at flowering stage. Two mutants which have lost the undesirable effects associated with the indehiscent character in UCR82-203 NS were later found to have lost the indehiscent character too, suggesting that the undesirable effects of the indehiscent (*id id*) locus are pleiotropic. Anther culture studies were undertaken with the objective of using doubled haploids for sesame improvement. Flower buds of 5 - 7 mm length incubated at 8 °C for 24 h plated on Murashige and Skoog medium supplemented with 10 mg l^{-1} 2,4-D and 2 mg l^{-1} of both IAA and BAP kept at 25 °C in the dark after plating recorded highest rate (46 %) of callus induction. Studies on the regeneration of haploid plants are in progress.

1. INTRODUCTION

Sesame (*Sesamum indicum* L.) is the major annual oilseed crop grown in Sri Lanka. It's average yields are low with a seasonal fluctuation from 378 kg/ha to 700 kg/ha [1]. There is much higher potential for this crop as some sesame varieties have recorded yields over 1 450 kg/ha [1, 2]. Low yields of sesame have been attributed not only to poor management, low inputs and environmental constraints but also to the lack of suitable cultivars with wide adaptability, high yield potential with tolerance to biotic and abiotic stresses [3 - 7].

Narrow adaptation of cultivars, indeterminate growth habit, shattering losses and pest and disease attack are some of the major constraints to increased yield in sesame [5 - 7]. In spite of the fact that sesame is an ancient crop, the available genes for indehiscent capsules, determinate growth habit, resistance to some of the major diseases and pests are very limited and very often have undesirable pleiotropic effects or linkages [3 - 7]. Therefore character improvement of these genotypes and additional variability in the adapted local varieties through induction of mutations could be rewarding.

The induced variability in different sesame genotypes for quantitative characters associated with yield and for morphological characters having breeding value are reported in this paper. Also reported are the results of drought resistance studies of promising mutants and sesame anther culture for the development of a protocol for efficient haploid production.

2. MATERIALS AND METHODS

2.1. Genotypes used for mutation induction

The cultivars that have a potential for further improvement were selected for mutagenic treatment. Due to wide variation reported in radio-sensitivity of seeds of sesame cultivars, optimal doses for seed irradiation were selected after growth reduction studies with MI 2 and MI 3 cultivars. For MI 2 variety doses in the range of 100 to 1 000 Gy were used and for MI 3 variety 100 to 1 750 Gy were used for radiation sensitivity studies. The irradiated seeds were grown in three replications in a randomized complete block design and germination percentage, plant height, survival percentage and seed yield were studied.

The cultivar MI 2 which has recorded the highest yield in Sri Lanka was used to further improve its potential yield and the seed quality. This cultivar has black seeds, basal branching habit and it produces one bicarpellate capsule per leaf axil. The cultivar MI 3 was also used in mutation breeding and is unbranched, has three capsules per leaf axil and white seeds. Both are recommended for cultivation in Sri Lanka. More details about these cultivars have been reported [1, 2, 8 - 10].

Seeds of UCR 82-203 NS, a cultivar with indehiscent capsules but with other undesirable side effects [4, 6, 11] were irradiated with gamma rays, treated with ethyl methanesulphonate and diethyl sulphate, with the objective of breaking any linkages as it has not been possible to separate the indehiscence from the undesirable traits so far [11].

2.2. Sampling and selection procedures

First formed five capsules of surviving M_1 plants were used to collect seeds for single plant progenies or making bulk populations for selection. Selection for shorter internodes, longer capsules, more erect branches (in mutants of MI 2 variety to reduce seed losses at harvest and to accommodate more plants per unit area), narrow leaf, increased pubescence, three capsules per leaf axil (in MI 2) were carried out in the second and third generations after mutagen treatment. Normal looking plants with increased number of capsules were selected for biometrical measurements and the best were carried forward. Details of selection procedures for quantitative characters have been reported [10].

In the third and subsequent generations, selected plants as well as plants grown in bulks from unselected material were screened and selected for seed colour and other desirable characters.

2.3. Yield evaluation

After preliminary screening in the breeding nurseries, the promising mutants were tested in larger plots with other promising breeding lines and with MI 3 variety as the control. The multi-locational experiments were conducted at the Research Farm of the Faculty of Agriculture at Mapalana in the low country of southwestern Sri Lanka; at the Field Station of the Institute of Fundamental Studies at Kengalla in the mid country intermediate zone, at Kurunegala in the low country dry zone and at the Government Farm, Batatha in the low country dry zone of southern Sri Lanka. The varietal screening methodology has been described in detail [8]. Some mutants were back-crossed to the parent variety or crossed with other material where further improvement was desired

2.4. Drought resistance studies

Different criteria were used to study the mutants for tolerance to water deficit.

2.4.1. Seed germination under high osmotic pressure

Seeds of twelve mutants and parent cultivars MI 2 and MI 3 were germinated in ordinary tap water, manitol solutions having osmotic pressures of 4,6 and 8 atm and polyethylene

glycol at 3, 3.5 and 4 atm pressure. Experiments were conducted separately for manitol and polyethylene glycol. Twenty five seeds per petri dish were used and five replications in a completely randomized design was used.

2.4.2. Leaf characters

The leaf disk method was used to measure the leaf area of field grown mutants.

Stomatal frequency was measured in three random plants using three leaves each from upper, lower and middle parts of the plants. Wax replicas were made randomly and the wax layer was peeled off, mounted on a microscope slide and the stomata within the field of view of the microscope were counted. The area of the field of view was determined using a calibrated slide.

Stomatal response to artificially induced water stress was monitored by measuring the guard cell dimensions at five minute intervals after detaching the leaves from plants growing under 75 % soil water saturation. Three leaves each from 3rd, 6th and 8th nodes of three random plants were used and the wax replicas were made at five minute intervals. Five guard cells were randomly selected in each field of view for measurements.

2.5. Anther culture

With the objective of using doubled haploids in the breeding of sesame, attempts are being made to develop a protocol for efficient haploid production from sesame anthers.

For establishing callus from sesame anthers, Murashige and Skoog (MS) medium [12] supplemented with different concentrations of 2,4 dichlorophenoxy acetic acid (2,4-D), 3-indole acetic acid (IAA) and 6-benzylaminopurine (BA) were used, as earlier studies have indicated the suitability of this medium [13]. Different concentrations of hormones and light regimes used have been described [14]. Anthers of MI 3 variety were used.

3. RESULTS

3.1. Varietal differences in radiation sensitivity

The growth reduction studies conducted with two recommended cultivars MI 2 and MI 3 revealed that MI 3 is more resistant to gamma radiation than MI 2 and that 50 % survival of germinated seeds was in the range of 750 - 1 000 Gy for MI 3 and 500 - 600 Gy for MI 2 (Fig. 1). The plant height also showed similar trends.

3.2. Selection of mutants and variability created

Selections in mutant populations were carried out for morphological changes which could contribute to increased yields as well as for quantitative characters. For this purpose bulk populations sampled from first formed five capsules of the M_1 plants as well as single plant progenies were used. Results of selection, heritability and correlations among characters in different generations have been reported elsewhere [10]. The variation observed among selections in preliminary screening of mutants in M_4 and M_5 generations is presented in Table I. At the preliminary screening stage the mutants recorded higher mean yield, plant height and number of capsules. The mean number of seeds per capsule was less in mutants selected from MI 2 variety than in the parent as only few selections could be made directly for this character. The mean number of seeds per capsule has also recorded an increase in the mutants of MI 3 variety although direct selection for this character was attempted only in few cases. This variety generally records a lower number of seeds per capsule (Table I).

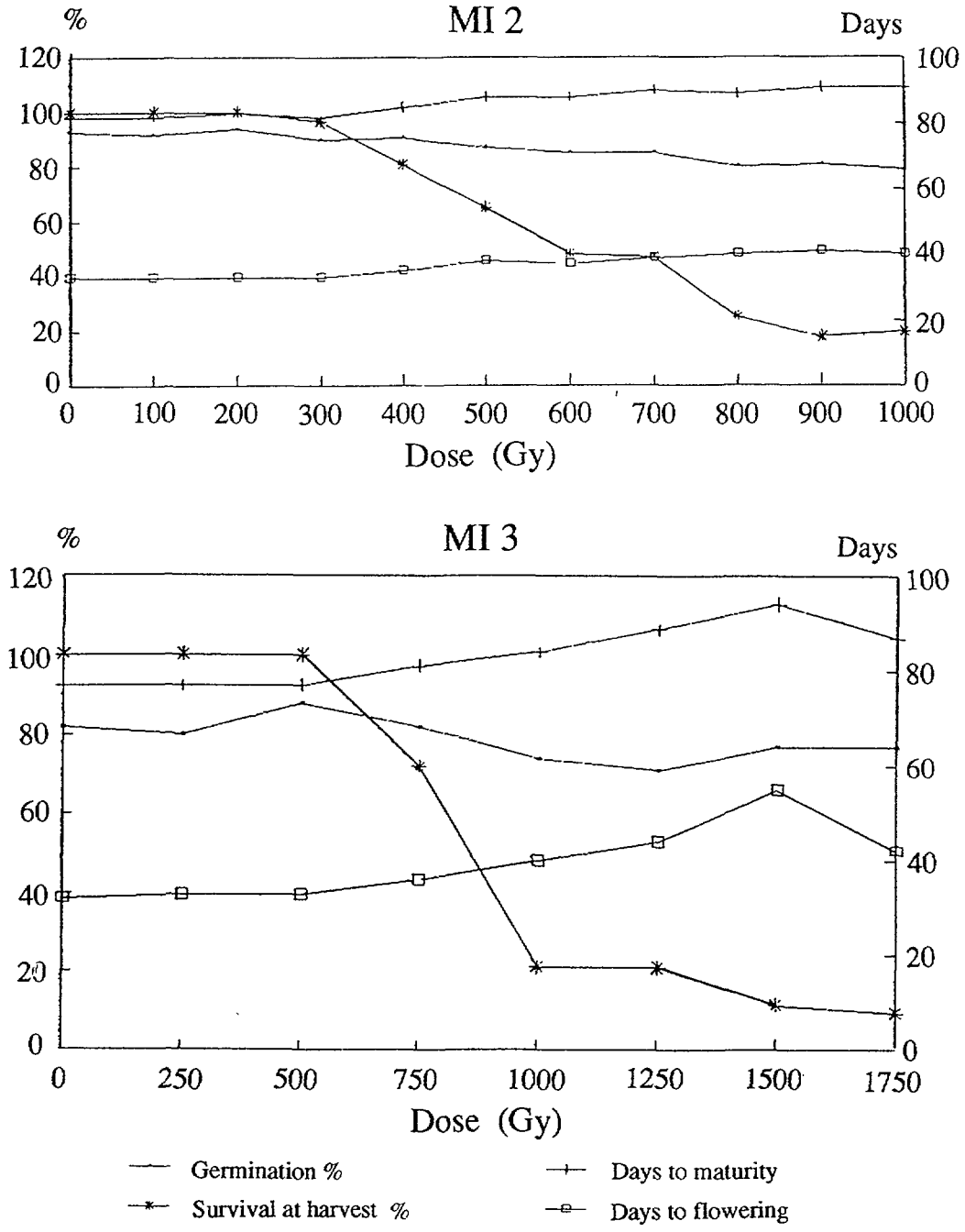

Fig.1. Varietal response to seed irradiation.

3.3. Performance of mutants in regional trials

The high yielding mutants were included in the major yield trials of the breeding programme and were tested in different locations along with the other high yielding breeding lines selected from the segregating populations and germplasm collection. The mean performance of the mutants compared with the control MI 3 variety at two or three locations during each growing season is given in Table II.

The mutants MB 29 and MB 33 have consistently outyielded the recommended variety MI 3 throughout the period of testing. Out of the six seasons of testing MB 13 recorded lower yield than MI 3 only during the northeast monsoon season of 1990/91. Due to relatively poor performance of MB 7 and MB 32, they were discontinued from the testing programme in 1992.

TABLE I. MEANS AND RANGES OF PERFORMANCE OF MUTANT LINES COMPARED WITH PARENT CULTIVARS

Materials	Age (day)	Plant height (cm)	Capsules per plant	Seeds per capsule	1000 seed weight (g)	Seed yield per plant (g)
Mutants of 'MI 2' variety						
43 lines in M_4[a]	93 (78-99)	119 (81-146)	54 (31-93)	59 (37-80)	3.3 (2.8-4.2)	8.4 (1.3-13.8)
MI 2	89	117	48	64	3.2	7.2
33 lines in M_5[a]	90 (83-96)	123 (90-137)	56 (28-69)	63 (44-82)	3.1 (2.7-3.5)	8.7 (3.8-10.2)
MI 2	90	131	53	69	2.9	8.4
Mutants of 'MI 3' variety						
78 lines in M_4[a]	91 (74-96)	98 (83-126)	41 (29-64)	63[b] (41-81)b	3.2[b] (2.7-3.9)	7.7[b] (3.5-12.7)
MI 3	85	94	35	58	3.1	5.8
35 lines in M_5[a]	88 (75-92)	95 (78-119)	42 (30-62)	57 (38-74)	3.3 (2.8-3.7)	7.4 (3.8-11.4)
MI 3	84	89	37	52	3.2	5.2

a : The ranges for mutants are given in paranthesis.
b : Only for 40 high yielding mutants selected visually.

3.4. Drought resistance of mutants

Although sesame is considered to be a drought resistant crop, drought at certain phases of growth can cause severe growth and yield reduction. Water stress at seedling and flowering stages are the most important in this respect. The mutants selected for improved yield and disease resistance in Sri Lanka [8,9] were tested for their characters related to drought resistance.

TABLE II. PERFORMANCE OF SESAME MUTANTS IN REGIONAL TRIALS

Treatment	Age days	Yield, kg/ha					
		Maha[a] 1989/90	Yala[b] 1990	Maha 1990/91	Yala 1991	Maha 1991/92	Yala 1992
MB 1	88	-	628	492	989	508	968
MB 7	85	-	721	684	725	339	-
MB 13	86	1 055	742	517	1 003	461	793
MB 29	86	-	-	1 011	925	555	655
MB 32	87	815	718	620	651	387	-
MB 33	86	-	-	865	1 123	475	804
MI 3(control)	85	561	684	824	871	440	728
Number of locations		3	2	2	3	2	2

a - Maha - Northeast monsoon season.
b - Yala - Southwest monsoon season.

3.4.1. Seed germination under increased osmotic pressure

Moisture stress can be simulated by increasing the osmotic pressure of water. Germination in solutions of high osmotic pressure is one way of estimating the tolerance of genotypes to moisture stress at seedling stage. Germination percentages of seeds of mutants in manitol and polyethylene glycol solutions are given in Table III. Seed germination in manitol was highest in MI 3 variety. Many mutants derived from MI 2 variety had greater capacity to germinate than the parent. Particularly noteworthy in this respect are MB-29w and MB 33.

There was a relationship between germination percentage in manitol and polyethylene glycol. Thus, the mutants MB 29w and MB 33 recorded higher germination percentage in polyethylene glycol solutions as well (Table III).

TABLE III. PERCENTAGE SEED GERMINATION IN MANITOL AND POLYETHYLENE GLYCOL SOLUTIONS

Treatments	Water	Manitol			Polyethylene glycol		
		4 atm	6 atm	8 atm	3 atm	3.5 atm	4 atm
MB 1	100	42	4	0	87	66	42
MB 1-1	100	39	7	0	85	68	53
MB 2	98	44	8	3	81	71	54
MB 7	100	58	14	6	91	51	45
MB 13	99	39	8	2	76	49	35
MB 29w	100	67	28	13	92	79	81
MB 30	100	48	10	4	80	67	45
MB 32	100	59	11	3	83	59	40
MB 33	99	63	19	8	86	76	70
C 8	98	57	10	4	78	62	54
C 10	100	38	6	0	79	63	52
C 37	100	22	4	0	86	78	56
MI 2	98	53	7	0	79	54	37
MI 3	99	94	74	30	91	69	53
LSD 0.05		Manitol 6.2			Polyethylene glycol 4.7		

3.4.2. Stomatal frequency and behavior

Stomatal frequency and behavior of stomata under water stress could give further indications of drought resistance of genotypes. Some of the mutants of MI 2 variety including MB 33 had fewer stomata per unit area than the parent cultivar (Table IV). Faster reaction of stomatal apparatus to water stress induction could be another indication of drought resistance.

Leaves at different stages of growth were studied in separate experiments but the reaction of stomata to simulated water stress was similar in leaves of different age in the same genotype. Therefore mean values of percentage decrease in width of stomatal aperture in some of the mutants studied is given in Fig. 2. Mutants MB 1-1 and MB 30 recorded more efficient closure of the stomatal apparatus to induced water stress, compared to the parent variety MI 2. The rate of closure of stomata of MB-29w was much slower (Fig. 2).

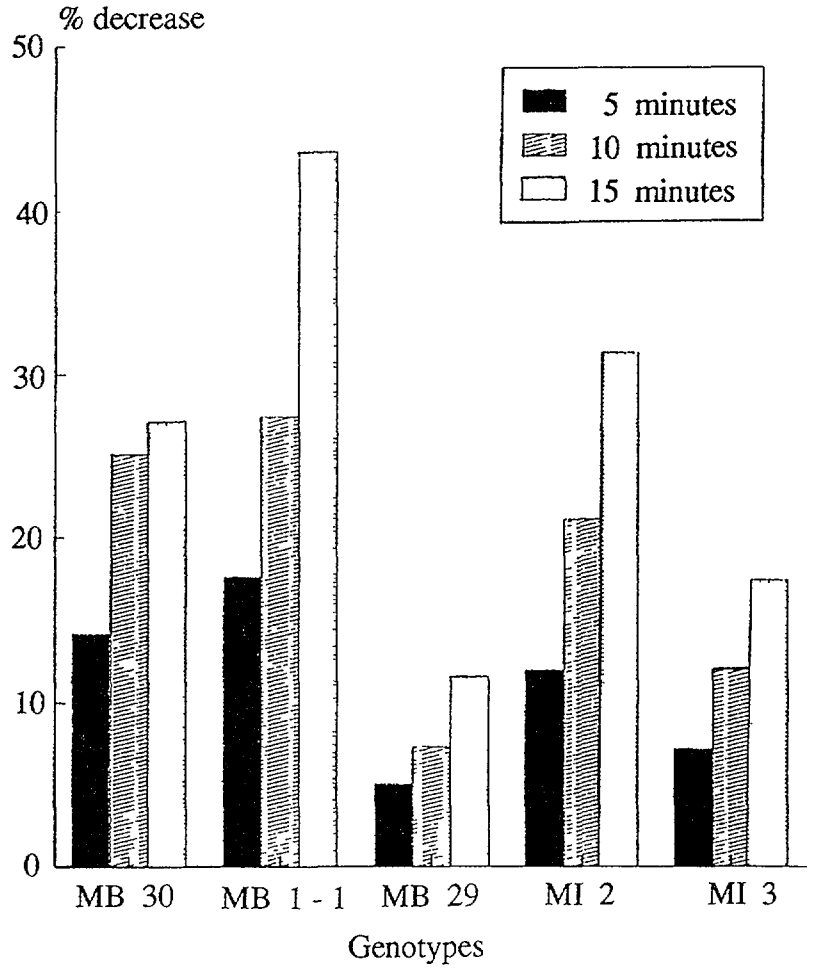

Fig.2. Percentage reduction in stomatal width after leaf detachment.

TABLE IV. SOME CHARACTERS RELATED TO DROUGHT RESISTANCE IN SESAME MUTANTS

Treatment	Leaf area, (cm^2)	Stomata per mm^2 (No.)	Shoot: root ratio by weight	Days to flower	Duration of flowering (day)	Leaf pube-scence[a]
MB 1	443	50	5.6:1	40	35	3.0
MB 1-1	430	43	5.4:1	41	35	2.7
MB 2	367	52	13.5:1	35	31	2.0
MB 29w	411	56	5.3:1	42	26	2.0
MB 30	510	73	7.6:1	36	31	2.0
MB 33	449	42	8.7:1	36	34	3.5
C 8	660	46	7.0:1	42	27	2.0
C 10	995	46	9.0:1	41	25	2.3
MI 2	438	56	12.6:1	36	33	2.1
MI 3	340	24	6.8:1	35	36	2.3
LSD 0.05	63	6.7	ns[b]	-	-	ns[b]

a : Glabrous = 0; Moderate = 5; Extemely high = 10
b : Non-significant

3.4.3. Other characters related to drought tolerance

The morphological and agronomic characters of the most promising mutants of MI 2 variety selected from the preliminary screening nurseries were studied in detail. Some of the characters which could contribute to increased drought resistance are presented in Table IV. The total leaf area of MB 2 was significantly less than MI 2. Many mutants had a leaf area comparable to the parent variety MI 2. Four mutants had significantly lower stomatal frequency than MI 2. MB 30 recorded a relatively higher number of stomata. The MI 3 variety which was used in later experiments on mutation breeding had less than half the number of stomata per unit area than MI 2 variety. It had a lower shoot/root ratio as well. Although not significant statistically, most of the mutants recorded a lower shoot/root ratio than MI 2 variety. The mutants MB 29w, MB-1 and MB 1-1 had the lowest shoot/root ratio among the mutants.

Drought during the flowering period can severely affect seed yield of sesame [3,4]. The shortest period of flowering was recorded in MB 29w and C-10. The leaf pubescence, though it did not exhibit much variation, was higher in MB 33, MB-1 and MB 1-1 (Table IV).

3.5. Results of seed irradiation of UCR 82-203 NS variety

In a population of more than 150 000 M_2 plants of UCR 82-203 NS variety representing about 40 000 M_1 parent plants, two mutants were recovered which have lost the undesirable effects associated with the indehiscent character. At maturity it was found that these mutants had lost the indehiscent character as well and were like a normal shattering variety. In subsequent trials they recorded 2-3 times higher yield than UCR 82-203 NS [15].

3.6. Isolation, confirmation and improvement of morphological mutants

The number of mutants of MI 2 and MI 3 varieties with changed morphological characters which could be effectively used in a breeding programme are given in Table V. These mutants have been confirmed in subsequent generations after selection. Details of these mutants have been described earlier [8].

TABLE V. NUMBER OF TRUE BREEDING USEFUL MUTANTS SELECTED AND CONFIRMED IN TWO CULTIVARS OF SESAME

Mutant character	Parent cultivar	
	MI 2	MI 3
Short internodes	5	2
Increased pubescence	4	1
Tetracarpellate ovary	6	3
Polypetalous corolla	1	-
Changed corolla colour	2	5
Early maturity	14	2
Late maturity	3	6
Branched stem	-	2
Long capsule	3	6
Short capsule	7	-
Three flowers per axil	3	-
One flower per axil	-	8
Changed seed colour	12	11
Brittle seed coat	1	-
Others	13	8
Total	74	54

It is necessary to decorticate the sesame seeds before processing for oil or use in confectioneries as the testa carries over 99% of the oxalates in the seeds. The testa imparts a slightly bitter taste to the meal, resulting from chelation of the calcium content by oxalic acid. Decortication also substantially increases palatability, digestibility and protein content of the meal [4,7]. Cultivars whose seeds can be easily decorticated will be useful in developing countries where machinary is not available for this purpose. A mutant isolated in the M_3 generation of MI 2 variety treated with ethyl methanesulphonate was found to have a brittle testa which can be easily removed by rubbing the dry seeds. This mutant was back-crossed to the parent variety and was also crossed with white seeded cultivars for selecting improved, brittle seed coat genotypes. The line MB 29w selected from a cross of MB 29 with local variety Sudu Thala has shown promise. The yield potential of MB 29 and MB 29w is similar to MI 2 and is higher than that of MI 3 variety.

The polypetalous corolla mutant (Table V) was found to be sterile and could not be maintained over several generations.

3.7. Sesame anther culture

Flower buds of 5 - 7 mm length and 36 - 48 h prior to anthesis incubated at 8 °C for 24 h gave the highest rate of callus induction. Continuous darkness after plating was better than a photoperiod of 12 h. The MS 5 medium recorded the highest rate of callus induction (Table VI) as well as callus formation after reculture (Table VII). MS 5 medium contained MS salts supplemented with 10 mg/l 2,4-D, 2 mg/l of both IAA and BA. Other media used in the studies and details of culture conditions for successful callus induction have been discussed [14].

4. DISCUSSION

4.1. Radiosensitivity

High level of resistance of sesame seeds to irradiation when germination is considered [16] and the differences in the radiation sensitivity of different sesame genotypes observed by other workers [16 - 20] are substantiated by our experiments with two morphologically different sesame cultivars. It is clear from the results that the MI 3 variety tolerates much higher doses of gamma rays than MI 2 variety considering germination after irradiation as

TABLE VI. CALLUS INDUCTION FROM ANTHERS OF MEDIUM SIZE (5 - 7 mm) FLOWER BUDS OF SESAME BY DIFFERENT MEDIA

Medium	Treatment	Number of anthers producing callus[a]	Callus induction (%)
MS1	Light	199	17
MS2	Light	80	7
	Dark	11	9
MS3	Light	0	0
	Dark	0	0
MS4	Light	60	5
	Dark	80	7
MS5	Dark	547	46

a : 1200 anthers were plated for each treatment.

TABLE VII. CALLUS FORMATION ON DIFFERENT MEDIA AFTER RECULTURE

Medium	Response of plated callus		Characteristics of callus
	Callus formation(%)	Size of callus[a]	
MS2	45.5± 0.7	+	brown, globular and hard
MS3	0	—	—
MS4	60.0± 1.7	++	yellowish soft
MS5	77.8± 1.8	+++	yellowish soft

a : + : All calli less than 1 mm in diameter
++ : More than 80% of calli 1-2 mm diameter
+++ : More than 80% of calli greater than 2 mm in diameter.

well as growth reduction studies. There seems to be a very high variation in growth reduction in sesame genotypes after irradiation and therefore different genotypes to be used in mutation breeding should be tested for radiosensitivity before deciding on doses.

4.2. Variability created through mutation induction

Easily identifiable, morphological mutants or those with resistance to diseases have been induced in sesame using mutation breeding methodology [9, 11, 13, 17 - 21]. Induced variability in quantitative characters has been described recently [10] and seems to be a promising approach from the results presented here. When selected for one yield component, a change in the other components generally occurs due to compensatory effects [8, 10]. Nevertheless, yield increase can be achieved in individual lines as well as in the mean yield of all selected lines if the population is large enough and selection pressure is high (Table I). Thus, except for number of seeds per capsule in MI 2 variety, all the other yield components have higher mean values in the selected mutant lines when compared with the parent. The range of variability is also quite high, offering many lines for advancing to yield trials.

The mutants selected purely for quantitative characters and those with changed morphological characters can be treated as exceptionally valuable material for further improvement of sesame because these mutants were induced in locally adapted, recommended and high yielding cultivars. These mutants have been included in the hybridization programme. The mutants MB 29w (white seeded brittle seed coat genotype) and MB 13 will be handed over to the Department of Agriculture for testing in the National Coordinated Variety Trials.

4.3. Drought resistance

The water requirements of different genotypes can differ at various growth phases of the plant. A genotype may be more tolerant to water stress at an early growth phase while a second may be more tolerant at a later stage of growth. Seeds of MB 29w and MB 33 have shown better capability of germinating at high osmotic potentials than other mutants. Low shoot/root ratio of MB 29w, MB 1 and MB 1-1 should be considered as advantageous when growing them in drought prone environments. The short period of flowering in MB 29w is also advantageous as drought at flowering stage can substantially reduce yields of sesame [3,4]. Shorter flowering period reduces the risk of drought and also facilitates synchronous maturity minimizing shattering losses [5, 6].

4.4. Indehiscence in sesame

The two mutants which have lost the undesirable effects of *id id* genotype were later found to have lost the indehiscent character as well. These were recovered from a population of about 150 000 M_2 plants. The indehiscent character is governed by a single recessive gene [11] and our objective was to break the linkage of the indehiscent character with the undesirable effects. The loss of the undesirable characters along with the indehiscent character implies that the undesirable effects are a pleiotropic in nature rather than due to very closely linked loci confirming the suggestions of some authors [11]. Therefore, search for other genotypes with improved seed retention should be intensified.

4.5. Callus induction from anthers

According to the only published results of anther culture in sesame, the Korean cultivars used have recorded 55.1 % callus induction when 25 mg/l of 2,4-D and 1 mg/l of BA were included in the MS medium [13]. In the present experiment, the MS 5 medium containing 10 mg/l of 2,4-D with 2 mg/l each of BA and NAA recorded a higher rate of callus induction than MS 2 medium with 15 mg/l 2,4-D and 1 mg/l BA. These results are consistent with the findings that the different genotypes may require different media for callus induction from anthers of the same species [22 - 24].

Shortening of the breeding cycle using doubled haploid technology can be achieved only if different stages take short periods [22 - 25]. All the media which were capable of producing callus were efficient in this context as they induced callus within 2 - 4 weeks. Nevertheless, MS 5 was the most successful medium where callus induction took place within 2 weeks and a greater proportion of anthers produced callus.

Acknowledgements : For their actual involvement in the conduct of experiments under this project, the author wishes to thank Mr. B.G. Wijewanse, Mr. J.P.S. Dissanayake, Miss. M.S.M. Fernando, Miss C. Umagaliyage, Mrs. R.A.S. Perera, Mrs. S. Subasinghe and Dr. K.K.D.S. Ranaweera.

Partial financial assistance by the International Atomic Energy Agency under RC 5442 RB and by the United States Agency for International Development under PSTC grant 6.106 is gratefully acknowledged.

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MODIFICATION AND ADAPTATION OF THE INDUCED DETERMINATE SESAME MUTANT BY CROSS-BREEDING AND ITS EVALUATION

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Abstract

The determinate sesame mutant which was induced by the author in the local Israeli variety "No. 45" by treating dry seeds with 500 Gy (50 krad) of gamma rays is described. This mutation which has not been described before has a unique plant architecture, large seeds and good quality. It can have an important role in developing improved sesame cultivars. Cross-breeding with the mutant has been undertaken by the author and by investigators in many countries who obtained seeds from the author. Advanced breeding lines are already available in Israel, R. of Korea and the USA. In yield trials in Israel, advanced (F_7 and F_8) selections gave yields exceeding or approaching one mt/ha. These yields, though significantly lower then those of two of the indeterminate lines are still quite respectable. It is concluded that the yield potential of the determinate selections should be improved. However, the lower yields could have resulted also from the fact that the population was too sparse. Spacing was according to the standard indeterminate type, while the determinate lines should probably be grown in denser stands.

1. DESCRIPTION OF THE DETERMINATE MUTANT

The determinate mutant "dt 45" was induced in the local Israeli sesame (*Sesamum indicum*) variety "No. 45", by treating dry seeds with 500 Gy (50 krad) of gamma rays [1]. No. 45 is a typical variety of the type growing in the Middle East. It was selected some fifty years ago from local land varieties. Its plants are fairly large and have several branches, usually basal. The original M_2 mutants, and later generations' plants [2,3] were much smaller than No. 45 and had 2 - 4 short branches [see also 4].

This mutation, with its determinate habit and novel plant architecture, is unique. Previously it was unknown in the entire germplasm of sesame. It is monogenic, recessive and stable in a wide range of environments [1, 2, 3]. The main stem and branches terminate with a cluster of 5 - 7 capsules, and the upper internodes are telescoped. In a study in Texas, USA [5] it was found that the determinate line had significantly greater seedling-root length then the indeterminate materials. This was manifested especially in higher temperatures (30 °C and 35 °C) for the number of lateral roots and for total lateral roots' length [5]. Very often, but not always, the apical flower in each branch has six fused corolla lobes instead of the normal five found in the other flowers on the same plant, and six stamens instead of the normal four or 4+1 found in the other flowers. The apical flowers have a symmetrical bell-shaped corolla (no lip). The upper clustered flowers, and later on the capsules, point upwards. In the apical flowers in each branch, the capsules are often quadricarpellate, while all other flowers in the plant (and the flowers of the source variety) are bicarpellate.

The seeds are large and plump as in the source variety, No. 45, and the seed set is good. Oil quality of the determinate line was compared to that of indeterminate ones in Texas, USA [6, 7]. In the determinate line, the oleic acid content was somewhat higher [6] but overall its oil quality was similar to that of the indeterminate cultivars. The fact that the determinate material had a higher seed weight led Brigham and Khan [6] to suggest that it should be utilized in developing high yielding varieties. Khan and Brigham [8] found considerable heterosis (F_1 exceeding better parent) in crosses of dt 45 with seven indeterminate lines of diverse backgrounds. The heterosis was manifested in seed yield, capsule number, capsule dry weight/plant and stem dry weight. In the M_3 some of the seeds germinated while still in the maturing capsules (vivipary). Apparently this was related to high moisture availability in

the soil at maturation. In subsequent generations, and in offspring from many hybrid combinations with dt 45, this phenomenon was not encountered, or was as rare as in other cultivars.

2. CROSS-BREEDING

A large-scale breeding programme with the mutant has been undertaken by the author in order to transfer the determinate mutant into the genetic backgrounds of many and different sesame cultivars from various regions, and to obtain determinate, adapted, high yielding, early, and good quality cultivars [2, 3]. In addition, seeds of the mutant were supplied to many sesame researchers in about 20 countries in Asia, Africa and North, Central and South America, who had the same objectives in mind.

In the author's large crossing program, a wide range of plant architecture types was developed: branched plants of various types, e.g. few or many branches, basal or upper branches; uniculm plants; plants with capsules only at the apex of the branches, and plants with capsules at the apex and in 2 - 3 nodes below. Also, lines with various leaf densities, textures and sizes were isolated, some very leathery, others fleshy, etc. In addition, the determinate mutant was crossed with lines having the *id/id* indehiscent character and with cultivars varying in seed size and color, etc. Thus, a wide range of character combinations was obtained.

Several years ago, in the USA, Dr. Raymond Brigham of the Texas A & M University, Agricultural Research and Extension Center, Lubbock, Texas, embarked on a large breeding program with the determinate mutant supplied by the author [4, 5, 6, 7, 8]. The author visited his nursery in the fall of 1992 and saw some very promising advanced determinate lines. Dr. Brigham is about to release determinate germplasm lines developed through cross-breeding with various materials adapted to his region (personal communication).

Breeding research with the determinate mutant is continuing in other countries, e.g. Thailand [9], China (personal communication - Tu Lichuan), R. of Korea (personal communication - J.I. Lee, C.W. Kang) and Venezuela (personal communication - A. Nava).

The determinate mutant was induced in a variety adapted to Israel's photoperiod (latitude 30 °N - 34 °N). Its requirements remain the same as those of the original source, No. 45. Therefore, when grown in considerably lower or higher latitudes its growth is affected. Furthermore, sesame is grown in the Middle East in the summer, which is free from rain. It is grown on stored moisture, in fairly dry conditions. Therefore, when dt 45 is transferred to humid regions with summer rains it may suffer from various diseases. Thus, the importance of cross-breeding is highlighted.

The modification of the plant's architecture by cross-breeding of the induced mutants with other lines, demonstrate clearly the potential and value of hybridization between mutants and other lines, as recommended by Micke [10]. Similarly, the disappearance of the vivipary phenomenon may be due also to the changed genetic background.

3. YIELD TESTS IN ISRAEL

Yield tests were conducted in the summer of 1991 in Sarid, near Nazareth. Two advanced (F_7) determinate selections (4104 and 4044) were compared with the following indeterminate local materials:

"No. 45"	-	A local, well adapted cultivar, selected from local land varieties in the 1940's by the late Dr. Y. Kostrinsky.
"Adie"	-	A locally adapted variety developed recently by Mr. D. Solomon.
"Yizrael 1"	-	A local selection made by Mr. G. Zur from a heterogeneous population established by Mr. H. Feldner.

In the summer of 1992 only the determinate Sel 4104 (F_8) was compared with the same materials.

The yield trials were planted in randomized blocks with 6 replications. The fields were well fertilized and trickle irrigated. Planting was on beds, 2 m wide, 3 rows/bed (60 cm between rows). Spacing within the rows was 5 cm. Each plot was 8 m long, one bed wide (2 m).

The 1991 field was planted on May 21 and harvested on two dates, according to the lines maturities (Table I). The 1992 trial was sown on May 18 and harvested on two dates (Table II). A 2 m long section of the middle row in each plot was hand harvested, threshed and weighed.

Table I. PLANT HEIGHT AND MEAN YIELDS OF TWO DETERMINATE (=Det) SESAME SELECTIONS AND THREE INDETERMINATE (=Indet) CULTIVARS AND SELECTIONS, SARID, 1991.

Cultivar or selection	Habit	Plant ht. (cm)	Days to harvest*	Yield, kg/ha	
				Mean**	Range
Yizrael 1	Indet	150	110	1980 a	1770-2120
No. 45	Indet	165	110	1540 b	1120-1810
Sel. 4104	Det	125	117	1080 c	890-1170
Sel. 4044	Det	120	117	890 d	730-1090
Adie	Indet	160	110	850 d	730-1020

* :Number of days from sowing to harvest.
** :Means marked by different letters differ significantly at the P=0.0001 level.

Table II. MEAN YIELDS OF A DETERMINATE (=Det) SESAME SELECTION AND THREE INDETERMINATE (=Indet) CULTIVARS AND SELECTIONS, SARID, 1992.

Cultivar or selection	Habit	Days to harvest*	Yield, kg/ha	
			Mean**	Range
No. 45	Indet	102	1 748 a	1 580 - 1 910
Yizrael 1	Indet	102	1 590 a	1 470 - 1 760
Sel. 4104	Det	111	1 210 b	1 020 - 1 420
Adie	Indet	102	1 193 b	980 - 1 320

* :Number of days from sowing to harvest.
** :Means marked by different letters differ significantly at the P=0.0001 level.

The determinate selections were shorter and somewhat later. The yields of the determinate lines were respectable, exceeding or approaching 1 mt/ha (Tables I, II). Still, their yields were significantly lower then those of No. 45 and Yizrael 1, but exceeded that of Adie (Tables I, II).

Two conclusions are drawn from these results. First, the yield potential of the determinate lines should be improved further. Secondly, the agronomic practices should be reconsidered. It is becoming evident that population density of the determinate lines should be increased, by narrowing the spacing between the rows.

Acknowledgement

I acknowledge with thanks the valuable contribution of Mr. G. Zur who was responsible for the yield trials.

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EVOLUTION OF IMPROVED VARIETIES OF SESAME THROUGH INDUCED MUTATION

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Abstract

Mutation breeding using gamma rays led to the development and release of two high yielding yellow sarson mustard mutant varieties, 'Safal' and 'Agrani', which are now commercially cultivated by the farmers of Bangladesh. In parallel to this breeding programme, mutation breeding was also applied to sesame, another important oil seed crop in Bangladesh. Variabilities in M_2 generation of sesame varieties of T-6 and S-30 were examined after treatment with gamma rays and a number of desirable mutants were selected including an indehiscent mutant from the local variety T-6. Six mutants of S-30 and two mutants of T-6 bred true in M_3 as earliness, longer capsule type, indehiscent and tolerant to waterlogged condition.

1. INTRODUCTION

Work on mutation breeding on oil seed rape started in the Plant Breeding Division of Bangladesh Institute of Nuclear Agriculture (BINA) since 1981 in collaboration with the International Atomic Energy Agency through a project, 2991/RB. Success has been achieved in this regard with the release of two high yielding mutant varieties, 'Agrani' and 'Safal'.

In the field of sesame breeding, development of indehiscent type and tolerant to waterlogged condition is very much needed by the farmers. Induced mutation work is in progress to achieve the objective mentioned for sesame under the coordinated research programme.

2. MATERIALS AND METHODS

2.1. Collection and evaluation of germplasm

Fifteen exotic strains of sesame collected from the USA and 13 local strains were given an adaptation trial with the local recommended variety T-6 as check in a randomized complete block design with three replications at two locations of Bangladesh during 1988-89 for selection of better materials for irradiation.

2.2. Seed treatment of sesame with gamma rays and M_1 studies

Dry seeds of the local black seeded variety T-6 and exotic white seeded variety S-30 were exposed to different doses of gamma rays and the seeds were sown directly in the field in a randomized block design with three replications.

Data on M_1 plants of different agronomic characters were recorded in the field. Since the effectiveness of radiation in M_1 plants were not satisfactory, the experiments were repeated in the next year and M_1 plants were harvested separately to grow M_2 plants.

2.3. Studies on segregation and selection of desirable mutants in M_2 and M_3 generations

M_2 plant progeny rows of sesame were raised under different doses along with original cultivar. The materials were carefully examined from seedling to maturity.

Selection preference was given to indehiscent and semi-indehiscent type, longer capsule type, more capsule per axil and on tolerance to waterlogged condition types. The M_2 selected mutants were further screened in M_3 along with the original cultivar and further selection was made.

3. RESULTS AND DISCUSSION

3.1. M_1 studies

High radio resistance was observed in sesame seeds as was observed in *Brassica* oil seeds [1]. Effect of gamma rays on germination and other agronomic parameters of T-6 and S-30 varieties of sesame are shown in Table I.

Table I. EFFECT OF GAMMA RAYS ON M_1 POPULATION OF BLACK AND WHITE SEEDED SESAME

Dose (Gy)	Germination (%)		Seedling height (cm)			Pollen sterility (%)	Plant height (cm)	Capsules/ plant (No.)	Capsule length (cm)	Seeds/ capsule (No.)	Days to maturity
	Sand[1]	B.paper[2]	14 days old		21 days old						
			Sand[1]	B.paper[2]	Field						
Strain S-30 (White seeded)											
0	80	82	6.5	5.2	7.9	8.6	118	52	4.0	67	95
400	82	88	6.4	4.6	7.0	40.9	114	56	3.1	62	96
500	78	83	6.1	4.6	7.0	35.0	111	56	3.1	60	96
600	79	63	5.1	4.3	5.0	38.0	101	55	3.1	60	97
700	75	84	5.0	4.1	4.6	40.4	100	50	2.9	54	97
800	75	82	4.3	3.2	4.2	52.2	95	39	2.6	54	97
900	65	69	3.8	2.6	3.2	60.2	93	35	2.4	52	97
Var. T-6 (Black seeded)											
0	75	94	6.0	4.6	7.0	9.6	110	68	2.3	53	93
400	71	93	5.9	4.5	6.3	18.2	109	72	2.2	53	94
500	73	93	5.3	4.1	6.1	10.5	109	66	2.2	46	95
600	76	87	4.9	4.1	4.9	24.6	104	66	2.2	44	95
700	82	92	4.9	3.9	3.9	35.2	96	62	1.9	42	95
800	73	85	3:6	3.5	3.5	48.2	89	58	1.9	38	95
900	64	65	3.4	2.6	3.1	35.5	87	55	1.8	33	95

1 : Germination in sand

2 : Germination on blotting paper

3.2. Selection of desirable mutants in M_2 and M_3 generation

M_2 seeds from the rest of the M_1 plants of the previous year of different doses of the varieties T-6 and S-30 were grown as plant progeny rows along with control at BINA farm in Mymensingh. A number of deviates from the control was observed before the development of true leaves, at seedling stage and at later stages. A series of chlorophyll and flower colour

deviates were observed suggesting that a number of different mutational event had occurred for both these characters. Mutation frequencies for both characters generally increased with increasing doses of gamma rays with an exceptional rise in mutation frequencies under 600 Gy in S-30 and 700 Gy in T-6. A saturation of mutation frequencies were observed for both of the varieties, between 600 and 700 Gy beyond which decrease in mutation frequency occurred. This was also stated by Fowler and Stefansson [2] while working with oil rape using different concentrations of EMS. Comparable results was also reported by Mackey [3] in wheat. A loss of M_2 plant vigour was also observed with increasing doses which was more acute in T-6 compared to S-30. Other variations observed were early flowering, shorter internodes, increased number of capsule per axil, variation in capsule sizes, rolled leaves, extreme distortion of growth habit mostly non-lethal, tolerant to *Macrophomina phaseoli*, shattering and waterlogged conditions etc.

Based on the phenotypic variation and on NMR (Nuclear Magnetic Resonance) screening for oil content a number of desirable mutants were selected for further studies in M_3 (Table II).

Table II. MUTANTS SELECTED IN M_2 POPULATION OF SESAME UNDER DIFFERENT DOSES OF GAMMA RAYS

Variety	Early flowering	Indehiscent type	Semi-indehiscent	Short internode	Long capsule	Increased no.of capsule/axil	Continuous bearing	Uniculm	Tolerance		Both S[3]&W[4]	Total mutant type	Population size
									WLC[1]	MP[2]			
S-30 (White seeded)	8	0	6	5	5	3	4	12	5	12	1	61	20 000
T-6 (Black seeded)	4	3	6	2	0	0	1	2	3	5	4	30	17 000
Total	12	3	12	7	5	3	5	14	8	17	5	91	37 000

1 : WLC : Waterlogged condition
2 : MP : *Macrophomina phaseoli*
3 : S : Summer
4 : W : Winter

To confirm true breeding behaviour 20 putative mutants of S-30 and T-6 were grown in plant to progeny rows. Five rows of respective original cultivar were also grown after every 20 plant progeny rows of mutants for comparison. The plant stand was normal during the whole growing period. The two early mutants of S-30 was found earlier by seven days from the original cultivar in M_3. Three mutants with long capsule (40 mm and above) were found to breed true. Other mutants segregated and further selection was made. Only two waterlogged tolerant mutants of T-6 bred true. Murty *et al.* [4] reported evaluation and establishment of true breeding mutants in M_2 to M_4 generations of sesame. Agronomic data relating to various yield attributing characters were recorded from 10 randomly picked plants of the mutants that bred true and the original line (Table III).

There was however, no significant differences in oil content of the M_3 material when compared with M_2. Mutant lines will be further evaluated during the next generation.

Table III. PERFORMANCES OF SESAME MUTANTS AND MOTHER VARIETIES M_3 GENERATION

Variety/ Mutant	Plant height (cm)	Capsule in main stem	Capsule in branches	Capsule length (cm)	Seeds/ capsule (No.)	Days to maturity	Oil content (%)	Reaction to M.P.	Mutant Characteristics[1]
S-30[2]	94	31	0	3.14	86.4	104	52	T	Susceptible to WLC
M-1	85	26	16	3.94	70.2	75	52	MR	Early maturing
M-2	95	25	21	2.98	65.6	80	51	MR	Early maturing & TWLC
M-3	60	21	11	3.30	68.0	76	50	MR	Dwarf, early maturing, long capsule
M-4	76	22	0	4.02	78.4	81	50	MR	Uniculm, early maturing, long capsule
M-5	92	30	0	2.88	78.8	87	51	T	Uniculm, early maturing, long capsule
M-6	85	20	18	2.66	62.0	86	51	T	TWLC, long capsule
T-6[2]	69	23	14	2.18	64.0	79	42	S	Susceptible to WLC
M-7	77	19	15	2.54	78.8	93	42	T	TWLC
M-8	98	25	10	2.44	66.0	93	43	T	TWLC

1 : WLC : Waterlogged condition, TWLC : Tolerant to waterlogged condition.

2 : The original cultivars as check.

Acknowledgement

The authors acknowledge with gratitude the financial assistance of the International Atomic Enery Agency through the RC. Nos. 2991/RB and 5432/RB.

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DOMESTICATION OF *Cuphea* THROUGH MUTAGENESIS

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Abstract

Cuphea plants have promising characteristics as unique source of medium chain fatty acids. Although several groups of researchers in the world are working to domesticate this promising plant species, it still has many unfavorable wild characters, preventing it from becoming a practical agricultural crop at the present stage. In the present research, mutation breeding methods were adopted to domesticate the species in hope of inducing genetic modifications, but retaining its fatty acid constitution. Among the problematic characters, indeterminate growth pattern and non-uniform maturity, dormancy/germination, shattering of seeds and other plant morphology or plant-architecture traits must be modified to adapt it to the modern agricultural system. Several species of *Cuphea* were evaluated as the initial material. Problems of seed germination and those encountered in soaking the seeds in water or aqueous chemical mutagen solution could be solved and several promising and interesting mutant lines could be obtained.

1. INTRODUCTION

Certain *Cuphea* species havde been used as flower and bedding plants. But the genus *Cuphea* (*Lythraceae*) represents an alternative source of lauric (C12:0) and capric (C10:0) fatty acids. Recently they have been the focus of a major domestication effort to develop them as new crop species. at present, the species present significant agronomic problems including indeterminate patterns of flowering and growth, seed shattering in ripening fruits, and viscid glandular hairs on stems, leaves and flowers.

Lauric acid is widely used in the manufacture of soaps, detergents, industrial products, and foods. Medium chain fatty acids (MCF), the major constituents of the seed oil, are of commercial and economic significance. Capric acid has numerous medical and nutritional uses, although it is currently of minor economic importance.

Like other wild species, *Cuphea* manifests seed dormancy. Dormancy decreases after ripening time, but the rate of the decrease varies between and within species and populations. Dormancy seriously impedes experimentation in certain species. Several breeding, genetics and cultural approaches have been utilized to remove the limitations to *Cuphea* domestication and agricultural production. Mutagenesis is one of the methods of *Cuphea* domestication.

2. MATERIALS AND METHODS

Seeds of *Cuphea wrightii* 670 and *Cuphea tolucana* 629 were obtained from the greenhouse of the Institute of Plant Genetics in 1991. The seeds were harvested from individual plants by hand and immediately dried at 20 °C in an open paper bag in the laboratory. The seed moisture content remained constant (from 5 % to 6 %) during storage. The harvested seeds were classified according to different colours of the testa; light green, dark green, light brown, middle brown, dark brown and black.

After classification the seeds were placed in 5 cm plastic Petri-dishes containing a piece of blotter paper soaked with water. Three replication of 30 seeds each were used for each treatment.

The germination tests were conducted in a day-night cycle of 12 hour light at 20 °C and 12 hour dark at the same temperature. After 14 days the germination was assessed as shown in Table I. The seeds were considered as germinated, when the radicle protruded through the coat. An identical procedure for the germination rate was carried out after different time of storage. Brown seed samples of *Cuphea wrightii* 670 were treated in 5 ml of gibberellin A_3 (GA_3), indole 3-acetic acid (IAA), sodium azide (SA)(NaN_3) with 1.0; 2.0 mM and hydrogen peroxide (H_2O_2) with 1 M and 2 M. The solution of NaN_3 was freshly prepared in 0.25 M phosphate buffer at pH 3. After soaking in the mutagen solutions for 3 hour at 20 °C, the seeds were thoroughly rinsed with water three times. The germination tests were conducted as mentioned before.

TABLE I. GERMINATION RATE OF *Cuphea tolucana* 629 AND *Cuphea wrightii* 651 SEEDS OF DIFFERENT COLOURS AFTER STORAGE

Colour of seeds	*Cuphea wrightii* 651							*Cuphea tolucana* 629						
	storage (months)							storage (months)						
	0	2	4	6	8	10	12	0	2	4	6	8	10	12
Light green	0.0	0.0	0.7	39.3	48.0	63.3	70.0	0.0	1.3	11.3	12.7	20.6	78.0	90.0
Dark green	0.0	2.7	24.7	80.0	85.0	88.0	90.0	0.0	2.0	46.0	47.3	50.6	88.0	92.0
Light brown	0.0	1.3	44.7	80.0	98.0	98.0	98.0	0.0	6.0	68.7	80.6	81.3	90.0	90.0
Middle brown	0.0	4.6	55.3	77.3	99.3	99.3	99.3	0.0	13.3	83.3	86.0	89.0	90.2	90.2
Dark brown	0.0	9.3	78.7	79.3	100.0	100.0	100.0	0.0	35.3	88.0	91.3	95.3	94.6	94.6
Black	0.0	8.7	80.7	84.6	98.6	98.6	98.6	0.0	40.0	92.7	100.0	99.3	98.6	98.6

The seeds of phenotypically stable mutant lines of *Cuphea tolucana* 629 in M_6 generation, were sown in the greenhouse at the space of 10 - 50 cm. Towards the end of their growth period following morphological traits were measured and counted: plant height, capsule length, number of branches and weight of 1000 seeds.

Bundles of flowers of *C. wrightii*, *C. tolucana*, *C. palustris*, *C.lanceolata*, *C. viscosissima* and *C. lutea*, were treated with two doses of MNU (N-nitroso-N-methylurea, 1.0 and 2.0 mM) for 2 hours at 25 °C. After the treatments the flowers were rinsed with running water. The pollen of *Cuphea* species was collected and stained with solution of carmine-glycerine (1:1) and the pollen fertility was examined.

The fatty acid composition was determined by gas liquid chromatography after trans-esterification to methyl ester as described by Thies [1]. The percentage of each single fatty acid was calculated as percent of the total fatty acid content.

2. RESULTS

The germination tests (Table I) showed a gradual increase in germination depending on the storage period and colour of the seed coat. After a long period of storage (12 months) no large differences were observed among the germination rates. But the germination rate of light green seeds diminished, when compared with brown and black seeds. It is very interesting that dark brown and black seeds of *C. tolucana*, showed a higher total germination rate after 6 months storage, whereas in *C. wrightii* the same results were observed two months earlier.

Sodium azide (2 mM) and hydrogen peroxide (2 M) increased the speed of germination of seeds stored for 4 months as compared to the control (Table II). After treatment with IAA there occurred a decrease of the germination rate. Seed treated with GA_3 showed a low increase in the speed and percent germination.

TABLE II. EFFECT OF GA_3, IAA, NaN_3 AND H_2O_2 TREATMENTS ON THE GERMINATION OF FRESHLY HARVESTED BROWN SEEDS *Cuphea wrightii* 670*

Treatment	% of germination			
	after 7 days		after 14 days	
Control - H_2O	11.3	4.5**	35.4	11.2**
GA_3 - 1mM - 3h	3.3	0.0	10.0	0.0
GA_3 - 2mM - 3h	33.3	5.3	44.5	11.9
IAA - 1mM - 3h	18.1	6.0	29.5	8.5
IAA - 2mM - 3h	11.7	7.2	20.4	6.7
NaN_3- 1mM - 3h	32.8	13.2	82.0	16.6
NaN_3- 2mM - 3h	17.6	13.2	72.0	12.1
H_2O_2- 1M - 3h	2.2	1.9	3.3	3.3
H_2O_2- 2M - 3h	89.0	7.6	97.8	1.9

* : Four months after harvest
** : Mean SD

A considerable number of mutants with different morphological traits were induced and detected, but only a few of them are given in Table III. All mutants showed significant increase in plant height, particularly mutants No. 1/86-17 and No. 1/86, as compared to the control (*C. tolucana*). The capsule length in all the mutant strains was generally altered as compared to *C. tolucana*, but only two mutants (11M-7 and 41-2) showed an increase of this trait. Regarding the average of branch number, it was larger in mutants as compared to the control. The 1000 seeds weight of mutants ranged from 1.0 g to 1.4 g and no variation coefficient was found in that case. The variation coefficient of the number of branches showed an increase. The variation coefficient of two traits, plant height and capsule length, was different, depending on mutants. Up till now we could not induce a non-shattering form.

As shown in Table IV, a decrease in pollen fertility and a change in pollen grain size were observed in *C. tolucana*, *C. palustris*, and *C. lanceolata* species at the stage of microsporogenesis following the MNU treatment. An increased number of pores were observed in *C. viscosissima* after treatment with MNU in stage of microsporogenesis. A small number (8) of interspecific hybrid seeds could be harvested.

TABLE III MEANS AND COEFFICIENTS OF VARIATION OF MORPHOLOGICAL CHARACTERS AND 1000 SEED WEIGHT IN M_6 MUTANTS AND IN THE SOURCE MATERIAL

Forms	Plant height		Capsule length		Number of branches		1000 seed weight	
	mean (cm)	c.v.%	mean (cm)	c.v.%	mean	c.v.%	mean (g)	c.v.%
C.tolucana	38.9	15.3	0.7	22.0	9.5	7.8	1.3	0.0
11M - 7	49.5	8.9	0.9	31.0	9.8	14.5	1.3	0.0
1/86 - 17	61.3	12.1	0.6	20.7	16.3	36.7	1.2	0.0
45 - 1	43.5	10.7	0.5	30.6	12.6	20.3	1.2	0.0
41 - 2	54.3	13.4	0.9	13.1	13.9	36.4	1.3	0.0
41 - 1	40.0	5.2	0.5	21.8	13.7	20.3	1.0	0.0
1/86 - 4	37.9	33.2	0.7	27.2	14.6	38.3	1.2	0.0
9 M	46.5	11.3	0.5	21.7	12.0	29.4	1.4	0.0
35 M	48.2	23.0	0.7	13.2	13.2	24.9	1.2	0.0
1/86	60.3	14.2	0.6	22.9	16.6	30.2	1.1	0.0

TABLE IV. POLLEN FERTILITY OF *Cuphea* SPECIES AFTER TREATMENTS WITH MNU AT THE STAGES OF MICROSPOROGENESIS

Forms	Pollen fertility	
	Control	After MNU treatment
C.wrightii	96,3	84,9
C.tolucana	97,2	84,6
C.palustris	97,4	89,0
C.lanceolata	87,8	59,9
C.viscossisima	98,4	93,2
C.lutea	98,8	94,8

Analysis of the fatty acids composition of variously coloured seeds (Table V) showed that the content of each fatty acid was similar in all the colour classes of seeds. Two mutant lines, M 41 and M 45, showed different medium chain fatty acids (MCF) content as compared to the source material (Table V). Using these materials, the inheritance of the gene and biochemical pathways of MCF synthesis could be studied.

TABLE V. VARIABILITY IN THE PERCENTAGE OF FATTY ACID COMPOSITION IN *Cuphea tolucana* AND MUTANTS

Forms	8:0	10:0	12:0	14:0	16:0	18:0	18:1	18:2
C.tolucana 629	0.5	25.4	60.1	3.9	1.7	0.2	2.1	5.1
C.t. light green	0.5	28.0	58.2	3.9	1.6	0.2	2.2	5.2
C.t. dark green	0.6	27.1	59.0	3.5	1.5	0.3	2.0	5.6
C.t. light brown	0.6	28.0	58.0	3.6	1.6	0.2	2.2	5.1
C.t. middle brown	0.5	29.1	56.1	3.2	1.8	0.3	2.9	5.5
C.t. dark brown	1.1	28.8	57.9	3.6	1.6	0.0	2.3	4.8
C.t. black	0.5	24.4	60.7	4.2	1.9	0.2	2.4	5.3
M - 1/86	0.5	23.6	61.4	4.1	1.7	0.2	3.0	4.8
M - 41	0.4	15.5	71.1	4.5	1.7	0.0	1.8	4.8
M - 45	0.3	15.6	70.5	4.8	1.7	0.0	1.8	4.4

3. DISCUSSION

Cuphea is clearly a good candidate to be introduced and developed as a new oil crop in Polish agriculture. Seeds of *Cuphea* are usually completely dormant for several months after harvest. Such prolonged dormancy is a special problem in rapid domestication of this species [2]. In a breeding programme designed to grow three or more generations per year, seed dormancy can be a major obstacle. The germination of *Cuphea* seeds immediately after the harvest increased to 100 % by removing the seed coat from the embryos. However, this is time and labour consuming. The mechanism by which the seed coat imposes dormancy on the species is poorly understood. Some investigators studying seeds of other species are of the opinion that the seed coat is the major permeability barrier for gases [3]. The present study on germination showed that a significant difference exists due to the length of the period of storage and colour of the coat. Similar results were obtained in Kentucky bluegrass by Phaneendranath *et al.* [4] who observed that seed maturation and reduction of dormancy in Kentucky blue grass was accompanied by changes in the colour of panicle branches, particularly from green to brown.

An increased germination rate after SA treatment was also observed by Fay, Gorecki [5], Upadhyaya *et al.* [6] and Adkins *et al.* [7], in *Avena.*

After treatments with chemical mutagens (MNU and SA) and gamma rays we could induce and select quite a number of mutants: earliness, changes in colour of flower and seed and dwarf forms. It was noted that all mutants are different from one another in morphological traits. All mutants showed a significant increase in plant height and number of branches per plant as compared to the initial form. Mutation studies [8, 9, 10, 11 and 12] showed that mutagenesis is a useful tool to alter morphological characters of *Cuphea* plants.

The major problem at harvest is seed shattering. Rain and wind cause seeds to fall out, dramatically reducing the yield. Development of non-shattering types is of the highest priority to assure maximum seed recovery independent of locally varying climatic conditions. Shattering is caused probably by an asymmetric growth of the placenta which is in accord with dorsiventral flower morphology. Probably a dominating characteristic of non-shattering of this genus impedes the induction of such traits.

For this reason we have tried to overcome shattering of the seeds by the use mutagenesis combined with interspecific crossing between different *Cuphea* species. Interspecific hybridization in combination with mutagen treatment at the stage of microsporogenesis would facilitate gene transfer, especially when removal of linkage effect was desired. Treatments with mutagens of microspores and macrospores might also allow breaking the compatibility barrier enabling interspecific transfer of genetic information. Transfer of specific paternal characteristics into a maternal background within the same species was made by Jinks *et al.* [13] in *Nicotiana rustica*. The possibility to achieve transfer of one or a few traits from one line to another by irradiation of pollen in maize was observed by Pandey [14].

The maturation stage of the seeds has no influence on the fatty acid composition. Thomson *et al.* [15] after studying the effect of seed maturity on fatty acid content of eight *Cuphea* species concluded that variations in seed maturity would not present any major constraints to commercialization of the harvested seeds.

Our domestication programme is in progress and we hope that the problems will be solved in the near future.

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INDUCTION, IDENTIFICATION, SELECTION AND EVALUATION OF SUNFLOWER MUTANT PLANTS

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Abstract

Mutation breeding of sunflower inbred lines could be profitable. This is the main conclusion of investigations carried out under the research programme with the IAEA. Many deviations observed as regards leaves, petioles, branching and heads, have no practical value. No drastic mutations have yet been reported for seed oil content. However, the data show that changes have been induced and continued screening is underway. Macromutations show maximum changes in means and variances of traits, and so, they are useful for breeding. Reconstruction of plant architecture using "short petiole" mutant, is in an advanced generation of backcrossing and selection in valuable inbred lines. The inheritance of short petiole trait has been analyzed. Irradiated pollen seems to be able to induce appreciable homozygous haploid plants using "in vitro" gynogenesis. Increasing attention will be given to the prospects of the genetic modification of sunflower oil and genetic manipulation of factors controlling disease resistance.

1. INTRODUCTION

The mutation methods in sunflower breeding have become more and more useful in connection with hopes of improving oilseed quality for nutritional uses and technical applications that substitute fossil petroleum for continuous supply and superior biodegradability of oleochemicals over petrochemicals in industrial and non-food uses [1].

Concerning resistance to diseases, one of the important objectives in sunflower breeding, a promising method is "*in vitro*" selection of resistant mutants and then, the regeneration of a resistant plant. Unfortunately, the regeneration techniques of this plant species are still in the research stage, including controlling of diseases resistance by transgenic method [2, 3]. Other objectives in sunflower breeding are : to obtain new sources of restorer lines for CMS, genetic tolerance to heat and drought, a new architecture of the plant and increased degree of self-fertility in valuable inbred lines [4, 5]. This report presents the results obtained in the recent years and some remarks on research priorities for the present and future programmes.

2. MATERIALS AND METHODS

In the experiments carried out within the frame of the contract with IAEA (5441/RB), the seeds were irradiated at the Seibersdorf Laboratory. The inbred lines: CG-3606, CG-3663, LC-1004, V-2012, one F_1 hybrid (black seeded branching type x white seeded branching type) and the hybrid *H. rigidus* x *H. annuus*, were used for seed treatment by gamma rays with doses: 100, 150, 200 Gy (^{60}Co). The M_2, M_3 and M_4 seeds were obtained by selfing. The mutant "short petiole" was transferred by backcross and selection to some valuable inbred lines.

Isolated plants of three hybrids: Select, Super and Felix were emasculated and pollinated with irradiated pollen in four doses: 300, 700, 800 and 1,000 Gy gamma rays. After 5 - 7 days the ovules were removed from the ovary and incubated on the medium MS "*in vitro*" under darkness at 29 °C.

A preliminary experiment was made to examine the differences among 18 hybrid plants of *H. rigidus* x *H. annuus*, by biochemical analysis of DNA using restriction enzymes, Eco RI and Bam HI. The protocols of DNA extraction and digestion by the enzymes are described in Maniatis *et al* [6].

3. RESULTS AND DISCUSSIONS

3.1. Plant injury after seed irradiation

The results reported previously on plant injury in M_1 revealed a decrease in germination and plant height [7]. A lot of variations or abnormal growth were observed in leaves, petioles, branching and heads [8].

3.2. Identification and selection of mutants

3.2.1. Deviation

In M_1 the plants presented many changes after seed treatment by gamma rays. Many morphological deviations, including earliness and plant height changes have already been reported previously [8].

3.2.2. Male sterility

The few male sterile plants detected, were checked by backcross, and they revealed a nuclear inheritance [8].

3.2.3. Seed oil content

Seed oil content is a polygenic trait with a strong additive effect of genes being markedly influenced by the environment. Breeding for such traits needs accumulation of favorable genes. Induction of mutations may be a promising method to improve the seed oil content, although it is a significantly time and labour consuming procedure.

There are two complementary ways of obtaining useful mutants: by using appropriate mutagens in suitable doses and effective screening methods, and by recombining mutant genes. This includes the transfer of mutated genes into different genetic background to exploit positive gene interaction.

The identification of mutants within sunflower inbred lines in advanced generations of selfing, should be based on comparison to control populations. The breeders should clarify the genetic background of a source before designating a segregant as a mutant.

The results of our investigations show that mutation induction in sunflower is not conclusive, and no drastic mutations have yet been reported for seed oil content. However, the data show that changes have been induced, and continued screening is underway. The seed oil content in selected M_3 progenies, was over 45.5 - 47.5 % (V-2012) and 50 - 54.2 % (LC - 1004) in comparison with initial inbred lines: 34 % (V-2012) and 39 % (LC - 1004)[8].

3.2.4. Macromutations

The screening process can be started in M_1 generation in terms of identification of the treated plants with optimum mutagenic damage. Screening of mutants in M_2 generation involves isolation of progenies segregating for macromutation and showing maximum change in mean and variance for economic trait in the normal looking plants [9]. The experiments with branched genotypes did not show severe plant injury in M_1 as in the previous experiment (TABLE I), so macromutant plants with chlorophyll deficiency sectors on the leaves or even on the whole leaf, were isolated. These plants presented light yellow flowers M_2 and M_3 (Table I). The percent of seed oil content ranged from 25.2 to 42.7.

TABLE I. THE RESULT OF MUTAGENESIS IN SUNFLOWER HYBRID F_1 (WHITE SEEDED BRANCHING x BLACK SEEDED BRANCHING) AFTER SEED IRRADIATION BY 150 Gy ^{60}Co GAMMA RAYS

	M_1				M_2		M_3	
No.of irradiated seeds	Germination percent (%)	No.of plants (1990)	No.of chimeric heads[1]	No.of variants[2]	No.of plants (1991)	No.of plants with light yellow flower	No.of plants (1992)	No.of plants with light yellow flower
1 213	67.3	816	19	44	4120	48	4240	144

1 : The chimeric sectors observed were dark, striped, not black as in the previous experiment.
2 : Chlorotic leaves.

3.3. "Short petiole" mutant in sunflower breeding

The mutant "short petiole" identified at Fundulea in the inbred 0-7657, after irradiation of the seeds, has a practical value for sunflower breeding. This trait permits the reconstruction of plant architecture with the view of increasing plant population per unit area. The genetic study demonstrated the high heritability of "short petiole" trait and the magnitude of the dominant gene effect indicated that this trait can be transferred to different genotypes, through backcrossing and selection (TABLE II). The inheritance is controlled by at least two dominant genes with cumulative effect [10]. The transfer of this trait into sunflower inbreds is underway.

TABLE II. GENIC EFFECT ESTIMATION AND STANDARD ERRORS FOR "SHORT PETIOLE" MUTANT TRAIT IN O-7657M x O-7704 SUNFLOWER INBRED CROSSES

Genic effects	Genic effect estimation	Standard error
Mean (m)	6.78	1.75
Additive (a)	5.23	0.19
Dominance (d)	8.79	5.15
Additive x additive (aa)	-1.80	1.14
Additive x dominance (ad)	-6.62	2.12
Dominance x dominance (dd)	-3.94	1.18

3.4. Gynogenesis by irradiated pollen

Hybrids and homozygous inbreds through gynogenesis have been obtained by utilization of irradiated pollen. The results presented in TABLE III show that the pollen irradiated with 300 Gy stimulated gynogenesis. Cytogenetic study revealed evidences of induction of diploids, haploids and mixoploids. The uniformity of seven progenies was appreciable as homozygous lines.

3.5. Molecular biological analyses

Following treatment of the total DNA of 18 hybrid plants (*H. rigidus* x *H. annuus*) by Eco RI and Bam HI restriction enzymes, common bands of the DNA were found in plants

TABLE III. THE INFLUENCE OF IRRADIATED POLLEN ON GYNOGENESIS IN SUNFLOWER HYBRIDS

Genotype	No.of ovules inoculated	No.of ovules developed	Embryos/ovules (%)	No.of 2n plants
Super-check	1 002	31	3.09	13
Super-70 Kr	88	1	1.13	0
Super-80 Kr	483	3	0.62	0
Select-check	1 173	64	5.45	20
Select-80 Kr	647	14	2.16	0
Select-100 Kr	250	-	-	-
Felix-check	638	22	3.44	15
Felix-30 Kr A	47	-	-	-
Felix-30 Kr B	45	10	22.22	37
Felix-30 Kr C	250	38	15.20	18
Felix-80 Kr	370	2	0.53	0

with the same origin. Also, the results suggested that it would be possible to show evidence of the sequences in hybrid plants originated in wild species *H. rigidus* by RFLP (Restriction Fragments Length Polymorphism) [11].

4. CONCLUSIONS AND PROSPECTS

The investigations carried out under the research contract with the IAEA, pointed out that mutation breeding of sunflower inbred lines could be useful. The results of the research on radiosensitivity, mutation rate, plant injury, chimeric pattern and even germplasm manipulation in sunflower, gave fundamental and useful information for mutation breeding programmes of this important oil crop.

The "short petiole" trait is a promising mutation because it permits the reconstruction of plant architecture with the view of increasing plant population per unit area.

Increasing attention will be given to prospects for genetic modification of sunflower oil, based on the studies on lipid biosynthetic enzymes and also to manipulation of factors controlling disease resistance. The irradiated pollen may be useful for transgenic procedure for sunflower plants as the source of dispersed DNA fragments.

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IN VIVO AND *IN VITRO* MUTAGENESIS AND RESEARCH FOR DOWNY MILDEW RESISTANCE IN OPIUM POPPY (*Papaver somniferum* L.)

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Abstract

Papaver somniferum L. (opium poppy) is an amenable system for *in vivo* and *in vitro* mutagenesis with respect to the desired traits, particularly the crude alkaloid productivity, qualitative and quantitative spectrum of various morphinan alkaloids, capsule size, leaf fringing, seed yield, content and quality of the edible oil in seeds, and resistance to downy mildew infection. The present study is primarily focussed on four macro and twelve micro- mutants, selected and advanced to M_4 generation following application of individual or combined doses of ^{60}Co gamma radiations and EMS on two inbred parent varieties of opium poppy namely, SHWETA and SHYAMA. Amongst the various mutagenic treatments, combined dose of 50 Gy + 0.4 % EMS was most potent in generating viable, visible and micro mutations. Inheritance studies revealed that the two macro-mutants i.e. Big Capsulated (BC) and Red Petaled (RP) had a monogenic pattern of inheritance, while a third macro mutant i.e. Pinnately-Cleft Leaved (PCL) possessed a quasiquantitative inheritance governed by plural genes. The fourth macro- mutant i.e. Dwarf Mutant (DM) emanates that simultaneous recessive mutations of closely linked genes or mutation of a major gene with pleiotropic effect on neighbouring genes account for the genetic basis of its origin. Field performance and assessment of these mutants over 4-5 generations for yield contributing attributes and disease reaction towards downy mildew disease caused by fungus *Peronospora arborescens* revealed that BC and micro-mutant M26/100/2 were the best morphine yielders while PCL, DM and micro-mutant M8/102/4 recorded 60-70% elevated level of resistance to downy mildew attack. Macro-mutant RP, on the other hand, registered highest seed yield and seed oil content (45.2 % d.wt.). To further broaden the spectrum of variations in opium poppy and to rectify the undesirable features associated with some of the mutant families, radiation treatments (with or without EMS) were superimposed on undifferentiated cell/callus cultures of such mutant lines. A highly efficient and reproducible protocol for the initiation of callus, its maintenance and growth and complete plantlet regeneration via shoot bud organogenesis has been developed. Medium formulation and cultural conditions for each of the morphogenetic phase i.e. callus proliferation, time and mode of mutagen application, shoot bud regeneration, rhizogenesis in excised shoots and hardening of the resultant plantlets have been standardized. The *in vitro* raised plants have been successfully carried forward to the field where they set viable seeds. Interesting variations with respect to disease resistance are at hand for field testing. Limited success has also been achieved in establishing dual cultures of poppy tissue cultures (callus, shoot buds, shoots and plantlets) and *P. arborescens* mycelium. The fungus, however, failed to sporulate during this *in vitro* association with the host tissues. In order to transfer the downy mildew and lodging resistance traits from a wild relative, interspecific hybridization between BC mutant of *P. somniferum* and *P. setigerum* (2n = 44) was carried out. The F_1 progeny was morphologically similar to *P. setigerum*, morphological segregants were recorded in F_2 with somatic chromosome number ranging between 20 - 36. A total of 15 elite selections (2n = 22) were made in F_2 which are being presently assessed in F_3 progeny for isolating homozygous lines. Two of the mutant lines i.e. BC and PCL, developed during the course of this investigation have qualified for release as new improved cultivars of opium poppy.

1. INTRODUCTION

Among the 400 000 or so species of plants known in the world today, *Papaver somniferum* is unique in its profound and diverse effects on humanity [1]. While on one hand poppy offers mankind freedom from pains, on the other it also enslaves mankind due to its addictive misuse. Even today, opium and its derivatives find extensive uses in modern medicines owing to their analgesic antitussive and antispasmodic action. Opium is the air-dried milky exudate obtained by the incision (lancing) of unripe capsules. It contains more than 25 alkaloids [2] belonging to two major classes i.e. (i) phenanthrene derivatives like morphine and codeine and; (ii) benzyl- isoquinoline derivatives like papaverine and noscapine (narcotine). The major alkaloids in crude opium are 4 - 21 % morphine, 0.8 - 2.5

% codeine, 4 - 8 % narcotine, 0.5 - 2.0 thebaine and 0.5 - 2.5 % papaverine. Morhine found its therapeutic use as narcotic analgesic, codeine is used in most of the cough syrups as analgesic and antitussive agent while papaverine is administered as a smooth muscle relaxant in treatment of angina pectoris and asthma [3].

Opium poppy is a major cash crop of the Indian subcontinent. India is the largest producer of opium in the World with nearly 70 000 ha of cultivation and 1 000 metric tons of opium production [3]. Previously, poppy was mainly cultivated for its therapeutic alkaloids but due to the increasing illicit transactions and production of narcotic drugs from poppy alkaloids coupled with rising addiction trends, the International Narcotics Control Board has issued a strict licensing on alkaloid production through the lancing of poppy capsules. Recovery of alkaloids from straw as such and the need for exploring the diversified uses of poppy crop have been recommended. Different poppy growing countries have evolved different strategies in this direction. While countries like Philippines have opted for a replacement of opium crop by *Chrysanthemum*, others like the USSR, Turkey, Yugoslavia and India have switched their interest from lancing-based opium production to straw-based alkaloid extraction and cultivation of poppy for its edible seed oil. Austria, Czechoslovakia, the Federal Republic of Germany, Hungary, Poland and Romania have been using poppy seed oil since a long time. The first (cold) pressing of the seeds yields a white edible oil of high culinary value while the second (hot) pressing furnished a reddish oil used for lamps, soap, and after bleaching, for oil paints. The edible oil is free from narcotic action and is also used in drug preparations for treatment of diarrhoea, dysentry and skin care.

1.1. Composition of poppy seed oil

Opium poppy seeds have a high lipid content (40 - 55 % of the dry weight). One poppy capsule contains 800 - 1 200 seeds (1 - 1.5 mm) which are non-shattering. The average seed yield is 1.2 - 3.0 t/ha. The non-polar lipids, particularly triacylglycerols, constitute a major portion (86 %) of the total lipids in the oil. The active period of oil synthesis lies between 15 and 20 days after flowering [4]. The relative percentages of polar lipids (phospho- and glycolipids), sterols and free fatty acids decline with advancing seed maturation. Though palmitic, oleic and linoleic are the major fatty acids at all stages of seed development there is a clear preponderance of linoleic acid (>70 %) in the oil pool and hence, poppy belongs to linoleic acid group of oil seed crops [5].

1.2. Downy mildew resistance in poppy - genesis of the present study

Like any other agricultural crop, *Papaver somniferum* is also attacked by an array of phytopathogens like fungi, bacteria, viruses and nematodes. Infestations of these microorganisms cause severe damage to the crop and adversely affect the yield and quality of alkaloid and seed oil. Amongst others, the majority of poppy diseases are caused by fungi belonging to different taxonomic groups [6]. These include :

- Seedling blight (*Pythium ultimum* and *P. mamillatum*)
- Leaf spot (*Alternaria brassicae*)
- Leaf blight (*Helminthosporium papaveris*)
- Powdery mildew (*Erysiphae polygoni*)
- Root rot (*Fusarium semitectum*)
- Capsule rot (*Alternaria somniferum*)
- Downy mildew (*Peronospora arborescens*)

Downy mildew of *P. somniferum* caused by *P. arborescens* is the most serious and widespread disease of poppy under the Indian climatic conditions. The infection is epiphytotic in nature and can be manifested at any stage of the life cycle of the host from seed germination to fruit-setting. It causes hypertrophy and curvature of the stem and peduncle and spreads upwards from the lower leaves. The entire leaf surface is found to be covered by a mildew coating consisting of conidiophores and conidia. In extreme severity, the infection spreads up to the stem and capsule resulting in the premature death of the plant [6]. Towards control measures for this obligate fungus, various approaches like crop rotation, wide spacing,

avoidance of low lying damp sites for cultivation, seed disinfection by spraying with 0.5 % Bordeaux mixture and other copper fungicides, application of Dithane Z-78, and Dithane M-45, have been recommended and practiced, but as yet complete control has not been achieved so far. Non-availability of the fungus in the absence of host plants (poppy being an annual crop of 4 months duration) and inability of growing *P. arborescens* on a synthetic medium are two major limitations in developing effective control measures for this important disease of great financial consequence. Need for breeding and developing varieties resistant to downy mildew in poppy is therefore, evident and merits serious scientific considerations.

Mutation breeding techniques have proved very successful in developing disease-resistant cultivars in many crop species [7]. The conventional *in vivo* mutation approaches have recently been supplemented with novel techniques of *in vitro* cell culture mutagenesis [25]. The latter primarily help in hastening the pace of screening large populations in a limited space and time. While the *in vivo* field experiments follow the usual procedure of subjecting the seed populations to various mutagenic treatments and then screening out the promising disease resistant mutants in the field, the *in vitro* approaches center around two main features of cultured cells i.e. exploitations of spontaneous or induced variability originating during undifferentiated state of growth of plant cells [8] and rapid screening of large cell population by challenging them with pathogen/toxin produced by causal micro-organism [9, 10]. Rapid strides in these directions have been made in case of many host-parasite combination (Table I). Disease resistant plants have been developed from cell cultures of sugarcane [11 - 13], potato [14 - 17], maize [18-20], *Brassica*, [21] and *Avena sativa* [22]. These developments have recently been reviewed [23 - 26].

TABLE I. LIST OF SOME OF THE WELL DOCUMENTED CASES OF HOST-PARASITE COMBINATIONS WHERE CELL CULTURE STUDIES HAVE RESULTED IN SCREENING OF RESISTANT STRAINS

Host plant	Pathogen
Sugarcane	Fiji virus
	Helminthosporium
Potato	*Alternaria*
	Phytophthora
	Erwinia
Tobacco	*Phytophthora*
	Alternaria
Rape seed	*Phoma*
Dianthus	*Fusarium*
Maize	*Helminthosporium*
Brassica napus	*Phoma*
Tomato	*Pseudomonas*
	Fusarium
Vicia faba	*Botrytis*
	Phytophthora
	Rhizoctonia

Some of the advantages of using cell culture techniques for the screening of disease resistant variant/mutants and to study the host-parasite relationships include: (i) exclusion of other contaminating micro-organisms; (ii) control of environmental parameters like temperature, light and nutrients; (iii) feasibility of inoculating host cells without wounding; (iv) control of pathogen inoculum vs host cell ratio; (v) the ease of application or removal of exogenous materials; (vi) ability of adding inoculum (spores, zoospores, hyphae etc.) directly in contact with host tissue(without allowing the culture medium to become source of nutrients for the pathogen) and (vii) the ability to follow, cytologically, the progress of infection and colonization of the host tissue by the pathogens [24, 26].

The present study got its genesis in light of the aforesaid developments and was aimed to obtain downy mildew resistant cultivars of opium poppy through induced mutagenesis. The programme was carried out under the aegis of an International Atomic Energy Agency (IAEA) coordinated research project on "Mutation Breeding of oil Seed Crops" and envisaged the application of induced mutagenesis through both conventional and novel cell culture techniques. The project was launched in June, 1989 with following broad objectives and work plan :

A. Development of *P. arborescens* resistant cultivars with high alkaloid/seed oil yields coupled with other desirable agronomic traits.

B. Initiation of callus cultures and development of protocols for plantlet regeneration.

C. Establishment of dual cultures of *Peronospora arborescens* and *P. somniferum* cells/callus, and refinement in *in vitro* mutagenesis and screening techniques for downy mildew resistance.

D. Induction of resistance for downy mildew at cell culture level and its transmission and manifestation at the whole plant level.

E. To work out the genetics of desired induced mutations.

F. To carry out interspecific hybridization between downy mildew susceptible *P. somniferum* and its wild, disease resistant relatives, *P. setigerum.*

This report enumerates the experiments and results obtained henceforth alongwith the methodology employed therein.

2. MATERIALS AND METHODS

2.1. General biology of the host and the parasite

P. somniferum (2n = 22) is a dicotyledonous plant belonging to the family *Papaveraceae.* The plant is an erect herb (30 - 100 cm high) with a thick pithy stem. The radical leaves are elongated, thick and soft. They are sessile, ovate or oblong with irregular lobes running down to the midrib. The reproductive stage is characterized by a drooping bud, having a hairy peduncle. Flowers are solitary, bisexual and regular. Petals are 4, free and generally white. The stamens are hypogynous, indefinite and arranged in several whorls. Carpels are many, united, with no style but have capitate stigma with 8 - 14 lobes. Fruit is a capsule. Seeds are numerous, reniform, white to pink or red in colour. They have a well developed endosperm filled with aleurone grains. After the fall of the petals, the capsule increases rapidly in size. By this time the laticifers show rapid branching. The latex is maximum at this stage which is also referred as "industrial maturity stage" of capsules. The latex is a cytoplasmic hydrosol containing numerous vesicles. These vesicles are the seat of alkaloid biosynthesis. Thus, they have the function of storage, biosynthesis and translocation of alkaloids.

In the present study, two improved inbred varieties of *P. somniferum*, namely SHWETA and SHYAMA, developed at CIMAP, under a genetic improvement programme of poppy, were used as parents. Both the base varieties are high latex producers with improved morphine content in the crude alkaloid [3]. They possess 56 - 83 % more opium (66 - 78 kg/ha) than the local cultivars (40 - 42 kg/ha) and have an average of 18 % morphine content in the crude alkaloid as compared to only 10 - 12 % in the local strains. However, both the varieties are highly susceptible to downy mildew disease.

The obligate fungus *P. arborescens* belongs to the family *Peronosporaceae* of the order *Peronosporales* (class *Phycomycetes*). The fungus is characterized by coenocytic mycelium that grows intracellularly within the host cells. Sporangiophores are typically dichotomously

branched with terminal branches sharply pointed. Sporangia is coloured and lacks apical papilla. Oospores are smooth and germinate by germ tubes. The fungus winters in old plant debris in soil [28].

2.2. Mutagenic treatment of the seeds and raising of M_1

Dry seeds of the parent varieties "Shweta" and "Shyama" were treated with gamma rays and ethyl methanesulfonate (EMS), either in single doses or in combinations, at the following dose/concentration rates :

A. Physical mutagen (gamma rays; ^{60}Co)
(i) 50 Gy (ii) 150 Gy (iii) 200 Gy

B. Chemical mutagen (EMS; 6h exposure)
(i) 0.2 % (ii) 0.4 % (iii) 0.6 % (w/v)

C. Combined treatment
(i) 50 Gy+0.2 % EMS (ii) 50 Gy+0.4 % EMS (iii) 50 Gy+0.6 % EMS

For each dose 10 g seeds were used. Seeds exposed to physical mutagen were sown in the field immediately after the treatment. In case of EMS treatments, the seeds were pre-soaked in distilled water (dH_2O) and were then exposed to EMS for 6h and subsequently sown in the field. For combined dose treatments, seeds were first irradiated with gamma rays (50 Gy), followed by soaking in dH_2O for 12h and treatment with varying concentrations of EMS for 6h. All mutagenic treatments were given as per the safety guidelines of FAO/IAEA Technical Report Series No.119. For each treatment 50 M_1 plants, of which 45 were normal looking and 5 were deformed/morphologically different, were randomly selected to screen micro- and macro- mutations, respectively. M_2, M_3 and M_4 generations were raised for the two classes of plants from each of the nine mutagen treated families, by growing selfed seeds of selected plants in progeny-row plots.

2.3. Callus induction and plantlet regeneration

Callus cultures were initiated using cotyledonary leaf and hypocotyl explants excised from 10-15 days old seedlings of two parent varieties and 4 macro and 12 micro mutant lines selected in M_1 and M_2 generations. Seeds were aseptically germinated on Murashige and Skoog [29] basal medium fortified with 0.7 % agar, 100 mg/l myo-inositol and 3% sucrose (henceforth referred to as MS), under low temperature (18-20 °C) and completely dark incubation conditions. For callus induction, explants were cultured on MS supplemented with 0.05 - 5.0 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), either alone or in combination with 0.1 - 1.0 mg/l Kinetin (Kn). For regeneration experiments, single and double combinations of various cytokinins namely kinetin, 6-benzylaminopurine (BAP) and 2-isopentyladenine (2-iP), and auxins namely indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and naphthaleneacetic acid (NAA) were made in MS at concentrations ranging from 0.1 - 5.0mg/l. Unless otherwise stated, the pH of the media was adjusted to 5.8 0.1 with 0.1N HCl/NaOH, before autoclaving at 121 °C (18 - 20 psi). The cultures were incubated at 25 3 °C under 2 500 - 3 000 lux intensity and 70 - 80 % RH.

2.4 Hardening of *in vitro* raised plants and field transplantation

The in vitro regenerated plantlets with 1 - 2 cm long roots were carefully removed from the culture vessels in winter season (December), thoroughly washed with water to clean off the adhering agar and planted in plastic pots (4" diameter) containing soil: sand: organic manure (1:1:1). The pots were covered with an inverted glass beaker to maintain high humidity around the young propagule for 10 - 12 days. The plants were initially irrigated with Hoagland [30] nutrient solution for one wk. After 20 - 25 days of hardening, the plants were shifted to the field where they were grown to maturity following standard agronomic practices.

2.5. *In vitro* mutagenic treatments

Calli of selected mutant lines i.e. BC, DM, M8/102/4 and two parental varieties were subjected to *in vitro* mutagenesis by exposing them to following mutagenic treatments :

A. Individual gamma rays doses
(i) 10 Gy, (ii) 30 Gy, (iii) 50 Gy, (iv) 70 Gy, (v) 100 Gy
(vi) 150 Gy, (vii) 200 Gy, (viii) 300 Gy and (ix) 400 Gy

B. Combined gamma and EMS doses
(i) 10 Gy+0.1 % EMS, (ii) 30 Gy+0.1 % EMS,
(iii) 50 Gy+0.1 % EMS, (iv) 0.1 % EMS (control)

Callus from stock cultures was plated on callus maintenance medium (MS + 1.0 mg/l 2,4-D + 0.1 mg/l Kn) in sterile petri dishes. Each petri dish (90 cm diameter) contained 12-15 callus pieces of 200 - 250 mg f.wt. each. For each radiation dose 5 replicated plates were irradiated. In case of combined treatments irradiated calli were aseptically exposed to 0.1 % EMS for 2 h. The EMS was prepared in liquid MS and filter sterilized before use. The EMS-treated calli were then washed 4 - 5 times with liquid MS medium before implanting on culture medium for incubation. Mutagenised calli were either cultured on callus maintenance medium for proliferation or on shoot bud regeneration medium (MS + 1.0 mg/l BAP) for organogenesis. Untreated calli of similar age were simultaneously maintained as controls.

2.6. Co-cultivation of host and parasite

To grow *P. arborescens* in culture, attempts were made through two approaches. In the first approach, the fungus was cultured on a synthetic potato-dextrose-agar (PDA) medium fortified with 5, 10, 15, 20, 25 and 50 % (w/v) host leaf extract or decoxitant. In the second method the fungal spores were co-cultivated on the surface of aseptically growing callus or regenerated plantlets. For both the approaches, sporangia (conidia) from freshly dehisced sporangiophores were collected from the infected plants in the the early hours of the morning and used for inoculations. For infecting poppy cultures with *Peronospora* spores the following techniques were employed :

A. Infected leaf pieces (1 mm^2) were placed on 3 - 4 weeks old callus cultures.

B. Only spores were dusted on callus.

C. Only spores were dusted on regenerated shoots or leaves of regenerated plantlets.

Since dusting of spores resulted in high rate of contamination in the cultures, "Serial Dilution" technique was followed. For this, spores of *P. arborescens* were suspended in double distilled sterile water and dilutions were made in such a way that spore density of 100-1 spores/0.1 ml was obtained. These suspensions were then used for doing inoculations on host tissues. Squash preparations or hand cut sections were cytologically examined to check the fungal growth in the host tissues.

2.7. Analytical techniques employed

Extractions and quantitative estimations of alkaloids were done following the procedure of Akhila and Uniyal [31]. Oil content in the seed was determined by solvent extraction in n-hexane using soxhlet apparatus [32]. For gas chromatographic study of the seed oils the methyl esters of the fatty acids of total lipids and triacylglycerol were prepared following the procedure of Porschmann [33].

3. RESULTS AND DISCUSSION

3.1. *In vivo* studies

3.1.1. Induction and screening of mutants

Populations raised from seeds treated with 9 mutagen treatments were studied in M_1 and M_2 generations. The quantitative variabilities were evident for various metric traits like capsule number/plant, straw yield, plant height, morphine content, seed yield etc. The two parent varieties were well differentiated by their relative degree of physiological damages (expressed in M_1) with regards to reduction in seed germination, seedling height, pollen sterility and phenotypic deformities. This mutagen sensitivity was more marked in Shyama than in Shweta. However, the potentiality order of the three mutagens for a specific parameter of physiological damage was identical in the two varieties as follows :

A.	Seedling height reduction	Combined mutagen > gamma rays> EMS.
B.	Seed germination, pollen sterility and phenotypic deformities	Combined mutagen> EMS>gamma rays

In Shweta, the mutation rate per unit dose of combined mutagen was 20 times higher than that of gamma rays and two times higher than EMS. In Shyama, this is about 8 times and 7 times more in EMS and combined mutagen treatment than in gamma ray, respectively. Both the treated varieties showed high mitotic and meiotic abnormalities such as chromosomal translocation, dicentric chromosome, sticky anaphase bridge, formation of differential segments in pachytene pairing, inversions, translocation, laggard behaviour etc. Apparently high physiological damage in treated Shyama was paralleled by its relatively high cytological damage over Shweta. Mean abnormal somatic and meiotic cells in M_1 of Shyama was 24.2 % and 37.8 % against the corresponding figures of 18.5 and 19.9 % of Shweta, respectively.

The two treated varieties showed differential response in M_2 for yielding chlorophyll and viable mutations (Table II). While Shweta exhibited chlorophyll mutations (i.e. albino, xantha, albino-xantha, xantha-alba, albino-viridis etc.) for 6 treatments, Shyama showed it for only 3 treatments. EMS doses were having great potential over the other treatments for manifesting chlorophyll mutations in Shweta, while in Shyama the maximum potentials for this were noted in 150 Gy exposure and 50 Gy + 0.4 % EMS. For inducing maximum visible mutation (i.e. frequency of chlorophyll + viable mutation) 50 Gy + 0.4 % EMS and 0.6 % EMS alone were most potent doses for Shweta and 50 Gy + 0.4 % EMS and 50 Gy + 0.2 % EMS for Shyama. The Msd values are depicted in Table II. It was therefore, evident that Shweta was superior to Shyama in generating high frequency of visible mutations.

Keeping in view the overall objectives of the project, 4 macro-mutants (i.e. red petaled (RP), big capsulated (BC), pinnately cleft leafed (PCL) and dwarf(DM)) and 12 micro-mutants (Table III) were selected for further investigations. These were advanced to M_3 and M_4 for studying the genetics of their inheritance and evaluation of their productivity-linked morpho-physiological attributes. The results of such performance trials are summarized in Table IV. Improvement in morphine content in PCL, RP and BC was 67.9, 123.4 and 136.2 %, respectively over the parental base. The 16 mutants were also assessed for their relative susceptibility/resistance towards *P. arborescens* infestation in the field and the results are summarized in Table V. Amongst other, fringed leaf mutant (PCL) and dwarf mutant (DM) could be characterized in M_3 as new genotypes having high *per se* morphine content of 0.86 % and 0.95 % respectively as against 0.46 % in the mother variety Shweta. They also showed very high level of resistance against *P. arborescens*. The other two macro- mutants (BC and RP) also registered very high morphine level of 1.07 % and 0.94 % but had high susceptibility for downy mildew infection.

TABLE II. MUTATION FREQUENCY IN M_2 SEEDLINGS RAISED FROM DIFFERENT MUTAGEN TREATMENTS IN *P. somniferum* L.

Treatment	Number studied		Mutation per Conf.		Total visible mutation (M_2 seedling)
	M_1 Plant Progenies	M_2 Seedling	Chlorophyll mutation (M_2Seedling)	Variable mutation (M_2Seedling)	
Shweta	485	8 770	0	0	0
50 Gy	475	7 580	0	0.53	0.53
150 Gy	428	5 960	0.034	1.04	1
F07					
200 Gy	306	6 874	0	0.93	0.93
0.2% EMS	455	9 920	0.05	0.73	0.78
0.4% EMS	468	5 250	0.133	1.69	1.82
0.6% EMS	370	4 740	0.928	1.52	2.45
50Gy+0.2%EMS	335	5 600	0.054	1.82	1.87
50Gy+0.4%EMS	326	6 366	0.895	2.48	3.37
50Gy+0.6%EMS	143	4 650	0	0.73	0.73
Shyama	496	8 627	0	0	0
50 Gy	488	7 665	0	0.34	0.34
150 Gy	436	5 762	0.139	0.38	0.52
200 Gy	337	6 910	0	0.36	0.36
0.2% EMS	325	8 540	0.058	0.33	0.39
0.4% EMS	318	9 625	0	0.27	0.27
0.6% EMS	212	6 344	0	0.46	0.46
50Gy+0.2%EMS	465	4 224	0	0.78	0.78
50Gy+0.4%EMS	266	6 170	0.081	0.58	0.66
50Gy+0.6%EMS	117	4 525	0	0.46	0.46

3.1.2. Genetics of induced mutants

Genetics of BC, RP, PCL (that were expressed in M_1) and DM (that was expressed in M_2) was worked out on the basis of their respective segregating patterns in M_2 - M_4 generations upon selfing and back-crossing with normal parents. While RP and BC mutants were obtained from parent variety Shweta (treated with 0.2 % EMS and 50 Gy gamma rays, respectively) the PCL was recovered from Shyama treated with 50 Gy + 0.4 % EMS. All the 3 mutants bred true in M_3 generation. The M_2 family of both RP and BC gave a phenotypic segregation of 3 mutants : 1 normal indicating a monogenic inheritance for both petal colour and capsule size wherein the mutant form is dominant. This was reconfirmed by crossing the mutants with untreated controls (Table VI). All F_1 hybrids were like the mutants. F_2 segregation in both the classes were in accordance with M_2 segregation. The values being 186:68 (normal) for RP and 163:62 (normal) for BC.

The M_2 family of PCL gave 5 phenotypic classes of segregants i.e. normal (N), lacerate-leaved (LC), pinnately cleft leaved (PCL), narrow pinnately cleft (NPC) and decumbent (DC); a grassy mutant having almost no lamina but a midrib only), in the ratio of 1N:4LC:6PCL:4NPC:1DC. The results of F_1 segregation of each of these families (except for DC because of its non-flowering and ultimate lethality) when crossed with normal untreated parents are summarized in Table VII. The results clearly showed that leaf shape in opium poppy is governed by two duplicate factors, each having cumulative function. If L_1 and L_2 represent two active factors with additive effect against their corresponding null alleles l_1 and l_2 then segregation in M_1 and F_1s may be explained as depicted in Table VIII. Hence, PCL mutant though expressed in M_1, is not a dominant mutation as was the case with RP and BC.

TABLE III. YIELD PERFORMANCE (kg/ha) OF DIFFERENT MACRO AND MICRO MUTANTS IN M_4 RELATIVE TO THAT OF PARENTS, 'SHWETA' AND 'SHYAMA'

Elite Selection*	Source	Yield of		Percent improvement over the parental base	
		Straw	Morphine	Straw	Morphine
I. Parent :					
18/188/1 (Shweta)	-	607.20	4.19	-	-
3/189/3 (Shyama)	-	762.96	5.79	-	-
II. Macro-mutants :					
25/1/2 (Dwarfmutant)	50Gy+0.4% EMS Shweta	744.48	7.05	22.61	68.27
3/2/2 (Red petal)	0.2% EMS Shweta	658.68	6.19	8.48	47,78
28/3/4(Big capsulated)	50Gy Shweta	762.30	8.16	25.54	94.68
12/4/2 (Pinnately cleft)	50Gy+0.4% EMS Shyama	504.24	4.34	Negative feature	At par with the control
III.Micro-mutants :					
M6/59/2	200Gy Shweta	619.08	6.07	1.96	44.81
M14/60/4	200Gy Shweta	645.48	6.13	6.30	46.36
M26/100/2	50Gy+0.2% EMS Shweta	749.10	6.89	23.37	39.26
M8/102/4	50Gy+0.2% EMS Shweta	696.30	8.63	14.67	106.08
M22/104/3	50Gy+0.2% EMS Shweta	630.30	7.06	3.80	68.49
M15/105/1	50Gy+0.2% EMS Shweta	737.22	8.11	21.41	93.56
M4/143/1	50Gy+0.2% EMS Shweta	570.90	5.25	Negative feature	25.36
M6/111/2	50Gy+0.4% EMS Shweta	678.48	5.97	11.74	42.51
M10/113/3	50Gy+0.4% EMS Shweta	650.10	5.79	7.06	38.09
M17/116/9	50Gy+0.4% EMS Shweta	730.62	7.16	20.33	70.89
M19/129/2	50Gy+0.6% EMS Shweta	645.48	8.91	6.30	112.61
M13/8/3	50Gy Shyama	704.88	6.91	16.08	19.14

*: First, second and third mumber with the selection representing S_2/M_2 family No. S_2/M_2 general selection No. and S_3/M_3 selection No., respectively.

TABLE IV. MORPHO-PHYSIOLOGICAL CHARACTERISTICS OF THREE INDUCED MACRO-MUTANTS OF OPIUM POPPY IN M_4 GENERATION

Metric Trait	Parent	Induced Mutants		
	(Shweta)	Red Petaled (RP)	Big Capsulated (BC)	Pinnately Cleft Leaved (PCL)
Plant height (cm)	95.89 0.99	108.98 0.46	100.54 0.79	102.83 0.45
Capsule number/plant	2.56 0.11	3.52 0.19	2.50 0.17	3.28 0.22
Capsule index	0.58 0.02	0.75 0.01	0.83 0.005	0.89 0.01
Straw weight/plant(g)	6.50 0.30	10.31 0.89	7.81 0.63	7.54 0.53
Seed yield/ main capsule (g)	2.41 0.15	2.72 0.12	2.98 0.12	2.72 0.19
Seed yield/plant (g)	4.56 0.23	8.62 0.61	6.98 0.52	6.49 0.46
Morphine content (%)	0.47 0.02	1.05 0.01	1.11 0.007	0.89 0.007
Pollen fertility (%)	92.62 0.14	86.95 0.20	90.80 0.17	82.28 0.26

TABLE V RELATIVE SUSCEPTIBILITY/RESISTANCE OF SELECTED MUTANTS OF OPIUM POPPY TOWARDS *P. arborescens* INFESTATION

Elite selections	Disease reaction towards downy mildew under field condition
I. Parents:	
Shweta-18/188/1	S (+++)*
Shyama- 3/189/3	S (+++)
II. Macro-mutants:	
25/1/2 (DM)	R (++++)
3/2/2 (RP)	S (+++)
28/3/4 (BC)	S (++)
12/4/2 (PCL)	R (+++)
III. Micro-mutants:	
M6/59/2	R (+)
M14/60/4	R (++)
M26/100/2	S (++++)
M8/102/4	R (+++)
M22/104/3	S (+++)
M15/105/1	S (+++)
M4/143/1	R (+)
M6/111/2	R (++)
M10/113/3	R (+)
M19/129/2	S (+++)
M13/8/3	S (++)
M17/116/9	S (++)

R : Resistance
S : Susceptibility
* : Increasing numbers of "+" denotes the degree of response

TABLE VI. M_2 AND F_2 SEGREGATION OF RP AND BC MUTANTS OF OPIUM POPPY

M_2/F_2	Mutant plants	Normal plants	X^2 (3:1 ratio)	P
M_2 RP	71 (Red)	27 (White)	0.340	0.5-0.7
M_2 BC	76 (Big)	31 (Normal)	0.900	0.3-0.5
F_2 PR x untreated control	186 (Red)	68 (White)	0.425	0.5-0.7
F_2 BC x untreated control	163 (Big)	62 (Normal)	0.784	0.3-0.5

It might have arisen from a recessive mutation at one of the two cumulative factors involved in the leaf shape and thus expressed a quasiquantitative mode of inheritance.

The dwarf mutant (DM) was recovered from the parent variety Shweta treated with 50 Gy + 0.4 % EMS. This was most interesting mutant in view of the primary objective of the project as it showed highest level of resistance towards downy mildew disease. Pollen sterility in this otherwise promising mutant, was the main limitation in its exploitation.

Besides high pollen sterility, the DM has shown that at least a dozen of characters were affected by the mutagenesis like height, number of capsule/plant, deformed ovary, less no. of stamens and short style etc. All the characters were transmitted from one generation to the

TABLE VII. M_2 AND F_1 SEGREGATION PATTERNS OF THE PCL MUTANTS AND ITS DERIVATIVES

M_2/F_1	Different leaf shape type					X^2	P
	N	LC	PCL	NPC	DC		
M_2	4	33	49	26	3	5.444 (1:4:6:4:1)	.2-.3
Heterozygous PCL x normal Shyama	26	64	35	0	0	1.368 (1:2:1)	.5-.6
Homozygous PCL x normal Shyama	0	55	0	0	0	-	-
LC x normal Shyama	46	39	0	0	0	0.576 (1:1)	.4-.5
NPC x normal Shyama	0	65	71	0	0	0.264 (1:1)	.6-.7

TABLE VIII. GENETICS OF PCL MUTANT OF OPIUM POPPY

A. M_2 ANALYSIS

UNTREATED PARENT SHYAMA

$(L_1\ l_1\ L_2\ L_2)$ or $(L_1\ L_1\ L_2\ l_2)$

|

Mutagen treatment

|

M_1 ($L_1\ l_1\ L_2\ l_2$: PCL mutant)

|

M_2 Segregation in a ratio of 1:4:6:4:1

i.e. 1/16 $(L_1\ L_1\ L_2\ L_2)$: Normal leaf (N)

4/16 $(L_1\ L_1\ L_2\ l_2,\ L_1\ l_1\ L_2\ L_2)$: Lacerate leaf (LC)

6/16 $(L_1\ L_1\ l_2\ l_2,\ L_1\ l_1\ L_2\ l_2,\ l_1\ l_1\ L_2\ L_2)$: Pinnately cleft leaf (PCL)

4/16 $(L_1\ l_1\ l_2\ l_2,\ l_1\ l_1\ L_2\ l_2)$: Narrowly pinnate leaf (NPC)

1/16 $(l_1\ l_1\ l_2\ l_2)$: Decumbent grassy leaf (DC)

B. CROSS HYBRIDIZATION ANALYSIS

(i) Heterozygous PCL x Normal

$(L_1\ l_1\ L_2\ l_2)$ $(L_1\ L_1\ L_2\ L_2)$

|

F_1 : 1 $L_1\ L_1\ L_2\ L_2$ (N)

1 $L_1\ L_1\ L_2\ l_2$ (LC)

1 $L_1\ l_1\ L_2\ L_2$ (LC)

1 $L_1\ l_1\ L_2\ l_2$ (PCL)

(i.e. 1N:2LC:1PCL)

(ii) Homozygous PCL x Normal

$(l_1\ l_1\ L_2\ L_2)$ $(L_1\ L_1\ L_2\ L_2)$

|

F_1 : $L_1\ l_1\ L_2\ L_2$ (LC)

(iii) LC mutant x Normal

$(L_1\ L_1\ L_2\ l_2)$ $(L_1\ L_1\ L_2\ L_2)$

|

F_1 : 1 $L_1\ L_1\ L_2\ L_2$ (N)

1 $L_1\ L_1\ L_2\ l_2$ (LC)

(i.e. 1N:1LC)

(iv) NPC mutant x Normal

$(L_1\ l_1\ l_2\ l_2)$ $(L_1\ L_1\ L_2\ L_2)$

|

F_1 : 1 $L_1\ L_1\ L_2\ l_2$ (LC)

1 $L_1\ l_1\ L_2\ l_2$ (PCL)

(i.e. 1LC:1PCL)

next in one block and hence inheritance pattern of DM indicated that simultaneous recessive mutations of a number of closely linked or neighboring genes, or mutation of a major gene having pleiotropic effect on yield and other major genes might have accounted for its origin.

3.1.3. Rectificaion of undesirable features of selected macro-mutants in BC and DM

Attempts were also made to rectify the negative traits associated with otherwise desirable mutants namely, BC and DM, through selection and hybridization. While BC was associated with severe lodging problem, DM had high pollen sterility index. Though selection in F_2 from the cross BC x normal parent gave further productive genotype, it could not provide lodging resistance. Similarly, efforts to break the undesirable association between pollen sterility and other morpho-physiological attributes of DM by cross hybridization with normal parent were also unsuccessful. It is probably due to very tight linkage among the mutated loci in question. Attempts were, therefore, made to multiply the plantlets from irradiated callus cultures of these two mutants and look for desirable somaclonal variations (for details see *in vitro* studies).

3.1.4. Pilot-scale trials for productivity assessments

During the course of this study emphasis was given to make systematic pilot scale appraisal of productivity of the induced mutant genotypes. A total of 12 micro-mutants and 3 true-breeding macro-mutants (BC, PCL and DM) were grown in replicated progeny blocks for assessing their straw alkaloid (morphine) productivity and resistance towards downy mildew attack. The per hectare alkaloid yield in these mutants ranged between 9.28 kg to 10.28 kg. The parent variety Shweta (included as check in the field trials) registered a corresponding yield of 4.64 kg. The percent improvement over the parental base in these mutants, therefore, varied between 100 - 121.5 %.

During 91 - 92 season the high yielding mutant strain BC was assessed for its productivity under final pre-release demonstration trial where it clearly established its superiority over the parent variety Shweta and another improved strain "Sanchita". The straw alkaloid content *per se* in BC was recorded to be 1.05 % against 0.94 % and 0.74 % of Sanchita and Shweta, respectively. Per hectare straw productivity in BC was 1375 kg as against 572 and 698 kg of Sanchita and Shweta, respectively. Per hectare seed yield in BC was 1476 kg while the corresponding figures for Shweta and Sanchita were 977 and 746 kg, respectively. The BC mutant is now ready for release as a new variety for commercial exploitation. Similar pre-release trial for PCL macro- mutant is being laid out during current planting season of 92 - 93.

As a part of general suggestion by the participants of the second RCM of this project, held at Cairo (Egypt; 16 - 20 Sept., 1991), the 16 selected mutant strains were also assessed for their seed oil productivity and the results are summarized in Table IX (DM was excluded because of the poor seed yield and quality). Analysis of the data revealed that seed oil content did not show a significant range of variation within the mutant population (31.6 % - 45.2 % as against 38.8 % in control variety). The macro-mutant RP registered the highest oil content of 45.2 % of seed dry weight, followed by 42.8 % in the fringed leafed mutant PCL. Preliminary TLC and GC studies indicated a pre-dominance of linoleic acid (65-70 %) in the oil.

3.1.5. Interspecific hybridization

In order to further improve the high yielding mutant BC for downy mildew and lodging resistance, interspecific hybridization was resorted to. For this, interspecific hybrids were raised by making crosses between BC ($2n = 2x = 22$) and the shattering type but disease and lodging resistance wild species of *P. setigerum* ($2n = 4x = 44$). The derived F_1 generation ($2n = 3x = 33$) from this cross was grown during 90 - 91 and F_2 during the period 91 - 92. While morphological features of F_1 were similar to *P. setigerum*, a spectrum of morphological segregants were recorded in F_2. Somatic chromosome number in F_2 segregants ranged from 20 - 36. Segregants with somatic chromosome number deviating from 22, were cytologically

TABLE IX. OIL CONTENT IN HIGH ALKALOID-YIELDING MACRO- AND MICRO-MUTANTS OF OPIUM POPPY

Mutant strain	Oil content (% d. wt.)
I. Macro-mutants	
12/4/2 (PCL)	42.8
28/3/4 (BC)	39.0
3/2/2 (RP)	45.2
B. Micro-mutants	
M/10/113/3	35.2
M/6/59/2	34.6
M/26/100/2	35.8
M/22/104/3	42.0
M/19/129/2	42.6
M/13/8/3	31.6
M4/143/1	34.2
M15/105/1	37.2
M8/102/4	41.8
M17/116/9	34.2
M14/60/4	37.4
C. Parent (Shweta)	38.8

unstable and lacked morpho-physiological fitness. F_2 selection was practiced for stable hybrid with desirable attributes and diploid chromosome number 2n = 22. A total of 15 elite selections have been obtained from F_2 generation, which are presently being grown in F_3 progeny beds for isolating homozygous lines.

3.2. *In vitro* studies

3.2.1. Raising of aseptic seedling cultures as explant source

Conditions were standardized for *in vitro* seed germination and raising of aseptic seedling cultures of both the parent varieties and 16 mutant lines. Continuous washing under running tap water for 24 h was found to be essential for high frequency seed germination. Basal MS medium without any hormone was found to be the best for seed germination (80 - 90 %). One of the important finding of these experiments was irrecoverable loss of seed germinability if cultures were kept in light during 24 - 48 h of culture initiation. Dark incubation at 18 - 20 °C was necessary for seed germination. Sufficient stock of aseptic seedling cultures was raised on MS to serve as explant source for callus induction.

3.2.2. Callus induction and maintenance

Efforts were made to devise an optimal growth medium for the induction of callus cultures. For this, cotyledonary leaf and hypocotyl segment explants from aseptically reared seedlings were excised and transferred onto MS supplemented with a range of 2,4-D (0.05, 0.10, 0.20, 0.40, 0.60, 1.0, 2.0, 3.0 and 5.0 mg/l) and Kn (0.1, 0.2, 0.5, 0.75 and 1.0 mg/l), either alone or in combination. Though presence of 0.75-1.0 mg/l 2,4-D in the medium was sufficient for eliciting the callus response in all the mutant lines, presence of low concentration (0.1 - 0.3 mg/l) of Kn in 2,4-D containing medium greatly improved the subsequent callus proliferation and growth. A stock of callus cultures has, thus, been generated on a medium containing MS salts + 3 % sucrose + 100 mg/l myo-inositol + 1.0 mg/l 2,4-D and 0.1 mg/l Kn, hereafter referred to as callus maintenance medium (CM).

Browning of explants and consequently of the medium was a severe problem encountered during callus induction phase. However, this was overcome by incorporating 40 mg/l of ascorbic acid (anti-phenolic agent) in the medium during 3 - 4 initial culture passages.

3.2.3. Plantlet regeneration from callus

When sufficient callus stock of each line was obtained on CM medium, efforts to induce complete plantlet regeneration from such calli got initiated. In all, nearly 250 single or double combination of various auxins (IAA, NAA or IBA at 0.01 - 10.0 mg/l level) and cytokinins (Kn, BAP, 2iP at 0.1 - 5.0 mg/l level) were tried. Out of these, medium comprising MS salts and vitamins + 100 mg/l myo-inositol and 1.0 - 2.0 mg/l BAP alone was found best for the maximum induction and proliferation of shoot buds in all the genotyes. The green nodular protuberances (shoot bud primordia) became visible on the callus surface within 8-10 days of transfer to BAP containing medium. By another two wks, leafy shoots measuring 2-4 cm in height developed from these buds. These shoots, however, did not form roots on BAP medium even after an extended culture passage of 8 - 10 wk. Reducing the salt strength in the medium to half and one fourth, with or without 0.1 - 2.0 mg/l IAA, NAA or IBA also proved a failure in eliciting a rhizogenic response in these regenerated shoots.

A survey of literature also indicated that though somatic embryogenesis and shoot bud proliferation from cell suspension and callus cultures of poppy have been frequently reported, regeneration of roots in such shoots has always been sporadic and unsatisfactory [34 - 42]. Hence, experiments were subsequently devised to see the influence of organic adjuvants and physical factors like light, water stress and temperature on root regeneration in poppy shoot cultures. Nearly 50 manipulations were made and it was observed that complete absence of organic adjuvants in the basal medium and low day/night temperature (18 °C/12 °C) for incubation are critical requirements for root initiation. Though rooting took place when shoots were incubated under such growth conditions on half strength MS medium without organics, the formation and growth of secondary and tertiary rootlets was optimum when 0.1 mg/l IAA or 0.1 - 0.5 mg/l IBA was also incorporated in the medium. Another important observation was that if the shoots regenerated on MS + 1.0 mg/l BAP were cultured for a brief period of 12 - 16 days on MS basal medium (devoid of phytohormone) prior to their placement on the rooting medium, the number and growth of regenerated roots could be markedly enhanced. A stock of completely regenerated plantlets of different lines was, thus, raised in this manner. Some of the cultures also showed *in vitro* flowering and capsule formation. In order to test the survival of such plantlets in soil, they were transplanted in pots and maintained in growth chamber (in summer) or in the glass house (in winter). They exhibited an establishment frequency of 70 - 80 %. The hardened plants were transplanted in field during 91 - 92 season where they recorded 50 - 60 % survival. Seven of the plants also flowered and set viable seeds. These seeds have now been sown to raise R_2 progenies. Besides, it is also aimed to transfer the second batch of 100 - 150 in vitro-raised plants in the field during 92 - 93. To the best of our knowledge this is the first study where complete plantlets, capable of soil transplantation have been produced in opium poppy cultures.

3.2.4. *In vitro* mutagenesis and screening

In order to broaden the spectrum of variation (particularly with reference to disease resistance) and rectification of undesirable traits of otherwise promising mutants i.e. BC, DM and MS/102/4, callus cultures of these lines were subjected to ^{60}Co gamma rays radiation treatments. Initially the doses tested were 50, 100, 150, 200, 250, 300 and 400 Gy. None of the lines survived beyond 100 Gy dose. The treatments were then narrowed down to 10 - 150 Gy. The LD_{50} dose was found to lie between 30 - 40 Gy, and doses beyond 70 Gy were lethal. The surviving sectors of the calli obtained on 10, 30 and 50 Gy were subcultured on fresh CM medium. After 15 days half of such cultures were subjected to second repeat exposure to respective irradiation dose to get rid of the escapees. Progenies of all the irradiated callus classes, thus obtained, were multiplied by repeated subculturing through a 4-wk culture passage.

Plant regeneration in different irradiated callus classes was achieved following the procedure outlined above. A decline in regeneration potential was noted with an increase in irradiation dose. This inhibition in shoot bud organogenesis was more marked in irradiated calli on which EMS (0.1 %) treatment was superimposed. The regenerants were screened *in vitro* with respect to their disease reaction following artificial infection with spore suspension of *P. arborescens*. Normal healthy looking plants are being carried forward to field for further screening and assessment during 92 - 93 (ongoing work).

3.2.5. Establishment of dual cultures

Experiments in this direction were commenced in 1990 with the onset of downy mildew disease in the fields of CIMAP experimental farm. Attempts to grow the fungus on synthetic PDA medium, either alone or after supplementation with host leaf extract/decoxitant, failed to provide any success in obtaining a pure culture of the parasite, reaffirming thereby the very strict obligate nature of this host-parasite complex. Experiments pertaining to efforts to grow the fungus as dual cultures on *in vitro* grown tissue of *P. somniferum*, on the other hand, gave rise to encouraging possibility. The fungal spores germinated and formed typical intracellular, aseptate mycelium (with characteristic dichotomous branches) following their dusting on the surface of all the *in vitro* grown host tissues i.e. callus, leafy shoots and leaves of regenerated plantlets. However, dusting of spores always resulted in the contamination of the cultures due to associated bacteria and other saprophytic fungi. Cleaning of *Peronospora* culture by successive transfer to fresh callus (uninfected) also failed to minimize the contamination problem. Addition of 200 - 250 mg/l Rose Bengal and 10 - 50 mg/l streptomycin in the medium could reduce the growth of associated contaminants but to a limited extent only. Besides, these treatments also adversely affected the mycelial growth of *P. arborescens*. "Serial dilution" technique was then followed. For this, spores of *P. arborescens* were suspended in double distilled sterile water. The suspension was then serially diluted with sterile water in sterilized screw cap vials (10 ml) with spore density measured at each dilution with the help of a haemocytometer. Dilutions were made in such a way that spore densities of 100 - 1 spores/0.1 ml were obtained. These suspensions were used for carrying out the inoculations on host tissues. The best results were obtained with suspension having spore density of 1 - 2 spores/0.1 ml. Callus tissues and young leaves of *in vitro* regenerated plantlets were the best host tissue to catch infection. Though, mycelial growth was very good, no fresh sporulation occurred when cultures were incubated at normal growth conditions i.e. 23 - 27 °C and 3 000 lux light intensity. Besides, these dual cultures also failed to survive beyond 2 - 3 wk of infection. Efforts are now reinforced in this direction to induce *in vitro* fructification of the fungus in such dual cultures and to work out the optimal host : inoculum densities for long term maintenance of these cultures.

4. CONCLUSION

The present study clearly demonstrates that genetic improvement of opium poppy through induced mutagenesis (both *in vivo* and *in vitro*) is feasible. A variety of mutants and somaclonal variations could be generated, screened and advanced to field trial levels particularly in relation to yield contributing metric traits and moderate to high level of resistance towards the most menacing disease of this crop i.e. downy mildew caused by *Peronospora arborescens*. Two of the mutant strains developed during the course of this study i.e. BC and PCL have qualified to be tested for commercial utilization as new improved cultivars.

Acknowledgements :

We are grateful to the Director General, International Atomic Energy Agency, Vienna (Austria) for the financial support to carry out this study, and to the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, IAEA (Vienna) to include this programme in the International Co-ordinated Research Project on "Mutation Breeding of Oil Seed Crops". We are also thankful to Dr. R.S. Thakur, Director, Central Institute of Medicinal and Aromatic Plants, Lucknow (India) to provide facilities and infrastructural support. Thanks

are also due to Professor A. Ashri and Dr. E. Amano (Coordinators of this programme) and Dr. P.S. Ahuja, Head, Division of Plant Tissue Culture, CIMAP (India), for their critical advise and guidance as and when required. Finally, we express our gratitude to the Council of Scientific and Industrial Research (New Delhi, India) and the Department of Atomic Energy (Bombay, India) for their prompt administrative support and advise.

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IMPROVEMENT OF CASTOR PLANT PRODUCTIVITY THROUGH INDUCED MUTATIONS

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Abstract

Ricinus communis L. seeds were subjected to physical or chemical mutagens to induce desirable mutations to be used in the development of cultivar(s) with improved plant type and other desirable characters for high yield. Optimization tests determined that 500, 600 and 700 Gy were the doses to be used in the field tests. The optimum conditions established by laboratory studies for the efficient use of chemical mutagens are puncturing the seed coat at the caruncle point of attachment, presoaking the seeds for 24 h and soaking the pretreated seeds in the chemical mutagen solution for 4 h. No chlorophyll mutations nor deformed leaves were observed in the M_1 of seeds treated with chemical mutagens. However, most of the mutations induced in the NaN_3 treatments could have been deleterious resulting in few surviving progenies in the M_2. Selections were made in the physical mutagen treated M_2 and M_3 progenies for the best plants with one or a combination of desirable characters, like earliness in flowering (50 days after emergence (DAE), or less), earliness in maturity (120 DAE or less), 100 cm plant height or less to the first raceme at maturity, 30 or more female flowers in the raceme and 25 or more developed capsules per raceme. Among the promising selections, the four mutants identified were 257-38-9 for spineless capsules, 9-1-7 for synchronized branching and flowering with uniform maturity of the capsules, 30-8 for dwarf and determinate plant with two racemes borne at the same node and 23-4 for large seeds compared to the original variety and more female than male flowers with a consequent large number of developed capsules. Development of a cultivar possessing all the characters of the four mutants could produce a dwarf, determinate, early and uniform in maturity castor cultivar desirable for dense planting as a cash crop.

1. INTRODUCTION

Ricinus communis L. is a potential cash crop. It is an easily adaptable crop growing successfully in tropical, subtropical and even some temperate zones [1]. It can be easily cultivated on well-drained soils and does not need high soil fertility.

Much of the harvested crop is derived from 'semi-wild' plants and not from commercially cultivated fields [1]. The various undesirable characters of the castor plant are the deterrents to its wide cultivation. These characters could be changed to encourage its wide commercial production and make it a high income cash crop of major economic importance.

Efforts to encourage castor plant production in the Philippines have been limited in extent and scope due to the major problems of low yield potential, the late and non-uniform maturity and tallness of most varieties grown. The sources of variability needed for the improvement of this crop are very limited. Therefore conventional breeding methods were not expected to accomplish considerable progress. Induced mutations could increase the variability and the appearance of desirable characters which could contribute to the high yield potential of this crop.

This research was conducted to develop castor plant cultivar(s) with improved plant type (compact plants with reduced number of branches) for better adaptation and yield performance as well as to develop dwarf, early and uniform in maturity cultivar(s) for dense population planting with the use of induced mutations.

2. MATERIALS AND METHODS

Newly harvested castor seeds, sun dried for three days and selected for uniform size were used.

2.1. Induction of mutation with physical mutagen

An optimization test was conducted in the laboratory. Two seed lots of Brazilian Tiger Brown castor, one with intact seed coats and another with the seed coats removed were packed 100 seeds to a bag in 20 polyethylene bags. These were irradiated with 0, 250, 350, 450 and 550 Gy of ^{60}Co gamma rays at the Philippine Nuclear Research Institute at Diliman, Quezon City. Germination, seedling height, root length and other morphological abnormalities were observed [2].

A second optimization test was planted outside the laboratory. Seeds of Brazilian Tiger Brown with intact seed coats were irradiated with 0, 700, 800, 900 and 1 000 Gy of ^{60}Co gamma rays. Evaluation was made on the same parameters and observations as in the first optimization test.

Based on the results of the optimization tests, Brazilian Tiger Brown seeds were prepared and irradiated with 0, 500, 600 and 700 Gy of ^{60}Co gamma rays. These were planted in the field, one seed to a hole filled with compost and set 75 x 50 cm apart in four replications on 10 October 1989. The procedure recommended for handling populations subjected to mutation treatments was strictly followed [2]. Observations and measurements were made for emergence, growth, survival, chlorophyll mutation and other morphological abnormalities, number of capsules per plant, number of seeds per capsule and sterility to assess plant injury in the M_1. Visual observations were made for desired characters.

Additional lots of Brazilian Tiger Brown and Aruna castor seeds irradiated with 0, 500, 600 and 700 Gy of ^{60}Co gamma rays were planted in the field in November 1990 to produce a large treated population. The same procedure used in growing the first lot was followed. The same parameters and observations were also gathered.

The first two racemes of all plants that grew to maturity were bagged for selfing and to prevent outcrossing [3]. Two seeds from each M_1 plant were bulked for the M_2 population. The other seeds were stored as remnant seeds.

The M_2 seeds were grown plant-to-row and set as in the M_1. The same procedure in raising the M_1 was followed. Data were gathered on (1) percentage germination, (2) percentage of plants with chlorophyll mutations, (3) percentage of plants with deformed leaves, (4) days after emergence (DAE) to flowering, (5) DAE to maturity of the first raceme, (6) number of days from flowering to maturity of the first raceme, (7) number of nodes to the first raceme, (8) number of developed capsules per raceme, (9) plant height in cm (from the base of the plant to the node of the first raceme) at maturity of the first raceme, (10) ratio of male to female flowers per raceme and (11) percentage of plants with sterile male flowers. Daily visual observations were made to identify desirable/mutant plants for selection.

Harvesting was made when all the capsules in the raceme have dried up and turned brown. Ten random seeds from each of 40 M_3 plant selections were set aside for the M_3 generation. All other seeds were stored.

The M_3 was planted in the field, plant-to-row, in holes filled with compost and spaced 1 x 1 m apart. The crop was maintained like the M_2. Data on (1) the percentage of germination, (2) DAE to flowering, (3) DAE to maturity of the first and second racemes, (4) plant height in cm (from the base of the plant to the node of the first raceme) at maturity of the first raceme, (5) number of nodes to the first raceme, (5) number of nodes from the first raceme to the second raceme, (6) ratio of male to female flowers in the first and second racemes and (7) number of developed capsules in the first and second racemes were

gathered. Visual observation was made for selection of desirable characters. All the seeds produced were set aside for the M_4 generation.

2.2. Induction of mutation with chemical mutagens

The castor seed coat is hard and impervious making the absorption of water and chemical mutagens in solution difficult. Besides, chemical mutagens are expensive and the procedure for their use is laborious and time consuming. This was pointed out during the First Coordination Meeting on "Mutation Breeding of Oil Seed Crops" [4]. Thus, it was stressed that the optimum conditions for the efficient use of chemical mutagens in castor seeds have to be established. Laboratory tests were, therefore, conducted on pretreatment methods for the seeds, presoaking duration and chemical mutagen soaking duration (simulated with acid fuchsin dye).

Brazilian Tiger Brown castor seeds were (1) scarified, (2) blanched and scarified, (3) boiled and (4) the seed coat punctured at the caruncle point of attachment (hilum) before these were soaked in water at different time durations. Half of the seeds thus treated were sown for germination test. The other half was soaked in acid fuchsin dye solution for different durations, then germinated.

Based on the results of the laboratory tests, the caruncles of newly harvested Brazilian Tiger Brown castor seeds were detached, punctured at the point of attachment and the seeds soaked in distilled water for 24 h. Two seed lots were prepared. The first seed lot was soaked in 0, 0.1, 0.5 and 1.0 % EMS solutions and the second seed lot in 0, 1×10^{-3}, 2×10^{-3} and 3×10^{-3} M NaN_3 solutions for 4 h at 20 - 25 °C with constant shaking. The seeds were thoroughly washed with running water for 4 h then air dried for 15 min before planting. The treated seeds were planted in the field dosewise with one seed per hill and spaced 0.75 x 0.50 m apart.

The procedure used in handling the physical mutagen treated M_1 was followed. The same way of making up the M_2 generation was also followed and the same observations and data were gathered. The M_2 population was grown as those in Study 1 and the same observations and data were gathered.

3. RESULTS AND DISCUSSION

3.1 Induction of Mutation with Physical Mutagen

The optimization test made in the laboratory had unusually high infestation of fungus. The seed lot with the seed coats removed succumbed to fungus. Data were obtained only from the lot with intact seed coats. On the other hand, the test planted outside the laboratory did not show any signs of fungal infestation.

The parameters gathered expressed as percentage over the control showed that seeds irradiated at 550 Gy and lower had more or less the same germination, seedling height and root length as the control (Table I). LD_{30} was noted to be at about 700 Gy while considerable seedling height and root length reduction occurred at 800 Gy. Drastic reduction of germination, 28 %, was observed at 1 000 Gy. At this dose, the embryos which showed signs of germination did not produce shoots and had roots that elongated to not more than 2 cm. Thus, the doses for the field tests were set at 500, 600 and 700 Gy.

In the field tests, chlorophyll mutations were observed in many plants in all dose levels in the M_1. Irradiation effects on affected characters increased with the dose. Otherwise, other characters measured appeared seemingly not to be affected. At all dose levels, some plants were observed to bear male flowers that were all sterile.

Among the M_2 plants, 40 were identified to possess one or a combination of desirable characters like earliness in flowering (50 DAE or less), earliness in maturity (120 DAE or

Table I. PERFORMANCE OF BRAZILIAN TIGER BROWN CASTOR SEEDS IRRADIATED WITH ^{60}Co IN OPTIMIZATION TEST

Treatment (Gy)	Germination (%)	Seedling height (%)	Root length (%)
0	100.00	100.00	100.00
250	96.65	92.78	96.41
350	100.00	84.03	89.74
450	97.82	83.51	94.69
550	96.69	91.96	88.53
700	89.91	68.92	69.71
800	98.71	54.06	63.55
900	82.84	56.11	69.85
1 000	71.79	-	-

less), plant height of 100 cm or less (from the base of the plant to the node of the first raceme) at maturity of the first raceme, 12 or more nodes to the first raceme, 30 or more female flowers per raceme and 25 or more developed capsules per raceme.

The high yielding 27 M_2 plant selections are presented in Table II. Most of the plants selected for the number of developed capsules had also high number of female flowers. Three plants (31, 37 and 244) had more female than male flowers and three plants (37, 189 and 197) had more than 40 developed capsules per raceme. Several selections had a combination of the desirable characters resulting to high seed setting and consequently, high seed yield. This could indicate that selection for high number of female flowers (30 or more per raceme) and for high number of developed capsules per raceme (25 or more) could contribute considerably to the high yield potential of castor.

Table III presents the performance of the best 24 M_3 plants. Seven of the selections, 200-32-8, 246-37-2, 189-28-7, 143-35-1, 101-13-5, 137-21-9 and 196-30-2 matured in three months. The earliest plant to flower as well as to mature was 200-32-8 from the 600 Gy treatment. It was also the shortest and had relatively high number of developed capsules in the raceme.

The plant selections were made primarily for earliness in maturity, short stature and large number of developed capsules in the first raceme coupled with the large number of female flowers in the raceme. Selections 9-1-7, 182-25-9, 182-25-10 and 191-29-9 had 30 or more developed capsules in the first raceme. Except for selections 153-24-6, 187-26-3, and 188-27-7 which were selected for their earliness and/or short stature, the rest of the selections had 20 or more developed capsules in the first raceme. Among these selections, two plants were identified possessing characters different from the normal. The line 9-1-7 had an unusual branching habit, two branches were produced at the same time, flowered and bore capsules that matured also at the same time (synchronized branching) while 267-38-9 had spineless capsules.

3.2. Induction of mutations with chemical mutagen

Neither scarification nor blanching with scarification hastened the absorption of water in castor seeds. Boiling, on the other hand, killed the embryo. Puncturing the seed coats at the caruncle point of attachment was found to preserve the viability of the seeds while allowing fast water absorption. The laboratory tests established that the best methods for the efficient use of chemical mutagens for induction of mutations are (1) puncturing the seed coat at the caruncle point of attachment to allow rapid entrance of water and mutagen solutions into the seed, (2) presoaking the seeds in distilled water for 24 h to stimulate mitotic activity and (3) soaking the pretreated seeds in the chemical mutagen for 4 h.

Table II. PERFORMANCE OF THE BEST M_2 PROGENIES OF ^{60}CO IRRADIATED SEEDS, CY 1990-1991

Plant number	Treatment (Gy)	Days from emergence to		Number of developed capsules per raceme	Number of nodes to the first raceme	Plant height at maturity of the first raceme (cm)	Ratio of male to female flowers per raceme
		Flowering	Maturity				
12	500	50	123	37	12	235	60:40
31	500	63	133	31	17	160	44:55
37	500	58	127	41	19	159	49:53
59	500	60	127	37	17	154	63:45
60	500	58	127	33	18	166	72:35
65	500	57	123	33	17	169	83:36
69	500	60	154	39	25	250	98:43
81	500	62	133	39	19	220	96:40
98	500	60	139	33	20	282	95:48
99	500	57	139	31	19	285	92:33
100	500	57	133	38	17	225	59:43
105	500	60	127	34	20	187	74:49
109	500	85	139	33	20	275	92:43
134	500	60	131	31	22	310	94:43
140	500	63	127	33	18	138	106:38
141	500	63	138	33	19	220	92:41
154	600	57	123	37	17	184	63:42
186	600	47	119	34	17	161	89:40
187	600	47	151	38	16	163	100:38
189	600	56	123	49	18	185	61:56
197	600	62	133	50	18	156	85:54
211	600	57	123	33	16	157	50:42
212	600	58	133	39	19	145	63:40
237	700	61	127	31	11	195	59:36
244	700	92	149	38	24	320	51:63
266	700	59	125	31	18	156	67:44
267	700	61	145	35	20	301	91:51

The M_1 of both chemical mutagen treated seeds showed neither chlorophyll mutations nor deformed leaves. Except for germination which was greatly affected, the other characters appeared unaffected. Few progenies of the seeds treated with NaN_3 grew to maturity. Most of the mutations induced by NaN_3 treatment could have been deleterious resulting to few surviving M_2 progenies.

The performance of the best M_2 progenies of treated seeds is presented in Table IV. These progenies had relatively high seed setting with 20 or more capsules per raceme.

3.3. Mutant Selections

Four mutants were identified and are listed in Table V. Three of these mutants were from Brazilian Tiger Brown and one from Aruna. Two mutants were M_3 selections and the other two from the M_2.

Table III. PERFORMANCE OF THE M_3 SELECTIONS OF ^{60}Co IRRADIATED SEEDS, CY 1991-1992

Plant number	Raceme number	Treatment (Gy)	Days from emergence to flowering	Days from emergence to maturity	Number of nodes to the raceme[1]	Number of capsules in the raceme	Ratio of male to female flowers in the 1st raceme	Plant height at maturity of the 1st raceme (cm)
9-1-7	1	500	82	152	18	34	125:34	93
84-12-10	1	500	57	128	16	28	75:28	98
	2		85	148	10	8	172:14	
101-13-5	1	500	54	120	16	26	171:43	93
	2		79	134	8	4	89:07	
103-15-5	1	500	57	126	17	27	184:36	101
	2		86	157	11	15	103:14	
137-21-9	1	500	70	120	20	21	49:25	95
143-35-1	1	600	57	120	16	21	180:30	90
153-24-6	1	600	68	129	17	19	137:19	85
	2		96	156	11	39	139:39	
176-22-2	1	500	62	128	17	23	89:28	98
	2		99	165	9	36	73:39	
182-25-9	1	600	56	128	15	34	114:39	88
	2		105	164	11	24	71:25	
182-25-10	1	600	57	127	16	30	164:37	92
	2		96	146	10	12	78:12	
184-23-1	1	500	71	134	18	23	187:23	135
	2		103	164	10	23	136:23	
187-26-3	1	600	62	129	17	13	106:13	90
	2		103	161	11	37	111:45	
188-27-7	1	600	61	130	17	12	113:12	103
	2		103	167	12	31	116:38	
188-27-9	1	600	54	125	16	25	132:42	82
	2		106	166	12	13	105:16	
189-28-1	1	600	63	133	15	29	132:42	85
	2		98	150	10	10	105:16	
189-28-4	1	600	61	134	17	25	109:29	70
	2		103	161	12	24	99:21	
189-28-7	1	600	53	118	15	25	140:25	85
	2		103	156	12	19	92:19	
191-29-9	1	600	61	129	16	34	129:34	84
	2		98	151	11	5	142:15	
196-30-2	1	600	51	122	14	24	69:24	80
	2		62	156	4	15	20:15	
200-32-8	1	600	46	107	16	29	82:29	74
230-34-2	1	600	63	129	17	21	133:24	94
246-37-2	1	600	73	116	14	23	178:26	84
267-38-2	1	700	57	123	16	20	125:27	109
267-38-2	1	700	58	164	16	4	58:04	55
268-39-2	1	700	61	124	17	26	111:30	84

1 : For the second raceme, number of nodes from the first to the second raceme.

Mutant 1, 267-38-9, was obtained from the M_3 plants of Brazilian Tiger Brown with 700 Gy irradiation. The M_2 of this selection was previously selected for the large number of developed capsules in the raceme. The unusual character, spineless capsules, was observed in the M_3. This character is desirable for handling capsules during harvesting and threshing.

Table IV. PERFORMANCE OF THE BEST M_2 PROGENIES OF EMS TREATED SEEDS, CY 1991-1992

Plant number	Treatment (%)	Days from emergence to		Number of nodes to the raceme	Number of developed capsules in the first raceme	Ratio of male to female flowers in the 1st raceme	Plant height at maturity of the 1st raceme (cm)
		Flowering	Maturity				
1-3	0.1	107	159	15	25	38:25	79
1-4	0.1	107	159	16	21	37:21	77
1-5	0.1	107	159	15	23	35:23	76
1-11	0.1	107	159	10	22	35:22	70
1-18	0.1	107	159	20	25	46:25	91
1-20	0.1	107	159	18	21	45:21	89
2-39	0.1	114	159	26	23	42:23	108
2-45	0.1	114	159	26	23	72:23	112
2-46	0.1	114	159	23	20	21:20	109
2-47	0.1	114	159	27	25	72:25	113
2-63	0.1	114	160	30	25	50:25	126
2-64	0.1	114	160	31	26	45:26	129
2-69	0.1	114	160	31	20	50:20	121
2-72	0.1	114	160	30	20	49:20	121
3-75	0.1	109	159	20	21	71:21	101
4-4	0.1	70	123	16	20	63:20	97
6-5	0.5	60	121	14	25	67:28	73
6-15	0.5	73	121	19	25	53:25	91
6-16	0.5	67	121	16	22	53:22	87
7-28	0.5	73	121	16	20	39:20	84
8-68	0.5	81	149	16	26	91:38	89
8-78	0.5	77	123	17	21	30:25	84
8-79	0.5	70	123	17	22	39:32	86
3-24	1.0	99	175	16	20	65:25	97

Mutant 2, 9-1-7, was obtained from the M_3 progenies with 500 Gy irradiation. The original variety, Brazilian Tiger Brown, normally produced a branch after every raceme. This mutant, however, had synchronized branching, produced two branches at the same time with both bearing flowers and the capsules maturing at the same time. Such synchronized branching is desirable for uniform maturity.

Mutant 3, 30-8, was obtained from the M_2 progenies of Brazilian Tiger Brown with 500 Gy irradiation. The plant was selected for its dwarf determinate growth habit and the production of two flower racemes in the same node. This character is desirable for uniform maturity and for dense planting.

Mutant 4, 23-4, was obtained from the M_2 progenies of Aruna irradiated at 500 Gy. Selection was made for the big seeds of the mutant compared to those of the original variety. In addition, more female than male flowers were borne in the unusually long raceme resulting to more capsules that matured and were harvested. Big-seededness and more female than male flowers in addition to the other good characteristics of Aruna make this mutant desirable for a cash crop.

Table V. PERFORMANCE OF MUTANT SELECTIONS, CY 1991-1992

Plant number	Treatment (Gy)	Generation	Days from emergence to		Number of nodes to the raceme	Number of developed capsules in the raceme	Ratio of male to female flowers in the raceme	Plant height at maturity of the first raceme (cm)
			Flowering	Maturity				
Mutant Number 1								
267-38-9	700	M_1	95	164	19	4	20:09	23
		M_2	59	129	18	31	67:44	156
		M_3	58	154	16	4	58:04	55
Control			95	140	15	5	28:07	97
Mutant Number 2								
9-1-7	500	M_1	69	148	16	3	32:12	25
		M_2	50	123	17	37	60:40	235
		M_3	82	152	18	34	125:34	93
			82	152	18	20	142:38	93
Control			95	140	15	5	28:07	97
Mutant Number 3								
30-8	500	M_1	115	186	18	1	28:04	5
		M_2	97	159	20	21	62:21	36
Contol			73	175	17	14	86:27	91
Mutant Number 4								
23-4	500	M_1	77	128	12	6	17:09	45
		M_2	43	109	15	79	30:180	71
Control			46	109	8	7	28:09	36

4. SUMMARY AND CONCLUSION

Induced mutations were utilized to increase the variability and the appearance of desirable characters that could contribute to the high yield potential of castor plant to encourage its wide cultivation as a cash crop of major economic importance.

Optimization tests conducted for the physical mutagen treatment of Brazilian Tiger Brown castor seeds showed that the seed coats should be intact for best germination. Seeds with intact seed coats irradiated at 550 Gy and below had germination, seedling height and root length more or less the same as the control. LD_{30} was shown to be at 700 Gy while drastic reduction in seedling height with negligible root and shoot elongation occurred at 1000 Gy. The doses for the field tests were, therefore, set at 500, 600 and 700 Gy.

The impervious seed coat of castor makes absorption of water and chemical solution difficult. Laboratory studies conducted established that the optimum conditions for the efficient use of chemical mutagens are puncturing the seed coats at the caruncle point of attachment, presoaking the seeds for 24 h and soaking the pretreated seeds in the chemical mutagen for 4 h.

Chlorophyll mutations and deformed leaves were observed in the M_1 of seeds treated with physical mutagen but not in those treated with chemical mutagens.

Selections were made for earliness in flowering and maturity, short stature, less number of nodes to the first raceme, large number of developed capsules per raceme and many female flowers produced per raceme. Forty plants selected in the M_2 of the first set of physical mutagen treated population with one or a combination of these desirable characters were advanced to the M_3. Two mutants were obtained from this population. Two other mutants were obtained from the M_2 of the succeeding set of seeds subjected to physical mutagen treatment. Three mutants were from Brazilian Tiger Brown while the other mutant was from Aruna.

Mutant 1 had spineless capsules which could facilitate handling during harvesting and threshing. Mutant 2 had synchronized branching and flowering with uniform maturity of the capsules. Mutant 3 had dwarf and determinate growth habit with two racemes borne at the same node. Mutant 4 had large seeds compared to the original variety and more female than male flowers which resulted to a large number of developed capsules.

These four mutants need to be studied further to determine the inheritance of the particular characters for which these were selected for. Further, cleaning of the mutants should be done to stabilize and maintain the lines. Also, crossing the mutants together could lead to the development of a dwarf, determinate, early and uniform in maturity cultivar desirable for dense planting as a cash crop.

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DEVELOPMENT OF IMPROVED COTTON CULTIVARS BY INDUCED MUTATIONS

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Abstract

Seeds of two superior Greek varieties namely Sindos 80 and 4S were irradiated with gamma-rays (100, 200 and 300 Gy) in order to create new genetic variation and consequently to develop lines resistant to *Verticillium* wilt. LD_{50} was found to be around 300 Gy for the two varieties. The treated seeds of both varieties along with the control were sown in 1992. From the two varieties used, 6 000 plants were obtained, some showing morphological differences compared to the control. M_2 seeds of 400 plants of M_1 generation were sown in small pots and placed in growth chambers. From these M_2 seedlings 0.23 - 0.24 % in both varieties were albino and about the same percentage were viridis. This gives an indication of the expected mutation frequency for other characters in the same genetic background.

1. INTRODUCTION

Cotton is one of the most important crops in Greece occupying the area of about 300 000 ha and yielding 800 000 tons of seed cotton every year. Greece is the main cotton producing country in EEC followed by Spain with a relatively lower production. Greece is the most northern country with sufficient cotton production. The climatic conditions are marginal for the demands of the cotton plant but the creation and propagation of varieties well adapted to the environment resulted in high yields and good fibre quality. The lint quality of Greek cotton is among the first, of Upland type, in the world.

The two major problems that cotton cultivation is facing are the short growing period, (especially in Northern Greece), and the *Verticillium* wilt disease. Therefore the most important characteristics expected for a new cotton variety must be earliness and resistance to *Verticillium*, combined with productivity and fibre quality. To combine all these characters has been very difficult, although traditional breeding methods have been employed in a continuous effort to endow cultivated varieties especially with resistance to *Verticillium*.

The early varieties are more susceptible to the disease. Thus 4S and Sindos 80, two well adapted productive varieties of Northern Greece and of excellent quality, are gradually restricted. Since there is no adequate way of chemical control of the disease the future of cotton cultivation depends on the development of the disease resistant varieties.

Besides the traditional breeding methods used so far, the method of mutation breeding was adopted recently to try to solve the problem. Genetic variation of the characteristics under consideration was practically limited within the available genetic resources. Therefore we tried to create genetic variation by induced mutation using gamma-irradiation [1]. Mutation breeding has been used successfully to improve other characteristics of cotton i.e. oil and protein percentage [2].

The mutation breeding programme started two years ago with the support from the International Atomic Energy Agency. The aim of the work is to develop varieties resistant to *Verticilium* wilt, with high productivity and good fibre quality.

2. MATERIALS AND METHODS

Seeds of the two early varieties Sindos 80 and 4S were subjected to gamma-irradiation of 100 Gy (14 min), 200 Gy (29 min) and 300 Gy (43 min).

After the treatment, the seeds were germinated to evaluate the optimal dose for induction of desirable mutations. Percentage of germinated seeds was used as index. After the preliminary dose-response experiments, the irradiated seeds of each variety were sown in a field in rows 10 meters long and 1 m apart. Untreated seeds of the same varieties were used as control. The total number of M_1 plants of each variety amounted to about 6 000.

Observations were made on the rate of seedling emergence, plant growth and boll setting. The bolls were collected and ginned and the seeds were kept apart.

In 1993 these M_2 seeds will be sown in a field contaminated with the *Verticillium* pathogen together with the control, the original susceptible varieties. If some promising mutant plants with the desirable character will appear, i.e. resistant to *Verticillium* etc., some flowers would be selfed and others would be back-crossed with the original susceptible variety for genetic studies and to clean undesirable mutant genes. When they are ready, the progenies of the crosses would be planted again in a contaminated field to confirm and evaluate their resistance to *Verticillium* wilt disease.

From the M_1 plants of the two varieties, 400 plants were selected at random and four seeds from each were sown in pots of 10 cm diameter and 15 cm depth. The pots were placed in growth chambers for germination.

The growth chambers were regulated for 14 hours days and 10 hours night and the temperatures were kept at 29 °C and 24 °C respectively while relative humidity was stable at 60 %. The seedlings were of M_2 generation. The aim of the experiment was to define the percentage of morphological mutations at the seedling level.

3. RESULTS AND DISCUSION

In Table I, the percentages of germinated seeds of the two varieties in response to the three doses of gamma-irradiation along with the control are shown. The dose of 100 Gy did not affect the germination very much in both varieties. The dose of 200 Gy decreased the percentages of germination in the both varieties.

The dose of 300 Gy reduced drastically the germination in Sindos 80 and 4S, the percentages being 58 % and 44 % respectively compared to the non-irradiated controls of 90 % and 91 %. Therefore the dose of 300 Gy for the two mentioned varieties and under the conditions of the present experiment is regarded as LD_{50}. The seeds treated with this dose were sown for further examination.

The germination in the field was somewhat lower. From the two varieties used, we could have totally 6 000 plants. In some of them growth was depressed and others were

TABLE I. SEED GERMINATION (%) OF VARIETIES SINDOS 80 AND 4S AFTER GAMMA-IRRADIATION

Treatments	Germination (%)	
	Sindos 80	4S
100 Gy	78	76
200 Gy	70	72
300 Gy	58	44
Control	90	91
$LSD_{0.05}$	13.6	18.1

TABLE II. PERCENTAGE OF M_1 PLANTS WITH MORPHOLOGICAL DEVIATIONS COMPARED TO THE CONTROLS.

Varieties and treatments	% Plant with			
	Small leaves	Low height	Long bolls	Sterile or semi-sterile
Sindos 80 (300 Gy)	10	12	4	3
Control	0	0	0	0
4S (300 Gy)	15	10	3	2
Control	0	0	0	0

TABLE III. NUMBER AND FREQUENCIES OF CHLOROPHYLL MUTANTS IN VARIETIES, SINDOS 80 AND 4S IN M_2.

Variety	No of seeds	No of seedlings	Albina seedlings	Viridis seedlings
Sindos 80	1 600	1 302	3 (0.23%)	4 (0.31%)
4S	1 600	1 241	3 (0.24%)	2 (0.16%)

sterile. Other plants had smaller leaves and height compared to the control, suggesting irradiation damages in the M_1 generation. The morphological deviants (%) found in M_1 are shown in Table II.

In 1992 these 6 000 M_1 plants were harvested. Cotton is basically a self-pollinated plant and the conditions of experiment secured self-pollination thus the segregation of mutant plant in the progenies could be expected.

In Table III, the observed frequencies of chlorophyll mutants at seedling stage in M_2 generation are shown. Four seeds each from the 400 M_1 plants of each variety were sown in the pots and placed in growth chambers. Some seedlings were albina or viridis (Table III) indicating the effectiveness of the mutagenic treatment.

Frequency of albino seedlings was about 0.23 - 0.24 % in both varieties. The frequencies of viridis were nearly the same. There were no albina or viridis seedlings in control. Although the samples from 400 plants were small, it gives a positive indication of the relative frequencies of mutations expected for other characteristics with the same genetic background.

In 1993 the harvested seeds from all the M_1 plants will be sown separately about 5 to 10 seeds from each so a wide range of progenies will be checked for possible resistance mutation to *Verticillium*.

The promising resistant plants found will be selfed and crossed with the original succeptible but superior variety. The progenies will be used for confirmation of the mutant character, the disease resistance, and then they will be integrated into the breeding programme and genetic studies, if affirmative.

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CONCLUSIONS AND RECOMMENDATIONS

This Coordinated Research Programme (CRP) for the improvement of oil seed crops included many projects on various crop species. Although the general objectives and some of the desired genetic improvements were common in terms of resistance or tolerance to biotic or abiotic stresses and higher and stabler yields with lower input of labour, these crop species differ considerably in the level of development, the amount of genetic and breeding knowledge available, specific objectives and suitable breeding approaches. Therefore it was deemed appropriate to divide the Conclusions and Recommendations section into crop chapters for the convenience of breeders and other users.

Each crop chapter consists of three parts, where applicable :

(1) crop specific problems or objectives;
(2) achievements of this CRP, e.g. recommended methods, mutant cultivars released and promising mutant lines obtained;
(3) general conclusions and recommendations as a contribution from the group.

Generally, it was demonstrated that mutation breeding is a very effective means to modify various traits, such as oil content and composition, plant architecture and physiological processes especially of resistance/tolerance to various stresses. It can contribute to widening the genetic variation necessary for breeding. It was also shown that mutation breeding is a practical method to produce the desired genetic variability, together with a wide range of additional favorable and unfavorable variation. The mutant alleles in the adapted variety may then be used directly by releasing an improved cultivar, or in cross breeding as the source of a desired gene.

As for the organizational aspects, the elements of this CRP were well conceived and executed. The aims and objectives set for each crop at the beginning of the programme were realistic as is evident from the significant progress made in the different crops. During the presentations and the discussion of the reports, it became evident that in formulating the second phase of this programme, or another CRP on oil seeds, care should be exercised to consider the general impact of a particular problem as well as the potential market size of the crop with relation to a particular trait, market prospect of a crop and adaptation to a wider range of countries.

Some participants, especially those working on relatively minor oil crops, felt the need and the importance of specialized training. Such training is very essential for those crop groups, because standard methodologies are often still lacking and must be developed by the researchers themselves.

Exchange of information, materials and techniques among the participating Institutes should be encouraged. The breeders should utilize the research network and linkage to the maximum and thus benefit more from the CRP and develop more efficient mutation breeding technology.

The researchers should characterize their induced mutants for their genetic basis and inheritance pattern, physiological parameters like photosynthetic efficiency, characteristics related to post harvest processing etc. Incorporation of molecular biology tools like DNA markers' mapping, DNA hybridization, ELISA etc. in research programmes will greatly help in breeding by facilitating rapid and reliable genotypic screening of heterogeneous populations.

The following general recommendations were made :

(1) Each research programme should be continued and, if needed, expanded to include *in vitro* culture as supporting techniques.
(2) Literature and related information on the specific crops should be exchanged or disseminated to the fellow researchers.

(3) A system should be set up by which seed exchange of promising lines or varieties can be availed of by the researchers.
(4) A listing of appropriate genetic markers helpful in mutation breeding of specific crops should be prepared. Breeders and researchers should be advised whom to contact for references and possible materials for their research.

1. *BRASSICA* GROUP CROPS

1.1. Crop specific problems and breeding objectives

This group includes Canola and standard rape and other *Brassica* oil seed crops, namely *B. campestris*, *B. napus*, *B. juncea* and *B. carinata*.

In both developing and industrialized countries there is a high priority for high yielding varieties with the desired oil and meal qualities. In developing countries, high oil yields are more important than high protein yields. Therefore, mutants containing more than 45 % oil and high yielding ability are required. Higher yields may be obtained in F_1 hybrids using genic-cytoplasmic male sterility (CMS), or self-incompatibility. Earliness, resistance to various stresses like pests, diseases, drought and shattering especially in *B. napus*, and all other traits stabilizing yield are highly desired. Some examples of the objectives are given below :

(1) *B. campestris* var. Yellow Sarson:
- Yield improvement
- F_1 hybrid using CMS
- *Alternaria* blight resistance
- Improved aphid resistance
- Salinity resistance through mutation breeding and interspecific hybridization with salinity resistant accessions of *B. napus*
- Canola quality for Bangladesh, China, India and Pakistan

(2) *B. napus*
- Yield improvement of double low i.e. Canola quality varieties
- Establishment of F_1 hybrids using CMS and self-incompatibility in winter and spring rapeseed.
- Lodging resistance through stem stiffness and short stature
- Early maturing genotypes (80 days) in Bangladesh, China, India and Pakistan
- Shattering resistance
- Resistance to *Alternaria*, *Cylindrosporium*, *Phoma*, *Sclerotinia*, *Verticillium* and virus diseases (main problem in China)
- Insect resistance, especially to aphids
- Drought resistance
- Adaptability of Canola germplasm to local conditions (Pakistan)
- Yellow seeds and high oil content
- Reduction of glucosinolate content to nearly zero
- High oleic Canola genotype
- In developed countries, also high erucic acid cultivars are desired, for industrial uses.

(3) *B. juncea*
- F_1 hybrids using CMS (China)
- Semi-dwarf types
- White rust (*Albugo candido*) and downy mildew (*Peronospora brassicae*) resistance for Asia
- Use of aphid resistance from RLM-514; T6342 (India)
- Studies on the nature of insect resistance; transfer of such genes to other *Brassica* spp.
- Canola quality for Pakistan, India and China
- Yellow seeded varieties

(4) *B. carinata*
- Early maturity
- Compact branching
- Semi dwarf types
- *Sclerotinia* resistance
- Insect resistance
- Canola quality

1.2. Achievements of the CRP

1.2.1. Mutagen treatment

The following methods of mutagen treatments were shown to be practical to improve genotypes of *Brassica* group crops through mutation breeding. But they should serve only as general guidlines since there are wide differences between genotypes within species.

(1) Irradiation with gamma- or X-rays

A. Seeds
- Dose: 1000 Gy - 1500 Gy for *B. napus* and *B. juncea*
  600 Gy - 900 Gy for *B. campestris*
  400 Gy - 800 Gy for *B. carinata*
  A 20% reduction in growth is recommended as the indication of optimal dose,
- Seed moisture: 8 % - 12 %
- Treated seeds should be of uniform size
- M_1 population size should be 3 000 plants/dose or more.

B. Pollen
- For *B. napus* doses of 20 - 30 Gy were employed.

(2) EMS
- Presoaking: 4 - 8 hours
- Concentration: 1 % - 5 %; Optimum around 2 %
- Treatment time: 6 - 10 hours
- Treatment temperature: 18 °C - 24 °C
- The samples should be shaken during treatment
- Post washing: 12 - 20 hours
- For each species and genotype the optimal procedure has to be standardized to avoid germination during the treatment.

1.2.2. Screening procedure

Appropriate screening for the desired mutants is very important in mutation breeding. The methods used strongly depend on the objective, or desired character. Some general recommendations and some specific objectives are described below.

(1) Size of populations to be used
10 000 - 50 000 seeds should be treated (M_1) depending on the mutant desired. About 10 seeds from each M_1 plant would be sufficient for the large M_2 population needed.

(2) Screening for disease/pest resistance
- ***Alternaria brassicae***
  Screening is done in the M_2 population under high natural epiphytotic conditions. The plants are scored for percent leaf area affected by the pathogen.
- **White rust** (*Albugo candida*), **Powdery mildew, Downy mildew**
  The screening procedure is the same as for *Alternaria*.
- ***Sclerotinia sclerotiorum***
  This pathogen attacks all *Brassica* species, particularly *B. carinata* and *B. napus*. M_2 material is screened both under artificial and natural epiphytotic conditions.

Artificial epiphytotics are created by spraying the plants with a spore suspension derived from apothecia placed in water. The inoculation is carried out in the late afternoon when the petals fall, early in the flowering period*.

- **Aphid resistance**
  M_2 progenies showing resistance in the field are screened also in the laboratory by the excised leaf method (antibiosis test). In this procedure the excised leaves are placed in petri dishes with a wet cotton swab. Then 3 - 5 newly born nymphs are introduced on these leaves and their percent survival, life span and fecundity (reproductive ability) are rated. Based on this test the relative tolerance of the selections can be confirmed.

(3) Developmental, morphological and yield traits

Traits such as dwarf, semi-dwarf, early, upright, stiff stems, long pods etc. are identified in the M_2. Progeny testing in M_3 and at times M_4 is necessary.

For some traits, such as a higher number of primary branches on the main stem screening may be more reliable in the M_3, with subsequent progeny testing.

For seed coat characters screening can first be practiced on M_3 seeds (M_3 embryos have M_2 seed coat, since the latter is maternal tissue).

(4) Screening for quality traits

To screen for fatty acid mutants the bulk method is recommended for field planting, but for analytical procedures half seeds or bulked seed samples have to be used, depending on the fatty acid of interest.

- **Erucic acid**
  M_2 seeds can be analyzed using the half seed technique to select as rapidly as possible for low or high levels. To breed for high yield performance and low erucic acid contents in China, well adapted varieties were irradiated. Only agronomically promising M_3 families were checked by gas chromatography for low C22:1. In this way, intensive backcrossing to regenerate high yielding genotypes was avoided.
- **Linolenic acid**
  Due to the lower content of linolenic acid and strong environmental influence, M_3 seeds should be analyzed by gas chromatography or by the thiobarbituric acid (TBA) test.
- **Oleic acid**
  Half seed analysis on M_2 seeds subjected to gas liquid chromatography (GLC) was successful in screening for high oleic acid mutants. Alternatively, M_2 seeds bulked per M_1 plant may be analyzed by GLC or NIRS (Near Infrared Reflectance Spectroscopy) to identify promising populations.

1.2.3. Output from the CRP in *Brassica*

In *B. juncea* white flower, yellow seed colour and narrow pod angle were induced. They are controlled by single recessive genes.

In *B. napus* low linolenic acid content is controlled apparently by several additive genes.

During this Coordinated Research Programme, the following five new varieties were released from the mutation breeding programmes in the participating countries:

B. juncea	: TM-2	India	Appressed pods
	TM-4	India	Large and yellow seed
B. napus	: Safal	Bangladesh	High yield, *Alternaria* and aphids resistant
	Agrani	Bangladesh	(same as above)
	26-1	China	High yield, low erucic acid, lodging and disease resistant

*cf. "Techniques for artificial contamination of oilseed rape in trial plots", CETIOM, 174, Ave. Victor Hugo, F-75226 Paris.

The following promising mutant lines are under evaluation; the superior ones will be released as registered cultivars :

B. juncea : 2 lines, *B. napus* : 23 lines

1.2.4. Recommendations for *Brassica* crops

(1) Well adapted, good cultivars which lack certain desired traits should be utilized in mutation breeding programmes.
(2) For oil quality improvement, e.g. Canola type, simple and quick analytical methods should be developed. Nuclear Magnetic Resonance (NMR) analysis and/or Near Infrared Reflectance Spectroscopy (NIRS) may be examined for their relevance.
(3) Disease and pest resistance may be improved by mutation breeding, and good screening techniques applicable to large segregating populations (e.g. the excised leaf method for aphid resistance developed at the Bhabha Atomic Research Centre, Bombay, India), should be used. Cooperation with plant pathologists and entomologists should be strengthened.
(4) Exchange of promising germplasm and literature should be made on direct request or through the international organizations and networks.
(5) On farm research on farmers' fields should be carried out to test the materials, demonstrate their attributes and to hasten the dissemination to the farmers of the new improved cultivars.

1.3. General conclusions and recommendations

This CRP has generated useful materials such as yellow seeded *B. juncea*, early maturing mutants in *B. napus*, *B. juncea* and *B. campestris* (Pakistan, India, Bangladesh), salt tolerant *B. campestris*, high oil containing *B. napus* and low erucic acid *B. napus* mutants adapted to China and Pakistan. Moreover, mutants showing drought resistance, low glucosinolate and high protein contents were selected in *B. napus*. Further progeny tests are necessary in some cases to confirm these results.

The induced mutants may lead directly to improved varieties or may be used in cross-breeding. In China, pollination control systems are under development for double zero materials. Both aspects are of essential importance for developing double zero hybrid cultivars. This requires the establishment of different gene pools.

To share experience in quality and resistance as well as hybrid breeding, mutual visits of the scientists should be encouraged.

To speed up breeding progress, participants should be familiarised with potentially helpful techniques, such as tissue culture, microspore culture and analytical methods for rapid screening, by intensive training in experienced laboratories.

Transnational seed exchange between the breeders will accelerate the breeding progress and should be encouraged.

2. SESAME (*Sesamum indicum*)

2.1. Crop specific problems

Breeding objectives and desired traits for sesame improvement are as follows :

2.1.1. Breeding objectives

(1) High seed yields.
(2) Semi-dwarf types for dense planting and mechanized harvesting

(3) Development of "Uniculm" (unbranched) types for intensive cropping systems and types with some basal appressed branching for less intensive cropping systems, particularly where the seeds are broadcast.
(4) Reduction of seed loss at maturity and harvest by developing non-shattering or semi-dehiscent cultivars, suitable for mechanical harvesting.
(5) Resistance to diseases and insect pests (see below).
(6) Investigation of hybrid vigor and male sterility mechanisms, in preparation for hybrid cultivars' production for high input conditions.
(7) Responsiveness to higher inputs.
(8) Higher harvest index.
(9) Larger seeds for confectionery purposes.

2.1.2. Desired traits

(1) Plant characters
- Early emergence at low temperature (as low as ca. 15 °C) for colder regions of cultivation.
- Early, vigorous seedling growth to compete with weeds.
- Long tap root with profuse secondary roots.
- Determinate habit.
- First capsules set low, 15 - 20 cm from ground.
- Basal appressed branching.
- Short internodes.
- Genic-cytoplasmic male sterility, or genic male sterility that can be environmentally controlled.
- Synchronous capsule maturation.
- Strong placental attachment of the seeds.

(2) Seed and oil quality
- Reduction of oxalate content of the testa (screening by microbial assay may be useful).
- Increased oil and protein content of the seed.
- Modification of fatty acid composition.
- Increased levels of antioxidants - sesamin and sesamolin.
- Easy dehulling of seeds for the confectionery market.
- Large, white seeds for confectionary uses.

(3) Reaction to environmental and biotic stresses
- Tolerance to salinity.
- Improved tolerance to drought.
- Shorter growing period with photo- and thermo-period insensitivity.
- Tolerance to waterlogging, particularly in varieties used on rice fallow.
- Resistance to *Macrophomina*, *Phytophthora*, *Fusarium*, *Sclerotium bataticola* and *Phyllody*. Methods of screening for resistance to one or more of these diseases which have been developed by researchers in this CRP should be made available to all participants.
- Research on MLO (mycoplasma like organisms) causing *phyllody* infection is needed and methods of screening for MLO resistance should be developed.
- Resistance to the leaf roller, *Antigastra catalaunalis*.

2.2. Achievements of CRP

2.2.1. Methods of mutagen treatment

Investigation under the present CRP confirmed earlier reports that sesame seed is highly resistant to irradiation and EMS. Also, it has been established that there exists a wide range of variation among genotypes for sensitivity for radiations and EMS. Therefore, it is desirable to conduct pilot dosage experiments on mutagen sensitivity of varieties newly

chosen for mutation breeding. Doses ranging from 200 - 700 Gy of gamma rays have been used in different varieties with success.

When using sodium azide as a mutagen, the seeds should be pre-soaked in water at 4 °C for 4 - 6 hours and soaked for 15 - 25 hours in water at 18 - 24 °C with Sörensen buffer solution (phosphate buffer). A 4 - 6 mM solution is used for treatment at 18 - 24 °C with shaking for 2 - 6 hours and washing in running tap water for at least six hours. For treatment with ethyl methanesulphonate the same method may be used employing 0.4 - 0.6 % solutions, but for longer exposures (up to 24 hours).

For inducing mutations affecting yield and its components, lower doses of irradiation or chemical mutagens should be employed. The potential to increase variability for quantitatively inherited traits affecting seed yield and its components using gamma rays has been clearly demonstrated.

2.2.2. Recommended screening procedures

At the harvest, every surviving fertile M_1 plant should be represented by 2 - 5 capsules. A 30 - 50 seeds' sample from them should be grown in M_2 either in progeny rows or in bulk depending on the objectives and budget. Mutants with tolerance to *Phytophthora* blight have been selected in bulk populations. Similarly the widely used determinate mutant was selected in a bulk M_2 population. On the other hand, most of the high yielding mutants isolated in the present CRP were selected in M_2 or M_3 progeny rows. Sesame being a diploid and generally self-pollinated, most of the mutants can be identified and selected in M_2 or M_3.

Sesame seed yields can be drastically affected by many soil borne diseases. Therefore screening for resistant/tolerant mutants should be done in highly infested fields for several successive generations. Screening for resistance to *Macrophomina phaseolina* may be done in the greenhouse by seedling inoculation as described in these proceedings.

Gamma ray induced mutants with tolerance to *Phytophthora* blight were isolated and further tested by field screening in highly infected disease nurseries in M_2 - M_5 generations. If the disease incidence is not sufficient in the disease nursery, sufficient inoculum may be added in the form of infected plants. Frequent irrigation may also help to build up the disease incidence to desired levels.

For *Fusarium* spp., *Sclerotium bataticola*, *Rhizoctonia solani* and *Phytophthora parasitica*, a method for making dry inoculant has been described in these proceedings. It may be used to inoculate seeds in pot experiments as well as in field experiments during the screening of mutants.

Suwonkkae, (Suwon 122), a high yielding selection from a cross of a local Korean cultivar and a mutant has recorded higher protein percentage and increased contents of essential amino acids. A brittle seed coat mutant induced under this CRP in Sri Lanka will be useful in facilitating village level decortication of sesame seeds, thus adding value to the farmers' produce.

The determinate mutant induced in Israel with gamma rays is widely used in the cross breeding programmes in many countries including R. of Korea, Venezuela, Thailand and the United States. Uniculm mutants induced in Egypt and Pakistan have shortened internodes as well, resulting in compact capsule setting and increased yields. Dwarf mutants induced in Korea have compact fruit setting and lower setting of the first capsule. They have recorded increased lodging resistance as well. A newly found progeny line from a determinate and indeterminate cross showed resistance to *Phytophthora* blight and *Fusarium* wilt and is promising with its higher yield ability due to longer capsules and determinate form. Semi-dehiscent mutants isolated in Egypt and Pakistan during this CRP could contribute to reduction of seed loss at maturity.

Some of the high yielding mutants isolated under the present CRP were found to be tolerant to several important diseases in subsequent screening. Tolerance to water logging and indehiscence in mutants developed in Bangladesh could contribute to increased seed retention and better capsules maturation.

2.2.3. Promising mutant lines obtained

Many mutant lines have been obtained in this CRP and the followings numbers were reported as promising breeding lines:

Bangladesh	: 3 lines	Egypt	: 20 lines
Pakistan	: 3 lines	Sri Lanka	: 4 lines,

Additional promising materials are being tested in R. of Korea, India, China and Israel.

2.3. Conclusions and recommendations

Various characters concerning plant architecture which can be induced by mutagens in locally adapted varieties can contribute to yield or quality improvement or both. These possibilities have been amply demonstrated in the present CRP.

Improvement of yield through selection for yield components e.g. low position of first fruit, number of capsules per plant, increased fruiting zone length and 1 000 seed weight in M_2 and M_3 plants has been possible. Low doses of irradiation have created more genetic variability in quantitatively inherited yield components.

Cooperation should be established with plant pathologists to improve the screening methods for resistance to biotic stresses and to develop new and better ones.

Mode of inheritance and genetic relation between seed color, its thickness and oil content should be carefully investigated as these characters contribute to the quality of seeds.

The use of tissue culture, particularly the method of doubled haploids could be a useful tool for fast and easier screening of mutants for their tolerance for disease, environmental stress etc. It is therefore recommended to develop methodology of anther and/or microspore culture for use in future breeding.

3. SUNFLOWER (*Helianthus annuus*)

3.1. Crop specific problems and breeding objectives

Since sunflower is an open-pollinated crop and much of its area is sown with hybrid cultivars, the main emphasis in sunflower mutation breeding should be placed on improvement and/or selection of inbred lines with superior agronomic traits. The priorities in this respect are the following:

(1) Development of inbred lines resistant to stalk, root and head rotting, such as white rot (*Sclerotinia sclerotiorum*), charcoal rot (*Sclerotium bataticola*) and brown rot or stem canker caused by *Phomopsis helianthi*. Induced mutations could be obtained for certain morphological characters related to host resistance, such as the "stay green" character of the stem, which has been shown to be correlated with resistance to *Phomopsis* attack.
(2) Improvement of the existing best inbred lines for pollen self-fertility, in order to develop highly self-fertile F_1 hybrids.
(3) Identification of additional pollen fertility restoration genes in order to develop new genic-CMS systems, based on different interspecific crosses, other than the only existing system, derived from *H. petiolaris*.

(4) Construction of a new morpho-physiological ideotype based on stem, leaf, petiol and head mutants which would be able to better explore the specific environmental factors and to cope with moisture and heat stress.
(5) Development of sunflower genotypes resistant to herbicides, especially those controlling weeds in maize and wheat which are grown in rotation with sunflower in modern cropping systems.
(6) Conversion of the existing valuable linoleic sunflower lines into high oleic lines, in order to get superior high oleic F_1 hybrids.

3.2. Achievements of the CRP

Mutation breeding of sunflower inbred lines could be profitable in modifying oil quality for nutritional uses and for technical applications and in obtaining resistance to diseases. No mutant varieties or hybrids were released at I.C.C.P.T., Fundulea, Romania yet.

However, the mutant "short petiole" was identified in 1984 at I.C.C.P.T. in the inbred line LC-07657 following seed irradiation with 150 Gy of ^{60}Co gamma rays. This "short petiole" mutant will contribute to the development of a new sunflower ideotype with compact foliage which would facilitate growing denser plant populations.

Four dwarf mutant lines were obtained also by seed irradiation with 100 - 200 Gy of ^{60}Co gamma rays in the period of 1982-1987. Three of them have been converted into cms lines and are being used in breeding dwarf and semi-dwarf F_1 hybrids. One dwarf mutant line carries Rf_1 gene for pollen fertility restoration. Two restorer inbred lines (CG-3306 and CG-3663) with recessive branching and high self-fertility (70 - 82%) were obtained in 1986 - 1989 at Fundulea-Romania by seed irradiation with 100 - 150 Gy of ^{60}Co gamma rays. These lines are being used in developing highly self-fertile hybrids with reliable seed setting in the absence of insect pollinators.

Oil content was improved by seed irradiation of two inbred lines (V-2012 and LC-1004), reaching the highest biologically possible level, in an inbred line (54.2 %). In this case, the mutation was expressed by the significant reduction of hull proportion (10 - 12 %).

3.3. Mutagenic procedures

The followings were found to be very effective.

(1) Dry seeds were irradiated at the IAEA Seibersdorf Laboratory, Austria, with 100, 150 and 200 Gy ^{60}Co gamma rays. The treated seeds were sown in Romania at Fundulea in the breeding nursery three weeks later. M_1 plants were self-pollinated at random.
(2) In experiments with irradiated pollen, isolated plants of three hybrids (Select, Super and Felix) were emasculated and pollinated with pollen irradiated with 300, 700, 800 and 1000 Gy gamma rays. After 3 - 7 days the ovules were removed from the ovaries and cultured on MS medium *in vitro* under darkness at 29 °C.
(3) All the seeds obtained from the self-pollinated M_1 plants were sown in the field nursery. The size of the M_2 progeny was 20 - 1 200 plants/M_1 plant. The sterile plants were pollinated with fertile pollen and the resulting F_1 hybrid plants were evaluated in order to establish the genetic nature of their male sterility.
(4) In M_2, earliness was examined by determining the number of days from sowing to flowering. Seeds for the advanced generations were obtained by selfing the selected plants. Selection of plants in M_2 was done by taking into consideration the seed oil content determined by NMR (Nuclear Magnetic Resonance). The self-fertility was examined by the number of seeds per head on bagged M_2 plants. Concerning the "short petiole" trait, the methodology of breeding consisted of the transfer of the mutant genes into other agronomically valuable inbred lines by backcross and selection.

The genetic study of "short petiole" trait demonstrated its high heritability. The mutant is controlled by a small number of dominant genes with additive effects.

Seed oil content is a polygenic trait with a strong additive gene effect and marked influence by the environment. Dwarf mutants are controlled by one or two recessive genes for short internodes. Therefore both parents should be homozygous recessive for the same dwarf locus in order to obtain a dwarf F_1 hybrid. This may be achieved by independent induction of such a dwarf mutant allele by mutagenesis, in the other parental line. Nuclear male sterility induced by mutation was found to be controlled by one recessive gene.

3.4. Conclusions and recommendations

Future research in sunflower should be focussed on the application of biotechnology *in vitro* with the purpose of developing haploid and dihaploid homozygous lines and cell mutant lines resistant to specific diseases and environmental stress. Embryo culture should be largely used in order to accelerate the incorporation of mutant genes into agronomically suitable sunflower genotypes.

4. OPIUM POPPY (*Papaver somniferum*)

4.1. Crop-specific results and recommendations

Poppy being a predominantly self-pollinated crop, homozygosity of the mutants can be maintained easily by bagging. In all seed handling procedures during the mutagenic treatments, sowing etc. the small seed size should be borne in mind. Also, thinning after germination is often needed. Appropriate population size in M_1 is 2 000 - 3 000 seedlings per treatment. M_2 planting should be done by the progeny line method.

Since a plant regeneration protocol has been developed for poppy callus during this CRP, *in vitro* mutagenesis, use of somaclonal variation and genetic transformation techniques can be utilized where well defined traits can be screened at the cell level i.e. biochemical mutants for specific metabolic blocks, disease resistance against toxins produced by pathogens, etc.

To make and popularize poppy as an oil seed crop (i.e. free from narcotic attributes) efforts to produce morphine-less cultivars should be intensified.

4.1.1. Achievements by mutation breeding

Useful mutants were obtained in this CRP with respect to the following traits:

(1) Improvement in morphine content : mutants having 18 - 22 % of morphine in the opium crude are at hand, as compared to 10 - 12 % in the parents.
(2) Improvement in total latex yield : Three mutants registering 100 - 121 % improvement over the local source varieties were obtained.
(3) Development of strains with alkaloid accumulation in the straw rather than in the latex : mutants with 97 % increase over the local source varieties are at hand.
(4) Increase in capsule size and seed yield : mutants with 51 % increase in seed yield over the source variety and mutants with 42.8 - 45.2 % seed oil content have been developed.
(5) Resistance against downy mildew : a fringed leaved mutant (PCL) that has shown about 70 - 80 % more resistance to downy mildew attack has been developed. Another mutant i.e. dwarf mutant (DW) also shows very high resistance to *Peronospora arborescens* attack but needs rectification of an associated adverse trait of pollen sterility (>70 %).
(6) Selection of somaclonal variation : protocols for complete regeneration of plants capable of field transplantation were developed. Somaclonal populations are in the field for screening in R_2 generation.

4.1.2. Achievements of the CRP

(1) Varieties officially released : One
Big capsulated (BC) mutant developed from the parent variety Shweta following the treatment of 50 Gy ^{60}Co gamma rays. The mutant has registered a 121 % improvement

in total crude yield, 50 - 55 % advancement in morphine content, 90 - 95 % improvement in straw yield and 40 - 45 % advancement in seed yield over the parental base.

(2) Varieties to be released : One
Pinnately cleft-leaved (PCL) mutant developed from parent variety Shyama after mutagenic application of 50 Gy + 0.4 % EMS for 6 h. The variety has a high oil content (42.8 %), 100 % improvement in latex yield and > 70 % increment in downy mildew resistance. It is under final pre-release trials.

(3) Improved breeding stocks developed : Two
Red petaled (RP) and dwarf mutant (DW) are useful breeding stocks for high oil content and morphine content *per se*.

4.1.3. New techniques/methodologies developed

(1) *In vitro* culture requirements particularly for inducing rhizogenesis in shoot cultures have been worked out. For the first time poppy plants capable of field transplantation have been produced. For initiating rooting at sufficient frequency and quality it was found that complete absence of organic adjuvants in a low nutrient medium, together with low day/night temp. (12 °C/16 °C) during incubation (4 000 lux light and 70 - 80 % relative humidity) are necessary. With this technique the poppy system is now ready for *in vitro* genetic modification strategies to be applied.

(2) For the first time, reasonable success has been achieved in establishing dual cultures of *Peronospora arborescens* and *Papaver somniferum* tissue cultures. The technique of serial dilution developed during this CRP will be useful in establishing such dual cultures of other obligate host:pathogen associations where mycotoxins are not involved.

4.1.4. Problems pending

Future breeding efforts (particularly of mutation breeding) in poppy should be directed towards the following objectives:

(1) Induction of cytoplasmic male sterility to produce and exploit hybrid vigor.
(2) Synchronized flowering and uniform capsule maturity to facilitate mechanized harvesting.
(3) Resistance to moisture stress and salinity, as most of the poppy growing areas are under the threat of secondary salinization.
(4) Selection of biochemical mutants (using cell culture mutagenesis) to elucidate and isolate genes or gene products involved in alkaloid biosynthesis. Such mutants with specific metabolic blocks will be useful in developing special chemotypes with accumulation of non-narcotic alkaloid i.e. codeine or papaverine or thebaine.
(5) Development of herbicide resistance, particularly for glyphosate, because weeding is a major expense in poppy cultivation.

5. CASTOR BEAN (*Ricinus communis*)

5.1. Crop specific objectives

The following objectives appear suitable for improvement by mutation breeding :

(1) A dwarf compact plant with a reduced number of branches,
(2) Early and uniform maturity.
(3) Pistillate materials for commercial hybrid production.

5.2. Methodology of mutagenesis

The following methods were effective:

(1) ^{60}Co gamma ray irradiation to castor seeds
- Use newly harvested seeds (1 - 2 months) and sundried for at least three days.
- Irradiation doses may be 500, 600, 700 Gy of ^{60}Co gamma rays. Non-irradiated control needed.

- M_1 population size may reach up to 8 000 to 10 000 plants.

(2) Chemical mutagen treatment for castor seeds.
   - Remove the caruncles and puncture the seed coats at the points of placentation.
   - Presoak the seeds in distilled water for 24 h with constant shaking (or place in a shaker).
   - Soak the preconditioned seeds in the freshly prepared chemical mutagen solution for 4 h at 20 - 25 °C with constant shaking.
   - Wash seeds thoroughly with running water for 4 h and air dry for 15 min. before planting.

5.3. Planting pattern and screening procedures in M_2

(1) Screen for agronomic characters and other attributes visually and in comparison with the control.
(2) For promising plant selections, plant all the seeds from the first two racemes.

6. *CUPHEA* (*Cuphea* species)

6.1. Crop specific objectives and problems

Cuphea could be a new oil crop, serving as a source of medium chain fatty acid (MCF) particularly of capric acid(C = 10 : 0) and lauric acid (C = 12 : 0). In the future it may become an alternative crop in Europe, substituting cereals.

Induction and selection of a non-shattering form of *Cuphea* is the main objective of our programme. The important non-shattering characteristic of *Cuphea* may be obtained by inducing a recessive mutation as occurred in many cultivated plants. The frequency of such induced mutants may be low. Perhaps as many as a million plants in M_2 and M_3 generations must be examined in order to obtain this mutant. For this purpose mutagenic treatment with N-nitro-N-methyl urea may be suggested, as well as other mutagens.

A protocol should be developed for effective chemical mutagenesis since presoaking and soaking treatments of the seeds are problematic.

6.2. Recommended methodology

(1) Following presoaking in water *Cuphea* seeds stick together, which makes sowing difficult. Pretreatment of the seeds in 50 % H_2SO_4 for 0.5 hr to destroy the stickiness was effective. The presoaked seeds should then be rinsed in running water for 18 - 24 hours.
(2) Optimal dose of chemical mutagens to induce variability :
   - Sodium azide : concentration; 1.0, 2.0 mM, soak for 3 hrs, at pH 3, using buffer solution;
   - N-nitroso-N-methyl urea : concentration; 1.0, 2.0 mM, soak for 3 hrs
   - EMS (cf. Dr. Knapp) or gamma rays 100 Gy or 150 Gy may be also effective.
(3) *Cuphea* seeds are usually completely dormant. Dark brown and black seeds are fully mature and give good germination after storage for 4 - 6 months. Green seeds germinated well only after a storage time of 12 months. Therefore it is best to use black seeds.

The germination of *Cuphea* seeds immediately after harvest increased to 100 % by removing the seed coat. However, this is too time consuming. Sodium azide (SA) and hydrogen peroxide (H_2O_2) increased the germination rate by 80 % to 90 % for freshly harvested seeds as compared to the control. By treating fresh *Cuphea* seeds with sodium azide it was possible to induce mutations and overcome dormancy simultaneously.

6.3. Screening procedures

The M_1 and M_2 were grown and harvested in bulk because large populations of M_2 and M_3 plants are needed to select non-shattering mutants. It is difficult to formulate how many seeds from each of M_1 plants should be harvested, as the number of initial cells in the seed embryos is unknown.

6.4. Achievements of CRP

(1) A mutant line with an increased number of branches per plant (No. 1/86) was induced and selected after treatment with sodium azide (SA).
(2) Mutant No. 41 showed an increased amount of medium chain fatty acid (MCF) particularly C-12 lauric acid, compared with the source species *Cuphea tolucana* 629 (control).
(3) Early mutant No. 1/86-17 matured about one week earlier than the original material.

6.5. Problems pending

(1) A good qualitative analysis of the fatty oil content will be the key in the screening of improvement of fatty acid content, especially for medium chain fatty acid in *Cuphea* seeds.
(2) Seed exchange of *Cuphea* to widen the genetic resources is very difficult because private firms which are active in such programmes, restrict the exchange.

7. COTTON (*Gossypium hirsutum*)

7.1. Crop specific objectives

(1) Genic-CMS to produce hybrid cotton cultivars.
(2) Resistance to diseases.
(3) Quality improvements in oil or meal by reducing unfavorable components.
(4) Improvement of fibre quality and quantity.

7.2. Method used and results

In the first phase of the mutation breeding experiments, chemical mutagens, ethylene imine and di-methyl sulfate, were used but no significant results were obtained. Then gamma-irradiation was used to improve the resistance to *Verticillium* wilt in some local varieties.

The dose of 300 Gy decreased germination of irradiated cotton seeds. For M_1 6 000 plants were obtained. The plants were sown in the field in rows 10 m long and 1 m apart, having about 10 plants per meter.

In the M_2 generation, ten seeds from each M_1 plant were sown in progeny rows in a field contaminated with *Verticillium* wilt. Chlorophyll mutants which segregated in the M_2 indicate that the mutagenic treatments were effective. The original susceptible varieties sown in the same contaminated field served as checks.

The resistance to *Verticillium* wilt will be evaluated as a percentage of the infected plants. The degree of infection will also be evaluated according to visual symptoms.

8. GROUNDNUT (Peanut) (*Arachis hypogaea*)

Groundnut is an important oil seed crop and some members of this CRP worked also on this material. Contributions made in the 2nd and 3rd RCM are integrated here.

8.1. Crop specific objectives

(1) Higher pod and oil yields.
(2) Plant types adapted to different agroclimatic conditions, i.e. to semi-arid and wet areas, to various temperature and photoperiod conditions.
(3) Higher harvest index.
(4) Resistance to plant and pod diseases.
(5) Resistance of the pods to colonization by *Aspergillus flavus*.

8.2 Objectives for mutation breeding

(1) Short duration varieties with synchronous pod setting.
(2) Strong pod attachment.
(3) Improvement of flavour and texture of seeds.
(4) Short seed-dormancy period.
(5) Oil quality modifications.
(6) Improved uptake of micro-nutrients, especially iron.
(7) Increased nitrogen fixation efficiency.
(8) Tolerance to environmental stresses, especially drought and salinity.
(9) Improvement of germination at low temperature (12 °C - 14 °C).
(10) Resistance to *Cercospora arachidicola*, *Cercosporidium personatum*, *Puccinia arachidis*, *Sclerotium rolfsii*, *Diplodia gossypina*, *Phytophthora* spp. and *Fusarium* spp.
(11) Resistance to bud necrosis disease (caused by Tomato Spotted Wilt Virus) and other viral diseases or their vectors.

8.3. Methodology of mutagenesis

Groundnut was one of the first crops to be included in mutation induction experiments. Already 33 cultivars have been released, as a result of induced mutations, directly or through cross breeding, as reported in the Mutation Breeding Newsletter No. 38 (1991). Inclusion of mutants in cross-breeding programmes has generated much variability and many varieties have been developed by this method. For gamma-irradiation, doses ranging from 100 - 250 Gy have been successfully used. For treatment with ethyl methanesulphonate, a solution of 0.4% concentration at 10 °C up to 24 h is recommended. Rinsing in running water for 2 - 4 h will be needed.

8.4. Screening procedures

The M_1 progeny should be sampled to include pods from all branches of all the surviving plants and may be carried forward as progeny rows or in bulk depending on the objectives and possibilities. Groundnut being a tetraploid, high yielding and other mutants may segregate at later generations and therefore screening should be continued at least up to the M_3 generation.

Screening methods for rust and *Cercospora* leafspot have been well documented in publications of ICRISAT. For *Cercospora arachidicola* and *Cercosporidium personatum* inoculation with spore suspension having 100 - 1 000 spores per ml may be useful. Advanced lines are scored on a 1 - 10 scale. A similar method can be used for screening for rust. Detached leaf technique in humid chambers has also been used successfully for preliminary screening of germplasm for resistance to rust and *Cercospora*.

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