SESAME IMPROVEMENT BY INDUCED MUTATIONS: RESULTS OF THE CO-ORDINATED RESEARCH PROJECT AND RECOMMENDATION FOR FUTURE STUDIES

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Abstract

The FAO/IAEA Co-ordinated Research Project has brought together sesame breeders from 11 countries. They, together with pathologists, agronomists and physiologists, have made considerable effort to advance the genetic improvement in sesame. The results and conclusions from this project cover the mutation techniques used for the genetic improvement of various aspects of sesame. These recommendations do not only deal with the application of mutation induction, but also with the wider plant breeding related objectives and methods to be considered for this semi-domesticated crop. It is clear that more advanced techniques can and should be incorporated in the process which would enhance the genetic improvement. Although five years is a relatively limited time in a plant breeding programme, the participants have been able to produce and make available a considerable pool of agronomically interesting mutant sesame germplasm. The participants in the CRP considered that, together with other specialists, plant breeders can gain fuller benefit from the mutations induced by radiation or chemicals. Work on these mutants must continue in co-operation/consultation with plant physiologists and pathologists, and with biotechnologists who may in the future be able to provide in the future methods for introducing beneficial traits from other crops into sesame. The sesame programme should include scientists from the Member States where sesame grows and scientists from developed countries who may have greater access to physiological and molecular research facilitites.

1. SESAME MUTANTS DEVELOPED THROUGH THE PROJECT

During this Co-ordinated Research Project a total of 142 mutants with agronomically useful characters were registered through different national sesame improvement programmes.

These mutants were developed through the use of chemical and physical mutagens as well as crosses with mutants. Doses and concentrations were all within the range of the recommendations given in this chapter. These were the following:

TABLE I. DEVELOPED CONFIRMED MUTANTS WITH AGRONOMICALLY USEFUL CHARACTERS ORGANIZED PER COUNTRY

Country	Mutant line name
Bangladesh	SM-5
	SM-7
China	95 ms-2
	95 ms-3
	95 ms-4
	95 ms-5
	95 ms-6
	95 ms-7
	CC-1
	DC-1
	MC-1
	MC-2
	MC-3
	WC-1
	YC-1
Egypt	EFM 92
	EXM 90
	Mutant 12
	Mutant 14
	Mutant 15
	Mutant 48
	Mutant 5
	Mutant 6
	Mutant 7
	Mutant 8
	Mutant 9
India	AUS 1138
	AUS 1198
	AUS 1207
	AUS 993

Country	Mutant line name
India (cont.)	AUS MS 1034
	DTF
	N-105 (Virescent)
	N-112
	N-113
	N-115
	N-147
	N-157
	N-169
	N-171
	N-238
	N-29
	N-57
	N-93
	NM-26
	NM-28
	NM-31 (Chlorina)
	NM-54 (Chlorina)
	NM-58
	NM-65
	NM-67
	NM-71 (Chlorina)
	NM-74
	NM-77
	NM-80
	NM-85
	NM-87 (Mosaic)
	NY-21
	NY-9
	PTM-1
	T-Leaf
	TMST-10
	TMST-11
	TMST-15
	TTL-3
	Y-55
Kenya	SIK MU 291/2
	SIK MU 296/1
	SIK MU 303/2/2
	SIK MU 353/6/1
	SIK MU 36/1
	SIK MU 55/1/1
	SIK MU 96/3
Pakistan	Pr.14-2 D-1
	Pr.14-2 EF-1
	Pr.14-2 EF-2
	Pr.14-2 HB-1
	Pr.14-2 HB-2
	Pr.14-2 MS-1
	Pr.14-2 MS-2
	Pr.14-2 MS-3
	Pr.14-2 PB-1
	Pr.14-2 PB-2
	Pr.14-2 PB-3
	Pr.14-2 PB-4
	Pr.14-2 T-1
	Pr.14-2 T-2
Pakistan (cont.)	Pr.19-9 D-1
	Pr.19-9 D-2
	Pr.19-9 EF-1
	Pr.19-9 HB-1
	Pr.19-9 MS-1
	Pr.19-9 MS-2
	Pr.19-9 PB-1
	Pr.19-9 T-1
	S-17 D-1
	S-17 EF-1
	S-17 EF-2
	S-17 MS-1
	S-17 MS-2
	S-17 S-1
	S-17 St-1
Rep. of Korea	SI84075-2B-23-1-1 (Suwon 144)
	SIM86029-2B-5-1-1-3-1(Suwon 158)
	SIM88H30-2B-68-1-1 (Suwon 155)
	SIM88H30-68-3-1
	SIM89101-2B-20-1-1
	SIM89JBE-2B-10-1-1-1(Suwon 157)
	SIM89JBE-2B3-1-2-1
	SIM89JE-2B-3-1-1-1
	SIM90HS2/3-2B-1-1-1
	SIM90HS2/3-2B-15-1-2
	SIM90HS2/3-2B-6-3-1
	SIM90JBS2/2-2B-1-1-1
	SIM90JBS2/2-2B-7-1-1
	SIM90JBS2/2-2B-7-3-1
	SIM91129-2B-6-1-1
Thailand	GMUB-1 (NS-1)
	GMUB-7 (NS-7)
	KU M 6005
	KU M 6011
	KU M 6015
	KU M 6021
	KU M 6026
	KU M 6040
	KU M 6041
	KU M 6045
	KU M 6051
	KU M 6054
	PMUB 19
	PMUB-1
Turkey	cc-?-1 (9430001)
	cc-?-2 (9413047)
	cc-?-3 (9422059)
	cc-?-4 (9413806)
	cc-?-5 (9415000)
	cc-?-6 (9511609)
	cc-?-7 (9531092)
	cc-?-8 (9542305)
	dt-?-1 (9411144)
	dt-?-2 (9412178)
	dt-?-3 (9512368)

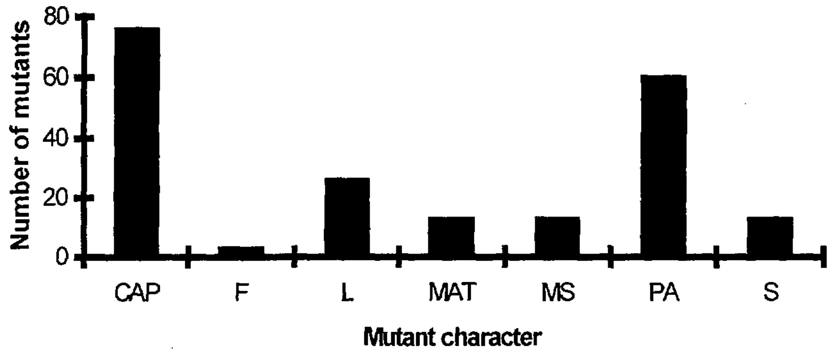


FIG. 1. Mutation induced characters in the developed, confirmed mutant sesame lines.

Various characters were induced mostly affecting the capsules (CAP), flowers (F), leaves (L), maturation (MAT), male sterility (MS), plant architecture (PA), and seeds (S) (Figure 1). Most mutations (76) were found for capsule related characters such as, 3-capsules-per-leaf-axil, shape, size, non/semi shattering, and capsule density on the stem. Also plant architecture was a frequently selected (60) character in mutant sesame lines, including short internode length, profuse branching, uniculms, and semi-dwarfs.

Sesame is a neglected crop from the plant breeding point of view, being in the second stage of plant domestication. This is part of the reason that the majority of the above mentioned characters are related to plant morphology. Also these traits are relatively easy to select for and this project from the start focused on these traits. A few mutants were reported with induced resistance to diseases, such as powdery mildew and some with improved seed oil content. Overall, more than 100 different traits were observed to have been the result of induced mutations in this project. There is still a long way to go before sesame will reach the desired production levels, but clearly the use of mutation techniques has shown to be a very useful tool in the genetic improvement of locally well adapted germplasm.

2. METHODS FOR MUTAGEN TREATMENT

The parent materials chosen for sesame breeding programmes using induced mutations should preferably be the best well adapted available varieties requiring improvement in one or two characters, which lend themselves to screening large number of plants. The treated varieties or lines should be homozygous and uniform, and if necessary selfed first for one or two generations to attain the above.

At the conclusion of this CRP the various mutagen treatments employed were reviewed and recommendations given, as follows:

— For **gamma rays,** doses ranging from 150–800 Gy proved successful in inducing useful mutations. Doses in the lower range were recommended for inducing desirable mutations with minimal simultaneous induction of additional, often undesirable mutations.

— With **fast neutrons'** irradiation of dry sesame seeds, preliminary results in Thailand showed that doses of 30 and 80 Gy were effective for the induction of useful mutations.

Treatments with **EMS** have also proved successful. The following protocol is recommended:
Pre-soak the seeds for 24 hours in water (preferably at a low temperature, 4°C); soak the seeds in EMS solutions of 0.4–1.0% v/v with a phosphate buffer (pH = 7) for 2 to 4 hours, with occasional shaking; post-wash in running tap water for at least 4 hours.

For **sodium azide** (NaN_3), the seeds should be pre-soaked in cold tap water (4°C) for 24 hours, then soaked in a 4–6 mM NaN_3 solution with Sörenson phosphate buffer (pH = 3) for 4–6 hours at 18–24°C, then post-washed in running tap water for at least 4 hours.

Since sesame seeds are small and fragile, they should be placed in nylon net bags (mosquito net size) for easy handling during pre-soaking, mutagen soaking and post-washing; labels indicating variety and mutagenic treatment details should be tied to the bags. Excess moisture should be removed using filter paper or paper towels, and the seeds should be planted in the field as soon as possible after the post-washing. Optimal conditions for germination of the treated seeds should be ascertained in all the various mutagenic treatments; this is especially critical when wet seeds are sown following post-washing.

3. MANAGEMENT OF M_1 AND SUBSEQUENT GENERATIONS

The handling of mutant populations in the different generations is important for efficient use of resources and enhanced probability of positive results.

3.1. M_1 generation

A maximum number of targets should be exposed to the mutagenic treatments in order to maximize the number of useful mutations. A large number of M_1 plants should be grown, and 2–5 capsules per plant harvested from the circumference of the main stem, so the required plot area and labour would be small. Whenever possible, precautions should be taken to minimize the risk of cross pollination (by insects) in the M_1. The M_1 plants can be harvested individually, if the M_2 will be grown in progeny rows, or in bulk (see below).

3.2. M_2 generation

The M_2 generation can be grown in progeny rows or in bulks, by varieties and treatments, depending on objectives, available financial resources and facilities. About 30–50 plants should be grown in the M_2 from each M_1 plant. The remaining seeds can be held in reserve for sowing in subsequent seasons. Previous experience has shown that desirable sesame mutants were identified when grown in progeny rows as well as in bulks. Progeny rows will furnish more genetic information. The M_2 plants should be amply spaced (between and within rows) to facilitate testing and screening during the season.

When putative recessive mutants are selected in the M_2 progeny, it is advisable to obtain seeds also from selfed normal sib plants where possible. Some of these plants may be heterozygous for the given mutations and will give in M_3 genetic segregations, thus furnishing information on their genetic control sooner then F_2 populations from crosses of the mutants with the source varieties.

3.3. M_3 generation

The M_3 generation should include progeny from individually selected M_2 plants to confirm the mutations' nature, and to study their breeding behaviour and agronomic value. M_3 plant progeny (bulks) should be planted to facilitate selection for various quantitative traits especially those affecting yield. In subsequent generations, the usual pedigree selection and evaluation procedures should be followed.

4. USE OF MUTATIONS IN CROSS BREEDING PROGRAMMES

The use of mutation techniques has proved very successful in inducing in sesame desirable mutations such as increased seed yield, earliness, modified plant architecture, disease resistance, seed retention, and high oil content. It is recommended to use wherever possible also the cross-breeding approach, involving 'local varieties x mutants', 'mutants x mutants', and 'mutants x introduced lines', in order to get new genotypes having more than one of the desired characters mentioned above. This is particularly valuable for building up promising ideotypes and for exploiting heterosis by developing hybrids between suitable parents (mutants) with good combining ability. It is recommended that while for qualitative characters selection could start with single plants in the F_2, for quantitative characters it should be initiated in the F_3 generation.

5. BREEDING OBJECTIVES IN SESAME IMPROVEMENT PROGRAMMES

Key potential mutant traits of importance for sesame improvement are: good seed retention, shorter plants, higher harvest index, shorter growing period, determinate habit, uniform maturity, and reduced biomass. Some important characters which are highly desired are described below.

5.1. Disease resistance

Disease problems tend to be country/region specific. Therefore, varieties that are resistant to the locally prevalent diseases (and at times pathogenic races) should be developed. Diseases that are known to be important in most countries/regions are *Fusarium oxysporum* f.sp. *sesami*, *Phytophthora parasitica* var. *sesami*, *Macrophomina phaseolina* ssp. *sesamica*, *Cercospora sesami*, *Alternaria sesami*, *Pseudomonas syringae* pv. *sesami*, *Xanthomonas campestris* pv. *sesami*, phyllody (Mycoplasma Like Organism = MLO), powdery mildew (*Oidium sp.* and others) and Sesame Mosaic Virus. In the Republic of Korea mutant varieties have been developed with resistance to Phytophthora; mutants resistant to it were induced also in Sri Lanka. However, the yield potential should be improved in these resistant or tolerant lines. The programme in Bangladesh has successfully induced tolerance for Sesame Mosaic Virus.

In general, it isrecommended that if possible, screening under disease pressure should not take place before the M_3 generation. It would be helpful to have the assistance of plant pathologists and to prepare visual aids for the purpose of better identification of the different diseases. Exchange of seeds for screening for resistance should be encouraged. If possible, the presence of different pathogenic races should be checked and thus obtain more critical information. It was also suggested to collect from host plants spores of certain fungal diseases every season, in order to maintain the locally prevalent mixture of races.

5.2. Pest resistance

Until now no efforts have been made to induce mutations for pest resistance, but identifying lines with tolerance or resistance to devastating pests in sesame such as *Antigastra catalaunalis* (webworm, leaf webber, capsule borer), sphingid moth (*Acherontia styx*), aphids and gall-midge would be very helpful.

5.3. Shatter resistance

Seed shattering before and during the harvest causes considerable losses in sesame. Mutations for seed retention (often monogenic) were critical in the domestication of most seed crops. A spontaneous indehiscent mutant (*id*) was discovered in 1942 in Venezuela by Langham (1946). However, due to its low yields and other undesirable side effects it has not been possible to use it in commercial varieties. Non-shattering mutants have been reported also in other crops.

Seed retention in sesame would be aided by determinate habit, i.e. that the plants would stop flowering, shed their leaves, and reach physiological maturity before their first capsules dry. Subsequently, the plants should dry as quickly as possible and release the seeds from the capsules in a way commensurate with the harvest and threshing methods.

Flowering and shattering are affected by branching, capsule length, capsule width, number of capsules per leaf axil and other characters. The preferred trait contributing to seed retention should be chosen according to the projected harvest method. Thus, if the crop is to be machine harvested good placenta attachment is necessary, but if the plants are shocked, this is not necessary. In fact, for manual harvest farmers would prefer no placenta attachment, in order to ease the threshing work.

The *gs* allele for seamless capsules and the *id* allele can be used only for the oil or food ingredients market and then only if the seeds can be processed in a timely manner to minimize the effects of seed damage from threshing. Much breeding work has been devoted to the development of productive *gs/gs* and *id/id* cultivars, adapted to combine-technology and with undamaged, good quality, whole seeds. These efforts have been unsuccessful so far, but still they should not be abandoned. It should be realized that the probability of success is low unless a breakthrough is found, e.g. a modifying gene or a change in the combine technology.

In the present CRP eight gamma ray (300–750 Gy)-induced mutants with indehiscent (closed) capsules were recovered in four different Turkish cultivars. Allelism tests are planned to determine if these mutants are in the same locus, the known *id* locus, or if there are different loci. In Thailand, irradiation with gamma rays (500 Gy) of two local varieties resulted in three shatter resistant mutant lines, all outyielding their respective parent varieties. Furthermore, in Thailand seven delayed shattering and shatter-resistant mutant lines were obtained following treatments with EMS (0.5–1.0%, 4 h).

Agreed terminology is proposed for determinate growth habit, maturity, and shatter-resistance in order to have a uniform frame of reference. Criteria need to be defined with time and additional experience.

5.4. Seed quality and contents

5.4.1. Oil yield

- To improve the oil yield two parameters have to be improved, that is seed yield of the crop and oil content in the seed.
- Oil content of the seed should be higher than 50%.
- There seems to be a correlation between oil content and seed colour; dark seeded varieties have lower oil content than the light seeded varieties, perhaps because the dark ones have thicker seed coats. However, breeders should aim at raising the oil contents of the dark seeded accessions, possibly by selecting for a thinner seed coat. It is suggested to attempt to increase oil production per unit area also through testing of promising lines under higher plant density.
- When screening for oil content, the seed samples (capsules) should be taken about 2.5 cm below the middle of the capsule bearing zone of the main stem of the plant. The seeds should be fully mature.

5.4.2. Oil quality

Mutations were used to induce changes in the fatty acid composition in sesame seeds in the Republic of Korea and could be attempted elsewhere, e.g. in lines with high oil content (>50%).

5.4.3. Antioxidants

Induction of mutations could be attempted for increased contents of lignans in the seeds and for their composition, e.g. relative amounts of sesamin and sesamolin and similar products. These substances have a wide variety of applications in the production of pharmaceutics, pesticides and other industrial end products.

5.4.4. Confectionery quality

The lines to be developed for confectionery uses should be screened for seed colour, size and shape, for flavour, and for seed coat thickness and texture, using specific descriptors developed together with the processors.

5.5. Harvest index

This is an important character that may be improved by modifying plant architecture. According to the various farming systems this would mean to develop cultivars that:

— are optimal in height (0.5–1.5 m),
— are uniculm for dense stands under high input conditions,
  or
— are medium branching with appressed branches for low input conditions,
— have high capsule density,
— form the first capsules at the height of 15–20 cm above ground for hand harvested crops,
  or
— form the first capsules at the height of 15–40 cm above the ground for mechanical harvesting, depending on the machinery and topography.

In several programmes within this CRP mutants were induced with distinct plant architecture modifications, e.g. reduced height, three capsules per leaf axil, uniculm, high capsule density and determinate growth habit.

5.6. Yield potential

To improve yield the important components to be considered are:

— Number of capsules per unit area. This should be given priority rather than number of capsules per plant.
— 1000 seed weight.
— Number of seeds per capsule. A representative sample of capsules from the top, middle and bottom part of the plant should be used to obtain a mean value for this parameter. However, the use of this parameter is debatable since eight-loculed plants have more seeds per capsule but they are not necessarily the highest yielding cultivars. Thus, the breeder should decide whether this is an appropriate parameter for his lines and conditions.

5.7. Adaptability

Cultivars to be developed should be adapted to the production systems of their prospective area(s) of cultivation.

— Short duration, early maturing cultivars that can be planted as a second crop (e.g. after rice in India, Pakistan, Bangladesh or after wheat or hay in Turkey, Israel) or as a single crop to exploit the short duration of the rainy season.
— Cultivars should be developed that are resistant or tolerant to the prevalent biotic and abiotic stresses.

5.8. Leaf morphology

This character depends very much on the population size and planting density. To increase photosynthetic efficiency, probably plants with appressed (at 45° angle to the stem) and lanceolate leaves should be preferred. Studies on stomatal anatomy would further enrich the knowledge on 'net accumulation rate' (NAR), and help select plants on the basis of leaf morphology and structure.

6. HYBRID VARIETIES AND HETEROSIS BREEDING

Sesame continues to be a high risk crop in many of the major producing areas. Through the classical breeding methods of selection, pedigree breeding, backcross and induced mutations, a major yield breakthrough was not achieved so far despite many efforts. Thus, sesame continues to be a poor competitor with other crops and is often relegated to the poorer fields.

Certain F_1 hybrid combinations in sesame resulted in marked yield increases. This was the case in studies conducted in China, India, USA and Venezuela. It appears that breeding hybrid varieties may give a yield breakthrough in sesame as seen in other self pollinated crops such as rice, barley, wheat and tomato. This can make sesame more remunerative and competitive. It may lead to its wider cultivation and to greater returns which in turn may lead to applications of higher inputs.

Hybrid varieties must be produced by crossing suitable inbred parents, with high general and specific combing ability. Once such inbreds are found, hybrid seeds can be produced in several ways:

1. By hand emasculation and pollination.
2. By spraying gametocides.
3. By using genic male sterility mechanisms in combination with hand pollination or honey bees as pollinators.
4. By using a genic-cytoplasmic male sterility system, with pollination as above.

In the case of sesame, the additional advantage is that for every successful pollination, a capsule containing 50–60 seeds can be obtained (a good stand is 250,000–300,000 plants/ha). Hand emasculation and pollination are useful in research but they are not feasible for commercial production. In India, the cost of emasculation and hand pollination was estimated to be US $ 10–12/kg for hybrid seed. For the farmers hybrid seeds must be produced on a large scale with lower cost which necessitates the use of male sterility. So far, only genic male sterility (GMS) has been found in sesame. A naturally occurring *ms* allele was found in Venezuela about 30 years ago. This is the one which is being used in China now. Mutations for male sterility were successfully induced in some of the cultivated varieties by four participants in this CRP following treatments with gamma rays (300–500 Gy) and EMS (1%, 2h).

The GMS can be used to produce hybrids but uniformly male sterile rows/plots (*ms/ms*) on which seeds will be produced cannot be obtained. Since, GMS is maintained by hybridizing male steriles (*ms/ms*) with isogenic but heterozygous plants (*Ms/ms*), the offspring segregates 1 male fertile: 1 male sterile. This would require early rouging of the fertile *Ms/ms* progenies. This operation could be feasible economically where labour is inexpensive and/or farmers can produce their own hybrids in an isolated plot or in a net house, rouging the *Ms/ms* plants, growing both the male sterile (*ms/ms*) plants of the A line and an appropriate pollinator, male fertile (*Ms/Ms*) C line, with good combining ability.

For large scale production of hybrid seeds by seed companies the above approach is not feasible, due to the high labour cost. Hybrid varieties could become economically feasible only through genic-cytoplasmic male sterility (GCMS). The search for GCMS should be continued. It should also be realized that searching for spontaneous or induced cytoplasmic mutants is difficult since it requires test crosses; self pollination of the M_1 and M_2 plants and screening their offspring are not sufficient in this case.

The following is recommended for future work:

I. The stability and seed set of the male sterile mutant lines induced in China, India and Turkey should be improved.

II. The induced male sterile mutants should be tested for allelism, to find out if more then one locus is involved.

III. Attempts should be made to obtain GCMS lines for which the following line of action is proposed:

— Cross cultivated species reciprocally with wild species of *Sesamum* (possibly *S. malabaricum*); cross male sterile F_1 interspecific hybrids with various germplasm lines representing different regions (pollen mixture); grow F_1 plants and search for male fertiles — presumably due to presence of a nuclear, genic restorer; self newly derived fertile hybrids; check offspring for Mendelian segregation of male fertiles vs. male

steriles. In such a way, it would be possible to find a restorer gene for the cytoplasmic male sterility generated by interspecific hybridization (see Figure 2).
— Existing *Ms/ms* lines be crossed with wild species.

"Split corolla" mutants were induced by the participants of this CRP from China, India, Sri Lanka, and Turkey and found naturally also in Venezuela. These mutants reduce self pollination drastically and can sometimes be used as female lines effectively. Their use as female parents in producing F_1 hybrids should be evaluated.

Crosses of some of the mutants induced in this CRP with the source parents and with other cultivars, gave F_1 hybrids showing very high heterosis for yield. The nature of this observed heterosis-like behaviour, which was noted also earlier in sesame and in other crops should be investigated further.

7. RECOMMENDATION FOR FUTURE RESEARCH IN SESAME IN COMBINATION WITH MUTATION TECHNIQUES

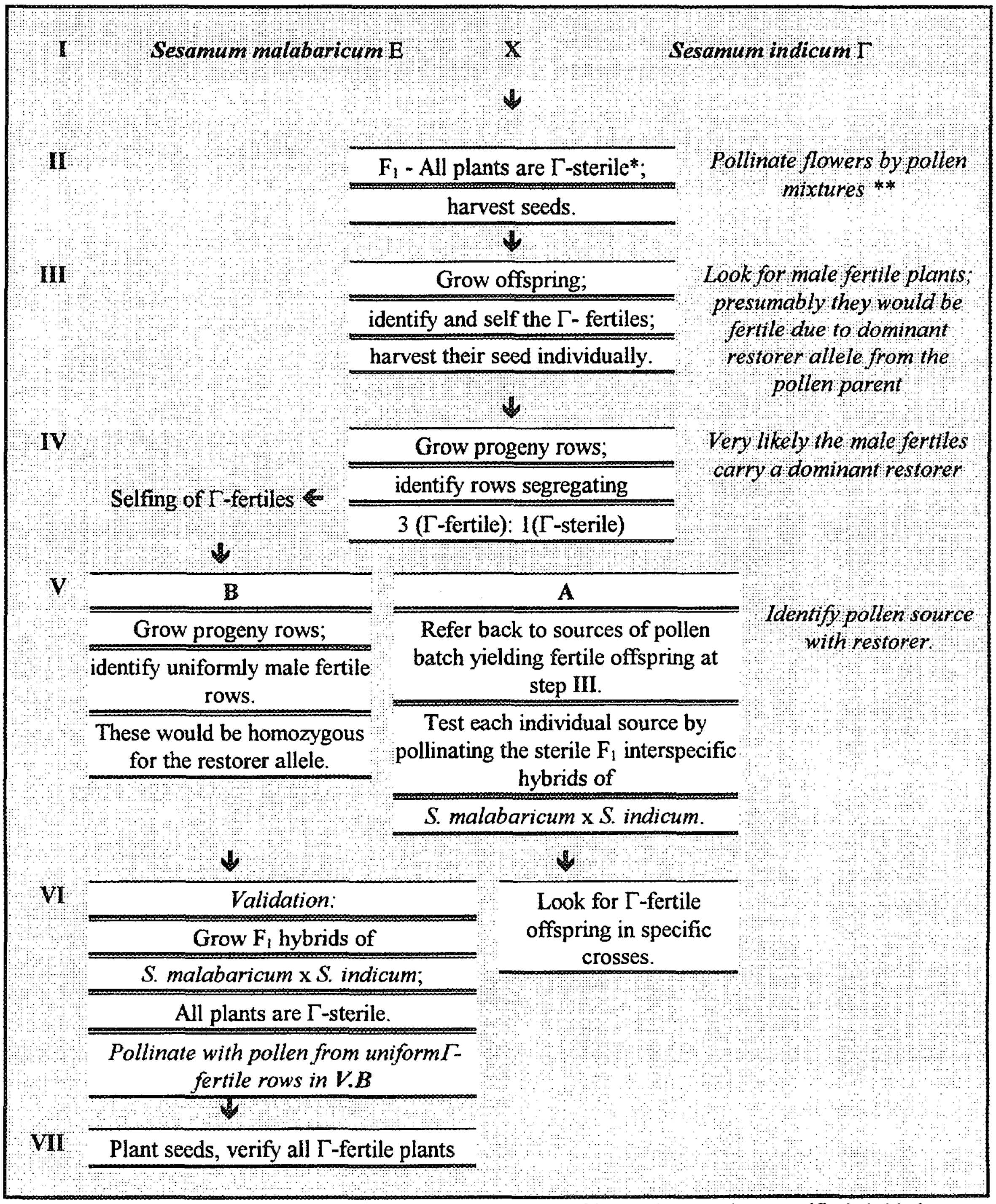
It was generally agreed that the induced mutants developed by the participants in this CRP have and will contribute further to improve productivity. However, it was realized that more sophisticated selection methods are required to take full advantage of the variation provided by the mutants. Involvement of three areas of expertise would make a substantial contribution to further sesame breeding research, namely physiology, pathology and biotechnology.

Mutations induced by radiation or chemicals provide variation in plant structure and function from which breeders can select plants having useful traits. Plant physiological and pathological approaches can provide deeper understanding and effective ways to identify characters or plants which can be useful in solving particular problems, thus facilitating more effective selection in breeding programmes. Biotechnology provides methods which enable:
— Screening for desired traits in the seedling or another early stage of plant development (allowing rapid screening for useful mutations of more plants earlier).
— Identification of plants with desired quantitative traits' genotypes, independent of environmental influences masking the traits' phenotypic expressions.
— Utilization of knowledge of gene action in some species for selection of beneficial traits in sesame.

Co-ordination of research in sesame breeding, physiology, pathology and biotechnology will therefore be very beneficial and a model for other crops. Four areas which are important for increasing the productivity and production of sesame in many parts of the world were identified:

A. Higher yields (e.g. increasing the harvest index).
B. Seed retention.
C. Tolerance to abiotic stresses (e.g. drought tolerance, waterlogging).
D. Pest and disease resistance and tolerance.

A. The harvest index of sesame, i.e. the proportion of energy invested in the seeds out of the total above ground biomass is low in relation to other crops. The harvest index may be improved by increasing light reception, photosynthetic efficiency, and by increasing the proportion of photosynthetic products directed towards the seeds. Under field conditions, the lower leaves are shaded by the upper leaves' canopy. Investigations on plant habit and architecture, and on leaf shape, size and angle to the stem could provide an understanding how sesame plants could capture light more efficiently. Mutations provide the necessary genetic variation to study these traits and their effects. Once light has been captured, more of its photosynthetic products must be partitioned towards the seeds, to increase the harvest index. In such studies radioactively labeled carbon from the uptake of CO_2 can be very useful.



* See Prabakaran, A.J. and S.R. Sree Rangasamy, 1995. Observations on interspecific hybrids between *Sesamum indicum* and *S. malabaricum*. I.Qualitative characters. Sesame & Safflower Newsletter 10: 6-10

** Use pollen mixtures drawn from diverse germplasm accessions and mutant lines. Preferably use many pollen batches each from a few identified sources.

FIG. 2. Suggested scheme for identification of restorer allele and GCMS development using interspecific crosses.

B. Reducing seed shattering has the most potential of all traits, to dramatically increase sesame production and to adapt it to mechanized harvesting. Substantial breeding efforts have addressed this problem and recently some anatomical and physiological work has commenced. A number of radiation and chemically induced closed capsule mutants and shatter resistant sesame lines have been selected but sesame varieties used in most areas of the world still shatter. Anatomical and physiological research designed to study the mechanisms leading to seed loss and its prevention, and to identify traits that can be used to select less shattering varieties, will benefit all sesame breeding programmes. More studies are required, particularly to compare the mechanism of non-shattering governed by the spontaneous indehiscent mutant (*id*), and those of other non- or semi-shattering mutants induced in this CRP.

C. Most sesame, whether in developed or developing countries, is grown under rainfed conditions where environmental conditions change drastically. In these conditions, drought is often the most serious yield reducing factor. Therefore, varieties of sesame with drought resistance must be developed. Recent work in other species has shown that methods such as isotope discrimination (the natural ratio of C^{12} to C^{13} in the plant) give a good measure of the water-use efficiency of plants. Certain characters such as leaf shape, leaf pubescence, mucilage glands, and stomatal conductance, control water loss from the plants. In the present CRP it was demonstrated that some of these characters are modified by radiation and supplement natural variation for drought tolerance. There is good evidence that cooperation between plant physiologists and plant breeders, utilizing mutation techniques, could enhance this tolerance further.

D. Breeding experience has shown that tolerance or resistance to pests and diseases can be enhanced in breeding programmes. Some plants with induced mutations have shown promising pest and disease resistance/tolerance, with at least two sesame varieties having induced disease resistance already officially released. Furthermore, the hybridization of different induced mutants with other lines, and between lines from widely different places of origin gave transgressive and unexpected levels of tolerance to diseases.

Molecular technologies

Currently, there is no molecular genetic research or molecular marker work on sesame. Sesame improvement could benefit markedly from the employment of the innovative molecular technologies that offer great potential to increase the efficiency of all breeding approaches. In other crops molecular markers have proved particularly successful when selecting for pest and disease resistance and complex traits, such as yield, quality and tolerance to abiotic stresses.

Since sesame is a neglected crop, grown almost exclusively in developing countries, where molecular approaches are not yet well advanced, the research efforts in this area will benefit primarily the end users in those countries. Co-operation of scientists from developing and developed countries in molecular studies on the nature of mutants such as determinate, closed capsules, disease resistance should be encouraged. The ultimate goal of this undertaking would be to characterize and map induced mutations of sesame using Amplified Fragment Length Polymorphisms (AFLPs) and other PCR based techniques. Mapping the mutant genes would be the first step towards map-based cloning of the genes and molecular marker assisted selection.

***In vitro* techniques**

Combinations of molecular and *in vitro* techniques have been used to incorporate specific traits into cultured protoplasts and tissues of many different plant species, and regenerate them then into whole plants. Induced mutations under *in vitro* conditions have specific advantages such as the option to screen very large cell populations within a limited space and to identify recessive mutations in haploid systems. *In vitro* techniques can also be used to:

i. Generate and identify new genetic variation in breeding lines, often via haploid production, using protoplast-, anther-, microspore-, ovule- or embryo-cultures.
ii. Produce somaclonal and gametoclonal variants with crop improvement potential.
iii. Rescue embryos following interspecific crosses.

Studies on sesame *in vitro* culture have been limited, but some initial success has been reported from China, India, Sri Lanka, Turkey, USA and Venezuela. Some protocols have been developed to produce somatic embryos from hypocotyl derived calli, plants have been regenerated following multiple shoot induction in seed cultures, and callus has been induced from anthers at the uni-nucleate stage of microspores. Attempts have been made to overcome disease problems through interspecific hybridization between the cultivated species and the disease resistant wild relatives *Sesamum alatum* and *S. radiatum* where by using embryo rescue a few progenies were obtained.

A major barrier to successful utilization of *in vitro* techniques for enhancing sesame breeding research is the transfer of sesame plantlets from tissue culture to the field. Only very few varieties of sesame have been examined for their regeneration ability; by testing more varieties some which are less recalcitrant might be identified. The participants feel that support for *in vitro* studies is vital to help overcome this problem.