Simplified Procedures for Development and Purification of A & R Lines in Cultivated Sunflower for Practical Utilization

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Selection of plants and development of crop varieties was practiced by man as an art in olden days. Hybridization and getting new recombinations was not taken up at that time because the role of sex was not understood. However, sex in animals is a long known phenomenon but sex in plants came to be recognized only after 700 BC. In 700 BC in Egypt and Assyria, artificial pollination of date palms was practiced regularly; but extending this approach of pollination for hybridization in a systematic manner was adopted only in 18th Century.

The understanding of the development of male and female gametophyte has been the subject of interest in the last century. The chronological sequence of the important events in understanding the gametes and in the conceptual development of the gametophytes in higher plants are presented below :

1676 - Millington, noted that anthers are functioning as male organ in plants

1677 - Leeuwenkoek, A.Van : invented microscope

1682 - Grew, N : Suggested that stamens are male organs and also explained the function of ovules and pollens

1694 - Camerarius, R.J. : With the aid of microscope he explained the sex in mulberry, castor and maize.

1716 - Mather, K. : Observed the effect of cross pollination in corn

1719 - Fairchid, D.: Produced first artificial hybrid

1727 - Vilmorin Company in France produced improved crop varieties

1761 - Koelreuter, J.G. : Made first artificial hybridization in plants between two varieties of tobacco and concluded that pollen parent also influence the characters

1761-1766 - Kolereuter, J.G, noted sterility of hybrids between *Nicotiana paniculata* and *N. rustica* but closely related types gave fruitful results by crossing.

1778 - Herbert, G. - Observed partial fertility in interspecific hybrids

1823 - Knight, noted that male and female contributes equally in first generation.

1854 - Naudin made reciprocal crosses and proved the identity of first generation.

1866 - John Gregor Mendel - Postulated law of heredity and segregation based on his experiments in garden pea

1877 - Strasburger, E. Suggested the term gametes

1884 - Strasburger, E. Fusion of male and female gametes - Synagamy

1898 - Navashion, M. Discovered double fertilization in higher plants - syngamy and triple fusion

1839 - Schleiden, M.J. - Demonstrated pollen tube entry in ovule

1900 - Correns, C.F.J., De Vries and Tsehermak E.Von - independently rediscovered Mendel's work Important events in Genetics

1901 - De Vries, H.: Discovered the phenomenon mutation

1904 - Rannig was credited for his idea of embryo culture

1906 - Bateson, W.: - Coined the term Genetics, Heredity, Variation and Genes

1911 - Johannsen, W.: - Coined the term genotype and phenotypes.

1917 - Morgan, T.H. et al - Established location of genes in chromosome of *Drosophila.*

1916 - Winkler, H.: - Proposed the term Genome

1916 - Shull, G.H.: - Suggested the term heterosis

1917 - Jones, D.F., - Proposed explanation for heterosis

1917 - Mc Fadden, E.S.: - Produced wheat x rye hybrid

1916 - Woodehouse, R.P. - Palynology - study of pollen grains

1933 - Rhoades, M.M.: - Cytoplasmic male sterility in corn

1934 - Dustin, P.: - Discovery of Colchicine

1940 - East, E.M.: Described self sterility system

1943 - Jones, D.F. and Clarke: - Described the inheritance of male sterility in onion and proposed a system to produce hybrid in onion.

1946 - Auerbach, E. and Lobson : showed that chemicals could induce mutation.

1950 - Gowen, J.W.: - Review of heterosis

1951 - Kihara, H.: - Reported first cytoplasmic male sterility in wheat

1966 - Smith, D.C.: - Plant Breeding and success - A symposium held at IOWA state University 1966 - Edited by Kenneth J.Frey.

Though hybridization work started systematically in 18th century; the hybrid era started in this century in different crops in different years. Release of varieties (hybrid derivatives) in all crops and hybrids in cross pollinated crops became popular. The hybrids were successful in crops where seed recovery *per se* was high. The artificial emasculation was posing a problem when cost benefit ratios were considered. But the identification of *CMS* systems in many crops and understanding / experience of hybrid vigour were the main reasons in the increase of area, production and productivity of major crops in millets, oilseeds, fibre crops etc;

The first report of *CMS* in few important crops have been given below.

Sl.No.	Year	Crop	Scientist
1	1931	Maize	Rhoades, M.M.
2	1932	Tobacco	East E.M.
3	1943	Onion	Jones D.F. & Clarke
4	1944	Tomato	Rick
5	1947	Carrot	Welch J.E & Cribal
6	1951	Wheat	Kihara.H
7	1954	Rye	Cehove Kaja
8	1954	Sorghum	Stephens and Holland
9	1955	Sugarbeet	Krappa
10	1956	Pearl Millet	Burton
11	1958	Chillies	Peterson, R.F.
12	1961	Cotton	Mayer and Mayer
13	1961	Cucumber	Barnes
14	1962	Rice	Kitamura
15	1963	Sunflower	Kinman
16	1966	Field Bean	Bonel *et al*

With this preview; the diversification of male sterility and restoration systems in *Helianthus annuus* has been discussed below;

Commercial cultivation of sunflower (*Helianthus annuus* L.) in India started in 1972 with the introduction of two Russian varieties - Paradovick (EC.68414) and Armavirskij 3497. Subsequently, the early maturing accession EC 101495 (Cernianka-66) was identified and released under the name "Morden" in the year 1979 for cultivation in Karnataka state and in 1980 at all India level. Sunflower cultivation suffered a set back during mid and late 70's due to poor seed set in the open pollinated varieties. The development and release of first ever sunflower hybrid in the country-BSH-1 gave a fillip and renewed the interest again in the crop. Thereafter, the area under sunflower has expanded rapidly. Presently sunflower is grown in India in an area of 2.7 million hectares with a production of 1.3 million tonnes of seed.

Genetics

Numerous genetic studies have been carried out in sunflower by workers all over the world. The crop is highly amenable to such investigations because of its *outbreeding nature, high reproductive ability, availability of cytoplasmic genetic male sterility system, photoperiod insensitivity, wide adaptation, and large diversity in the germplasm* (Seetharam, 1996).

Self-incompatibility

Sunflower is an obligate outbreeder because of protandrous nature of disc florets and presence of various degrees of self-incompatibility.

Limited information available on the inheritance of this character indicates that it is sporophytic in nature and its quantitative genetic inheritance is complex.

Petrove and Piskov (1985) found that self-fertility is genetically determined.

The filling percentage showed wide variation from 0 per cent seed set to 100 per cent fertility due to presence of self-incompatibility.

Cytoplasmic Male Sterility and Fertility Restoration

The discovery by Leclercq (1969) of cytoplasmic male sterility in sunflower and subsequent identification of genes for fertility restoration (Enns *et al*., 1970; Kinman,

1970; Vranceanu and Stoenescu, 1970; Leclercq, 1971) resulted in the widespread production of sunflower hybrids.

The earlier studies indicated a single dominant gene restorating fertility in cytoplasmic male-sterile *(CMS)* lines.Further studies revealed the presence of different types of gene action-two independent complementary dominant genes (Fick and Zimmer, 1974b; Reddy and Thammiraju, 1977) in the control of fertility restoration. Dominguez-Gimenez and Fick (1975) indicated that the inheritance of fertility restoration is more complex and up to four non-allelic genes may be involved in this process.

The presence of restorer genes is not common among cultivated sunflower but more common among wild sunflower (Dominguez-Gimenez and Fick, 1975).

Several new sources of cytosteriles have been reported in sunflower (Fick, 1978; Whdelan, 1981; Heiser, 1982; Serieys and Vincourt, 1987).

CMS F : *Helianthus petiolaris*

CMS PF : *Helianthus petiolaris* ssp *fallax*

CMS I : *Helianthus annuus* ssp. *lenticularis*

CMS/CMG 3 : *Helianthus maximiliani*

" GIG 1 : *Helianthus gigantus*

" PET 2 : *Helianthus petiolaris*

Heterosis Breeding

* There are numerous reports of marked heterosis for both seed yield and oil content.
* Since the mid-1970s, there has been a marked shift in breeding emphasis from population improvement to heterosis breeding and exploitation of hybrid vigour.
* Cultivation of hybrids imparts a few distinct advantages over that of open-pollinated varieties.

* Hybrids have more production stability, are well suited to high-input agriculture, have high self-fertility resulting in higher seed set even in areas where pollinators are not abundant and show uniform growth and maturity.

Interspecific Hybridization :

* Successful crosses are reported in the genus *Helianthus* with and between polyploid levels involving annual and perennial species.

* Hybridization did not depend on morphological affinities.

* The wild species of sunflower are highly variable for many agronomic characters.

* They are an excellent source of resistance to many major diseases and pests of sunflower.

* The sources of resistance have been successfully transferred to the cultivated types in the USSR.

* The boom of hybrid sunflower all over the world during the last two decades is largely due to *CMS* sources contributed by wild annual *H.petiolaris*.

* Several additional sources of male sterility have been identified in other wild species that could be used in *CMS* diversification programmes.

* Virupakshappa *et al.* (1991) have reported a number of maintainer and restorer lines for some of the new *CMS* sources.

The successful spread of any crop involving 3 lines (A/B/R)) depend on the strong breeding approaches viz., continuous programmes for synthesis of stable A lines with maintainers (B) and production of restorers (R).

Reasons for lack of purity in parental lines :

I. A&B Lines :

The cytoplasmic male sterile (CMS) line or female line loses purity due to the presence of following types of plants in varying proportions.

- Pollen shedders and
- Off-types

Pollen shedders : The two main reasons why pollen shedders occur in A line

1. Mixing of B line seeds in A line and
2. Out crossing of A line with any other line carrying restorer genes; more often due to contamination of B line.
3. Rarely by breakdown of male sterility.

How B line seeds mixed with A line

During breeder and foundation seed production of A line, it is a recommended practices to sow A and B lines in 3:1 row proportion. Since A and B lines look alike (ISOGENIC LINES) there by every possibility of B seed mixing with A seeds at various stages-harvesting, processing, packing, storage etc.,

Reasons for failure of the fast spread of hybrids

i. Failure to multiply and supply of quality seeds of parental lines in large quantity

ii. Negligence in the maintenance of genetic purity of parental lines

iii. Mechanical mixtures

iv. Mutations

v. Minor genetic variations and

vi. Natural crossings with undesirable genotypes and off types

In commercial hybrid seed lots problem of multi headed (R) plants leads to variations for height, flowering and maturity.

Development of Inbreds, Cytosteriles and Restorers

* Inbred lines must be developed to exploit heterosis.
* There are varying degrees of self - fertility and incompatibility that complicate the task of developing inbreds.
* Marked differences are seen within and between populations for self-fertility. It is therefore natural, to expect some failures during the development of inbreds.
* Most of the open pollinated varieties bred in the USSR show a high degree of self-sterility. So greater problems and failures are to be expected while attempting development of inbreds from such populations. On the other

hand, a variety like Morden which shows relatively higher degree of self-fertility, is more useful in inbred development.

* As in other cross-pollinated crop plants, the procedure of selfing phenotypically desirable plants in open-pollinated cultivars is common in sunflower.
* Selfing is to be continued for five to six generations till the desired level of uniformity is reached.
* Selections could be practiced both within and among progeny rows looking into desirable plant characters and agronomic attributes.
* After reaching the desired level of uniformity in the selfed lines sibbing could be practiced in subsequent generations.
* Both genetic and cytoplasmic sterility systems are available for use in heterotic breeding programmes.
* But cytoplasmic male sterility and restorer systems have been more useful in developing hybrids than genetic male steriles of self-incompatibility systems.
* The inbreds with good *GCA* could be converted into male sterile by repeated backcrossing with known *CMS* lines.
* The most popularly used *H.petiolaris CMS* source has been found to be stable under Indian conditions.
* The restorer genes appear to be scanty within the group of cultivated sunflowers.
* They are available in abundance in wild and weedy forms of *H.annuus* and in many related species.
* However a complex nature of fertility restorer behaviour in certain materials.

Under this context, the steps involved for the development of inbreds, their maintenance and conversion programmes have been given in nutshell.

I. DEVELOPMENT OF INBREDS

* Inbreeding and Heterosis started in 1950s Putt in Canada, Habura and Schuster in Germany, Gundeav and Jdonaf Volf in Soviet Union

* **Resource Materials**

1. Adopted varietal population - OPVs
2. Local varietal populations

3. Intervarietal hybrids, composites, synthetics
4. Single cross and multiple cross hybrids
5. I.S. hybrids
6. Gene pools

II. MAINTENANCE OF INBREDS

1. Difficult and demands personal attention.
2. Both selfing and sibbing to be practiced.
3. Incompatible lines to be maintained by sibbing.
4. Uniformity for highly heritable characters is important for distinctness, stability and uniformity.

III. EVALUATION OF INBREDS

1. Combining ability to be tested in S3 and S4 generations.
2. Polycross test and test cross method could be adopted for *GCA.*
3. Polycross suitable for self-incompatible lines.
4. Lines to be tested for *GCA* are sown in isolation in replicated block design and allow for open pollination polycross test.
5. The progenies of each line are sown in comparative trials and *GCA* values assessed for yield.
6. Line x tester analysis is suitable for assessing combining ability of large number of inbred lines.

CHARACTERISTICS OF GOOD TESTER

1. Tester should be genetically diverse from the lines tested.

2. To identify an inbred line (parents) with maximum *SCA* in a situation when one parent of a single cross / 3 way hybrid is to be replaced; the opposing line/lines already in use would be the tester.

3. Heterogenous testers exhibit minimum line x tester interaction and also hybrid x location/ hybrid x year interaction. Average effects can thus be assessed with less time and expenditure.

STEPS FOR LINE X TESTER ANALYSIS OF COMBINING ABILITY

METHOD I :

1. Lines to be tested are sown in isolation in alternate rows method for the lines and testers (Crossing Block).

2. Have separate crossing blocks for different testers.

3. Enforce pollination between lines and tester in a controlled way.

4. Induce male sterility if necessary in the lines using GA3 and 100-150 ppm.

5. Harvest sufficient quantities of seed for each combination.

6. Comparative yield trial with check hybrids for *GCA* assessment for oil content and yield.

7. Open pollinated cultivars, synthetics, composites, inbreds and hybrids could be used as testers.

METHOD 2 :

1. Use *CMS* lines with good combining ability.

2. Pollinate *CMS* lines with mixture of pollen from the lines .

3. Evaluate the resultant hybrids.

4. This method has the advantage of permitting simultaneous evaluation for fertility restoration also.

Selected lines having good *GCA* are tested for *SCA* by crossing in all combinations (Diallel crossing).

A general correlation between yields of inbred lines and their crosses exists. So visual selection of inbred lines is effective in producing lines with high combining ability.

IV CONVERSION OF INBREDS :

1. *CMS* LINE DEVELOPMENT

1. Line having good *GCA* and *SCA* is converted into *CMS* line.
2. Already available *CMS* is used as donor of cytoplasmic male sterility.
3. Atleast 6 generations of backcrossing is needed for conversion.
4. A few lines might be difficult to stabilise particularly those developed from commercial cultivars as these lines may possess genes for partial fertility restoration (Modifiers)

2. RESTORER DEVELOPMENT

Lines with good *SCA* can be converted into restorers also.

METHOD 1 :

1. Conventional method involves crossing a non-restorer line with a known restorer line and then selecting plants having restorer gene for back crossing.
2. This method is tedious as it involves alternate study of restoration and back crossing.

METHOD 2 :

1. Cross involved with known restorer.
2. Cross the resultant hybrid with *CMS* line.
3. Fertile plants are back crossed to the inbred.

4. Continue back crossing for another 4-5 generations.
5. The fertile plants thus obtained are selfed to produce an inbred line with Rf genes.
6. Fertility restoration genes in cultivated sunflowers is rare though not totally absent.

DEVELOPMENT OF INBREDS THROUGH HAPLOIDS

1. Chromosome duplication of haploids will resolute in 100 % homozygous lines.
2. Main advantage being the quickness with which the inbreds are obtained.
3. Haploids occur among twin seedlings at a frequency of 0.64 - 4.76 per cent.
4. Effective mechanisms of producing haploids at will artificially are not yet available.

BREAKDOWN OF MALE STERILITY

1. Occurrence of pollen shedders with normal fertility is not due to break down of sterility.
2. *CMS* breaks down due to genotype x environment interaction might result in production of fertile pollen in small percentage.

3. This could be avoided by understanding the effect of temperature and humidity on *CMS* stability.

Production of hybrids :

The lines that well, possessing good general and specific combining ability, are converted in *CMS* and restorer lines. They are subsequently crossed to produce single-cross hybrids, which are evaluated in multilocation trials before their release for large-scale cultivation.

Breeding Researches in India since 1972

***** Sunflower breeding started in India in 1972 under the All India Coordinated Research Project on Oilseeds.

* During the last 20 years many open pollinated varieties have been developed and

this has widened the varietal base.

* The high-yielding variety 'Surya' was evolved by mass selection using 'Latur bulk' as base material at Akola in Maharashtra. This variety was released in 1980 and

recommended for cultivation in Maharashtra.

* A list of varieties and hybrids released since 1972 for cultivation in different parts

of the country has been appended.

* The value of hybrids and the importance of heterosis breeding in this crop was recognized quite early.

* Experimental hybrids were developed during 1974 -75 at Bangalore and their performance was compared with Russian varieties then under cultivation (Seetharam *et al.,* 1977).

* The hybrids were distincly superior to the check variety in both seed and oil yield.

* BSH.1(*CMS* 234A/RHA 274) was the first sunflower hybrid to be released in the country.

* During the last five years, four more hybrids have been released for cultivation in different parts of the country.

Maintenance breeding for populations :

Pustovite method of seed reserve : (1964)

* Progeny evaluation

** Controlled pollination of best families

Salient steps involved :

1. Selection of large number of plants (10000) from the open pollinated populations.

2. Seeds of the selected plants are analysed for oil content, husk per cent etc; in the lab, finally 10 % of the populations (plants) selected.

3. Progeny evaluation : A portion of the seeds from each selected plant is sown in RRT (2 reps). with controls at every 10th row.

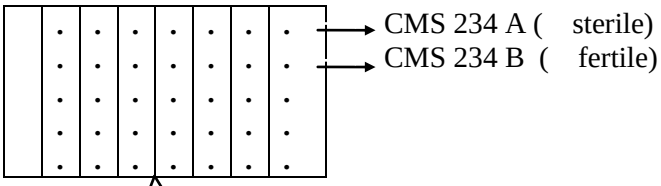
4. Select best progenies (families) performed well in 1st season. Repeat the trial for these best using a portion from the remnant seeds.

5. Remnant seeds of the elite plants giving the best families are sown in a spatially isolated multiplication field during the next season for pan mixis.

6. Each entry is harvested, tested, analysed and bulked family wise and raised for **super elite nucleus seeds.**

Maintenance breeding and upgradation of A line through respective B line

Cycle 1 : **(eg : CMS 234A via 234B)**



- Paired crossing block
- Do 250 paired crosses between A/B
- Simultaneously self all the 250 B plants

Cycle 2 :

NRRT

250 A/B
(F_1)

- Observe for flowering
- Observe for pollen shedders
- Fix the **best crosses**

RRT

S_1 of 250
CMS 234 B

- Use a little seed for S_1(.)
- Observe for phenotype
  - Uniformity
  - flowering
  - head traits
- Assess for yield and oilcontent
- Fix the best S_1 families (.)

(.) Based on the results fix the best B plants and bulk the remnant seeds (S1) from Cycle 1.

Cycle 3 :

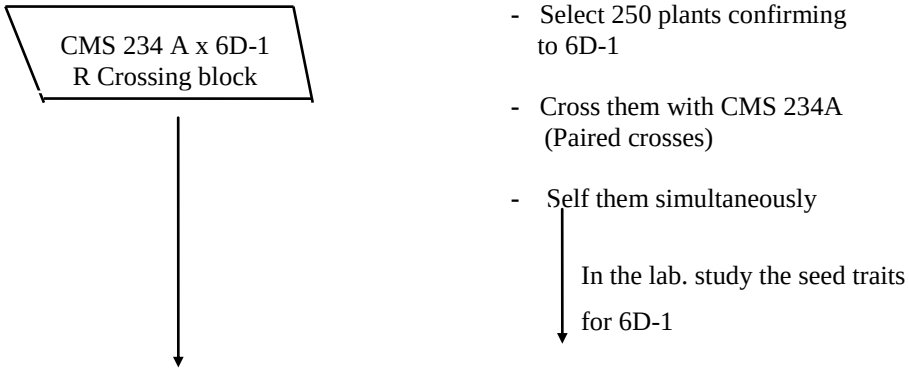
Raise the bulked remnant seeds of B and raise them in isolation. Allow for panmixis. Resultant seeds from **super elite nucleus seeds of B.**

Cycle 4 :

Breeder seed increase of CMS 234 B from super elite nucleus seeds.

Improvement of R line (ex : RHA 6D-1)

Cycle : 1 **6D-1 Bulk crop**



Cycle : 2 Field evaluation :

Evaluation for heterosis	Evaluation for purity in RRT
- NRRT - heterosis scored - **evaluate for sterility*** - select the best hybrids and fix the **best R plants(.)**	- Use a portion of the seeds only (*) - Evaluate S1 families for uniformities in .. genetic purity Seed. yield Oil content and fix **best R plants (.)**

(.) Based on the field and lab. evaluations select the best performing R plants.

Cycle : 3

Bulk the remnant seeds $(S_1)^{(*)}$ of the best R plants from cycle.1 and raise them in isolation. Allow for panmixis. Resultant seeds form the **"Super elite nucleus seeds of 6D-1 (R)".**

Cycle : 4

Use the super elite nucleus seeds of 6D-1 for the production of **Breeder seeds of 6D-1**.

PRODUCTION OF SEEDS FOR DIFFERENT CATOGERIES

Selection of superior plants :

From a population of open pollinated variety, large number of plants (in 1000s) are selected on the basis of different characters like height, girth of stem, diameter and inclination of head. Selected plants are harvested separately and evaluated for (i) seed filling, (ii) yield (iii) 1000 seed weight and (iv) oil content.

The variability present for each character in the selected plants are found out and for each trait norms are fixed. Individual plants which pass through the fixed norms, i.e. Mean ± 2 S.E. are selected. Roughly 20 % of the superior plants (Elite plants) are retained and advanced to the next stage.

2. Study of progenies of superior plants.

The seeds of the selected plants are sown in progeny rows (after retaining portion of seeds in them). Based on the performance of the families for the above characters, as compared to the checks, about 50 % of the families found superior to the checks are selected for growing in the seed nursery.

3. Production of super-elite seeds

The remnant seed of the superior families are mixed in equal quantity and sown in isolation as panmictic population for raising the seed nursery. Great care should be taken in roguing all the undesirable plants and susceptible to pests and diseases before flowering. The seeds obtained from this crop forms the super-elite stock.

4. Production of elite seeds

The super-elite seeds are raised in isolation to produce elite seeds. Method of sowing, rouguing, harvesting are just similar as in seed nursery. Upto this stage the seeds are handled by the breeders.

5. First reproduction seed

Elite seeds are sown in isolation to produce first reproduction seeds. Crop has to be rogued 2-3 times, once before flowering, during flowering and before harvest of the crop. This is produced in state seed farms.

6. Commercial cultivation seed

First state reproduction seeds are distributed for commercial cultivation.

Distinct merits of sunflower

The reasons for sunflower making rapid strides in the oilseeds scenario in the country as compared with other oilseeds crops are:

* Wider adaptability to wide ranging agro-climatic situations.
* High yield potential of the hybrids.
* Suitability for cultivation in all seasons due to its day neutral nature.
* Being a short duration crop, it can fit into various inter and sequence cropping systems.
* Remunerative market price due to high quality oil.
* Low seed rate and high seed multiplication ratio (1:80).

Advantages of sunflower hybrids

Hybrid sunflower offers following distinct advantages over open pollinated varieties.

* Hybrids have more production stability and are suited for input intensive agriculture.
* They are superior in their seed filling ability and are comparatively more self fertile.

* The crop stand is uniform and facilitates easy harvesting. The harvested produce is also uniform.

* They are more tolerant to disease and pests.

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MAINTENANCE AND UPGRADATION OF B & R LINES

Steps involved : Cyclic And continuous process of raising

- paired crossing block and crossing.
- simultaneous selfing
- lab analysis
- fixing best A/F_1 through NRRT
- fixing best selfed families through RRT
- raising the bulked remnant seeds of B / R in isolation

↓

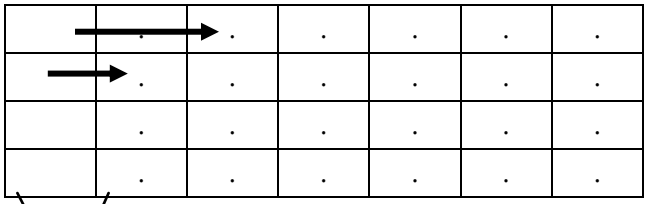
Super elite nucleus seeds of B / R.

A line : CMS 234 A

R line : R 6D-1

Maintenance breeding upgradation of A line through respective B line

Cycle 1 : (eg. CMS 234A via 234B)



CMS 234A (sterile)
CMS 234 B (fertile)

Cycle 2 :

- Paired crossing block
- Do 250 paired crosses between A/B
- Simultaneously self all the 250 B plants

NRRT	*RRT*
250 A/B	S_1 of 250 CMS 234B

- Observe for flowering
- Observe for pollen shedders
- Fix the best crosses

- Use a little seed for S1(.)
- Observe for phenotype
- Uniformity
- Flowering
- Head traits
- Assess for yield and oilcontent

Fix the best S1 families (.)

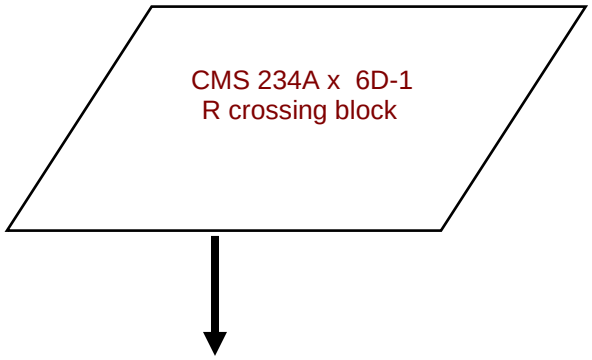
(.) Based on the results fix the best B plants and bulk the remnant seeds (S1) from Cycle

Cycle 3 : Raise the bulked remnant seeds of B and raise them in isolation. Allow for panmixis. Resultant seeds from **super elite nucleus seeds of B.**

Cycle 4 : Breeder seed increase of CMS 234 B from super elite nucleus seeds.

Improvement of R line (ex : RHA 6D-1)

Cycle : 1



6D-1 Bulk crop

- Select 250 plants confirming to 6D-1
- Cross them with CMS 234A (Pairedcrosses)
- Self them simultaneously

Cycle : 2 Field evaluation :

- In the lab, study the seed traits for 6D -1

Evaluation for heterosis	Evaluation for purity in RRT
♬ NRRT ♬ heterosis scored ♬ **evaluate for sterility *** ♬ Select the best hybrids and fix the **best R plants (.)**	♬ Use a portion of the seeds only (*) ♬ Evaluate S1 families for uniformities in ..genetic purity Seed yield Oil content And fix **best R plants (.)**

(.) Based on the field and lab. evaluations select the best performing R plants.

Cycle 3 : Bulk the remnant seeds $(S_1)^{(*)}$ of the best R plants from cycle.1 and raise them in isolation. Allow for panmixis. Resultant seeds from the "Super elite nucleus seeds of 6D-1 (R)".

Cycle 4 : Use the super elite nucleus seeds of 6D-1 for the production of Breeder seeds of 6D-1

R line maintenance (6D –1) :
Plant height (cm) in hybrid and R line

	1996		1997		1998		1999		2000		2001	
P. crosses (No.)	201		123		205		198		185		175	
	A/R	R	*A/R*	R	A/R	R	A/R	R	A/R	R	A/R	R
Minimum	124.0	78.0	120.0	80.0	123.0	82.0	135.0	83.0	132.5	84.0	130.5	82.7
Maximum	171.0	138.0	168.5	130.5	165.5	132.0	170.0	128.5	168.0	127.0	165.0	123.8
Mean	140.9	111.9	139.3	108.7	138.5	110.6	141.3	108.7	140.5	107.3	142.3	106.5

Note : ✹ **Range in R line is narrowed.**

✹ **Mean for F_1 & R line maintained.**

R line maintenance (6D –1) :
Head diameter (cm) in hybrid and R line

	1996		1997		1998		1999		2000		2001	
P. crosses (No.)	201		123		205		198		185		175	
	A/R	**R**	*A/R*	**R**	**A/R**	**R**	**A/R**	**R**	**A/R**	**R**	**A/R**	**R**
Minimum	**8.5**	**6.5**	**9.7**	**8.6**	**10.2**	**10.0**	**10.4**	**10.5**	**10.6**	**10.8**	**10.9**	**11.2**
Maximum	**17.3**	**17.5**	**18.6**	**18.5**	**18.8**	**17.9**	**19.3**	**19.8**	**19.2**	**19.5**	**19.8**	**20.0**
Mean	**14.1**	**11.7**	**14.5**	**13.0**	**14.9**	**14.1**	**14.3**	**14.5**	**14.5**	**14.8**	**14.7**	**15.1**

Note :

- **Minimum value; increased.**
- **Range is reduced.**
- **Medium size of the head is maintained.**

R line maintenance (6D –1) :
Seed yield / plant (g) in hybrid and R line

	1996		1997		1998		1999		2000		2001	
P. crosses (No.)	201		123		205		198		185		175	
	A/R	**R**	*A/R*	**R**	**A/R**	**R**	**A/R**	**R**	**A/R**	**R**	**A/R**	**R**
Minimum	21.5	13.1	22.4	14.2	25.8	15.7	26.8	18.5	26.3	18.9	27.5	20.4
Maximum	52.6	42.5	54.5	43.3	55.6	45.8	56.3	46.3	56.5	47.4	55.8	48.1
Mean	48.5	26.5	49.2	29.8	50.0	31.4	50.3	32.5	50.8	33.6	51.4	34.3

Note :

- **Range is narrowed in R line.**
- **Upward trend in mean for F_1 & R line.**

R line maintenance (6D –1) :
Oil content (%) in hybrid and R line

	1996		1997		1998		1999		2000		2001	
P. crosses (No.)	201		123		205		198		185		175	
	A/R	**R**	*A/R*	**R**	**A/R**	**R**	**A/R**	**R**	**A/R**	**R**	**A/R**	**R**
Minimum	30.4	33.2	30.8	33.5	31.2	33.8	32.0	34.1	32.2	34.3	33.0	34.6
Maximum	41.1	43.5	42.3	43.8	42.6	44.0	43.1	45.5	43.4	45.8	43.8	46.1
Mean	37.3	40.4	37.8	40.8	38.1	41.2	38.6	41.4	38.8	41.8	39.5	42.3

Note : ✹ **Minimum oil content; increased.**

✹ **Mean oil content; increased.**

CMS 234A maintenance :
Plant height (cm) over the years

	1996		1997		1998		1999		2000		2001	
P. crosses (No.)	195		194		275		263		141		130	
	A x B	**B**	**A x B**	**B**	**A x B**	**B**	**A x B**	**B**	**A x B**	**B**	**A x B**	**B**
Minimum	83.0	78.0	82.0	80.0	84.0	82.0	86.0	83.0	85.5	82.0	84.6	86.0
Maximum	153.0	125.0	152.0	124.5	145.0	130.0	139.0	134.0	128.0	125.0	125.0	131.0
Mean	108.4	105.0	110.2	108.6	107.6	105.3	103.8	109.5	110.7	105.8	108.6	110.4

Note : ✹ **Range in B line narrowed.**

✹ **Mean height of A and B maintained.**

CMS 234A maintenance :
Head diameter (cm) over the years

	1996		1997		1998		1999		2000		2001	
P. crosses (No.)	195		194		275		263		141		130	
	A x B	B	A x B	B	A x B	B	A x B	B	A x B	B	A x B	B
Minimum	6.5	6.0	7.4	8.2	8.1	9.3	9.2	9.7	9.4	10.1	9.6	10.5
Maximum	14.5	12.5	16.3	17.0	16.7	17.5	16.9	18.1	16.5	17.9	17.4	18.4
Mean	9.1	9.2	10.4	10.8	10.9	11.4	11.2	11.8	11.4	11.6	11.8	12.1

Note :

- **Minimum and maximum values increased.**
- **Shift in range.**
- **Mean head diameter; increased.**

CMS 234A maintenance :
Seed yield / plant (g) over the years

	1996		1997		1998		1999		2000		2001	
P. crosses (No.)	195		194		275		263		141		130	
	A x B	**B**	**A x B**	**B**	**A x B**	**B**	**A x B**	**B**	**A x B**	**B**	**A x B**	**B**
Minimum	**8.7**	**9.5**	**9.4**	**12.3**	**10.3**	**12.5**	**11.4**	**13.2**	**12.3**	**13.4**	**13.0**	**12.9**
Maximum	**40.2**	**36.7**	**46.8**	**38.4**	**47.2**	**39.6**	**48.6**	**45.7**	**48.5**	**46.2**	**49.6**	**50.1**
Mean	**26.7**	**24.9**	**27.9**	**28.2**	**28.5**	**30.1**	**29.3**	**31.4**	**29.7**	**31.8**	**30.1**	**32.4**

Note : ✹ **Shift in range for positive side.**

✹ **Mean values; increased.**

CMS 234A maintenance :
Oilcontent / plant (%) over the years

	1996		1997		1998		1999		2000		2001	
P. crosses (No.)	195		194		275		263		141		130	
	A x B	**B**	**A x B**	**B**	**A x B**	**B**	**A x B**	**B**	**A x B**	**B**	**A x B**	**B**
Minimum	**31.7**	**32.0**	**32.0**	**32.1**	**34.3**	**33.3**	**33.8**	**33.5**	**34.0**	**33.8**	**34.1**	**34.0**
Maximum	**42.1**	**41.6**	**42.4**	**42.0**	**42.5**	**42.1**	**42.3**	**42.0**	**42.6**	**42.3**	**42.7**	**42.4**
Mean	**38.1**	**37.9**	**38.3**	**38.2**	**38.6**	**38.4**	**38.5**	**38.3**	**38.7**	**38.5**	**38.8**	**38.9**

Note : ✹ **Variability maintained.**

✹ **Mean oil content maintained.**

Upgradation and character maintenance through paired crosses over the years in CMS 234 A, B and R (6D-1)

		A		B		F_1		R	
		1996	2001	1996	2001	1996	2001	1996	2001
Head diameter (cm)	Range	6.5-14.5	9.6-17.4	6.0-12.5	10.5-18.4	8.5-17.3	10.9-19.8	6.5-17.5	11.2-20.0
	Mean	9.1	11.8	9.2	12.1	14.1	14.7	11.7	15.1
Seed yield per plant (g)	Range	8.7-40.2	13.0-49.6	9.5-36.7	12.9-50.1	21.5-52.6	27.5-55.8	13.1-42.5	20.4-48.1
	Mean	26.7	30.1	24.9	32.4	48.5	51.4	26.5	34.3
Oil content (%)	Range	31.7-42.1	34.1-42.7	32.0-41.6	34.0-42.4	30.4-41.1	39.5-43.8	33.0-43.5	34.6-46.1
	Mean	38.1	38.8	37.9	38.9	37.3	33.0	39.5	42.3