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HYBRID SEED PRODUCTION IN COTTON

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PREFACE

India is the pioneer country for the commercial cultivation of hybrid cotton. The 1st commercial hybrid - Hybrid 4 (H- 4) was released in 1970 from Main Cotton Research Station of Gujarat Agricultural University, now Navsari Agricultural University for cultivation in the state of Gujarat. Lateron, several hybrids were released for cultivation in different agro- climatic zones of the country. By now 60 hybrids have been released by public sector. In addition - Bt cotton hybrids have been developed and released for cultivation by various seed companies.

Complete information on practical hybrid seed production methodology (including steps in emasculation & pollination) are not available from any single source. The prime objective of writing this bulletin is to provide comprehensive information about cotton hybrid seed production including challenges in Bt hybrid seed testing.

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Director

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Introduction

Cotton is a major fibre crop of global significance. It is grown in tropical and sub-tropical regions of more than 80 countries. The major cotton producing countries are China, India, USA, Pakistan, Uzbekistan, Turkey, Brazil, Greece, Argentina, Australia and Egypt contributing about 85% of global production. India has the largest area under cotton cultivation in the world (9.1 million ha.) and grows all four cultivated species namely, G. hirsutum, G. barbadense, G. herbaceum and G. arboreum on commercial scale. The major cotton growing states are Punjab, Haryana, Rajasthan, Madhya Pradesh, Maharashtra, Gujarat, A.P., Tamil Nadu and Karnataka. However, the productivity of cotton in India is low due to its rainfed cultivation in major areas (66%) and inadequate use of quality inputs.

India is the pioneer country for commercial cultivation of cotton hybrids which covers more than 50% of the cotton area. Cotton hybrids have 50% higher productivity than varieties. Moreover, hybrids have wider adaptability, high degree of resistance to biotic and abiotic stresses and better fibre quality. Hybrids can be developed with comparatively lesser time frame than straight varieties. The hybrids are highly productive and have uniform fibre quality.

Among the major cotton producing countries, India and China are the only two in which hybrid cottons are being cultivated on a large scale. India is the pioneer country in the world for development of cotton hybrid for commercial cultivation. The first intra-hirsutum hybrid cotton Hybrid - 4(H-4) was released in 1970 from Main Cotton Research Station, Surat of G.A.U. by Dr. C.T. Patel. This was followed by the development of world first inter-specific hybrid Varalaxmi in 1972 from U.A.S., Dharwad by Dr. B.H. Katarki. Thereafter, development of hybrids got momentum and numerous location specific superior hybrids were released in the country. The successful hybrids earlier developed were of inter or intraspecific new world cotton which are susceptible to pests and diseases compared to Asiatic cotton. Asiatic cotton are hardy and better tolerant to adverse field conditions. Hence the work on desi cotton hybrid development was intensified and the development of first Asiatic cotton hybrid G.Cot DH 7 (G. *herbaceum* X G. *arboreum*) was widely acknowledged. Interspecific hybrids between G. *hirsutum* and G. *barbadense* showed very significant hybrid vigour and such hybrids not only improved the lint yield but also the lint quality. The cultivation of conventional F1 hybrids significantly increased the lint yield over the best cultivars available in upland and Asiatic cottons. The hand emasculation and pollination resulted in high cost of hybrid seed production. Discovery of male sterility systems in cotton by Justus and Leinweber in 1960 and also a stable cytoplasmic genetic male sterility system by Meyer in 1973 opened new vistas for cheaper hybrid seed production due to elimination of emasculation process. Suguna was the first hybrid developed through exploiting genetic male sterility (Gregg male sterile) in public sector and MECH 4 in private sector. At present hybrids cover more than 50% of total cotton area in India and contribute about 60% to the national cotton production.

TYPES OF HYBRIDS

In Cotton hybrid seed production is done either by conventional hand emasculation and pollination or by nonconventional (male sterility based).

Conventional Hybrids

Majority of the hybrids released so far are conventional ones. Development of such hybrids involve three steps viz. (i) Identification and growing of male and female parents, (ii) emasculation of female parent and (iii) pollination of female parent with identified male parent. Cotton is an often cross pollinated crop. The average outcrossing is 6%. The pollen is heavy and sticky and hence cross pollination occurs only by insects i.e. honey bees and bumble bees. In diploid cottons conventional method is highly uneconomical since boll setting is low due to small flower size and brittle pedicel.

Male-sterility based hybrids

The development of hybrids using male sterility eliminates the process of emasculation since the anthers are sterile in female parent without pollen. Thus the cost of hybrid seed production can be reduced. However, pollination has to be done manually. In cotton, mainly two types of male sterility such as genetic male sterility and cytoplasmic genetic male sterility are used for seed production.

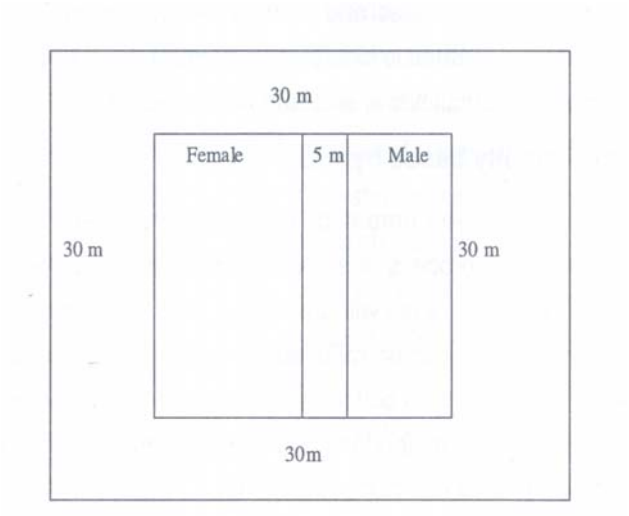
AGRONOMY IN HYBRID SEED PRODUCTION

Selection of site:

Soil- Medium to heavy deep well drained soil is considered ideal for seed production.

Isolation distance

Maintenance of genetic purity for certified seed production of conventional or male sterility based hybrids depends upon the use of safe isolation distance. The cotton hybrid seed production plot should have **30 m** isolation distance on all sides from other fields. The isolation distance between parents should be minimum 5 m as shown below. The other field standards tobe met for certification given in table 3.



Fertilizer dose of 100: 50: 50 or 150: 75: 75 kg/ha NPK depending on the soil fertility has to be given out of which 50: 50; 50 or 75: 75: 75 Kg/ha NPK as a basal dose and remaining 50 or 75 Kg N in 2 - splits at square initiation, flowering, boll development stage to be given.

Fertilizer

Recommended dose for rainfed cotton is 60:30:30 NPK per ha. Basal dose of Suphala (2 bags containing 100 kg suphala has NPK - 15: 15: 15) is given @ 200kg/ ha. The remaining 30 Kg of nitrogen is given in the form of urea @ 32.6Kg/ha each in 2 splits at flowering and boll formation stage.

Raising of Parental lines

If male sterile lines are used as parents for producing hybrid seed, following eligibility requirements are to be considered:

- An inbred line to be eligible for certification shall be from a source such that its identity may be assured and approved by the certification agency.
- Hybrid seed to be certified should be the progeny of two approved inbred lines one of which shall be male sterile.
- An inbred line shall be a relatively true breeding strain resulting from self pollination with selection.
- The foundation class seed shall consist of an approved male sterile line to be used as female parent and an approved inbred line to be used as a male parent for the purpose of producing hybrid seed.
- A male sterile line shall be a strain carrying cytoplasmic genetic male sterility which sheds no viable pollen and is maintained by the normal sister strain (2) which is used as a pollinator.
- The certified class seed shall be the hybrid seed to be planted for any use except seed production.

Sowing

Breeder/ foundation seeds of selected hybrids. Female and male parents are planted in the same field in separate lots with 5 m isolation between parents and 30 m from other cotton crop. The sowing dates of parental lines are so adjusted in such a way that there is synchronization of flowering in female and male parent and there will be continuous supply of pollen till the crossing season is over. Staggered planting of male is generally done depending on the date of flowering in male and female.

Hybrids	Female	Male
H x H (Both parents flower at the same time)	100%	50% along with female, 50% 7-10 days after sowing
H x B / Diploid Multinodal - (*G barbadense*) Female flowers early and Male flowers late	100%	50% along with female, 50% 7-10 days after sowing
H x B Uninodal (*G. bardadense*)	100%	10% along with female 30% one week after 30% two weeks after 30% three weeks after

The spacing and planting ratio to be followed for parental lines of different hybrids is given in table:

Table 2: Seed rate and spacing for female and male parents of conventional, GMS (desi) and CMS hybrids

Type of hybrid	Female Parent (0.67ha)	Male Parent (0.33 ha)
(H x H) Planting ratio	Seed Rate: 3.0-3.5 Kg/ha Spacing: 90cm x 90cm cm/120cm x 120cm (one plant/hill) 2	Seed Rate: 8-10Kg/ha Spacing: 90 x 30 cm/ 90 cm x 60 cm. 1
(H x B) Planting ratio	Seed Rate: 3.0-3.5 Kg/ha Spacing: 90x90 cm/120x120 cm (one plant/hill) 2 1	Seed Rate: 8-10 Kg/ha Spacing: 60 x 30 cm 1 (Multinodal sympodia) 1 (Uninodal sympodia)
Diploid Spacing Planting ratio	Seed Rate:10Kg/ha Spacing: 90x90 cm/90cm x 60cm (one plant/hill) 2	Seed Rate: 10 Kg/ha Spacing: 90 x 20 cm/ 90 x 10 cm 1

The plants in these seed production plots are to be thoroughly examined for purity, and off-types if any should be removed

Plant Protection Measures: Sucking pests and boll worms are the major pests of cotton. The attack of sucking pests (jassid/aphids and thrips) occurs during early growth stages of the crop and can be effectively controlled by spray of Confidor @ 100 ml/ha. During flowering, spray of Endosulphan @ 2L/ha can manage the attack of boll worms on squares and flowers. In case of severe infestation Avaunt @ 500 ml/ha need to be sprayed. Towards boll setting stage control of pink boll worm can be achieved by spray of Thiodecarb @ 1 Kg /ha. Grey mildew, alternaria and bacterial blight are the major diseases affecting cotton. Spray of Bavistin @ 10g in 10 litre water controls the grey mildew and spray of streptocycline 1g along with 20g copper oxychloride in 10

litres of water controls the bacterial blight.

Irrigation: Irrigation by alternate furrow method has been found beneficial whenever required.

Physiological Disorders

a Leaf reddening: The primary reason for leaf reddening has been found to be short supply of nutrient especially nitrogen to leaves. Foliar spray of DAP @ 2%, urea @ 2 % or MgSo4 @ 1 % has been found to overcome this problem.

b Potassium deficiency: Supply of K is important in attaining high number of retained bolls and thereby increasing the hybrid seed yield. Foliar spray of K in the form of Potassium chloride @ 1 % is to be given during flowering to boll formation at 15 days interval.

c Square/flower/boll shedding: Emasculation injury, sudden climatic change, ethylene production which results into formation of abscissic acid which ultimately cause dropping of squares/bolls/flowers. External application of auxin (NAA) @ 10 ppm during crossing period is beneficial for better fruit set, and increased kapas yield.

STEPS IN HYBRID SEED PRODUCTION (Conventional)

I) Emasculation of female parent

This is a highly skilled, laborious and cumbersome process. Different emasculation methods have been devised which can be adopted depending upon the flower types. Flower buds which are likely to open the next-day are chosen for emasculation for which the best time is after 1 P. M. This can be done by either of the following methods:

A. Doak method or thumbnail method:

This is the most successful method used in hybrid seed production of tetraploid cottons wherein 40 to 50% or more seed setting is obtained. The method involves removal of corolla along with anther sheath by giving shallow cut at the base of the bud with thumb nail and removing corolla and anther column in one jerk twisting action (Doak, 1934). Care should be taken to ensure that the white cover membrane of the ovary is not damaged or removed during this operation as this affects the boll setting. It should also be verified that no anther sac remains at the base of ovary at the time of emasculation. This will cause selfing and cause genetic impurity by increased number of seed of the female parent. Emasculated flower buds are generally covered with tissue paper bag (9cmx7cm) so as to prevent contamination from foreign pollen. If no open flowers are left over in the field of female parent, bagging may be avoided but marking of emasculated flower helps in identification during pollination Though commonly adopted, this method is not suitable for developing diploid or desi hybrids since the flower buds of these are small and the style is short and brittle rendering the method unsuitable in large scale seed production of hybrid seeds. Unless appropriate alternative method is developed, the seed cost for this type may become too prohibitive to become an economic feasibility.

Fig: 1 Doak method of emasculation

A. Bud selection **B. Removal of corolla and anther sheath**

C. Emasculated flower bud **D. Covering with red bag**

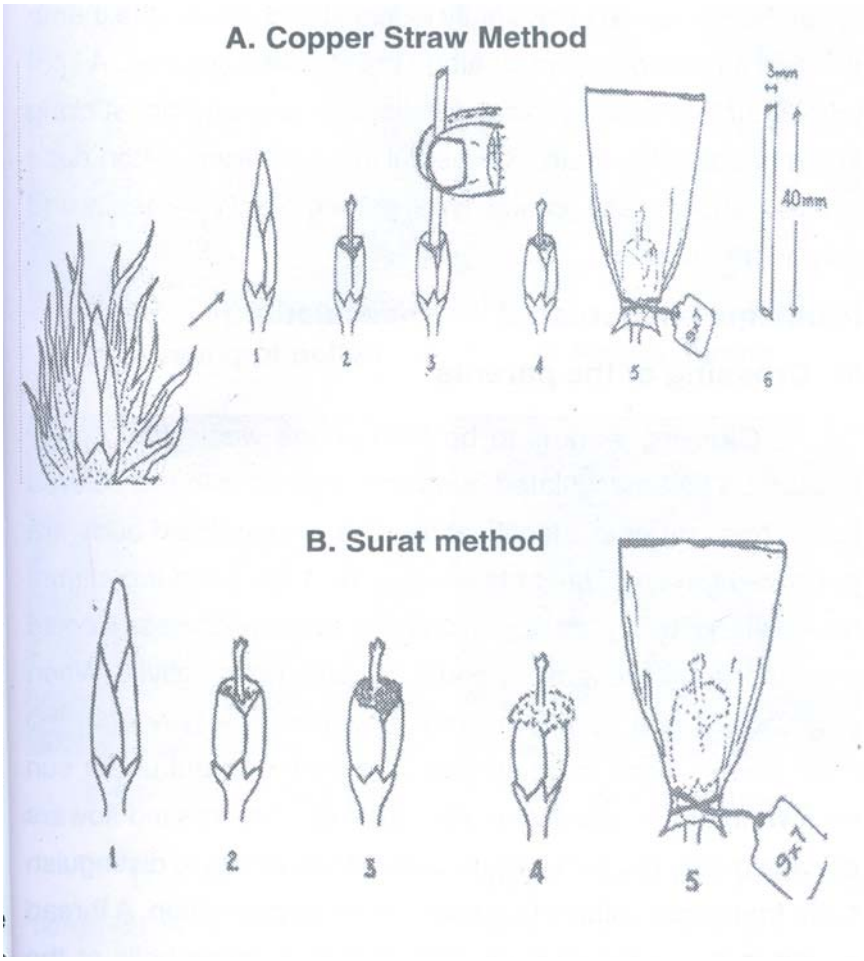
B. Pinching off of top of corolla /Surat method

The method is useful for emasculation of diploid flowers where the top portion of flower bud is pinched off using thumb and first finger nails by hand so that the tip of stigma get slightly exposed and the bud is covered with mud. As the buds mature the stigma tip extend sufficiently for enabling pollination. In genotypes where stigma tip exsertion is comparatively low, instead of cutting the top portion of corolla, the entire corolla is removed and lint dipped in mud is applied on unopened anther sacs. The pollen is applied next morning (Mehta et al, 1983). It is an easy method of emasculation for diploid flowers though chances are there leading to selfed seed production. Hence production of hybrids with high genetic purity (90%) cannot be guaranteed.

C. Straw tube/copper straw method

The top of the corolla of bud is pinched off and a piece of straw tube is used by inserting it on the style so as to separate all anthers from anther column and leaving the tube in the same 1 position till pollen is applied next morning (Hays et al, 1953). However during the process of emasculation relatively more time is required for inserting the straw tube exactly on the style so that anthers would get dislodged.

Fig: 2 Copper straw method and Surat method of emasculation



1. Bud 2.Top petal portion removed 3. Inserting straw/ Smearing mud paste 4. Bud with straw inserted/smeared with mud and stigma exposed 5. Bud covered in red tissue bag

D. Petal removal and brushing off anthers:

Only flower petal is removed by thumb nail method and the anthers brushed off by lightly touching and moving the thumb and first finger down and up along the staminal column.. A light tapping of the flower pedicel will dislodge any anthers sticking in the bracts. This method is useful in *herbaceum* cotton buds where anthers are of granule type, get removed very easily and drop to the ground

Problems encountered in emasculation:

II) Crossing of the parents

Crossing work is to be started one week after flower initiation. The emasculated buds are covered with red colored tissue bags for easy identification. The emasculated buds are pollinated the next day between 8 and 11 am because stigma receptivity is maximum during this period. Nevertheless it could be extended till 1.00pm depending on stigma receptivity. When crossing is done during the months Oct and – Nov end, the male flowers need to be spread on a cloth and put under sun for few hours for effective pollen dusting. The crossed flowers are again covered with a white colored tissue bag to distinguish them from emasculated bud awaiting cross pollination. A thread is tied to the pedicel for identification of crossed bolls at the time of picking. Fertilization occurs after 12-30 hrs of pollination and hence the crossed buds should remain covered for 3-4 days after pollination.

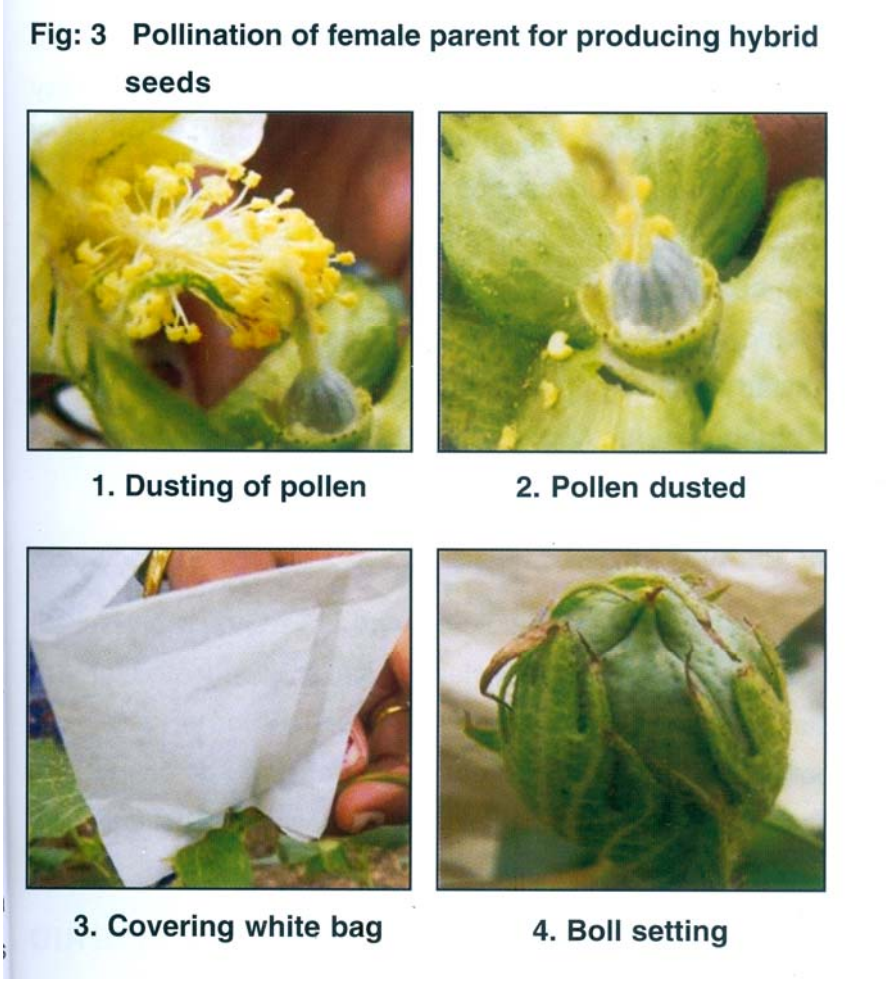


Fig: 3 Pollination of female parent for producing hybrid seeds

If proper isolation is given, and early morning removal of open flowers in female parents is assured bagging before and after is not required. All these operations such as emasculation and pollination are to be undertaken by properly trained persons under the supervision of qualified technical staff. One person can emasculate and pollinate about 250-400 flowers a day during

peak flowering phase. For good setting and boll development, it is necessary that crossing programme should be confined to about 10- 14 weeks after the commencement of flowering. Light and frequent irrigation should be given during crossing and boll development. Nowadays tieing of thread to pedicel after crossing is also avoided by removing bracts, it is better if only one bract is removed and two bracts are intact.

III) Picking

Only the completely opened bolls should be picked, collected in bags, properly cleaned and subsequently stored in gunny bags after proper tagging. Damaged or un-developed bolls/locks should be separated and removed before sorting. The good kapas fit for obtaining seed should be sent for ginning and subsequent seed certification.

SEED STANDARDS FOR CERTITIED HYBRID COTTON SEED

Hybrid seeds produced after meeting the above field requirements should meet the following seed standards as per Indian minimum seed certification standars

Table 3: Minimum Seed standards for hybrid seed certification

Particulars	Standards (Certified seeds)
	Hybrids
Pure seed (minimum)	98.0%
Inert matter (maximum)	2.0%
Other crops (maximum)	10/Kg
Weed seeds (maximum)	10/Kg
Germination (minimum)	70%
Moisture (maximum)	10.0%
For vapour proof containers (maximum)	6.0%
Genetic purity (for those produced by emasculation) Female Self plants Offtypes	90.0 8.5% 1.5%

HYBRID SEED PRODUCTION (MALE STERILITY BASED)

Use of this system enables us to overcome problems of emasculation since the flowers are already male sterile. However, pollination needs to be performed manually as described above.

Use of Cytoplasmic Genetic Male sterility

In this system, a combination of both nuclear genes and cytoplasmic genes determine the fertility or sterility. The sterility of G. *arboreum* and G. *anomalum* is not stable and is influenced by environmental factors. The CMS line of G. harkensii cytoplasm is highly stable and fertility restoration is also possible which is being widely used for hybrid seed production. The following plan is being used for maintaining cytoplasmic genetic male sterility and for production of fertile F1 hybrid seed for commercial cultivation

1) Maintenance of eMS' A'iine : 'A' CMS lines X 'B' isogenic line for pollen fertility

2) Maintenance of 'B'line 3) : Selfing since pollen is fertile

3) Maintenance of 'R' line : Selfing since pollen is fertile

4) Hybrid seed production : A X R

The A, Band R lines are maintained by as given above and are planted in separate blocks.

Demerits of CGMS system

Availability of limited number of restorers is a major drawback in this system of male sterility. Transfer of undesirable characters along with sterile cytoplasm is also a major concern in development of CGMS lines. Problem of fertility restoration and undesirable cytoplasmic effect could be overcome through use of genetic male sterility.

Fertile Sterile

Use of Genetic male sterility

GMS lines are generally simple in inheritance and controlled by one or two pairs of nuclear recessive or dominant genes. The lines controlled by recessive genes are used to produce hybrid seed. Maintenance of genetically double recessive male sterility is done by crossing male sterile plants by pollen obtained from corresponding heterozygous male fertile plants. The major drawback in this is that the cross population segregates into male sterile and male fertile plants in the ratio of 1:1 (male sterile: male fertile plants) The male fertile plants have to be rogued out at

flowering stage. This results in very low stand of the (50% stand reduction) male sterile plants reducing the hybrid seed yield. One way to overcome this is to sow two or three seeds per dibble and fertile plants thinned out at the flowering stage to ensure that enough male sterile plants are left. A total of 17 GMS lines have been identified. Out of these ms_{14} and ms_5ms_6 are being utilized for hybrid seed production in India and China.

Demerits of GMS system

Maintenance of male sterile population in GMS system of hybrid seed production is difficult as compared to cytoplasmic genetic male sterile system and also less quantity of seed is produced. Moreover, sterility source under reference is unstable. Low ginning in male sterile line which is not inherited in F1 hybrids is also advantageous in obtaining higher proportion of hybrid seed.

Table: 4- Hybrids released from 2000-2006

Hybrids	Species	Year of release	Yield (q/ ha)	Duration (days)	Area of cultivation	Released from
G. Cot MDH 11	AA (GMS)	2001	27	140	Gujarat	GAU, Surat
AKDH 7	AA (GMS)	2001	15	170	Vidharba	PDKV, Akola
CISAA 2 or CICR HY 2	AA (GMS)	2004	25	175	North zone	CICR RS, Sirsa
CSHH 198	HH	2004	27	165	North zone	CICR RS, Sirsa
Raj DH 7	AA (GMS)	2001	28	170	Rajasthan	RAU, Sriganganagar
AAH 1	AA (GMS)					Hissar

HH- Intra-hirsutum, AA- Intra- arboreum

LIMITATIONS OF HYBRID SEED PRODUCTION:

There are four main problems of hybrid seed production in cotton such as high cost of hybrid seed (conventional and male sterile hybrids), high cost of cultivation, difficulty in seed production (diploid hybrids) due to very poor seed setting (about 25%) and presence of neps and mots especially in interspecific hybrids. The availability of pure hybrid seeds in time is also a major limitation due to the requirement of Grow-out test for genetic purity which does not fit in the period between seed production and planting in the next season. The high cost of conventional hybrid seeds is due to requirement of emasculation and pollination which can be reduced to some extent through use of male sterility. But the yield of male sterility based hybrids is 10-15% lower than conventional hybrids since restoration of sterility is again a problem. Whichever method is selected for hybrid seed production, its commercial production depends on the development of an economical and reliable method to ensure adequate pollen transfer from fertile flower to sterile flower. Since cotton pollen is very heavy and sticky it is not easily

transferred by wind, and requires insects especially honeybees for pollen transfer.

In addition to above there is also the problem of parrot beak boll formation due to low dusting of pollen when same flower is used to pollinate more than 4 female flowers

HONEY BEES AS POLLINATORS IN HYBRID SEED PRODUCTION

In cotton, insects are the natural agents for pollen n transfer. The honeybees (Apis mellifera) are major pollinators. Id These pollinating agents when used in male sterile hybrid seed production enable us to significantly reduce the cost of hybrid. Studies on bee visiting and factors affecting bee visiting during pollination has been done.

The visits of honeybee to cotton flowers vary according to location, season and dayhours. It is known that 50 pollen grains per stigma are enough to fertilize all the ovules (McGregor, 1976), in insect pollinated cultivated crop plants. On an average 10 bees per 100 cotton flowers are reported to be sufficient to practically coat all stigma with pollen. The yield of seed cotton in A line also depends on the bee population and distribution of bee colonies. It was found that 13-15 bee colonies are adequate for economical seed production. However, studies conducted in the Central zone indicated that hand pollination was better than insect or bee pollination for boll setting and seed quality. The reasons attributed to this are shift in visit of honey bees from cotton to other more rewarding crops in the area, higher gossypol content in cotton which may affect the foraging behaviour and the heavy pesticidal schedule used for the control of pests.

Few basic studies show that sucrose content in the flower nectar especially in CMS flowers affected significantly the honey bee population density and might be a key factor for higher hybrid seed production in CMS lines (Mofett et al, 1976; Wang et al, yet to be published). Among various sugar components of the cotton nectar, only sucrose showed highly positive and significant correlation with visiting honey bee frequency. Thus breeding lines with higher sucrose content is needed for increasing bee visits which in turn can increase the boll setting per plant and decrease the number of aborted seed, resulting in higher yield of hybrid seed.

FACTORS AFFECTING YIELD AND QUALITY OF HYBRID SEED:

1. The planting ratio of male and female parents.
2. No: of male flowers used in pollinating one female flower. Using one male flower to pollinate more than three female flowers is found to decrease the hybrid seed yield and quality.
3. Staggering sowing pattern of parents. If staggeraing is not followed as required seed yield will decrease due to failure in synchronization.
4. Stage and period of crossing : Seeds produced after crossing during the second fortnight (15 days) of flower initiation has the highest germination compared to later produced hybrid seeds.
5. Vigour of female parental seed.
6. Pollen production ability of male parent.
7. Position of boll set on the plant. eg- early crossed bolls generally developed at lower portion remains for longer period, absorb more nutrients and develop seeds with high seed index.

Problems during emasculation: Bract damage during emasculation may lead to production of seeds with low seed index. Damage to style and stigma also cause low seed set.

MANAGEMENT TECHNIQUES AND HYBRID SEED PRODUCTION

Extensive studies were carried out at C.I.C.R., Nagpur on various management techniques such as controlled fruiting, restricted pollination and plant trimming to increase the hybrid seed production in various GMS hybrids.

Controlled Fruiting and restricted pollination:

It was observed that the reduction in pollination period from 60 to 30 days followed by the removal of fruiting forms other than pollinated flowers and developing cross bolls, at specific intervals significantly increased the boll setting percentage maintaining the yield level similar to the 60 day pollination period. Reducing the pollination period during rainy season would make available the hybrid seed by December providing sufficient time to conduct grow-out test which in turn will enable the timely availability of hybrid seed in the market.

Plant trimming and pruning:

Pruning of sympodia and removal of basal monopodia along with aged leaves allows us to manage a second off-season crop (summer) and a third (second rainy season) crop. The trimmed crop responds well to irrigation and nutrient inputs and is different from a ratoon crop in that the incidence of pests and diseases is less. Also well adaptive crop morphoframes enable a further reduction of pollination period from 30 to 17 and 13 days respectively in the subsequent two crops. This management technique could be adopted wherever the irrigation facility is available to increase the per unit time productivity of hybrid seed cotton.

Mixed planting of parental lines:

Mixed planting fashion of parental lines has also been found to increase hybrid seed production instead of the normal spaced row planting method. This helps in reducing the distance between CMS and restorer lines in the seed production field, thus increasing the pollen transfer from fertile to sterile flowers.

GENETIC PURITY TESTING IN COTTON

Seed parameters including germination (Blotter method or Tetrazolium), physical purity (by visual examination), moisture content, seed health and vigour (stress tolerance) are tested in the Laboratories whereas genetic purity is assessed commonly by a grow-out test conducted in the field.

Grow - out test

This is the standard test performed to determine the genuineness (purity) of seed of a variety or hybrid. Submitted sample size for GOT in hybrid cotton is 1000gm. The test crop should be raised along with authentic control according to the field size specified in minimum seed certification standards Le. a total of 400 plants in 4 replications should be observed and compared with a minimum of 200 plants from control sample. Observations are to be made during the full growing period. Identifying characters of each hybrid help to distinguish between a true hybrid and a female parent or an F2. The plants which are obviously of other cultivar are counted and recorded.

With the development of more and more hybrids including transgenic hybrids, it is difficult to distinguish them on the basis of plant morphological characters alone. There are other complementary tests which can distinguish genotypes efficiently, quickly, reliably and can be conducted in the laboratory itself such as seed image analysis, protein and isozyme markers etc. Molecular markers such as SSR, ISSR and AFLP are still more reliable being more in number and stable.

ECONOMICS OF HYBRID SEED PRODUCTION

The economics of hybrid seed production is worked out by adding expenditure on various items such as land lease, cost of preparatory tillage, cost and sowing of parental seed, registration and inspection charges, cost of fertilizer, hoeing and weeding, irrigation charges, plant protection, emasculation and pollination, picking of crossed bolls and transportation to gin and ginning charges. The income is estimated from sale of hybrid seed and lint and thus net profit per ha is worked out.

Table 5: Economics of hybrid seed production in cotton (per hectare).

	Particulars	Expenditure rupees
A.	Expenditure	15,000
1.	Land Lease	2000
2.	Preparatory tillage and sowing	3000
3.	Seed of parental lines registration and inspection charges.	10,000
4.	Fertilizers	3000
5.	Hoeing & weeding	3000
6.	Irrigation	10,000
7.	Plant Pro1ection	40,000@ Rs. 80/ day for 10 females
8.	Emasculation & Pollination	3000
9.	Picking of crossed bolls	1000
10.	Transportation to gin & ginning	90,000
	Total Expenditure	
B.	Income	
11.	Income from hybrid seed (6 q/ ha x Rs. 30, 000/ q)	1,80,000
12.	Income from lint (3 q/ ha x Rs 4000/ q)	12,000
13.	Total Income	1,92,000
14.	Net Profit	1,02,000
15.	Production cost of hybrid seed per Kg	150

OTHER APPROACHES

USE OF GAMETOCIDE INDUCED MALE STERILITY

There are various chemicals available which can artificially induce male sterility. These chemicals are called gametocides. This method of inducing male sterility is fast and inexpensive compared to backcross method and there is no necessity to use maintainer lines. The effective chemicals used in various crop plants are Sodium Methyl Arsenate, Zinc Methyl Arsenate, Naphthalene Acetic Acid (NAA), Gibberellins, Ethrel, Maleic Hydroxide and FW 450. In cotton, latter two have been effective in inducing male sterility, maleic hydroxide for upland cotton and FW 450 for *arboreum* cotton. Highest pollen sterility has been found to be caused by 1.5% FW-450 applied twice i. e before bud initiation and during bud initiation. (P. Singh and Sanjeev Singh – 1999). However, in cotton large scale trials with these gametocides have not been encouraging because these chemicals cause both male and female sterility. However the pollen abortion is incomplete and erratic and there are adverse effects on ovule fertility resulting in low seed setting.

Properties of an ideal gametocide

- It should be selective in inducing male sterility with affecting ovule fertility.
- It should give consistent/reproducible results
- It should be economical with simple application method
- It should be safe with minimum side effects on plant growth

THERMOSENSITIVE GENETIC MALE STERILITY

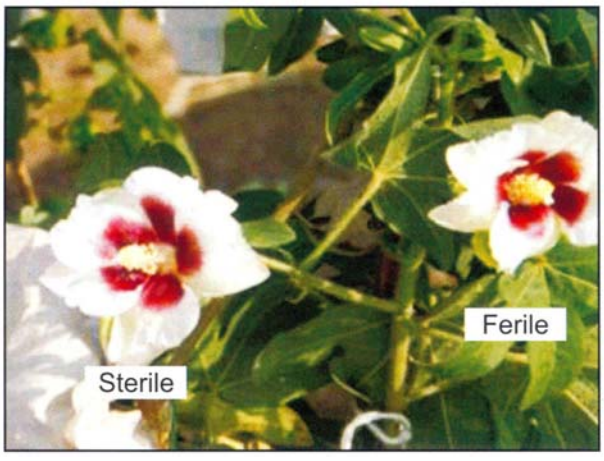
The discovery of environment sensitive genetic male sterility system which involves photoperiod sensitive genic male sterility and thermo-sensitive genic male sterility in rice laid the foundation for replacing the three line system. In cotton GMS lines of G. *arboreum* were observed showing sensitivity to different temperature regimes especially under lower minimum temperatures (<18^{0}C) where there is a sterility breakdown. Histological studies showed that male sterility in cotton is post meiotic and that microspores are not released after the tetrad stage due to non degradation of callose wall surrounding the tetrad. Later the microspores inside become shriveled and aborted. Callase enzyme mediates this degradation process of callose wall releasing microspore from tetrad which is active under reduced temperature and inactive at higher temperature expressing the fertility and sterility respectively.

Merits of the system

(1) It helps to overcome the problems of crossing for maintenance of parental lines
(2) It helps to overcome the problem of linkage drag due to transfer of sterile cytoplasm
(3) Prevents the problem of low restoration since any fertile line could be used as a pollen parent.

Demerits of the system

(1) Any sudden change in the environmental factors will influence the sterility of EGMS leading to more contamination.
(2) The multiplication of EGMS lines and hybrid seed production will be restricted by space and season.



Expression of altered sterility in a TGMS line of arboreum

APOMIXIS

Apomixis is a phenomena observed in crop plants enabling production of seed without meiosis and fertilization. It has considerable significance to breeders as a mean of genetic fixation and multiplication of heterotic genetic combination. Apomixes help to fix the hybrid vigour once a desirable combination has been selected and made. The hybrid seed could be multiplied and maintained just like a straight variety. Bt cotton seeds being too expensive development of apomictic Bt hybrids will prove economical since the seeds need not be purchased every season. This is also called as one line method of hybrid development. In cotton, development of apomictic lines is in progress and possibilities of evolving apomictic Bt hybrids is being explored.

IPR FOR HYBRIDS AND THEIR PARENTS

The Intellectual Property Rights for crop varieties/ hybrids/parental lines is granted through Plant Variety Protection. India has enacted a unique legislation on Protection of Plant varieties and Farmers' Rights (PPV & FR) in 2001 to provide a legal framework for Plant Breeders' and Farmers' Rights. The Act entitles royalty to the breeder whenever his variety is produced, sold or marketed by others. Researchers have the right to use the variety for further research without prior approval of PBR holder provided that such use does not involve repeated useof protected variety as a parental line or multiplication for commercial purposes. The act also entitles the farmers to save, use, sow, re-sow, exchange, share or sell produce including seed of a protected variety. In hybrids the parental lines used for developing the hybrid will be granted protection. The basis for grant of protection will be its Distinctness, Uniformity and Stability (DUS).

Distinctness

The variety must be clearly distinguishable by one or more essential characteristics from any other variety whose existence is a matter of common knowledge at the time when protection is applied for.

Uniformity

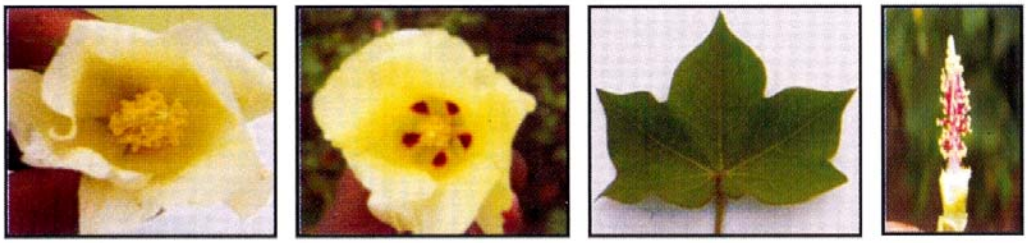
The variety should be uniform in its relevant characteristics subject to the variation that may be expected from the particular features of its propagation.

Stability

The variety should be stable so that its relevant characteristics remain unchanged after repeated propagation. Stability is tested by growing for two more generation at one location or growing for one generation at more than two locations.

For new genotypes to be tested for DUS, its essential characteristics are to be compared with those of other existing varieties. Hence existing varieties need to be characterized and documented based on the National DUS Test Guidelines developed for each crop. Accordingly

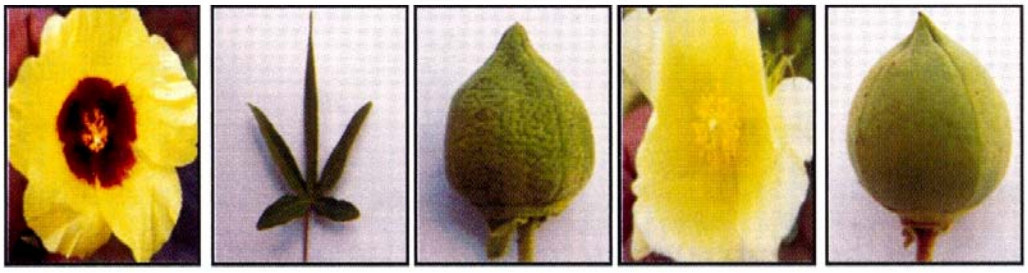
the varieties are observed for - characters including seedling, leaf, flower, lint and seed characters. Work on characterization and documentation of existing varieties/parental lines along with hybrids has been already initiated under the leadership of Directorate of Seed Research. So far, 22 hybrids along with their parents have been characterized. Utility of DNA markers particularly SSR markers for establishing distinctness is also being studied.



H 4
Cream Flower

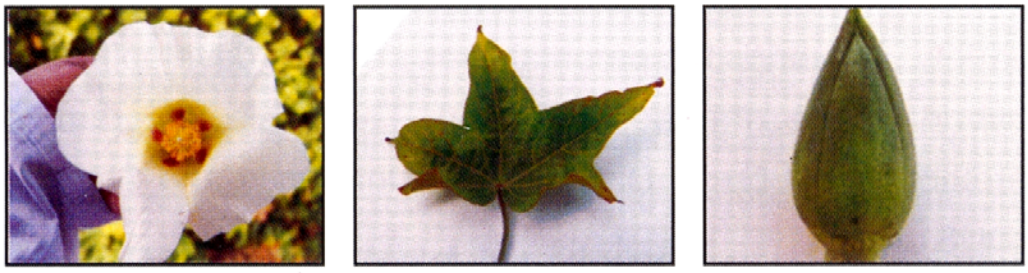
PKV Hy 3
Petal spot; light yellow flower

PPKV Hy 5
(Palmate leaf)
Pigmented anther filament



PKV DHI
Yellow flower, petal spot, Digitate leaf, Oval shaped boll.

NHH 44
Light yellow flower, Round Shaped boll



Sruthi
Cream, Petal spot, Semi- digitate leaf, Elliptical Boll

USE OF BIOTECHNOLOGY

Development of transgenic plants through genetic engineering is one of the most promising approaches currently available to improve the quality, diversity and yield of agricultural crops. Plant cells or organs are manipulated at the molecular level for introduction,

integration and expression of specific characteristics of foreign genetic material in a host plant thus enabling us to produce seeds of new crop varieties with desirable traits.

BT COTTON/ TRANSGENIC COTTON

In Bt cotton toxin producing gene has been utilized from the soil bacterium Bacillus thuringiensis. The Bt gene is very effective against the American Boll worm (Helicoverpa armigera) the major pest of cotton. In India, Bt cotton was first approved for commercial cultivation by the Central Government in March 2002. Initially three Bt hybrids viz; MECH 12, MECH162, MECH 184 were released for commercial cultivation. By now 133 Bt cotton hybrids (Private) have been released for cultivation in three cotton growing zones of India. Normal hybrids are slowly being replaced with Bt hybrids owing to their superior performance. At CICR work on genetic transformation has yielded development of Bt transgenics in NHH44 (hybrid) as well as hirsutum and desi varieties which will prove economical to farmers. Bt gene is being incorporated in the parental lines (either male or female or both) used for development of hybrids. Difference in expression levels of toxin in the plants as well as seeds of Bt hybrid when male parent alone or female parent alone or both used as Bt parent need to be studied from seed testing perspective. With growing demand for Bt hybrids Government has formulated certain laws for seed quality control of such hybrids.

By Hybrid Grow out Telt

Once Seed Bill, 2004 starts operational all Bt cotton hybrids will have to undergo genetic purity testing by a field grow out test. Genetic purity testing of Bt cotton hybrid using Grow out test is confronted by various issues such as :

- Determining the threshold level for Bt expression in the hybrid. Significant variation for CrylAc expression have been reported in different By cotton hybrids despite having a common gene insertion event. Intra-olant and in-seasonal variability in cry 1 Ac expression levels has also been reported in Bt cotton. The crylAc expression is found to decline progressively over the crop growth with toxin levels falling below the critical level of 1.9mg/g after 110 days.
- Identifying the ideal plant part which need to be examined for cry protein expression. This is because expression of crylAc is found to be highly variable in different plant parts. Highest level was found in leaves of seedlings followed by squares, boll rind and flowers.

Under Seeds Rules, 1968, Central Government have recognized in 2003 Central Institute for Cotton Research laboratory of ICAR as Referral Laboratory for detection of Bt gene. The detection kits developed at CICR has been recognized by the Government as a standard test for Bt detection in GM seeds. As per the rule all seed testing laboratories are to perform Bt purity testing along with genetic purity testing and the following standards has been specified for the same:

Submitted sample size: 25g

Working sample size: 10 seeds

For Bt transgenics Bt toxin level (minimum)	450 nanog/sq.cm or /g seed
Purity for Bt protein expression	90% (9 out of 10 seeds tested should be Bt+ve)

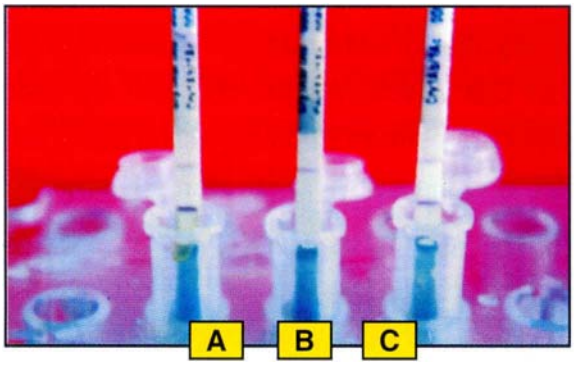
Field and seed standards for Bt hybrid cotton crop is yet to be provided in the certification schemes. Till then the same standards provided in the text earlier may be followed.

Bt detection methods:

Three Bt detection methods, viz; (i) Bt express (ii) Bt detection (iii) Bt quarantine are in common use. A brief description of these methods is presented below:

Bt Express:

This is an instant check method where a strip provided with the kit is dipped into a vial containing seed crushed with supplied buffer. If two bands develop at the top it indicates presence of Bt gene Cry1 Ac and Bt positive. If only one band develop at the top end of the strip it indicates that the sample is Bt negative. It is a very easy method that can be used even by farmers besides laboratories and industries.



Bt Express Strip Test for Cry 1 Ac protein

A & C - Cry 1 Ac positive plant; B - Cry 1 Ac negative plant

Bt Detect: This is to know the presence of Bt gene at molecular level which could be done only by the trained persons in laboratories.

Bt Quant: This is an ELISA kit facilitating a precise quantification of Bt gene and again done by trained personals in Seed Testing Laboratories. Depending upon the capabilities of a laboratory several hundred of samples can be processed per day.

FUTURE PROSPECTS

In India, remarkable progress has been made in the development of commercial cultivated hybrids and also in the Seed Production Technology of such hybrids. Future work on hybrid cotton seed technology need to be directed towards following thrust areas.

⇒ In diploid hybrids the seed setting is very poor (about 20%). Hence efforts have to be made to improve seed setting in diploid hybrids.

⇒ In future, more emphasis has to be laid on the development of CGMS Based hybrids which will help in providing hybrid seeds to farmers at cheaper rate.

⇒ Research on use of two line and one line and method of hybrid development and seed production needs to be intensified.

⇒ Most of the presently available Bt hybrids are conventional. There is need to develop CGMS based transgenic hybrids.

⇒ To have a successful seed testing programme on Bt cotton it is essential to understand the level of toxin expression in seed coat for differentiating F2 seeds from F1 hybrid seeds.

⇒ Difference in transgene expression in direct and reciprocal Bt crosses i.e. Bt x NBt and NBt x Bt and Bt x Bt to solve the problem of varied expression in same hybrid while testing

⇒ Level of toxin expression in seeds when more than one transgene is present

⇒ Expression of toxin levels on different qualities of same seed lot.

⇒ Study on the effect of Bt gene on various seed vigor and storability

⇒ Efforts are also needed to further improve the planting value of hybrid seeds especially with respect to enhancing shelf life during seed storage.

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