

Participatory On-Farm Breeding of Organic Cotton



Foreword

The 'Manual for Participatory On-Farm Breeding of Cotton' aims to assist trainers in conducting instructional and practical training courses on participatory plant breeding (PPB) in their respective communities and focus areas. It will also serve as a facilitator handbook to help organise their cotton breeding programmes in a participatory manner.

The guide is directed to those involved in planning and implementing participatory breeding activities in organic cotton. This includes farmer associations, field workers, extension officials, non-governmental organisations (NGOs), research centers, and universities.

This manual on PPB is based primarily on the direct experience of FiBL staff derived from several years of implementing PPB of organic cotton and related farmers' workshops. It also features insights from FiBL's master students exploring participatory methodologies and the fast-growing source of relevant scientific literature and guidelines on participatory breeding and participatory research in general (e.g. Gonsalves et al. 2005a, b, c, Ceccarelli et al. 2009, Ceccarelli 2019).

The manual aims to provide the relevant information and instructions for field activities and workshops in one document. It is imperative for readers to know that this guide does not completely account for all methods available for PPB and that socioeconomic aspects like trust-building processes, finding common language and mutual understanding, the role of the facilitator etc. are not included in this manual, as this is not specific for PPB but of general importance for all participatory multi-actor research and innovation processes.

The document is intended to share our specific experiences in PPB in collaboration with smallholders, researchers, breeders and value chain partners gained during our participatory cotton breeding projects Green Cotton (www.greencotton.org) and Seeding the Green Future (www.SGF-cotton.org) with our partners and a broader audience.

The manual can serve as a blueprint for replication and upscaling of PPB in different regions of India, in different countries and also extrapolate to other crops.

The objectives of this manual are:

- Compile training modules to train the trainers in PPB and empower farmers;
- Introduce the facilitators to the concepts and methodologies specific to PPB;
- Take the user through the important steps and techniques for implementing participatory breeding and training programs in cotton;
- Provide a practical illustration of widely adopted participatory breeding methodologies;
- Provide examples and formats for conducting different training activities;
- Discuss key issues in participatory plant breeding of organic cotton.

The guide begins with a brief introduction of Participatory Plant Breeding (PPB) in section 1, followed by seven sections on participatory cultivar evaluation including management of replicated mother trials and on-farm baby and pilot trials; making of crosses to create genetic diversity; plant selection in segregating populations (F2 – F8); maintenance breeding and multiplication; organic seed production and seed quality; lastly, commercialisation of cultivars to shed some light on the cultivar release process for both, the public and the private sector in India with emphasis on farmers and breeders.

Different workshops and trainings outlined are not just limited to the cotton crop, they can be followed by any institution, small group or an individual working on participatory plant breeding. This manual also has the potential to be translated into different local languages thereby catering to different audiences, maximising its outreach and widespread adoption.



Contents

1. Participatory Plant Breeding	4	6. Organic Seed Production and Seed Quality	56
1.1 Why Participatory Plant Breeding?	4	6.1 Goals	56
1.2 Cotton production in India	5	6.2 Role of farmers in organic seed production	56
1.3 Concerns about loss of cotton genetic diversity	6	6.3 Organic seed production of inbred lines	56
1.4 Call for action	9	6.4 Special case of organic hybrid seed production	61
1.5 Outline of different stages of Participatory Plant Breeding	10	6.5 Workshop on seed production and seed quality	62
2.1 Goals	13	6.6 Training on field monitoring and inspection	66
2.2 Activities in the field	13	7. Commercialisation of Cultivars	72
2. Participatory Cultivar Evaluation	13	7.1 Seed distribution systems in India	72
2.3 Workshop on cultivar evaluation	19	7.2 Formal seed sector	73
2.4 Training in management of on-farm trials	25	7.3 Informal seed sector (farmer bred and released varieties)	76
3. Crosses to Create Genetic Diversity	33	7.4 Cottonseed multiplication	76
3.1 Goal	33	7.5 Potential non-GM organic cottonseed supply chain models	78
3.2 Activities in the field	33	7.6 Summary	80
3.3 Workshop on crosses	34	References	81
4. Plant Selection in Segregating Populations (F2–F8)	37	Imprint	83
4.1 Goals	37		
4.2 Introduction	37		
4.3 Activities in the field	38		
4.4 Workshop on single plant selection	41		
5. Maintenance Breeding and Seed Multiplication	46		
5.1 Goals	46		
5.2 Different seed categories	46		
5.3 Maintenance breeding production of nucleus and breeder seed	48		
5.4 Training on rogueing	53		

1. Participatory Plant Breeding

1.1 Why Participatory Plant Breeding?

The development and release of a new variety is a time intensive process. This may take a decade, or even more from the first crosses to bringing a promising cultivar on the market, available to farmers for cultivation. As a result, by the time a new cultivar is released to address one concern or challenge faced by farmers (yield, productivity, disease, pest resistance etc.), a new problem has already arisen.

Every year, following the process of conventional breeding, several varieties are officially released, but only a few are considerably adopted by farmers. Adaptation to local farming conditions and context is a key factor behind the adoption of any cultivar. Both are often not considered or simply ignored when addressing the issues of high productivity.

Contrary to conventional breeding, Participatory Plant Breeding (PPB) relies on consistent and harmonised efforts that endeavour to actively engage the farmers (including female farmers) in selecting genotypes from a wide range of continuously evolving genotypes derived from crosses

between breeding material and/or genebank accessions (Figure 1). PPB has evolved mainly to address the difficulties of poor farmers in developing countries where the availability of seeds and/or adoption of promising cultivars is low or negligible (Walker, 2007).

PPB, as described in this manual, is characterised by decentralised on-farm selection and sharing of decision power among farmers, breeders, value chain actors and research partners (Figure 2). PPB approach is widely seen as having advantages for use in low yield potential, high-stress environments. PPB is most often applied when specific trait and local adaptation is sought, and adoption of modern varieties is low (Ceccarelli & Grando, 2019).

PPB programs are very diverse, the tasks and responsibilities of different stakeholders involved are not pre-defined, and limited to one. Scope of collaboration amongst various participants and their roles may also vary according to the methodology adopted in a particular PPB program (Weltzien et al. 2003, Walker et al. 2007). For example, some partners may be responsible for arranging promising plant material crosses, while other partners may be responsible for implementation of multi-location trials.

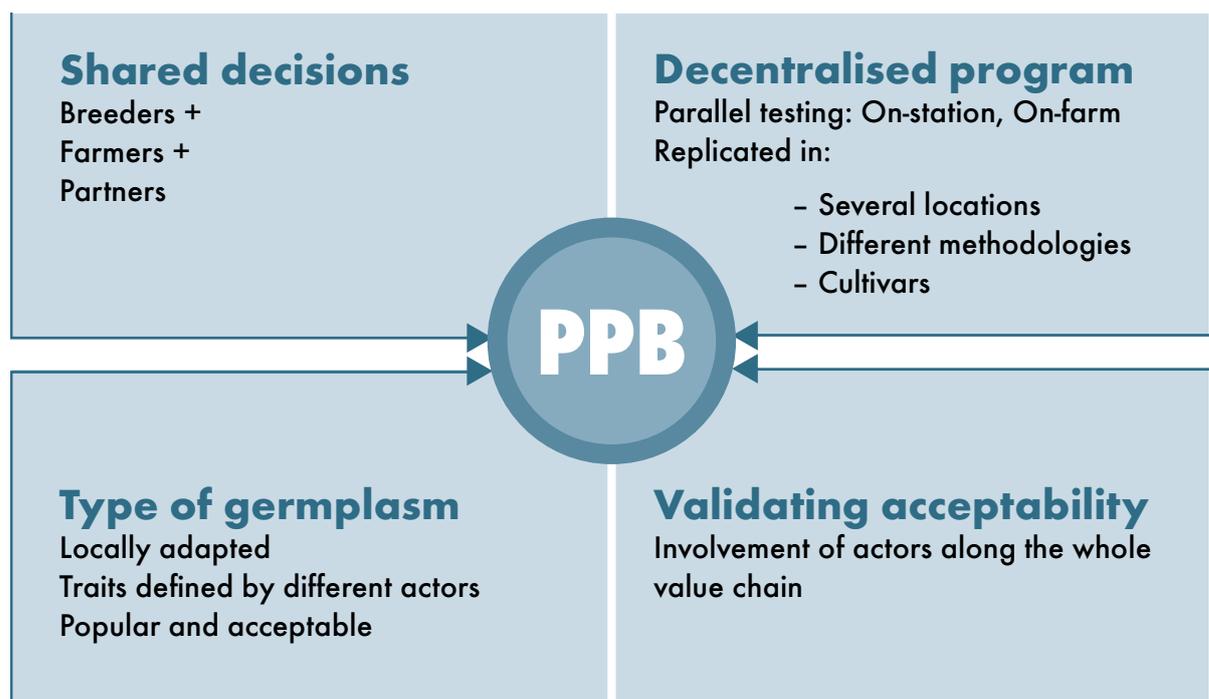


Figure 1. Features of participatory plant breeding

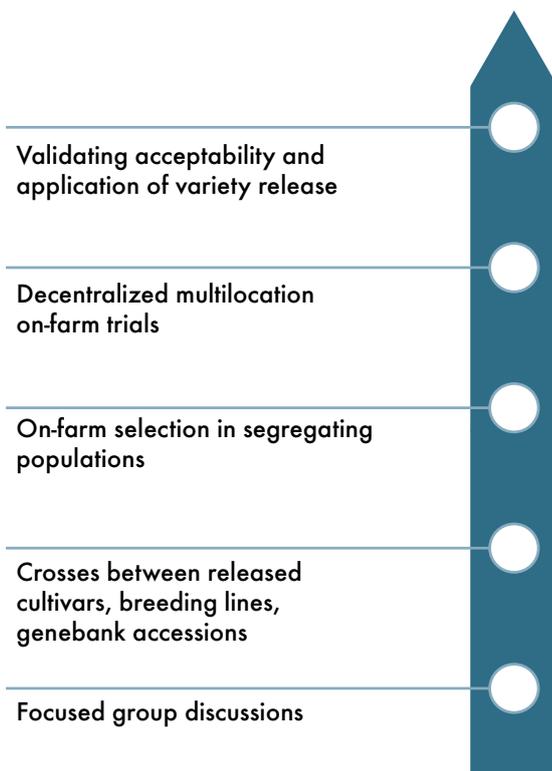


Figure 2. Process flow of PPB

A successful participatory program must be gender-inclusive and should ensure the unbiased involvement of both genders. PPB not only empowers the participating stakeholders but also creates numerous opportunities to make decisions at different important stages during the varietal development process. PPB supports the establishment of a healthy and stable seed system of adapted cultivars for smallholder farmers working in marginal and stress-prone environments, ultimately contributing towards sustainable food production, at the same time reducing the risk base from unforeseen circumstances.

1.2 Cotton production in India

Cotton is produced in more than 100 countries. It is one of the major agricultural crops as it represents the backbone of the world's textile industry. India is the largest cotton producer accounting for 22.7 % of world production (ICAC 2020). Cotton is a cash crop in India, accounting for a substantial portion of the rural economy and livelihood for six million farmers, mostly smallholders. Besides, the adjacent textile industry provides direct employment to over 36 million people and the export of textiles make up nearly one-third of all foreign exchange earnings of India (CICR 2010). Today 80 % of global cotton is genetically modified either by insect-resistant genes of Bt and/or herbicide tolerance (ISAAA 2017).

Cotton belongs to the Malvaceae family and its genus *Gossypium* includes 45 diploid ($2n = 2x = 26$) and five allotetraploid ($2n = 4x = 52$) species. The four species in cultivation are *Gossypium herbaceum* and *G. arboreum* (desi cotton), both diploid and originally from Asia and Africa and tetraploid *G. hirsutum* (American upland cotton) and *G. barbadense* (Sea Island Cotton) with extra-long fibres from the American continent. India is unique in the sense that it grows all four cultivated species, whereas worldwide only *G. hirsutum* dominates production, which is available in a large number of hybrid varieties.

In India, a number of local 'desi' varieties are grown beside the 'American hybrids'. They are usually more resistant to pests and drought, but most have a short staple length (< 28 mm) and thus fetch lower prices in the market. There is a large number of different cotton varieties available in the seed market, and research stations and seed companies continually release new varieties. Most of them are bred for producing high yields under high-input conditions using fertilisers, pesticides, and irrigation. However, the majority of cotton (60 %) is grown under rainfed conditions with sowing according to the onset of the monsoon in June or July, while only 40 % is produced under irrigated conditions with early sowing in May (summer sowing). Delayed sowing of three weeks can cause a yield reduction of up to 30 %.

Cotton is usually cultivated as an annual crop with a rather long vegetation period of around 175 to 225 days. Pollination usually occurs within a few hours after the flower opens its five petals. Most cotton varieties have self-fertile and self-pollinating flowers (Lanting et al. 2015) with the anthers producing pollen close to the stigma. Consequently, although cross-pollination may occasionally occur, it is very limited (OSGATA 2014). As cotton pollen is heavy, cross-pollination happens mostly with the help of pollinator insects such as honeybees and pollen beetles (Lanting et al. 2015).

Cotton is sensitive to frost and requires warm temperatures between 18 °C and 30 °C for optimal vegetative growth. Naturally, it is a perennial plant although it is now usually cultivated as an annual crop. Cotton requires a minimum of 500 mm water and high levels of nitrogen during vegetative growth. The conditions best for cultivation are deep and well-drained soils with good nutrient content since waterlogging can critically affect the crop's performance (OCCW 2012). Furthermore, the high number of insect pests is one of the main limiting factors in cotton cultivation. Among the important pests are jassid (*Amrasca biguttula*), aphid (*Aphis gossypii*), white fly (*Bemisia tabaci*), spotted bollworm (*Earias vittella*), pink bollworm (*Pectinophora gossypiella*), American bollworm (*Helicoverpa armigera*), various feeding caterpillars and also increasingly, the mealy bug (*Phenacoccus solenopsis*) (MoEF 2012). Due to the high yield losses triggered by the severity of these pests, conventional cotton cultivation consumes high amounts of chemical pesticides (45 % of all pesticides used in India), presenting huge implications for the environment as well as the health of the producers (Aktar et al. 2009).

In addition, the high quality of cotton fibre is decisive for fetching a good price in the market. The main fibre quality requirements of the textile industry are as follows:

- Maturity: 0.86 to 1.00
- Fibre length: 28 to 31 mm
- Micronair: 3.7 to 4.2 (up to 4.5 possible)
- Short Fibre Index: 6 to 7.5
- Strength of fibre: 28 to 32 p/tex, tested by HVI modus, or 22 to 24 g/tex, tested by ICC modus
- UR: 80 to 83, tested by HVI modus, or 43 to 45, tested by ICC modus
- Elongation: 5.9 to 7.6 (better are: 6.8 to 7.6)
- Neps in relation to maturity

1.3 Concerns about loss of cotton genetic diversity

It is increasingly believed that the reason for recent stagnation and/or decline in cotton yield and fibre quality in India is mainly due to the declining trend in genetic diversity of released cultivars and breeding stocks, the emergence of new menaces (such as minor pests becoming major pests) especially after the introduction of Bt cotton hybrids, a steep decline in the cultivation of desi cultivars and greater exposure to environmental challenges, such as drought, heat and salinity.

Research on genetic diversity of cotton cultivars and some of its related species conducted in several global laboratories including India using a range of molecular markers have clearly shown the narrowness of the genetic base of cotton cultivars (Boopathi et al. 2014).

To broaden the genetic base through participatory breeding programs, the genetic diversity among available germplasm is a prerequisite.

1.3.1 Dominance of GM hybrids

India is the country that most rapidly adopted genetically modified cotton (GM-cotton) carrying a gene of the bacterium *Bacillus thuringiensis* (Bt) which produces a specific toxin to combat bollworm larvae (Figure 3).

In 2002, Indian farmers grew only about 50,000 hectares of Bt cotton, but adoption increased rapidly over the decade (Figure 4) so that by 2008, 7.6 million acres were planted in Bt cotton, representing 82 % of all cotton planted in that year (Pray et al. 2011).

Since 2013, local traders offer only genetically-modified (GM) cotton seed that is explicitly excluded in organic farming. Since the demand from conventional farmers for non-GM seed has eroded to a significant extent, there is little interest by private seed companies to further invest in this sector. Farmers have also lost their traditional knowledge of seed production. Hybrid seeds must be purchased each season, and therefore organic cotton farmers today depend on a diminishing supply of non-GM cotton seed. At the same time, India is the number one contributor to the world's organic cotton supply (Textile Exchange 2019). Therefore, organic smallholder farmers are facing increased difficulties to find cultivars suitable for organic cotton cultivation.

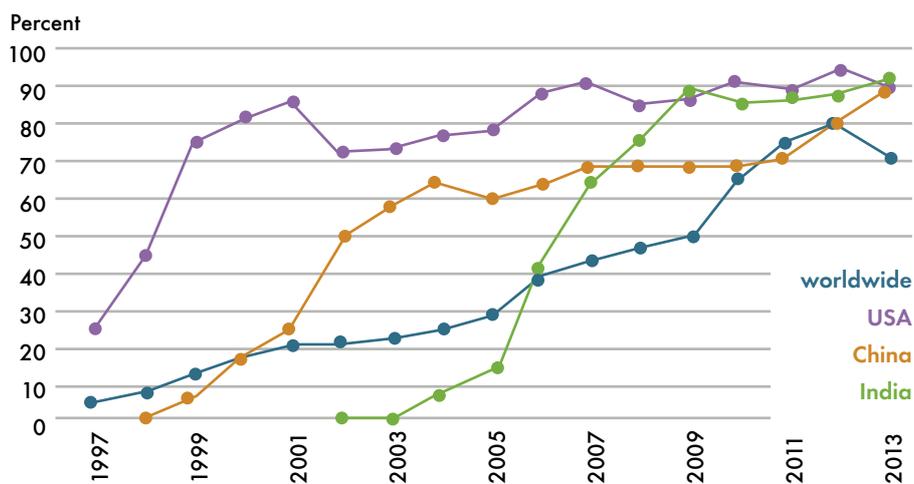


Figure 3. Adoption of Bt cotton hybrids in India compared to other leading cotton producing countries

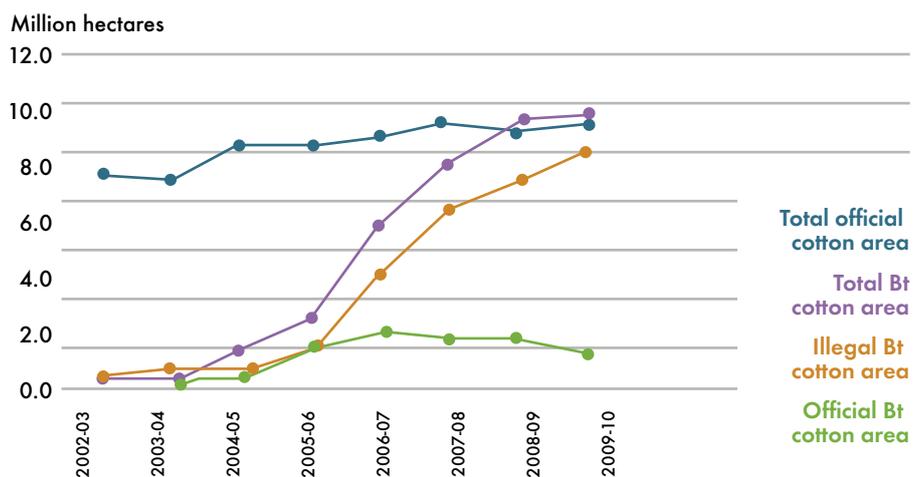


Figure 4. Development of Bt cotton area in India from 2002/03 to 2009/2010

1.3.2 Loss of traditional desi cotton cultivars

The history of cotton cultivation goes back thousands of years in India, where indigenous short-staple cultivars (*G. arboreum* and *G. herbaceum*), so-called Desi cotton, were cultivated providing sought-after characteristics such as hardiness, pest resistance and drought tolerance. During British colonial times long-staple cultivars from the American continent (*G. barbadense* and *G. hirsutum*) were introduced to India, being more suitable for the new spinning mills. With the release of the first

cotton hybrid in the early 1970s and the subsequent boom of high-yielding *G. hirsutum* hybrids, the area under indigenous Desi cotton reduced dramatically in India (Figure 5). Furthermore, in 2002, the Indian government approved genetically-modified (GM) cotton, which within a few years totally revolutionised the Indian cotton sector (Menon 2003). Currently, more than 95 % of the cotton area in India is covered by *G. hirsutum* hybrids carrying Bt gene(s). The four cultivated cotton species show high diversity in traits (Figure 6).

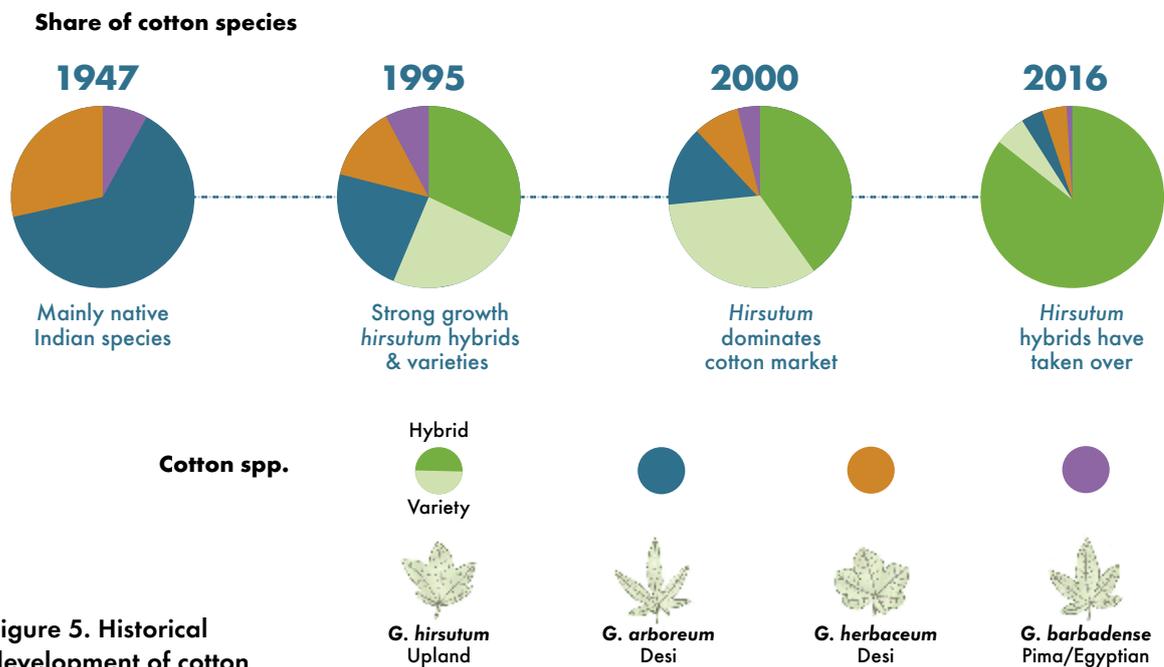


Figure 5. Historical development of cotton species cultivated in India

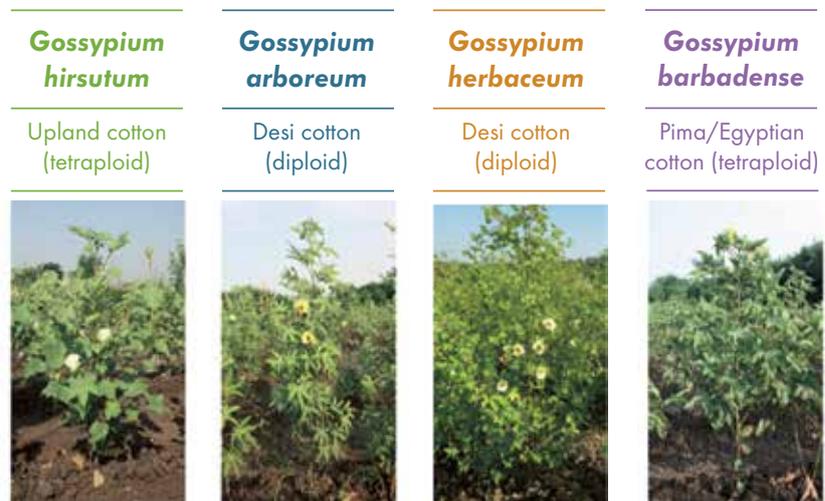


Figure 6. Cotton species cultivated in India

Long-staple *G. hirsutum* has high yield potential and a wide range of adaptation, whereas long-staple *G. barbadense* meets higher fibre quality (Table 1). However, both long-staple species are more susceptible to pests and diseases compared to Desi cotton (CICR 2010). Short-staple Desi cotton is not only more tolerant to pests and diseases, but also to drought and therefore more suitable for rainfed areas (Eyhorn et al. 2005b). However, its boll size

is small and the fibre is coarse and short (CICR 2010). Therefore, *G. arboreum* was crossed with *G. hirsutum* to improve the fibre quality of desi cotton. This resulted in a few varietal lines and hybrids that reached a fibre length of 28 mm, which is the threshold of the textile industry. Short staple cotton is demanded for surgical cotton due to its good water holding capacity.

Table 1. Description of different cotton species cultivated in India

Cotton species	Share of global production	Fibre length	Origin	Recent cultivation	Height
<i>G. hirsutum</i>	90 %	26 – 28 mm	America	Worldwide	1.5 – 2 m
<i>G. barbadense</i>	2 %	< 28 mm	America	Worldwide	2 – 3 m
<i>G. herbaceum</i>	< 4 %	< 25 mm	Africa	Mainly Asia	up to 1.5 m
<i>G. arboreum</i>	< 4 %	22 – 28 mm	Asia	Asia and India	2 – 3 m

1.4 Call for action

Although the traditional diploid cotton species *G. arboreum* and *G. herbaceum* have several advantages with regard to biotic and abiotic stress resistance, they largely remained neglected after the introduction of Bt cotton, and their cultivation dropped within ten years from 20 % to less than 5 %. However, these cotton species have shown to be especially suited for low external input conditions under organic farming and are morphologically distinct from present Bt-*hirsutum* hybrids which enables easy identification of GMO contamination existing in the field. Without a secure supply of GMO-free cotton seed, organic cotton production and the income of smallholder farmers in India has been severely threatened.

Fast action is needed to re-establish GMO-free seed supply chains and breeding programs to support organic and low input cotton farmers in India. Participatory plant breeding offers a great opportunity for developing locally adapted cultivars as well as for maintaining and increasing genetic diversity (Weltzien et al. 2003). To improve the access of organic cotton farmers to high-quality non-GM cotton seeds, a national workshop on 'Disappearing non-GM cotton – ways forward to maintain diversity, increase availability, and ensure the quality of non-GM cotton seed' was organised in Dharwad in June

2011. The goal was to involve the joint expertise and knowledge of breeders, organic farmers, advisors, and representatives of the textile industry along the whole market chain. The resulting Dharwad declaration towards safeguarding the heritage of Indian Desi cotton, maintaining genetic diversity, avoiding GM contamination and supporting the organic farmers with suitable cultivars was important to create public awareness and initiate first projects (Messmer et al. 2014; 2017).

Following the declaration of a broad stakeholder alliance FiBL initiated and coordinated the Cotton Cultivar Evaluation Project (2011 – 2017), the Green Cotton Project on participatory cotton breeding (2013 – 2017, www.greencotton.org) and the successive project Seeding the Green Future (2017 – 2022, www.sgf-cotton.org) to improve access to quality organic seeds of adapted GMO-free cultivars in the short term, to develop improved organic cultivars through PPB and to secure the integrity of organic cotton throughout the supply chain in the long term.

This manual aims to guide you through the different processes of it decentralised farmer-driven PPB from cultivar evaluation, crossing, selection of improved genotypes up to the development of cultivars specifically suited to local growing conditions, their maintenance and seed multiplication.

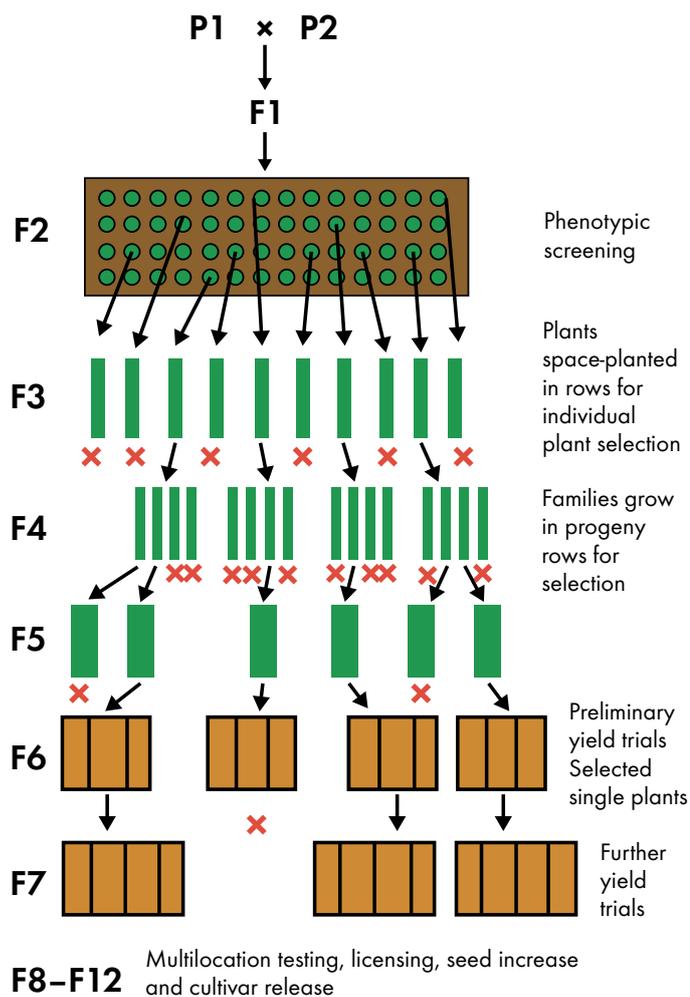


Figure 8. Breeding scheme for cultivar development and release

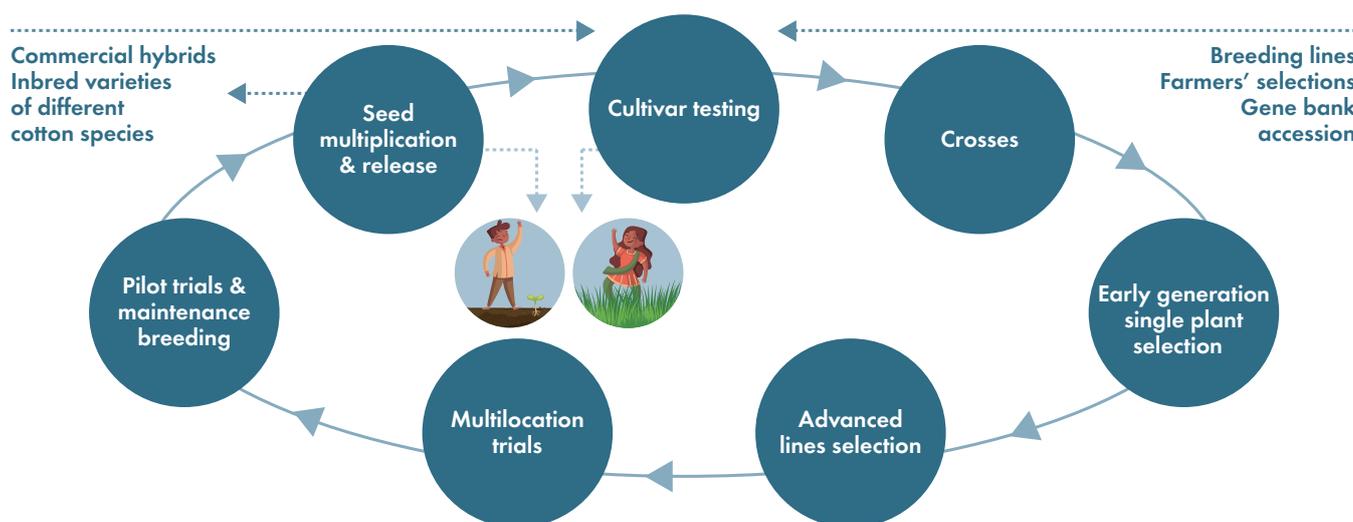


Figure 7. Important steps in participatory plant breeding

1.5 Outline of different stages of Participatory Plant Breeding

A PPB project is divided into several tasks addressing all major steps from crossing, selection, cultivar testing, multiplication, up to the distribution of non-GM cotton seed to the farmers (Figure 7). Sourcing available GMO-free cotton cultivars (inbred lines and hybrids), breeding lines, farmers selections and genetic resources followed by participatory cultivar evaluation under real-world conditions in farmer's fields is the first step to identifying suitable cotton cultivars for commercial production as well as for making crosses to increase genetic diversity (Jarvis et al. 2000).

Crosses between selected cultivars are conducted to increase genetic diversity, combine traits of different parents (e.g. resistance trait of parent A with fibre quality of parent B), and to develop superior progeny by crossing parental lines with similar traits (e.g. crosses between two parents both with high resistance to sucking pest) in order to combine different genes for the same traits (Figure 8). The derived seed of such crosses is an F1 plant like a hybrid cultivar. It is homogeneous and shows hybrid vigour, so-called heterosis effects.

Only the progeny of these F1 plants, i.e. the F2 generation will segregate and is the first generation where individual plant selection can start.

In the early generations, the level of heterozygosity is still high. As cotton is predominantly a self-pollinating species, the heterozygosity level will reduce from each generation to the next until they are stabilised lines in the F5 or F6 generations (Table 2). As long as there is more than 10 % heterozygosity there is still some remaining hybrid vigour and the progenies will continue to segregate. Depending on the different breeding method, pedigree or bulk selection, a farmer needs to trace which progenies are derived from which individual plant

that was selected. It is also important to record from which crosses the largest number of progenies were selected to identify superior parental lines for making future crosses.

In our breeding program, single plant selection is performed in the early generations of segregating populations (F2 until F5). Due to limited amount of seeds per selected plant, this often allows sowing only for one row (Figure 8) at two contrasting growing conditions in the following generation (mother plant → daughter row).

Only when the progenies are sufficiently homogeneous (from F5/F6 onwards) seeds of all plants from one daughter row can be harvested

Table 2. Different breeding activities from crossing till release of a variety

Generation	Heterozygosity level (rest hybrid vigour)	Segregation level	Selection method	Breeding task
Parental lines	0 %	No	Choice of parents used for crosses	Crosses
F1 derived from crosses	100 %	No	No selection, only multiplication to F2	Multiplication
F2	50 %	High	Selection of individual plants	Individual plant selection (IPS)
F3	25 %	High	Selection of individual plants	IPS
F4	12.5 %	Medium	Selection of individual plants	IPS
F5	6.3 %	Low	Selection of individual plants	IPS and initiation of maintenance breeding
F6	3.2 %	Low	Selection of all progenies derived from 1 row	Lines testing and multiplication
F7	1.5 %	Very low	Selection of all seeds from one plot	Lines testing in multilocation trials (MLT) & on-farm baby trials
F8	1 %	Very low	Selection of all seeds from one plot	Lines testing in multilocation trials (MLT) & on-farm pilot trials
F9	< 0.5 %	Very low	Selection of best candidates for commercialisation	Official cultivar testing & seed multiplication

together (mother plant daughter row → granddaughters plot). This amount of seed allows the testing of yield potential in the advanced generations (F6–F9). Availability of higher quantities of seed from each generation enables the establishment of several multilocation trials with replicates, a large number of on-farm baby trials of best candidates and the on-farm pilot trials of 0.1 to 1 acre.

In parallel, the maintenance breeding should be started. This is important to stabilise the potential cultivar candidates by strictly following plant to row seed multiplication, removal of off-types in the field and careful harvest of these Nucleus seeds. This seed is the basis of seed multiplication for commercial production. Most promising cultivar candidates will enter into focused testing for official variety release or for commercialisation of Truthfully Labelled Seed (refer to section 7.4.1)

As indicated in Figure 7, participatory plant breeding is a cyclical process, that needs to be repeated each year to constantly develop new cultivars that can address the challenges from new pests and diseases, the increased weather extremes anticipated due to climate change and global warming. Therefore, it is important to empower farmers to drive this process.

Empowerment of farmers is achievable through active engagement of on-farm breeding activities and action research, also through specific workshops and mutual exchange of farmers knowledge and scientific results. Interested farmers shall obtain the opportunity to follow a 'farmer breeder curriculum'. The different workshops on participatory cultivar evaluation, hybridisation, individual plant selection, and seed multiplication described in this manual form an integral part of this curriculum. The workshops support lead farmers, trainers, facilitators and advisors in conducting similar workshops with male and female farmers which is key for local capacity building (Zahumensky 2014).

In addition to these technical aspects, special emphasis should be placed on building trust and cooperative networks which are committed to succeed with this multi-actor approach. A neutral facilitator is key, one who can moderate in case of conflicting interests between partners, ensure timely implementation of trials, exchange of information and material among partners, compilation of results and interpretation of data, all that supports informed decision-making process among involved parties. Care must also be given to the socio-economic assessment of potential bottlenecks for participation of farmers in the process, empowerment of female farmers, adoption of new cultivars, dissemination and knowledge transfer beyond the project partners. For further reading see Gonsalves et al. 2005 a,b,c, and Pimbert 1991.

2. Participatory Cultivar Evaluation

2.1 Goals

- Explore the potential of genetic diversity available, including different cotton species.
- Identify and prioritise traits important for farmers, cotton pickers, ginners, spinners and textile industry.
- Give recommendations for commercial production of GMO-free cultivars in respective regions and farming conditions.
- Select interesting cultivars for making crosses to develop improved cultivars through PPB.

2.2 Activities in the field

- Determine the most important traits and threshold for farmers, cotton pickers, ginners, spinners and textile industry.
- Source cotton germplasm of all four cotton species: inquire at different universities, cotton institutes, seed companies and traders, farmers' associations, gene banks and conduct online inquiry for information and seeds of GMO-free cultivars suitable for different cotton-growing regions (Central, Southern and Northern) in India.
- Select most promising cultivars based on prioritised traits.
- Select soil type and growing conditions which are most representative of farmer's practice.
- Identify interested farmers to engage in participatory cultivar testing.
- Identify farmers' fields that would suit for on-farm testing.
- Design and conduct replicated on-farm field trials with 20 to 50 cultivars of different cotton species at several locations (mother trials) (Figure 9).
- Design and conduct unreplicated on-farm or on-station trials with four to six selected cultivars involving many farmers to cover a large range of growing conditions (baby trials) (Figure 9).
- Assess agronomic traits, stress tolerance, yield and fibre quality parameters in both mother and baby trials.
- Visual evaluation of cultivars in mother and baby trials.
- Cross-verification of statistical analysis of mother and baby trials with farmers' experience.
- Selection of promising cultivars to verify results in the following season.
- Coordinate order for seed.

2.2.1 Replicated mother trials

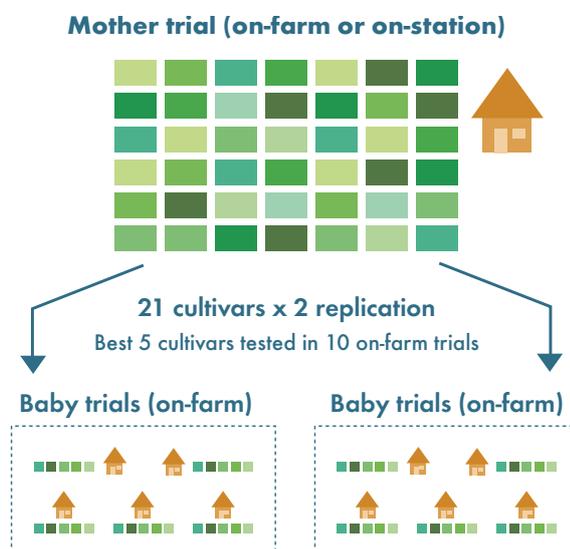


Figure 9. Participatory on-farm / station mother and baby trial plan

- GMO strip test of seeds of all entries before planting.
- At least 15 or more cultivars including *G. arboreum* and *G. hirsutum* cultivars and commercial check (main focus on varietal lines) with at least 28 mm fibre length
- Identification of suitable fields for trial (avoid slope, trees and grazing areas)
- Two to three replications (Figure 10, page 14), randomised complete block design with border rows around the whole trial at least at two representative growing conditions (e.g. rainfed light soil and irrigated fertile heavy soil).
- Four to five row plots (20 feet long) with 100 plants per plot for yield assessment with one separator row (e.g. okra, hibiscus, finger millet) in between plots to avoid mixtures during harvest of the plots.
- Plant density shall match soil and cultivar type.
- Trials on heavy soil: summer-sown; irrigated; spacing 3 × 1 feet for varietal lines, 3 × 2 feet for hybrids (Figure 11).
- Trials on light soil: monsoon-sown; rainfed or limited irrigation; spacing 2 × 1 feet (Figure 11).
- Use three seeds per planting hole, and resow seven days after first planting in case the seeds fail to germinate. Thinning to one plant after 10 to 14 days.

Replication 1	Plot No	101	102	103	104	105	106	107	108	109	110
	Unique code	SGF_701	SGF_706	SGF_705	SGF_008	SGF_303	SGF_001	SGF_719	SGF_301	SGF_306	SGF_718
	Plot No	120	119	118	117	116	115	114	113	112	111
	Unique code	SGF_008	SGF_319	SGF_002	SGF_010	SGF_305	SGF_003	SGF_318	SGF_006	SGF_323	SGF_321
Replication 2	Plot No	121	122	123	124	125	126	127	128	129	130
	Unique code	SGF_008	SGF_706	SGF_319	SGF_718	SGF_301	SGF_322	SGF_303	SGF_306	SGF_705	SGF_318
	Plot No	140	139	138	137	136	135	134	133	132	131
	Unique code	SGF_321	SGF_010	SGF_006	SGF_003	SGF_323	SGF_719	SGF_001	SGF_305	SGF_002	SGF_701
Replication 3	Plot No	141	142	143	144	145	146	147	148	149	150
	Unique code	SGF_008	SGF_322	SGF_718	SGF_001	SGF_010	SGF_719	SGF_323	SGF_318	SGF_301	SGF_306
	Plot No	160	159	158	157	156	155	154	153	152	151
	Unique code	SGF_706	SGF_305	SGF_705	SGF_701	SGF_002	SGF_303	SGF_319	SGF_321	SGF_003	SGF_006

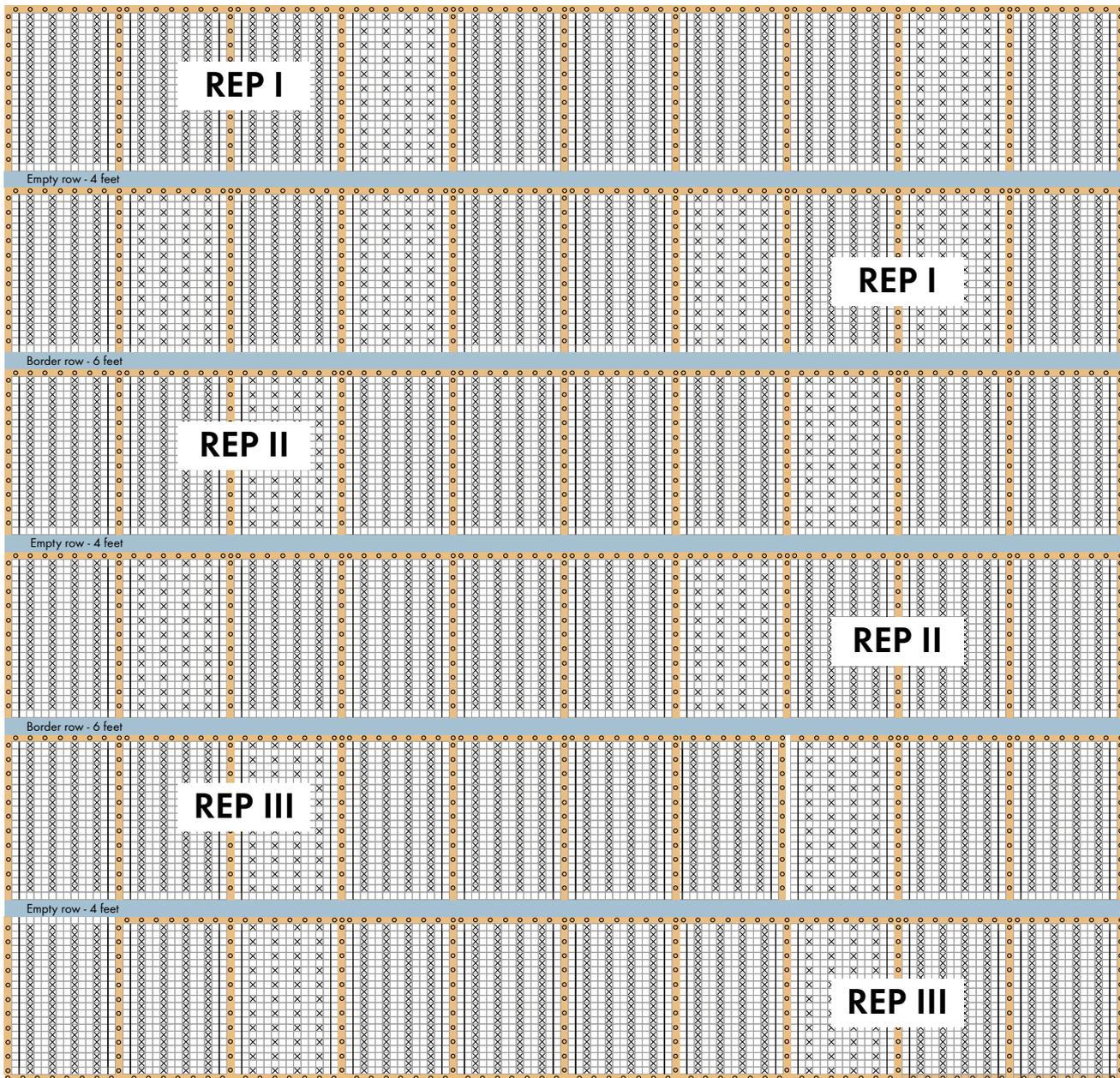


Figure 10. Field layout of replicated mother trials under organic condition

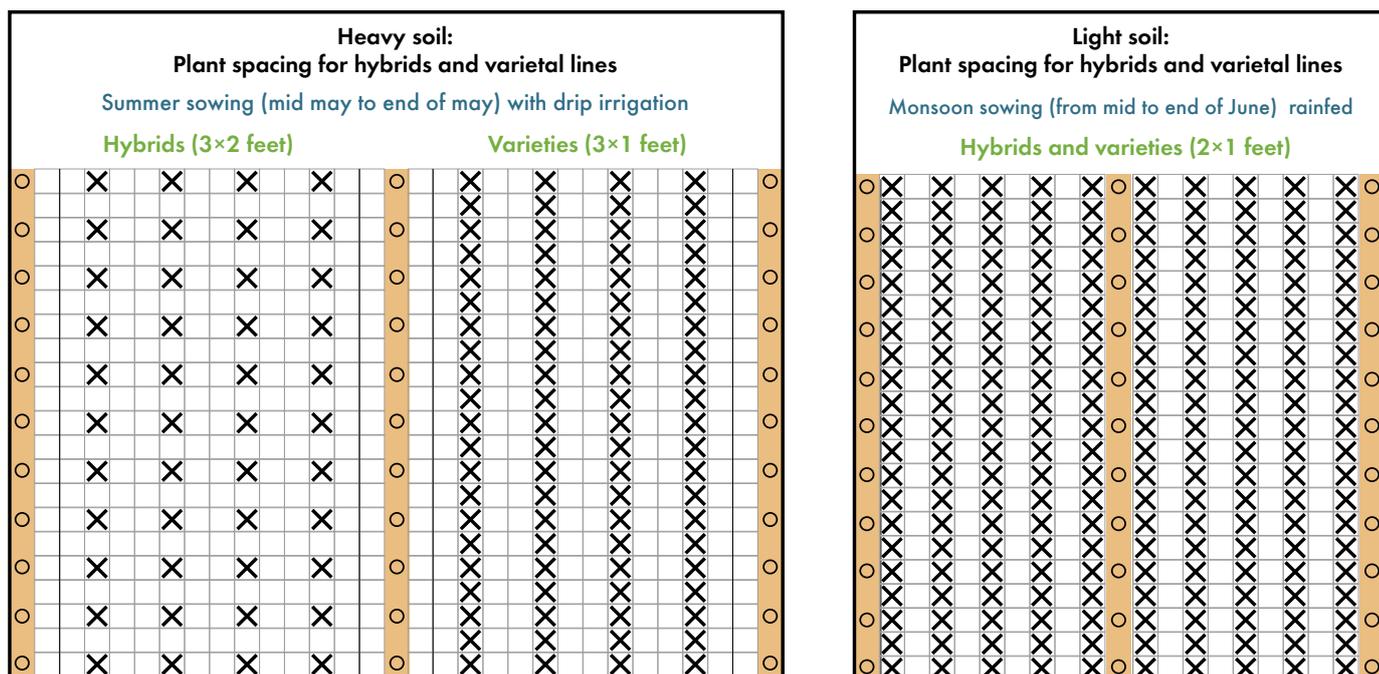


Figure 11. Spacing pattern for mother trials under heavy and light soil conditions

- One to two prunings of *G. arboreums* at the height of 3 feet and 4 feet to avoid plants that are too tall and bend.
- Assessment according to field book and agreed protocol in given excel format (see Table 3) for agronomic traits, Ginning Out Turn (GOT) and fibre quality (Snapp 2002).
- Conduct separate harvest in three periods (Oct, Nov and Dec).
- Determine ginning out turn and fibre quality of bulk of first and second harvest.
- Record details of soil, climatic, crop rotation, fertilisation, pest, weed control, irrigation and other field operations.
- Timely and proper management is essential, special care must be given to weed management.
- At least 100 plants in 2-row or 50 plants in 4-row plots with one separator row (e.g. oca, hibiscus, or finger millet) to separate from remaining cotton field of farmer.

2.2.2 On-farm baby trials

- Distribution of seed of promising cultivars (own breeding lines, public or commercially released cultivars).
- GMO strip test of seeds of all entries before planting.
- At least five cultivars including *G. arboreum* and *G. hirsutum* cultivars and one commercial check (main focus on varietal lines) with at least 28 mm fibre length.
- Check is repeated twice on both sides of the trial and identical for all baby trials across years.



On-farm baby trial labeling and coding chart

Table 3. Parameters to be recorded from established on-farm trials

Parameter	Heavy / light soil	Unit	Growth stage	Times	Remarks		
Resistance							
Diseases	Alternaria blight	Plot level	Score 1 – 5	Vegetation period	1 – 3 ×	Record the data for obvious disease and/or pest infestations by scoring the general visual impression at plot level, with each evaluator doing at least one repetition. In case of heavy infestation, make a first assessment and then a second assessment two weeks after.	
	Bacterial blight	Plot level	Score 1 – 5	Vegetation period	1 – 3 ×		
	Rotten plants	Plot level	Score 1 – 5	Vegetation period	1 – 3 ×		
	Others	Plot level	Score 1 – 5	Vegetation period	1 – 3 ×		
Pests	Jassids	Plot level	Score 1 – 5	Vegetation period	1 – 3 ×		
	Aphids	Plot level	Score 1 – 5	Vegetation period	1 – 3 ×		
	White flies	Plot level	Score 1 – 5	Vegetation period	1 – 3 ×		
	Thrips	Plot level	Score 1 – 5	Vegetation period	1 – 3 ×		
	Spotted bollworm	Plot level	Score 1 – 5	Vegetation period	1 – 3 ×		
	American bollworm	Plot level	Score 1 – 5	Vegetation period	1 – 3 ×		
	Pink bollworm	Plot level	Score 1 – 5	Vegetation period	1 – 3 ×		
Deficiencies	Caterpillars (Leaf dam.)	Plot level	Score 1 – 5	Vegetation period	1 – 3 ×		
	Mealy bug	Plot level	Score 1 – 5	Vegetation period	1 – 3 ×		
	Yellowing	Plot level	Score 1 – 5	Vegetation period	1 – 3 ×		
Morphology	Reddening	Plot level	Score 1 – 5	Vegetation period	1 – 3 ×		
	Number of plants	Plants per plot	Number	Harvesting period	3 ×	Count plants once in each picking period	
	Homogeneity	Plot level	Score 1 – 5	Oct./Nov.	1 ×	1 = homogeneous, 3 = medium, 5 = heterog.	
	Plant height	Plot lev. (av. 5 rows)	cm	Oct./Nov.	1 ×	5 random plants in LS and HS	
	Canopy diameter	Plot lev. (av. 5 rows)	cm	Oct./Nov.	1 ×	5 random plants in LS and HS	
	Leaf shape	Plot level	Score 1 – 5	Oct./Nov.	1 ×	1 = hirsut., 3 = medium, 5 = arbor. (okra) like	
	Hairiness of leaves	Plot level	Score 1 – 5	Oct./Nov.	1 ×	1 = very hairy, 2 = hairy, 3 = medium, 4 = less hairy, 5 = not hairy	
Yield Data	Hairiness of stem	Plot level	Score 1 – 5	Oct./Nov.	1 ×	4 = less hairy, 5 = not hairy	
	3 picking periods	Gross plots		3 picking periods		Keep each picking separate (quality analysis)	
	Date of picking	Per plot first picking	Date	Oct./Nov.		Keep each harvest separate	
	Plants per plot	Per plot first picking	Number	Oct./Nov.		Keep each harvest separate	
	Harvested balls	Per plot first picking	Number	Oct./Nov.		Keep each harvest separate	
	Weight of picked balls	Per plot first picking	g	Oct./Nov.		Keep each harvest separate	
Easiness of Picking	Maximum ball size	Per plot first picking	mm	Oct./Nov.		Keep each harvest separate	
	Bending of plants	Plot level	Score 1 – 5	Nov./Dec.	1 ×	Bending of plants (1 = no, 5 = heavy)	
	Position of most balls	Plot level	Score 1 – 5	Nov./Dec.	1 ×	1 = top, 3 = middle, 5 = bottom	
	Boll size	Plot level	Score 1 – 5	Nov./Dec.	1 ×	Boll size (1 = big, 5 = small)	
	Compartments	Plot level	Number	Nov./Dec.	1 ×	Number of compartments of cotton ball	
	Ball openness	Plot level	Score 1 – 5	Nov./Dec.	1 ×	Ball openness (1 = good, 5 = poor)	
	Lint easy to pick	Plot level	Score 1 – 5	Nov./Dec.	1 ×	Lint easy to pick (1 = fast, 5 = slow)	
	Boll dropping	Plot level	Score 1 – 5	Nov./Dec.	1 ×	Boll dropping (1 = little, 5 = many)	
Quality	Lint dropping	Plot level	Score 1 – 5	Nov./Dec.	1 ×	Lint dropping (1 = little, 5 = much)	
	Ginnery	Seed index	1 st + 2 nd , 3 rd picking	Value	Harvest end		First and second picking together, third picking separate
		Lint index	1 st + 2 nd , 3 rd picking	Value	Harvest end		
		Ginning out turn (GOT)	1 st + 2 nd , 3 rd picking	Value	Harvest end		
	HIV Testing	Fibre length	1 st + 2 nd , 3 rd picking	Millimeters	Harvest end		
		Fibre strength	1 st + 2 nd , 3 rd picking	Value	Harvest end		
		Maturity	1 st + 2 nd , 3 rd picking	Value	Harvest end		
Uniformity		1 st + 2 nd , 3 rd picking	Value	Harvest end			
Visual Scoring	Finesse	1 st + 2 nd , 3 rd picking	Value	Harvest end			
	Micro-naire	1 st + 2 nd , 3 rd picking	Value	Harvest end			
Monitoring	Researcher		Score 1 – 5	Harvest		5 = very good, 4 = good, 3 = medium, 2 = bad, 1 = very bad	
	Farmer		Score 1 – 5	Harvest			
Monitoring	Cultivar on station		Photo	Harvest start / end			
	Cultivar on farm		Photo	Harvest			

- Plant density (Figures 12 and 13) shall match soil fertility and cultivar type and farmer's practice:
 - Trials on heavy soil: summer-sown; irrigated; spacing 3 × 1 feet for varietal lines, 3 × 2 feet for hybrids.
 - Trials on light soil: Monsoon-sown; rainfed or limited irrigation; spacing 2 × 1 feet.
- Use three seeds per planting hole, and resow seven days after planting in case the seeds fail to germinate. Thinning to one plant after 10 to 14 days.
- One to two prunings of *G. arboreums* at the height of 3 feet and 4 feet to avoid plants that are too tall and bend.
- Trial management according to farmer's practice.
- Assessment by the farmer (with support of the researcher) according to field book and agreed protocol of the following information presented in spreadsheet (Excel) format: agronomic traits, GOT and fibre quality. Soil and climatic data, crop rotation, fertilisation, pest and weed control and irrigation is also recorded.
- Timely weed management is essential.
- For baby trials a colour coding that matches the label in the field with the label of the harvesting bag helps to avoid any confusion.

2.2.3 On-farm pilot trials

- Distribution of seed of promising cultivars and candidates (own breeding lines, public or commercially released cultivars) with at least 28 mm fibre length.
- GMO strip test of seeds of all entries before planting.
- Each field used for pilot cultivation will be divided into two parts: at least 0.1 acres will be cultivated with the new cultivar, the remaining area will be planted with commercial cultivar used by the farmer (Figure 14).
- At least 2 to 3 on-farm sites per cultivar.
- Research will assess yield in three windows of 5 × 5 m for the new cultivar and the check.
- Plant density shall match soil fertility, cultivar type and farmer's practice:
 - Trials on heavy soil: summer-sown; irrigated; spacing 3 × 1 feet for varietal lines, 3 × 2 feet for hybrids.
 - Trials on light soil: Monsoon-sown; rainfed or limited irrigation; spacing 2 × 1 feet.
- Three seed per planting hole, resowing seven days after planting, thinning to one plant after 10 to 14 days.

Cultivar	Cultivar type	Cultivar type
Suraj 1	HV	green
	AV	orange
	HV	blue
Mallika 207	HH	purple
Suraj 2	HV	green
	HV	yellow
Namaskar 81	AH	pink

The order of cultivars will be randomised for each field.

Additions:

- at least one *arboreum*
- at least one to two *hirsutum* inbred lines
- at least one *hirsutum* hybrid

- Heavy soil: Summer sowing (mid May to end of May) with drip irrigation
- Light soil: Monsoon sowing (mid to end of June) rainfed
- 7 stripes with 2 rows × 50 plants of 4 rows with 25 plants = 100 per cultivar
- 300 seed, about 40g per cultivar and on farm trial
- Each cultivar has different colour code, labelled accordingly with colour ribbons on plant and harvesting bag.
- Suraj is included as *hirsutum* varietal check twice (Suraj 1 and 2) to estimate the homogeneity of the field.
- Mallika is included as hybrid check.

Figure 12. On-farm baby trial instructions

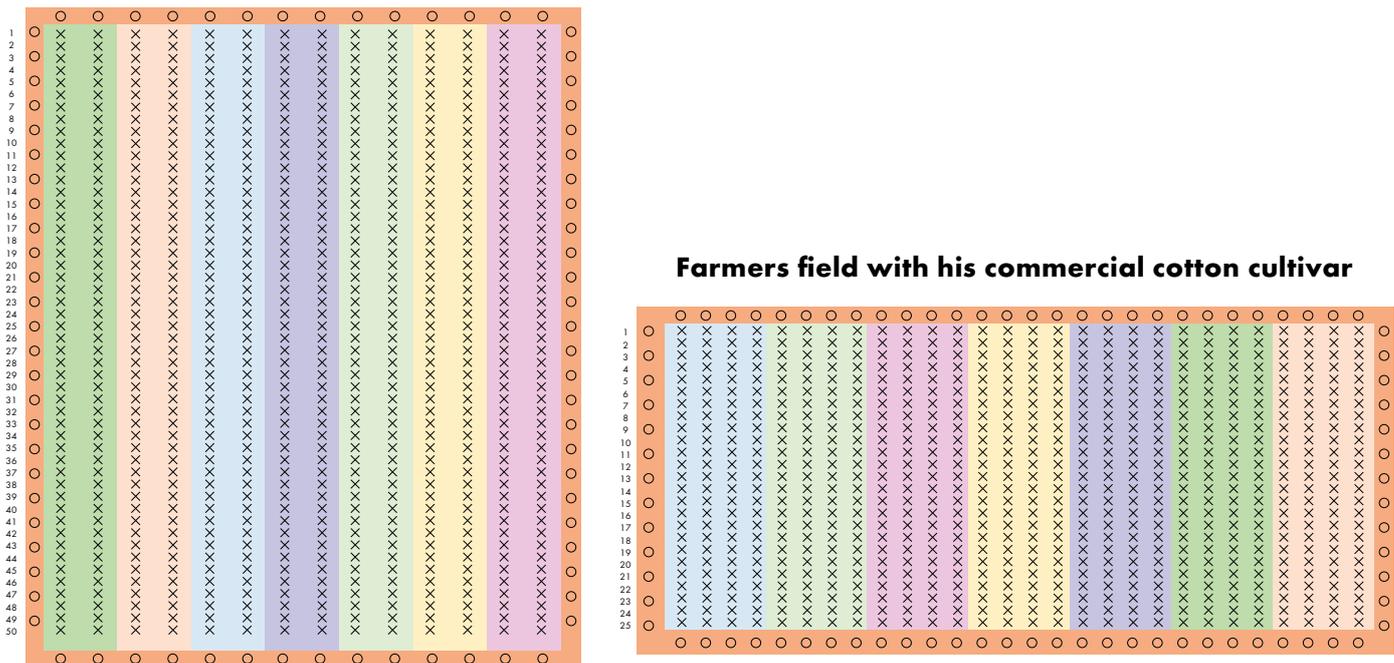


Figure 13. Field layout on-farm baby trial

- One to two prunings of *G. arboreums* at the height of 3 feet and 4 feet to avoid plants that are too tall and bend.
- Management according to farmer's practice.
- Assessment by the farmer (with support of the researcher) according to field book and agreed protocol of the following information presented in spreadsheet (Excel) format: agronomic traits, GOT and fibre quality. Soil and climatic data, crop rotation, fertilisation, pest and weed control and irrigation is also recorded.
- Staff will determine fibre quality data.
- Timely weed management is essential.

Templates for mother, baby and pilot trials can be obtained on the Seeding the Green Future project website www.sgf-cotton.org.

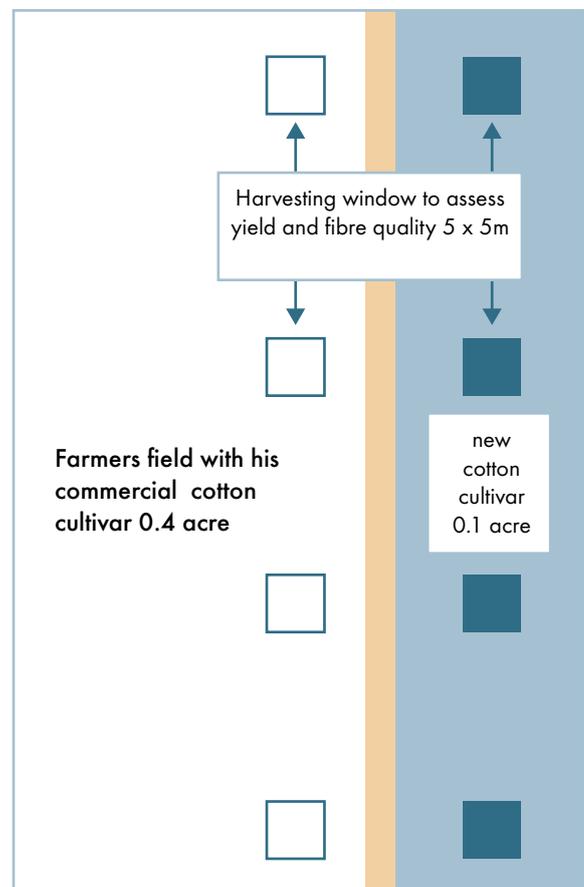


Figure 14. Field layout plan for on-farm pilot trial

2.3 Workshop on cultivar evaluation

2.3.1 Learning objectives

- Understand relevance of selecting appropriate cotton cultivars for specific growing conditions.
- Learn characteristics of different cotton species and cultivar types.
- Mutual understanding from male and female farmers and other stakeholders of the cotton value chain on the desired cotton characteristics.
- Identification and ranking of traits of cotton cultivars relevant for different growing conditions.
- Understand where information on the performance of specific cultivars can be found.
- Learn how to select cultivars that best suit your farm.

2.3.2 Right cultivar for the right site at the right time (3R's)

Farmers have different priorities for the selection of cotton cultivars than researchers; hence, it is important to involve farmers when setting priorities, evaluating cultivars or selecting promising plant types in early generations to breed for specific adaptation and their suitability for organic and low input farming. As cotton is a cash crop, it is also important to consult the value chain, for the specific fibre quality needed for the textile industry.

The initial identification of the most important varieties and their preferred traits is done during small focus group discussions with key local leaders, practising farmers and with active participation of women. The selected crop varieties should fit in the agro-ecological conditions of the community, also respecting women's preferences for crop species and varieties that matches the need of the textile industry.

Organic farmers are more interested in robust varieties that are resistant or tolerant to pests and produce satisfying yields with less external supply of nutrients. Some varieties, however, combine the advantages of the 'desi' varieties (hardy, drought resistant, resistant to most of the sucking pest complex) with those of the *hirsutum* varieties (high yield, long fibres) (Figure 15).



Varietal evaluation in progress

Selecting the right cotton varieties

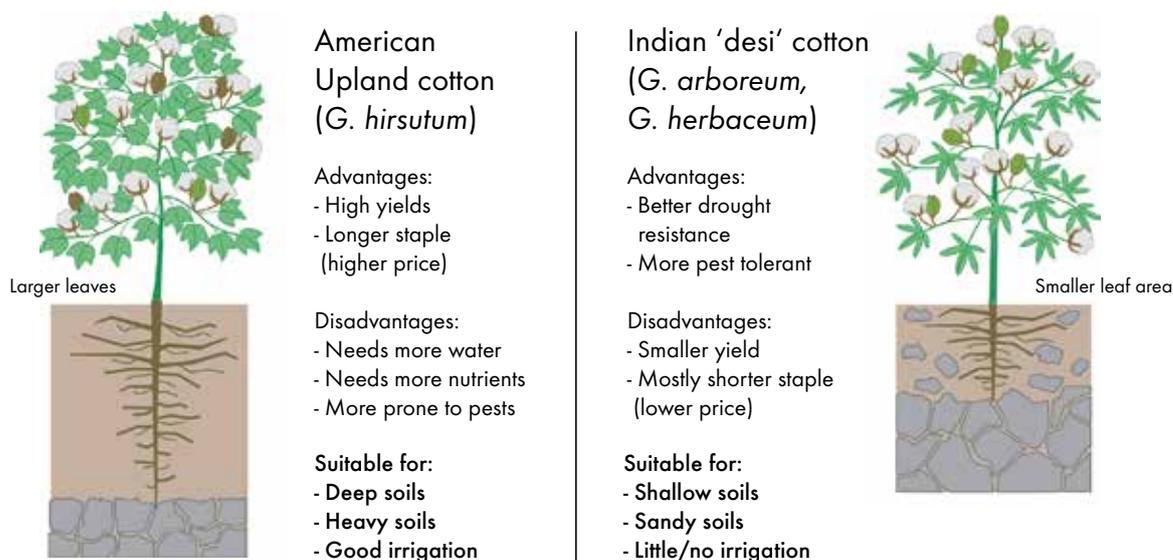


Figure 15. Comparison of American upland and Indian desi cotton

2.3.3 Characteristics to look for differentiating amongst cotton cultivar or species

Different varieties can be of great interest, especially for organic farmers with less irrigation availability. To select the most suitable varieties, farmers should select a cultivar or species considering the site conditions (soil quality, rainfall, availability of irrigation water, etc.) as well as the conditions

of the farm (availability of manure, possibility for pest management through organic practices, etc.) Where irrigation is a constraint and rainfall is erratic, it is preferable to use varieties that require less water (e.g. those with smaller leaf area). Also, farmers need to consider the buyers' requirements concerning staple length and other fibre quality aspects. Table 4 summarises the traits of different cotton species.

Table 4. Traits of different cotton species

Cotton species	<i>G. arboreum</i>	<i>G. herbaceum</i>	<i>G. hirsutum</i>	<i>G. barbadense</i>
Plant type	Perennial much branched shrub	Sub-shrub	Small annual shrub	Perennial shrub or annual sub-shrub
Height	1.5 – 2 m	1 – 1.5 m	1 – 1.5 m	1 – 3 m
Leaves	Pubescent/ sub-glabrous	Sparsely hairy and rarely glabrous	Glabrous to densely heavy board	Glabrous
Flowers	Yellow with purplish-red petal spot	Small and yellow with a purple center	Yellow and petal spot usually absent	Yellow and petal spot present
Boll	Round to tapering	Small and round	Round, oval or elongated larger	Narrowly ovoid to elongate
N° of locules	3 – 4	3 – 4	3 – 5	3
N° of seeds/ locules	6 – 17	11 – 10	5 – 7	5 – 8
Seeds	Small with short fuzzy hairs	Large with short fuzz and lint	Copious hairs and a thick fuzz	Bald with no fuzz

2.3.4 Training in participatory cultivar evaluation

- Participating farmers should be divided into groups according to; (i) gender, as female farmers have different tasks to perform in the field than male farmers and hesitate to speak openly if men are present (Pimbert, 1991) (ii) growing conditions, most representative growing conditions for organic cotton farmers in India are; (a) cultivation on fertile, deep clay soil with irrigation-summer sowing (heavy soil), and (b) cultivation under shallow sandy soil without irrigation depending on monsoon sowing (light soil). Cultivar performance strongly depends on sowing time, soil fertility and water availability. Different cotton species and cultivars are suited to different regions, environments, soil type and other important factors responsible for the cotton cultivation.
- Identify and define one person from the respective groups to report back results to the whole group.
- Participatory identification and prioritisation of important cotton traits for organic cultivation, under irrigated and rainfed conditions should take place within the designated groups. Inquire among the farmers about the locally available cotton varieties and which traits are most important for male and female farmers (e.g. morphology, yield potential, adaptability to local conditions, resistance to pests and diseases), ginners (e.g. ginning outturn), spinners (e.g. fibre length, fineness), and textile industry (e.g., fibre strength, yarn counts, colour). The groups will use different brainstorming tools (whiteboard, posters, drawings, paper etc.) to identify cotton traits, which will later be displayed on a board.
- Presentation of the results from each group, note results on a board or large paper, discuss similar and different characteristics of suitable varieties according to the local growing conditions. Trainer or value chain partner will present wish list and threshold of the industry. Trainer might add other important cotton traits not considered so far by farmers.



Grouping of participants



Brainstorming session



Sowing under light and heavy soils



Presentation of group results

- Visit on-farm mother or baby trial to demonstrate the mentioned traits in the field. To clearly understand the preferred characteristic, farmers are asked what is a good expression and what is a bad expression of a given trait. It is very important that all have a common understanding of the listed traits before prioritising the traits.
- Each farmer, according to his/her experience and opinion, will select the most important and preferred traits by placing coloured sticky pointers on their preferred trait (Table 5). Each farmer can use a maximum of two pointers for a single trait. Farmers of each group will receive different coloured magnets/pointers. This will help to summarise scoring of the identified traits for each group.



Visit to on-farm trials



Selection of important traits using coloured ribbons

Table 5. Cotton plant traits rating sheet

Evaluation on: _____

Sr n ^o	Traits	Top five traits	Remarks
1	Good germination		
2	Continue flowering and flush		
3	Big boll size		
4	Good number of bolls		
5	Good puffing		
6	Easy picking		
7	Locule retention / non-dropping (<i>Arboreum</i> sp.)		
8	Tolerant to biotic stresses (pests and diseases)		
9	Tolerant to abiotic stresses (e.g. drought, waterlogging)		
10	Good lint quality		

Signature: _____

- It is important that farmers are exposed to different cotton species and cultivar types. The following cultivar types are grown in India: *G. hirsutum* (HV), *G. arboreum* inbred lines (AV), *G. herbaceum* (HeV) and *G. barbadense* (BV) inbred varietal lines (see Figure 6), the commonly grown *G. hirsutum* hybrid (HH), *G. arboreum* hybrid (AH) and *G. hirsutum* × *G. barbadense* (H × B) inter-species hybrids, as well as *G. hirsutum* compact lines bred for machine picking. Inbred lines are of special interest as they allow farm-saved seed production thus, strong reduction of seed costs while, *G. arboreum* lines are preferred as they do not intercross with Bt-*hirsutum* hybrids, have greater tolerance to sucking pests and stable performance even under rainfed and marginal growing conditions. For this purpose, different GMO-free cultivar types collected from various sources shall be grown in different planting density and management options for demonstration to farmers. It is important that all these cultivars should at least give 28 mm fibre length. In succession, a brief group discussion will be conducted to discuss advantages and disadvantage of these cultivar types like *G. arboreum* vs *G. hirsutum* and inbred lines vs. F1 hybrids. It is suggested to explore which cultivars different farmers or farmer groups would like to try in their own fields.



Farmers exposure to different cotton species

- After instruction from the trainer, each farmer will conduct a visual assessment of cultivars and/or advanced breeding material of different cultivar types in on-farm mother or baby trials. Each farmer will be asked to select her/his most preferred cultivars within the different cultivars exhibited during the field activity as per their preference for important traits. For this purpose, every farmer will receive five coloured cloth ribbons to mark the cultivars she/he likes most or

the ones she/he would wish to plant next year on their field (Table 6). Each group (female, male, heavy soil, light soil) has a different colour coding. Sometimes, it is helpful to give out a second set of coloured ribbons to mark those cultivars that farmers do not approve. Afterwards, the trainer needs to count the different coloured ribbon per cultivar.



Visual assessment of cultivars

- Meanwhile, each farmer will use a cardboard indicating three to five cultivars he likes most, the expression on a scale from 1 (no expression), 3 (weak expression), 5 (medium expression), 7 (strong expression) up to 9 (maximum expression) for those traits have been prioritised before.



Cultivar selection using coloured ribbons

- Finally, cultivars selected by most farmers will be discussed within the groups and farmers will be asked to mention the main reason for his/her selection. Results of all groups will be presented in the plenum. Farmers will nominate at least four to five cultivars to be further examined under their conditions in farmer managed on-farm trials during next year season.



Small group discussion on cultivar selection

- This exercise should be repeated at least twice at beginning of boll bursting to identify tolerance to various biotic and abiotic stressed and before final harvest to estimate yield potential. The impression and preference of cultivars may differ during different vegetation periods and in contrasting soil types (e.g. heavy soil, light soil) as performance of cotton cultivars depend very much on local growing conditions, irrigation and fertilisation level.
- Farmers will obtain a certificate for attending the course as part of the 'farmer's breeder curriculum'.

Table 6. Selection of five top most preferred cultivars using ribbons by male and female participating farmers divided into groups on sites with heavy soil and irrigation (HS) and light soil and rainfed conditions (LS)

Sr. n°	Name of cultivar	Cultivar type	Heavy soil & irrigation (HS)			Light soil & rainfed conditions (LS)			Grand total of both trials (HS & LS)
			N° of ribbons marking the plant in replication		Total n° marked by male & female farmers	N° of ribbons marking the plant in replication		Total n° marked by male & female farmers	
			I Male	II Female		I Male	II Female		
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									



Recognising farmers for their efforts

2.3.5 Summarising activities

1. On-farm trials with different cotton cultivar types under irrigated, fertile soil with summer sowing (heavy soil) and under rainfed, shallow soil with monsoon sowing (light soil) to represent most typical growing conditions of local cotton farmers.
2. Discussion on cultivars (*G. hirsutum* & *G. arboreum*) most preferred by the participating farmers and reasons behind their preference.
3. Field visit to identify and learn about the important traits / characteristics relevant to yield and market.
4. Farmers' preference for cultivar and cultivar types observed during field visit by rating important traits during cultivar evaluation.

2.4 Training in management of on-farm trials

2.4.1 Learning objectives

- To learn about establishing on-farm trials.
- To understand the basics of designing and execution of on-farm trials.
- To follow best management practices for carrying out trials successfully.

2.4.2 Why an on-farm cultivar trial?

The best performing cultivars of the research station may not be suitable for differing managements, soils and environments. So, on-farm trials are done to test the cultivars adaptability to different farm conditions.

It is better to have several cultivars tested on a farm to address the changing needs of the market as well as to tackle the biotic and abiotic stresses (late monsoon, pest outbreaks, and deficiency of nitrogen) and each cultivar is more or less adapted to different ones. We focus on the yield and also the stability of yield and matching the market quality criteria, thanks to the diversity.

These trials should allow each farmer to test at least five cultivars each year, and then decide if she/he wishes to grow some of these lines in the near future.



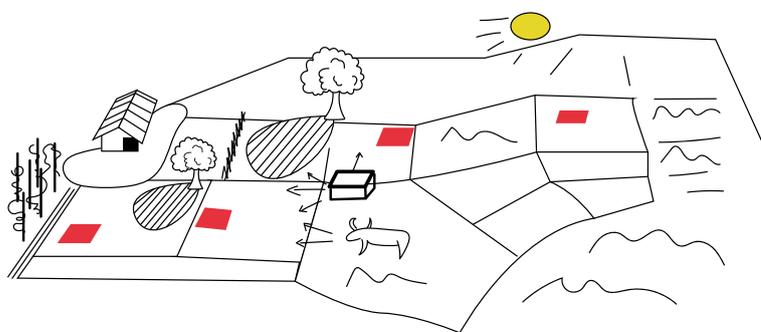
Discussion with farmers on management of on-farm trial

2.4.3 Which cultivars should I use?

Each farmer can start a trial preferably with three to five cultivars and one common check which is repeated twice. The number of plants per cultivars for a trial depends on the time, land resources and farmer's commitment. It should be at least 50 plants per cultivar, preferably in double rows. It is important that the management of the on-farm trial is conducted according to good agricultural practice. It is better to do timely weeding of three cultivars plus checks than to have a weedy trial with ten cultivars. But there must be a minimum of three cultivars and the check for comparison.

The staff can provide promising cultivars from the research trial, corresponding to the demand of the farmer. Both hybrids and varieties of *G. arboreum* should be included.

A meeting day could stimulate the exchange among farmers on different cultivar types and varieties in the area. The staff has to help the farmers to set up the trial, mark the different cultivars in the field, separate them from the remaining trials (e.g. with one row of okra or finger millet), harvest and keep them in harvest bags with different colour coding. Farmers can be trained to measure yield per picking and counting of cotton bolls. Research staff will provide a data sheet and collect information from each farmer on cotton management (soil type, sowing time, sowing density, replanting, thinning, fertilisation, irrigation, weeding, pest control, etc). Also, farmer's preference or ranking of the cultivars including the checks will be collected. The research staff will maintain the varieties by storing them in seed banks, removing off-types, checking fibre quality and GM contamination by performing the strip test before sowing and at harvest (for strip test see chapter 6.5).



Set the trial (in red) far from borders, compost, shadow, grazing and waste-throwing zones. Avoid the slopes. Integrate the trial in a field.

Figure 16. Site selection for the trials

2.4.4 Where can I set my trial?

The on-farm trial should be set in the cotton fields of the farmer. The soil must be homogeneous, and must represent the soil type of the farmer's field. Management (particularly nutrient input) should be representative of soil type and farmer's practice. If the trial has received more nutrients than farmers' fields, farmers are likely to select cultivars not adapted to their practices. The field should be leveled without any slopes and protected from grazing animals.

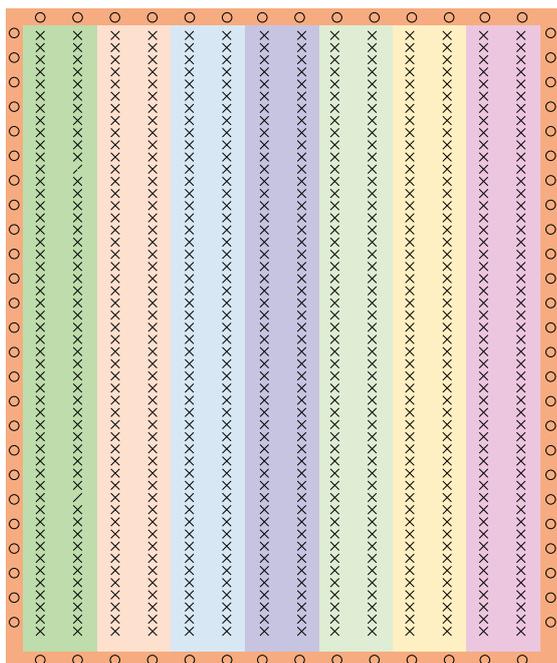
To select a homogeneous field one can look at the weed emergence pattern in the field; a difference in emergence pattern of weeds could be used as an indicator for the difference in the soil and/or soil moisture dynamics, which can directly or indirectly affect the performance of genotypes.

Then, pay attention to these points (Figure 16):

- Avoid any shadow of nearby trees and shrubs.
- Avoid proximity of compost area, animals or a waste dumping zone, as the gradient of organic matter can have a strong influence in the outcome of the trials.
- Avoid competition of border rows, for example, a row of tall pigeon pea as border row can have a strong detrimental effect due to competition of nutrients, water and sunshine.
- Avoid slope of the field, the organic matter often flows to the bottom.

2.4.5 How do I set my trial?

You will have a small plot integrated into the cotton field. In the best case, you have two replications per field. However, this is often not feasible. To allow statistical analysis, at least one check (always the same cultivar across on-farm trials and years) should be planted in replication (see 2.2.2 on-farm baby trials). The cultivars should be randomised and planted in double rows (Figure 17). You have to put the rows randomly (not the hybrids always on the left and the *arboreum* on the right for example). The randomisation should be different in the different on-farm trials.



A small replication (two lines of each) and a randomisation do not require much more work, but can prevent selection errors due to soil heterogeneity.

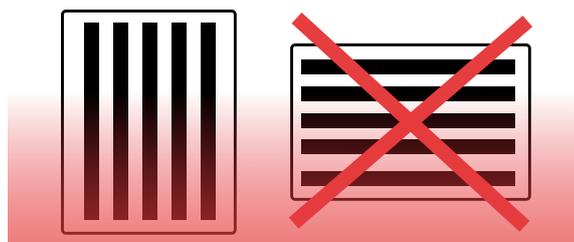
Figure 17. Replication and randomisation

If there is a slope or a possible gradient (due to shadow, organic matter or border), you must give the right orientation to the rows (Figure 18).

The distance between the plants can be discussed and will depend on the soil type (light or heavy soil), cultivar type (*hirsutum* > *arboreum*) and farmer's practice including his/her weeding method.

To facilitate exchange with other farmers and the communication with the staff, each cultivar should have a small label, colour or sign to denote it. Therefore, the varieties are easily associated with a name, collection bag, etc.

In the case of heterogeneity, it is good to have a cotton border, with the cotton of the field (Figure 19).



The red gradient could be a slope, a water or an organic matter gradient.

Figure 18. Field orientation in relation to gradients

2.4.6 Challenges of on-farm trials

You have to evaluate the cultivars of the current trial several times throughout the season. As you have only the check replicated, small inhomogeneities in the field might cause confounding effects with the cultivar.

Potential risks:

- Eliminating a cultivar because its row is affected by inhomogeneous soil e. g. soil compaction or waterlogging during monsoon or increased occurrence of weed in that particular row compared to the rows of other cultivars.
- Selecting a bad cultivar because the row had particularly good soil, e. g. increased dose of fertiliser compared to the other cultivars, more water due to leaking drip irrigation.
- Losing the trial due to delayed weed management based on time constraints of farmers or interrupted irrigation due to lack of water or power supply.
- Look at the border cotton to detect heterogeneity (Figure 20).

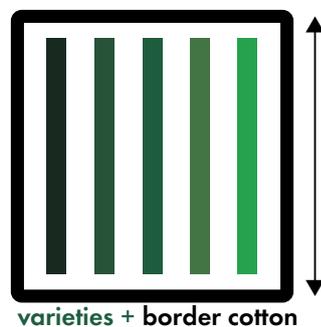
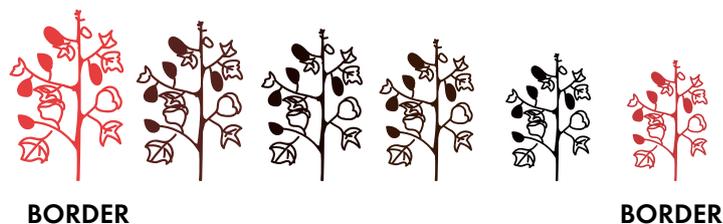


Figure 19. Placement of the border cotton



The height of the cultivars and the border cotton indicate a gradient. Without the border cotton, it would be difficult to see that cultivar 4 is smaller, because there is a gradient in soil properties.

Figure 20. Relevance of the border cotton

- Having incomplete or unreliable data on cultivar trial and its pedoclimatic conditions due to infrequent visits from the farmer and facilitator as a result of the scattered nature of on-farm trials.
- Keeping the farmers motivated to continue with trials on their field for the entire crop duration, making sure that they do not uproot them because the plants' performance or anticipated yield is not promising.
- Assessment of yield from just one harvest period.

To ensure the cultivar performs consistently, it should be tested for at least two years in a similar agroclimatic zone.

To improve statistical power, the different on-farm trials can comply as replication if the same check is included in each on-farm trial, twice. This allows a very good assessment of the different cultivars and prediction of their suitability for the diverse growing conditions.

Take the seeds of the best plants from each row (if only one or two plants are good, take seed preferentially from them), to have enough seeds for next year's sowing. However, only use this seed in the next season after ruling out the possibility of GM contamination through cross-pollination. For small amounts of available seed and large samples, Bt strip tests are the most cost-efficient. The measurement can be done (i) most reliably on the seed before sowing, (ii) on the fresh leaves of young plants, as Bt

is reduced during vegetation period and also during drought (please note that manufacturers give no guarantee for testing leaf samples), and (iii) on a sample of harvested seeds of selected plants. Be cautious that spraying Bt as organic plant protection will also result in a positive signal in the strip test. Before carrying out the Bt strip test, first, ascertain the quality of the Bt strips with seed or leaves from Bt cotton. Poor storage conditions and high temperature can deteriorate the quality of the strip test.

If the cultivar performs consistently well across many locations in two seasons it can be promoted to be cultivated on a larger scale as a pilot trial in a farmer's cotton field (0.1 acres, next to the commercial cotton production), while individual plant selection and maintenance breeding continue in parallel.

Besides agronomic performance, yield, and yield stability, it is also important to test that the fibre quality fits the demand of the textile industry. Certain cultivars show variable fibre length and micronaire (fibre fineness) based on growing conditions, whereas other cultivars consistently show high fibre quality. Cultivars with poor average fibre quality compared to the check will be discarded and not tested further.

2.4.7 Source of good quality seed, testing and preparation

In advance, order seed of cultivars with desired fibre quality (above 28 mm length and mic between 3.7 and 4.2) and good performance under organic conditions.

- Selected seeds should be genetically pure with high germination percentage and vigour.
- While selecting the seed variety, care should be taken to select the varieties preferred by the farmers in a particular area.
- Good quality, healthy and uniform sized seeds from a reliable source should be selected.

Good seeds are pure (of the chosen variety), full and uniform in size, viable (ensure more than 80 % germination) and free from weed seeds, seed-borne diseases, pathogens, live insects, or other matter particles.



On-farm assessment of yield

2.4.8 How do I manage my trial?

Don't put more compost on the trial than in the field; otherwise you will select cultivars adapted to high input. If you spray an organic repellent, spray it on all the rows (not only the rows affected by a pest).

Don't overspray, as it will limit the selection of the most pest-resistant cultivars.

It is important that weeding is done regularly and in time (do not overlook even if there is a lot of work in the field). Proper weed management is decisive for the quality of all organic cultivar trials.

In the case of *G. arboreum* first nipping is recommended at about three feet plant height and a second at four feet to avoid lodging of plants, as they can even grow over six feet in fertile soils.

Most importantly, follow timely recording of your management operations, plant observations and harvests from on-farm trial fields in the provided data sheet templates. If you re-sow some plants or some rows, remember to inform the staff.

Invite your neighbours over and explain your engagement in participatory cotton cultivar evaluation and breeding. Motivate them to take up on-farm trials on their farms.

Exchange your experience with the staff and other farmers involved in PPB during field visits in November/December and the planning meeting in March / April each year.

Land preparation

- The surface of the field should be made as leveled as possible. A well prepared/leveled field reduces the efforts in crop establishment and care and increases yields. Leveled land also improves water coverage which:
 - Reduces the amount of water required for land preparation.
 - Improves crop establishment and care.
 - Decreases the time to complete tasks.
 - Results in better crop stands.
 - Reduces weed problems.
 - Results in uniform crop maturity.
- Soil condition in the selected field should be suitable for the crop.
- The field should be ploughed thoroughly without any lumps.
- Land preparation should be done early while incorporating green manure or animal manure in the field to enhance the nutrient content of the soil.
- Organic manures like farmyard manure, compost and vermicompost can be used to enhance soil fertility.
- Land preparation should also ensure that weeds are substantially removed to prevent excessive growth of weeds during the early growth phases of the cotton crop.



Incorporation of organic compost



Manual preparation of ridges and furrows

Field plan and labeling

- Label each cultivar on a field plan where a clear direction is indicated (like north, south).
- Install permanent metallic labels for each cultivar that needs to be cultivated.
- If it is possible, assign each cultivar a different colour.



Field plan and labeling

Sowing

- Planting should be done as soon as the rainy season begins to ensure that the planted seeds get adequate moisture for germination and growth.
- The ideal spacing depends on the soil type and the irrigation facilities.
- Where soils are light, and little irrigation water is available, the spacing can be narrower (e.g. 3 × 2 feet) than in heavy soils and well-irrigated land (e.g. 3 × 3 feet).
- Sow the cotton seeds at a depth of 3 to 5 cm and cover them with fine soil to avoid germinating seed to dry out.



Sowing in progress

- Depending on the seed quality and cost, three to four seeds are sown per spot.
- Re-sow cotton in gaps where the seeds did not germinate or the seedlings has been destroyed, within two weeks after the emergence of the young cotton plants.
- Thinning is done when the plants are 6 to 10 cm long, leaving the strongest two plants per hill.

Weed management

- Weed management strategies in cotton include proper crop rotation, timely soil cultivation, proper sowing density and ploughing to remove the weeds.
- For proper weed control, monitor the field at least every 10 to 14 days.
- The field does not need to be kept free of weeds throughout the season.
- In the initial stage of crop growth, weeds take up nutrients which otherwise would be lost through leaching. These nutrients are returned to the soil and made available to the cotton crop when the weeds are cut and decomposed. Weeds should be cut before seed setting.
- They also serve as trap crops, distracting pests from the cotton plant.



Hand weeding

Homogeneity of cultivars

Off-types or heterogeneity of traits might occur in certain cultivars. This should be recorded as it indicates that the cultivar is still segregating and that further generation advancement and purification still needs to be done.



Identifying off-types in the trial field

If there are only one or two off-type plants (that may have resulted from carryover of some seeds during processing or sowing of that cultivar), these cultivars need to be removed.

Water management

Application of compost and organic manure helps increase organic matter content in the soil, which is known to improve soil structure, hence increasing water infiltration and water retention in soil allowing crop to sustain better during the dry period. During the first 6 to 7 weeks after sowing, irrigation should be moderate to avoid too much vegetative growth and to encourage cotton roots to penetrate deeply into the soil.

The cotton crop is very sensitive to waterlogging, which causes increased boll shedding, thus affecting yields. For monsoon-sown cotton in India, the first irrigation should not be given until August, after the first square buds have formed.

Minimum tillage and shallow soil cultivation (hoeing) reduce water evaporation from the soil. Covering the soil with mulching materials helps to preserve humidity in the soil and to prevent water loss while enhancing increased biological life in the upper-parts of the soil.

Drip irrigation system enables farmers to start cotton cultivation before the onset of the rainy season, to bridge dry periods and to protect at least part of their fields from drought.

Use of low-cost drip system allows farmers to install drip-irrigation systems with lower investment



Field irrigation using drip irrigation system

costs, but the cheaper systems are usually less durable.

Active rainwater harvesting through pits or trenches leading to wells can help to recharge groundwater levels and therefore improve the availability of irrigation water.

Fertilisation, pest and disease control

- Fertilisation, pest and disease management should be done according to the farmer's practice which is representative of the different growing conditions for summer and monsoon sowing.
- Do not aim for optimal but representative growth conditions to avoid the often-observed high yield potential in perfectly managed on-station trials which cannot be realised under the farmer's practice. You want to select with the farmers for their benefit.

Picking of cotton

- Provide correct labels with unique sample numbers for each plot. Indicate the cultivar by using the colour of the bag and take all measures to avoid mixing.
- Supervise and monitor picking regularly to avoid mixtures.
- Pick the cotton after the morning dew has dried, so that the cotton is dry and less prone to fungus when stored.
- Only pick fully matured bolls.
- Use the bulk of first and second flush picking (i.e. picked up until the end of November) for fibre



Picking of the cotton flush.



Ginning in progress using hand gin

analysis as the quality drops during the end of season and results of fibre quality are only due in January.

- Use third and fourth picking until uprooting for total yield assessment.
- The picked cotton, when completely dry, should be stored in a dry place and protected against insects and rats in steel storage boxes, if not ginned immediately.
- Through the entire organic cotton processing chain, it is important to avoid contamination through mixing of seed by separating organic from conventional cotton

Ginning, fibre quality testing and storage

- Harvest of first and second picking period (up to the end of November) will be ginned on small manual gins.
- Fibre analysis can be conducted in-house, at a university or in contract labs. All partners should use the same testing facility. At least 100 g of lint is needed for detailed fibre analysis, 300 g for testing yarn quality like number of counts.
- Storage should be done off the ground and protected from rain or standing water. Seed cotton as well as seeds must be stored in closed metal boxes or plastic drum at a dry place to protect against insects, rats and squirrels.

Data recording and data management

- All assessments must be conducted according to instructions to compare results across locations, years and partner organisations.
- Enter all recordings in provided field book and transfer data to a computer in the provided electronic template as soon as possible. Use a program for double data entry to exclude errors during transcripts.
- Perform easy descriptive statistics and outlier tests to identify errors whilst plants are still in the field.

2.4.9 Summarising activities

1. Selection of ideal field for conducting on-farm trials.
2. Facilitation of meeting with farmers for the selection of preferred varieties.
3. Set up of optimal trial design with the same check replicated twice.
4. Setting up on-farm trials following management instructions.
5. Carry out timely field operations and practices.
6. Recording of crop management, cultivar observations and harvest from the on-farm trial.

3. Crosses to Create Genetic Diversity

3.1 Goals

- Conduct crosses to increase genetic diversity for various traits.
- Combine traits of different parental lines to one cultivar.
- Out-perform parental lines by transgressive segregation.

3.2 Activities in the field

3.2.1 Crossing

Cross breeding is a major step in plant breeding, including PPB to increase the genetic diversity of many different traits by combining genes of two or more parental lines. Only a broad genetic diversity allows for selection and development of improved cultivars. Based on the results from on-farm mother trials, parental lines that were adjusted to organic growing conditions or have other important traits like tolerance to bollworm or sucking pests, shall be selected and such crosses are designed to combine best traits of each parental line. It is preferable to cross many different lines to achieve high genetic diversity, e.g. $A \times B$, $C \times D$, $E \times F$, $G \times H$ involving eight parents in four crosses, instead of $A \times B$, $A \times C$, $A \times D$, $B \times C$, $B \times D$, $C \times D$ involving only four parental lines in six crosses. It is also possible to conduct three-way crosses $(A \times B) \times C$ or double-crosses $(A \times B) \times (C \times D)$. In the case of elite parental lines, it might be worthwhile to conduct reciprocal crosses $A \times B$ and $B \times A$ as female parent provides cytoplasm as well as extrachromosomal genes to the progenies.

- Crosses should be conducted in both species *G. hirsutum* and *G. arboreum*. Use for crosses only cultivars with fibre length >28 mm.
- Each year at least 20 to 50 new crosses should be planned according to defined breeding goals to obtain 20 to 50 segregating populations in the F2 generation.
- Test parental lines with Bt strip or PCR tests to avoid any Bt contamination in the initial breeding material.
- Plant parental lines of 20 to 30 plants every two to three weeks to allow synchronous flowering of male and female parents. Crossings can be conducted in the field during normal growing season, under-protected tunnels in off-season or in temperature regulated poly houses.
- At least 20 flowers should be emasculated and pollinated, ideally not all from the same plant (see details below).
- Keep some extra emasculated flowers unpollinated and unprotected to estimate outcrossing rate due to insects or wind. Low seed set indicates little risk of outcrossing at that site.
- Keep some extra emasculated flowers unpollinated but protected to check the efficiency of your crosses. If all anthers have been timely removed during emasculating, no seed setting should take place.
- Additional flower buds and flowers shall be removed and the crossed bolls regularly monitored to avoid loss of seed or labels.
- Monitor crosses and selfed flowers weekly to avoid loss of bolls, drop of labels or mixtures during the season.



Crosses conducted using promising parents



Cotton flower buds

- Bolls spoiled by rains, or damaged by insects, or otherwise damaged, should be picked separately and discarded.
- Take great care when harvesting the bolls from the crosses.
- The picked seed cotton from crosses should be processed manually but only when it is completely dry.
- Remove all the fibre from the F1 seed manually. Do not use mechanical ginning as there is a risk of small cracks in the seed caused by the pressing from rolls.
- The goal is at least 20 F1 seeds per cross. All F1 seeds are bulked together and stored in dry, cool place, safe from insects and animals in a glass jar or sealed metal box.

3.2.2 Selfing

To maintain high purity of parental lines and to provide pure Nucleus Seed as a starting point of seed multiplication, selfing is another important technique for cotton breeding, to avoid any out-crossing due to insects or wind.

- Select cotton flower buds before opening and protect them either with small bags as used for crossings or by sealing the flower bud with wool/thread to prevent flower opening.

For capacity building in PPB, it is important that farmers/staff are well trained in crossing and selfing. For further instructions on selfing, please see on page 50 of this manual.

3.2.3 Multiplying obtained F1 seed to obtain segregating F2 populations

F1 seed is like a hybrid with a high level of heterozygosity; therefore, F1 seeds cannot be selected. Firstly, F1 seed needs to be self-pollinated to obtain segregating F2 populations. This can be done in a subsequent season, or if possible, under-protected conditions in the off-season to speed up the breeding process. The goal is to obtain at least 300 to 500 F2 seeds to start bulk or pedigree selection (see Chapter 4.3.1).

3.3 Workshop on crosses

3.3.1 Learning objectives

- Inform about breeding process and importance of crosses to increase genetic diversity.
- Technical training in emasculation, pollination and selfing.

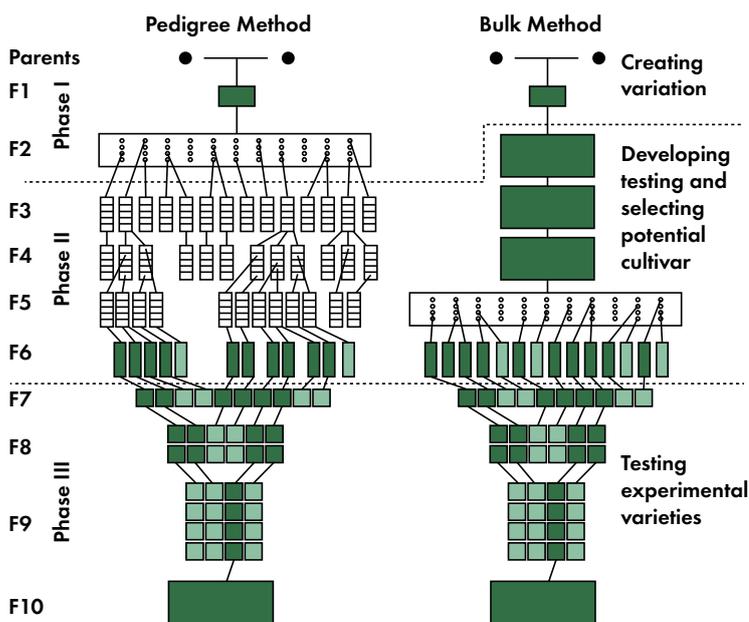
3.3.2 Training in crossing

Materials required

- Breeding schemes
- Scheme for hybrid production
- Paper bags (red and white colour)
- Magnifying glass
- Tray
- Thread
- Notebook and pencil etc

3.3.3 Technical instruction in crosses

- Explain why we need crosses and link to F1 hybrids.
- Explain mass selection, pedigree and bulk selection (Figure 21).
- Discuss selection of parental lines that shall be crossed with each other to achieve segregating populations with a chance to develop improved cultivars. In general, we try to combine positive traits of two different parental lines, or to improve one specific trait by crossing of elite plants, e.g. two highly resistant plants, with each other.
- Based on the planned crosses, the parental lines should be sown three times, each with two weeks difference to achieve synchronous flowering of male and female parental line. Make sure parental lines have been tested before sowing to be GMO-free with Bt-strip or PCR tests.



adapted from Osei et al. 2014

Figure 21. Schematic example of hybridisation methods



Bud selection

- Mature flower buds of healthy and vigorous mother plant are selected for emasculation before flower opening (i.e. candles).
- Perform manual emasculation by removing anthers from the selected buds to avoid self-pollination. Petals together with anthers (male organs) are removed without damaging the stigma and pistil (female organ) in the middle of the flower with help of thumbnail or scissors. The best time for emasculation is 2 to 6 pm one day before flower opening. The emasculated buds are covered with a paper bag and marked with red colour to prevent natural out-crossing.
- In the case of **genetic male sterile (GMS) plants** that gain more and more attention to ease hybrid seed production, the flower bud also needs to be protected, but there is no manual emasculation



Emasculation



Collection of flowers from parental lines

- necessary as plant produces non fertile pollen. Thus, self-pollination is genetically excluded.
- **Pollination of emasculated buds** is performed the next morning by dusting the pollen of flower from male parent. Collect the flowers of male parent, remove petals of flowers and keep in water glass. Preferred time for pollination is in between 8 to 11 am, as stigma receptivity is observed to be maximum during this period. Generally, 3 to 4 buds of female parent are pollinated by one flower of the male parent.



Pollination



Farmers practicing crossing techniques

- Following pollination, the red paper bags are replaced by white paper bags to protect the pollinated flower. A thread is tied on the pedicel of crossed bud for identification of crossed bolls and parental lines at the time of picking.
- To test successful emasculation, bag a few emasculated flower buds without pollination, and mark them with a label. There should be no seed set; otherwise your emasculation was not complete.

3.3.4 Precautions to be observed

- After pollination, always tie a cotton thread on the pedicel of flower bud for proper identification of crossed bolls to enable easy picking and to avoid mixing at the time of harvest.
- Unemasculated and unused flowers of the same plant should be removed on a daily basis to retain actually crossed flowers (bolls) on the female parent.
- If daily removal is impossible, unemasculated and unused flowers should be harvested separately from crossed flowers (bolls) on the female parent to avoid the mixing of cotton at harvest which could lead to contamination.
- Destroy leftover collected male flowers after use.

3.3.5 Summarising activities

1. Introduce farmers to breeding schemes.
2. Technical instructions in emasculation, pollination and selfing.



4. Plant Selection in Segregating Populations (F2–F8)

4.1 Goals

- Introduction of pedigree selection involving farmers and other stakeholders.
- Development of improved and locally adapted cultivars for organic production derived from crosses and farmers' selection in contrasting environments.
- Combine natural and human selection for breeding cultivars with high resilience under stress conditions considering breeding goals prioritised by farmers, ginners, spinners and textile industry.

4.2 Introduction

Selection, as a breeding procedure, involves identification and propagation of individual genotypes or groups of genotypes from mixed populations, or from segregating populations derived from specific crosses of two or more parental lines. Selection is the process of planned improvement in the performance of specific cultivars for certain traits through conscious choice. Unless genetic variation can be identified and distinguished from environmentally caused variability within the mixed population, selection may not be effective in isolating the desired genotypes.

The sources of variation may be a natural mutation, defined crosses, segregating populations or natural outcrossing. Commonly used selection methods in handling the segregating population developed through natural or manual hybridisation are pedigree, bulk or mass selections (Osei et al. 2014). The crop ideotype consists of several morphological and physiologically desirable traits which contribute to improved yield or any other desirable characteristics compared to prevalent crop cultivars. The major challenge of breeding is to select genotypes that combine different traits simultaneously. This reduces selection gain for a single trait.

4.2.1 Major objectives in cotton breeding

Major objectives in cotton breeding are as follows:

- Consistently high and stable seed cotton yield (e.g., high number of bolls, less monopodia).
- Resistance or tolerance to biotic stresses especially against a broad range of pests (e.g. bollworm, aphids, jassids, whitefly, thrips etc.) and diseases (e.g. damping off disease, mildew).
- Resistance or tolerance to abiotic stresses (e.g. drought, flooding, cold).
- Better adaptability to low external input growing conditions and different soil fertility levels (e.g. nutrient and water use efficiency).
- High seed vigour and fast youth development (e.g. good germination, fast growth and soil coverage).
- Easy picking (e.g. big bolls, good boll opening, no lint dropping, tall plants).
- High production of lint (e.g. high ginning out-turn).
- Improvement in fibre quality (>28 mm fibre length, 3.7–4.5 micronair, >29 fibre strength, etc.).
- Adaptation to mechanical harvesting (e.g. synchronous flowering, compact plant type, leaf drop).



Selecting an ideal plant type

4.3 Activities in the field

4.3.1 Single plant selection in early generations (F2–F5) of segregating populations

- Plant 5 to 10 segregating populations obtained by own crosses (see Chapter 3) or derived from parents in the F2 to F5 generation on-farm under irrigated heavy soil with summer sowing and on-farm under rainfed light soil condition with monsoon sowing.
- Plant at least 200 to 500 individual plants per population and site, with 2 feet distance between plants (Figure 22).
- Populations should be separated by a separator row (e.g. okra, finger millet).
- Do not use intensive pest control or nutrient application to increase stress level and natural selection pressure.
- Individual farmers and breeders in each population will mark the most promising plants with a coloured ribbon based on visual observation on plant health, earliness, tolerance to sucking pests and bollworms, number of bolls, boll size, boll opening, lodging tolerance etc.
- Number of selected plants can vary between populations, as some parents match better than others.
- Only selected plants will be harvested. All bolls of one plant will be bulked to obtain F3 seed. Keep track from which population and plant, the seed is derived.
- Use small gin to obtain fibre and determine fibre length as this is a highly heritable trait.
- Discard all selections with fibre length <27 mm for *G. arboreum* and <29 mm for *G. hirsutum* populations.
- The remaining selections (around 5–10 % of total F2 plants sown in the field) will be used to grow the F3 generation in the same target environment.
- Again, record from which population / cross the plant was derived and from which F2 plant. Label the F3 seed accordingly e.g. F3_Pop A (Rocky × Amazon)₄, i.e. F3 seed derived from F2 plant #4 of the population A, derived from crossing Rocky (female) with Amazon (male parental line).
- F3 seed from each line is sown in one row in the following season (around 20 seeds per plant).
- Selection procedure will be repeated as described above to obtain F4 seed.
- This is followed by one or two additional selection cycles until the F5 or F6 seed is obtained.

Keep records on the pedigree e.g. F6_Pop A (Rocky × Amazon)_{4_1_2_1}, i.e. F6 seed derived from population A of cross Rocky × Amazon, #4 plant in F2, #1 plant in F3, #2 plant in F4 and finally from #1 plant in F5.

- Based on this coding system it is possible to trace back the origin and identify related plants. For example F6_Pop A (Rocky × Amazon)_{4_1_2_5} is a sister line to the one mentioned above as they share the same F4 plant #2 (i.e. they have the same grandmother), while F6_PopA (Rocky × Amazon)_{4_3_2_1} only shares the selected plant in the F2 generations, thus they share the same great-great grandmother. If in doubt, it is advisable to select progenies from genetically diverse populations and origins to cover a greater range of traits.

4.3.2 Selection of advanced generations (F6–F8)

- From F5 or F6 onwards, the plants should be almost stable with heterozygosity of between 3.1 % to 6.3 %.
- This allows farmers to plant one to four row plots (depending on seed availability) of selected F6 line in one or two replications (Figure 23) at the given target environment as described in the mother trial in Chapter 2.
- Plot trials allow much more detailed assessments of agronomic traits and a first reliable estimation of the yield potential.
- Plants with promising performance will be selected for further testing in F7 and F8. Also, maintenance breeding will start to produce Nucleus Seed.
- For this reason, healthy and vigorous plants that correspond to the characteristics of the whole plot will be marked and selfed (see Chapter 3).
- From the F7 generation, sufficient seed will be available to test performance at multilocation trials with both heavy and light soil type.
- From F8 onwards sufficient data and seed should be available to enter into on-farm baby trials and first pilot trials (see Chapter 2).
- Most promising candidates will be forwarded for seed purification and multiplication (Chapters 5 and 6) and testing to obtain either officially released cultivars or licence to sell Truthfully Labeled Seed (see Chapter 7).
- Do not use pest control and limited fertilisation to have a more stable environment.

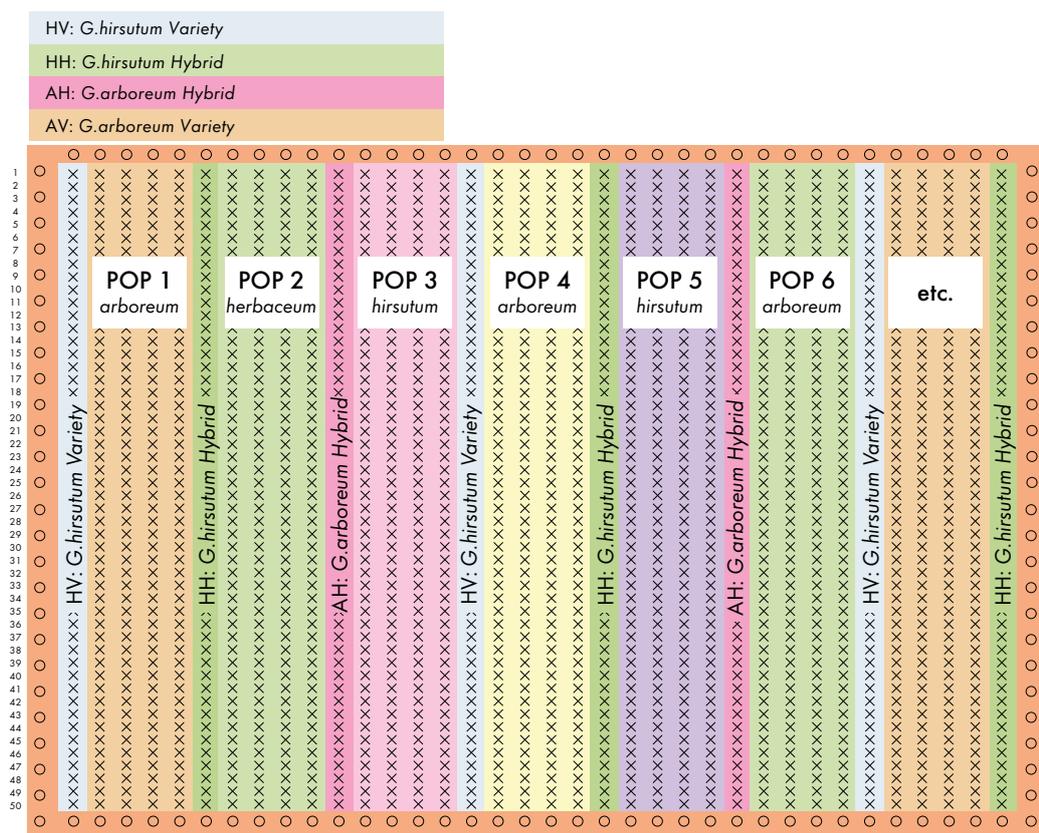


Figure 22. Trial design for early generations of segregating populations

Procedure for early generations trial of material F2 to F5

- **Site selection:** Select one heavy soil and one light soil to select plants under different growing conditions
- **Sowing period:** Summer sowing (mid May until the end of May) on heavy soil, with drip irrigation; monsoon sowing (mid to end of June) on light soil, rainfed
- **Sowing:** Sow 4 rows of cotton with at least 200 plants per population (4 rows \times 50 plants = 200 per population), if you have sufficient seeds. Sow 3 seeds per planting hole to avoid gaps (600 seed, about 60 g per population and site). If you have limited seeds plant only one seed per planting hole.
Add standard cultivar to compare performance of segregating material and allow for individual plant selection (IPS) at harvesting time.
Use manure or Trichoderma treatment to enhance seed health.

- **Plant spacing:** 3 \times 2 feet for heavy soil and 2 \times 1.5 feet for light soil to see the performance of individual plants.
- **Separator rows:** Separate the populations with a separator row of morphologically different cotton species (e. g. *G. herbaceum*, *G. barbadense*), or alternate *arboreum* and *hirsutum* populations or with a check line. Trial should be surrounded by other morphologically different cotton species (e. g. *G. arboreum*, *G. herbaceum*, *G. barbadense*), or oca / millet or pigeon pea, which must be cut back to avoid border effects.
- **Thinning:** 10–14 days after germination, thin plants, leaving only one plant per planting hole.
- **Gap filling:** No gap filling, as later sown plants will be smaller and difficult to evaluate.
- **Plant protection:** Check weekly for weeds, pests and diseases and treat immediately according to organic guidelines.
- **Cutting top:** Cut arboreum cultivars back twice (at 3 feet and later at 4 feet) to avoid tall plants.

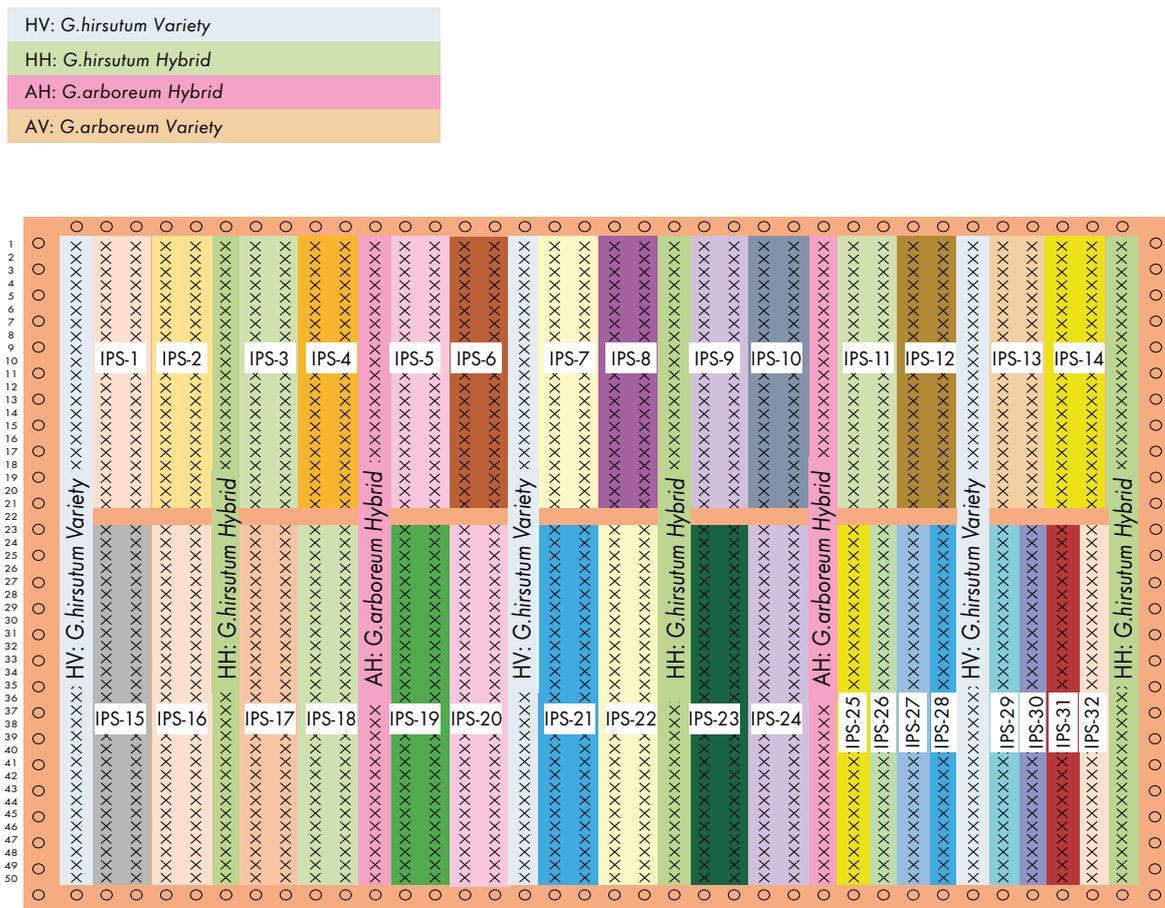


Figure 23. Trial design for advanced generations of segregating populations

Procedure for advanced generations trial of material F6 to F8

- Test your promising advanced lines in the different generations F4 to F6.
 - Plant from each individually selected plant (IPS 1–32).
 - 1–2 rows per IPS of 2–30 plants up to 90 seeds, about 5–10 g per IPS and site
 - Surround the trial by morphologically different cotton species (e.g. *G. arboreum*, *G. herbaceum*, *G. barbadense*), or oca/millet or pigeon pea, which must be cut back to avoid border effects.
- If your material F6 to F8 is already homogeneous, you can enter it into an on-station trial with four row plots of 2 replicates.
- **Site selection:** Select one heavy soil and one light soil to select plants under different growing conditions
 - **Sowing period:** Summer sowing (mid May until the end of May) on heavy soil, with drip irrigation; monsoon sowing (mid to end of June) on light soil, rainfed

- **Sowing:** Use 3 seeds for each planting hole to avoid gaps. Add a standard cultivar to compare performance of segregating material and allow for individual plant selection. Use manure or Trichoderma treatment to enhance seed health.
- **Plant spacing:** 3 × 2 feet for heavy soil and 2 × 1.5 feet for light soil to see the performance of individual plants.
- **Thinning:** 10–14 days after germination thin plants, leaving only one plant per planting hole.
- **Gap filling:** At the time of thinning, close gaps, either by replanting additional seedlings or by resowing.
- **Plant protection:** Check weekly for weeds, pests and diseases and treat immediately according to organic guidelines.
- **Cutting top of arboreum:** Cut arboreum cultivars back twice at 3 feet and later at 4 feet to avoid tall plants.
- (IPS) at harvesting time

4.4 Workshop on single plant selection

4.4.1 Learning objectives

- To understand the importance of selecting the ideal plant type.
- To critically examine and identify important traits in individual plant selection aligned to the specific breeding objectives.

4.4.2 High and stable fibre yield

The high yield of high-quality fibre (lint) is the ultimate objective of cotton breeding. The yield of a cotton plant can be determined by the number of bolls, size of the bolls, and percentage of lint per seed cotton yield (ginning outturn, GOT). The number of bolls can be a major contributor to the final yield. For plants to be high-yielding, they must be prolific and should set a minimum number of bolls required for potential yield.

Lint production is affected by the number of sympodia (i.e. branches carrying cotton bolls) in comparison to the number of monopodia (i.e. branches that do not produce cotton bolls). The occurrence of bollworm and sucking pests (e.g. jassids, thrips, whitefly, aphids) causes considerable yield loss due to boll dropping or damage. Cotton yield is also challenged by the limited availability of nutrients especially, nitrogen and water. A long vegetation period of cotton usually results in higher cotton yield but, also incurs higher labor costs due to increased number of pickings. The ginning outturn is determined from the weight of the lint that is obtained from a given weight of seed cotton. Larger seed weight results in lower GOT. Diploid desi cotton species like *G. arboreum* and *G. herbaceum* have smaller 100 seed weight and thus higher GOT than the tetraploid *G. hirsutum* and *G. barbadense*. However, farmers are currently getting paid per kg of seed cotton, not taking into consideration the higher GOT of the desi cotton.

4.4.3 Fibre quality

Cotton fibre is the major commercial product from cotton. The fibre develops within bolls consisting of three to five locules. Average compartments of tetraploid cotton is four, whereas desi cotton have generally three compartments. In contrast to *G. hirsutum* and *G. barbadense* that show upward boll opening, the *G. arboreum* and *G. herbaceum* have downward opening bolls susceptible lint dropping. Cotton fibres are separated into two groups according to fibre length (Lint and linters/fuzz).



Selection of plants for high fibre yield



Selection for long staple length and fibre quality

The spinning performance of cotton fibre is associated with the length, strength, and fineness of the fibres. These characteristics vary in different type of cotton cultivars (see table 2). Special instruments are available that accurately measure these quality parameters in cotton fibre samples.

a. Length

Fibre length is an important factor which directly contributes to the quality of yarn. Variation in the length of the cotton fibres can be found within a cultivar and even within a single boll. Uniformity in staple length improves spinning performance, increases the utility of the cotton, and reduces waste. The range of fibre length is limited within species. While *G. barbadense* produces fine fibre up to 38 mm, *G. hirsutum* varies between 24 to 32 mm, while *G. arboreum* and *G. herbaceum* have shorter fibres between 18 to 26 mm. Only few *G. hirsutum* introgression lines of *G. arboreum* reach fibre length of 28 mm, which is highly demanded by the textile industry.

b. Strength

Fibre strength is important in determining yarn strength. Cotton from cultivars that produce weak fibres is not considered desirable by the industry. The structure of the inner layers of the cotton fibres affects its tensile strength. Weak fibres are difficult to handle in manufacturing processes.

c. Fineness

Fibre fineness is associated with the perimeter or diameter of the fibre, and the thickness of the fibre wall. Cotton fibres from some cultivars feel soft and silky; fibres from other cultivars feel coarse and harsh. The difference in the way cultivars feel is determined by the fineness or coarseness of the fibres. As the fibre consists of a single cell, fibre length and fineness are also correlated with each other. The optimal fineness is between 3.7 to 4.5 micronaire. *G. hirsutum* are often below and *G. arboreum* are often above that optimal range.



Selection for fibre strength and fineness



Plant selection for resistance against biotic stresses

4.4.4 Tolerance to biotic stresses

a. Pest tolerance

Bollworms are the most deleterious pest of cotton, especially under irrigated conditions (Vonzun et al. 2019). Early maturing cultivars can escape a severe attack of pink bollworm, but remain vulnerable to the attack of American and spotted bollworm. Early bollworm attacks cause a severe drop of square heads and bolls, and great damage to infested bolls. Sucking pests have become predominant in the last decades, destroying the photosynthesis active leaf area, resulting also in severe yield losses. Due to the harder leaves and leaf hairiness, the *G. arboreum* genotypes are relatively less susceptible to sucking pest compared to *G. hirsutum*, while *G. barbadense* is most sensitive. Selection of cultivars with high tolerance to the different bollworms and sucking pest should be conducted under environments with high natural pest pressure. For example, high sucking pest attacks occurred under shallow soil in rainfed conditions, while bollworm attack is often observed in irrigated heavy soil. Thus, it may be necessary to select different environments based on the tolerance of the different pests.

Selection should be based on characteristics that suppress insect population development, such as hairy leaves or high gossypol content in the square. These characteristics have been used in breeding for resistance.

b. Disease tolerance

Several diseases are associated with the cotton plant, like damping-off disease. Breeding for host-plant resistance has been an effective method of control against major pathogens. Development of multi-disease resistance has also received much attention in the breeding for resistant cultivars. Healthy plants with high vigour, least prone to infestation, must be screened and selected.

c. Weed suppression

It is ideal to select vigorous cotton plants that grow faster, and can achieve good soil coverage to suppress upcoming weeds.

4.4.5 Tolerance to abiotic stresses

a. Low availability of nutrients

Cotton has a long vegetation period and demands a lot of nutrients for the production of seed and fibre. Limited supply of nitrogen results in less developed plants with few bolls that fall very much behind their yield potential. As provision of plant available nitrogen is generally a challenge in organic agriculture, while phosphorus can be fixed to soil

due to high pH value. Thus, cultivars should be selected that develop good root systems and produce stable yields even under nitrogen and phosphorus deficiency. Cultivars should also have symbiosis with plant growth-promoting rhizobacteria and mycorrhiza and convert nitrogen into seed instead of increased vegetative growth. Plants that use nutrients efficiently will have less boll drop compared to normal plants under limited nutrient availability. Based on the geology, there are two main cotton-growing conditions in India, one on fertile deep clay soil with nutrients from rivers and those on shallow sandy soils in hilly regions, where most of fertile top soil has already been eroded. Nutrient use efficiency combined with improved organic fertilisation regimes is extremely important to achieve good yield level under sandy soils.



Plant selection for abiotic stresses

b. Waterlogging and Drought Tolerance

Water is often a limiting resource for cotton production in dry areas of the world. Limited sources of irrigation water and higher fuel costs for pumping is causing breeders to look for cotton strains with more efficient water use under drought conditions. Selection should vary according to the availability of irrigation water. As sowing time has a big impact on yield potential, different cultivars are required for irrigated cotton production for summer sowing (May, beginning of June) and rainfed cotton production with delayed monsoon sowing (end of June to end of July).



Plant selection for drought tolerance

For rainfed conditions

- Medium plant height (90 to 120 cm) and more compact growth habit allowing higher plant density.
- Smaller leaf area and thick leaves with sparse hairiness to prevent transpiration.
- Deep roots.
- Withstanding logging during monsoon.
- High water and nutrient use efficiency after monsoon for early and synchronous ripening.
- Medium to large boll size.
- High degree of resistance to pests and diseases.

G. arboreum has higher water use efficiency due to okra-type leaves, thicker leaves and improved root systems compared to *G. hirsutum*. It is therefore very well adapted to rainfed conditions.

For irrigated conditions

- Medium plant height (90 to 120 cm) with terminated plant growth.
- Sympodial plant habit making pyramidal shape for wider plant spacing.
- High harvest index to avoid too much vegetative growth of monopodial branches without bolls.
- Tolerance to waterlogging during monsoon.
- Early maturity to avoid high incidence of bollworms (150 to 165 days).
- High level of tolerance to bollworm and sucking pests, as pest incidence is much higher under irrigation with 2 to 3 crops sown per year than under rainfed conditions.
- High nutrient use efficiency especially for nitrogen and phosphorus.
- Fast germination, high vigour and fast soil coverage to suppress weeds.
- *G. arboreum* needs to be trimmed once or twice due to its indeterminate growth habits to avoid tall, bending plants which hamper the picking of bolls.



Gossypium herbaceum with salt tolerance

c. Salt tolerance

Genetic differences to salt tolerance during late growth stages have been observed in cotton strains grown in saline soils. Salt tolerance during germination, early growth, and during late vegetative growth has been observed specifically in *G. herbaceum*. Therefore, this species is mainly grown in coastal regions. It is important to select healthy, vigorous plants in segregating populations grown under saline conditions.

d. Strong wind

The ideal plant selection must focus on identifying short plants with short fruiting branches, single-boll set, early fruiting and early maturity and most importantly, the seed cotton that adheres tightly on the boll at maturity. These are all characteristics desired in storm-proof cotton cultivars.

4.4.6 Early maturity

Flowering of the cotton plant is indeterminate, with bolls set over a period of time. Small compact plants, small bolls and seeds are generally associated with earliness in a cotton cultivar. Earliness is influenced by;

- how early the cotton plant begins to set squares and to flower.
- how rapidly the new flowers develop.
- the length of time required for the bolls to mature

Rapid fruiting and early maturity reduce losses to pests by escaping the most susceptible vegetation period, especially for bollworms. Early maturity also increases production efficiency by reducing the use of fertiliser, protective chemicals, or irrigation water. Therefore, it reduces the overall cost of cotton production. In addition, it allows farmers to plant a second food crop after cotton. The long production period also guarantees higher yield potential as the plant can compensate for pest and disease attack or unfavorable weather conditions.

Therefore, certain farmers prefer long duration crops. While modern *G. hirsutum* hybrids are selected for earliness, desi cotton usually has an indeterminate growth and longer vegetation period.



Plant selection for early maturity

4.4.7 Easy picking

For farmers, especially female farmers, big boll size, four to five compartments and good boll opening is of great importance as it influences picking efficiency (kg seed cotton/ picking day). Bolls need to open sufficiently to permit cotton to fluff, and be easily removed from the capsules. On the other hand, cultivars must stick well enough to the capsules to avoid lint dropping during a storm or late harvest. A medium tall plant, with many sympodia, synchronous ripening, non-lodging on fertile soils, bolls spaced along the main stem and set high enough off the ground would be the ideal. In general, upward opening bolls of *G. hirsutum* and *G. barbadense* are easier to pick than downward opening bolls of *G. arboreum* and *G. herbaceum*. Hybrids in general have larger boll size than varietal lines in all crop species.

4.4.8 Training of farmer in single plant selection

- Facilitator will explain pedigree selection and discuss the visual assessment of the most relevant traits on a single plant basis and their reliability based on the heritability of the traits, determined in replicated mother trials in multiple locations. For example, yield can hardly be assessed on a single plant level, while fibre length is quite predictive from single plants.
- Farmers are divided into different groups (e.g. female and male farmers and growing conditions) and made to visit experiment plots of the segregating populations in respective growing conditions (e.g. heavy soil with irrigation and summer sowing and rainfed cultivation in medium or light soil with monsoon sowing), which is representative or most similar to their own farm
- The different farmer groups get ribbons with different colour coding. They will mark only those plants that are well adapted to the growing conditions and which perform above average. In general, not more than 5 to 10% of the plants should be marked.
- The selection made by male and female farmers will be compared, as well as the difference in selection between research staff and farmers' groups. The selection made by participants will be considered in the breeding workshop.
- Through this workshop, male and female farmers should be encouraged to provide land and time resources to start a selection of segregating populations (F2 to F5) or already advanced lines in on-farm baby trials on their farm or within the network of other partners.

4.4.9 Summarising activities

1. Explanation of pedigree breeding.
2. Discuss the most important traits under their conditions (see chapter 2) and the ones that can be assessed on a single plant basis in early generations.
3. Field visit to segregating populations under contrasting growing conditions.
4. Tagging of best plants based on a visual selection in the field.

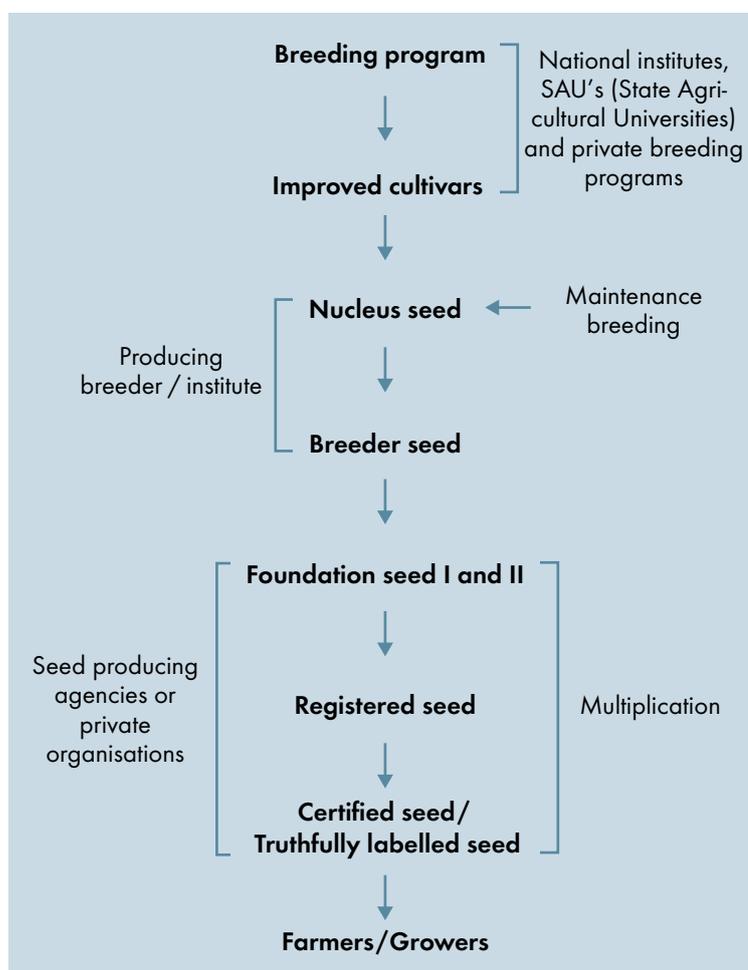


Selection of plants suitable for manual picking

5. Maintenance Breeding and Seed Multiplication

5.1 Goals

- Guarantee genetic purity and stability of candidate varieties or breeding lines to ensure that progenies show identical characteristics and performance.
- Keep varieties free from outcrossing and Bt-contamination.
- Provide purified nucleus and breeder seed for commercial production of certified seed or truthfully labelled seed.
- Guarantee uniformity and stability, which are essential requirements for official variety release.



adapted from Virender Singh Lather, ICAR 2017

Figure 24. Breeding program, seed types and responsible agencies

5.2 Different seed categories

The Indian Seed Programme is backed by a strong crop improvement program in both the public and private sectors. This program addresses the generations' system for seed multiplication limitations by utilising a phased system. Certain norms are followed for the production of pure seeds. The seeds of notified varieties are multiplied through a well-defined seed chain recognised by the Indian seed system, including nucleus, breeder, foundation and certified seed (Figure 24) (Prasad et al. 2015). Notified varieties that passed the state-wise or all India variety testing protocol concerning Distinctness, Uniformity and Stability (DUS) and Value for Production and Use (VCU) are legally protected by the plant breeder's right. The whole process of notification and quality seed production of notified varieties is regulated by the provisions of the Seed Act of 1966. The bags of different classes of seed are identified with specific coloured tags as per section 5 of the Seed Act (Table 7).

After variety release, quality of breeding lines must be maintained to ensure high performance across many generations. Therefore, it is vital that maintenance breeding starts from selected single plants, which show all the characteristics of the released variety. Seed from one plant is multiplied for a maximum of two generations to obtain nucleus and breeders seed.

Nucleus seed

The nucleus seed is the origin of the selected variety and must be maintained and multiplied without any impurity. It is obtained from a single or a few selected healthy plants grown in an isolated plot. Typically, the breeder, responsible for the development of the variety, produces it. Phenotypic observations such as plant size, growth habit, colour and shape of various plant parts, days to flowering, maturity, etc. are taken into account, observed and recorded by the breeder for variety release and maintenance breeding.

This stage is the most critical phase in the seed development because any erroneous selection of the nucleus seed plants would adversely affect the successive generations. Once these plants are selected, the flowers are isolated to ensure self-pollination. The seeds of each plant are individually collected and ginned to obtain plants for the row maintenance breeding process.

Table 7. Definition of the different seed categories

	Definition	Produced by	Produced for	Quality requirements
Nucleus seed	<ul style="list-style-type: none"> Original seed source of the cultivar 	<ul style="list-style-type: none"> Breeder of the cultivar 	<ul style="list-style-type: none"> Breeder of the cultivar 	<ul style="list-style-type: none"> Purest seed category
Breeder seed	<ul style="list-style-type: none"> Produced from Nucleus seed Only small quantities 	<ul style="list-style-type: none"> Breeder of the cultivar Usually kept with breeder/ research institution Responsibility for overall production lies with SAU 	<ul style="list-style-type: none"> Public sector seed production agencies Private seed producers have free access to released public sector cultivars 	<ul style="list-style-type: none"> Quality control lies with the breeder No seed certification done Monitoring by All India Coordinated Cotton Improvement Project (AICCIP)
Foundation seed	<ul style="list-style-type: none"> Progeny of breeder or of foundation seed itself 	<ul style="list-style-type: none"> Seed produced from breeder seed by selected growers under close supervision 	<ul style="list-style-type: none"> Companies Seed producer organisations Public research institutions 	<ul style="list-style-type: none"> Production from foundation seed is permitted for stage 1 and 2 if genetic purity is maintained 50 m isolation distance must be maintained for cultivation
Certified seed	<ul style="list-style-type: none"> Progeny of foundation seed or of certified seed itself 	<ul style="list-style-type: none"> Grown by seed producer organisations and farmers 	<ul style="list-style-type: none"> Sold for commercial crop production 	<ul style="list-style-type: none"> Not allowed to exceed three generations beyond foundation stage 1
Truthfully labelled seed	<ul style="list-style-type: none"> Uncertified seeds can be labelled and sold as truthfully labelled Seed 	<ul style="list-style-type: none"> Grown on large scale by private seed companies 	<ul style="list-style-type: none"> Sold for commercial crop production 	<ul style="list-style-type: none"> Not certified Requirements need to be controlled by the seed producer organisation

Source: Marty 2013 based on, Government of India, Virk (1998)

Breeder seed

The harvested nucleus seed is used for the production of breeder seed in isolation. The offspring of the individually harvested single plants are grown in single-row plots next to each other and actively selected for varietal typical traits. The harvested seed of all selected plants generates breeder seed, which is the direct offspring of the nucleus seed. The original plant breeder of the breeding programme or institution directly controls the maintenance breeding by selecting seeds with the desired qualities for certification as breeder seeds.

The genetic purity of breeder seed must be maintained at 100 percent. The entire production process is monitored by the scientists and officers of the Seed Certification Department and the representatives of the National Seed Corporation. The responsible seed certification agency issues a golden yellow colour certificate for this category of seed.

Foundation seed

Multiplication of seeds for commercial seed production starts with breeder seed. The maximum of three generations of multiplications are allowed to

be certified as organic seed. The first offspring from the breeder seed is called foundation seed. Its production must be approved by a certification agency. The genetic purity of foundation seed should be maintained at 99.9 percent.

The National Seed Corporation, the State Farms Corporation of India (SFCI), has the responsibility to produce foundation seed which satisfies the demand of public varieties. Private seed producers can also produce foundation seed. Seed certification agencies issue a white colour certificate for foundation seed.

Registered seed

Registered seed is the progeny of foundation seed and is approved and certified by a certifying agency. This seed is suitable for the production of certified seeds. It is the second generation of breeder seed, also sometimes called foundation seed stage II. The genetic identity and purity should conform to the particular crop's specified standards. A purple colour certificate is issued for this category of seed.

Certified seed

Certified seed is the progeny of foundation seed or registered seed, and must meet minimum national seed certification standards. A certification agency supervises and approves the production of certified seed which must conform to the Department of Seed Certification definition of uniformity and purity.

The seed of this class can be produced by the State and National Seed Corporation or private seed companies. Certified seed can also be produced on the farms of enterprising growers under the supervision of authorised agencies. A person or company who grows and distributes the certified seeds as per the procedure and specifications of the certification agency is called certified seed producer.

This is the commercial seed which is available to farmers. Its genetic purity should be 99.5 percent. Seed certification agencies issue an azure blue colour certificate for this category of seed.

Truthfully labelled seed

Farmers or private seed companies can produce truthfully labelled seed. This seed is only tested for its physical purity and germination. However, companies are required to maintain field and seed standard as per the Seed Act. Seed inspectors can take samples for checking the seed quality. Using

this method, any farmer can produce seeds and market them as truthfully labelled seed. Under the Seed Act, the seed producer and the seed seller are responsible and liable for the quality of seed. Truthful labelling is compulsory for notified varieties, but seed certification is voluntary. The seed certification agency issues an opal green colour certificate for this category of seed. This is the second type of commercial seed available to farmers.

5.3 Maintenance breeding production of nucleus and breeder seed

To maintain the performance of selected cultivar candidates or breeding lines and to provide a pure seed (nucleus, breeder and foundation seed) for successful seed multiplication of non-GM cotton seed (Figures 25 and 29), the following critical points need to be considered:

- The breeding line must be at least in the F7 generation.
- The seed source derived from single plants has been tested to be GMO-free.
- Geographic or physical isolation of the field for seed multiplication.
- Cross-pollination and seed mixture with Bt cotton and any other cotton cultivar through selfing and border plants has been prevented (Figure 27).
- Regular inspection and removal of off-types in the field (rogueing).
- Careful picking of mature balls and processing of seed cotton to obtain nucleus and foundation seed for production of commercial seed (see Chapter 6).

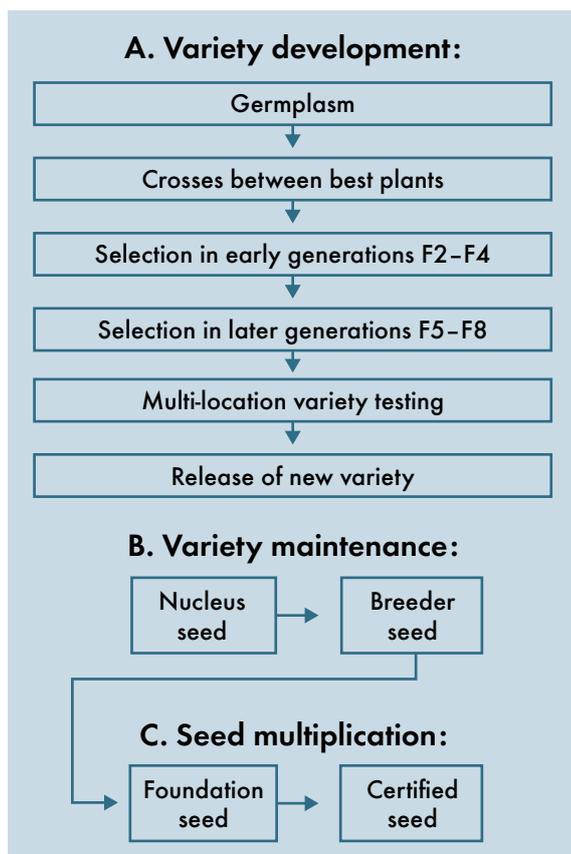


Figure 25. Schematic steps of variety development, maintenance breeding and seed production

5.3.1 Production of nucleus seed

Nucleus seed is the handful of original seed selected from the individual plants of a particular variety for maintenance and purification by the originating breeder. A qualified plant breeder supervises the multiplication and maintenance to provide breeder seed. This seed has the highest genetic and physical purity.

As illustrated in Figure 26, the maintenance of purity of nucleus and breeder seed can only be realised if each year the most representative healthy plants are selected and selfed to restart plant to row multiplication. Therefore, continuous maintenance breeding, including purification and GMO testing (see Chapter 6.6.3) in isolation, is necessary for the annual production of nucleus seed. The multiplication rate from nucleus seed to breeder seed is around 10. Thus, 100 m² of nucleus seed production will result in 1000 m² breeder seed and a 1 ha foundation seed production area.

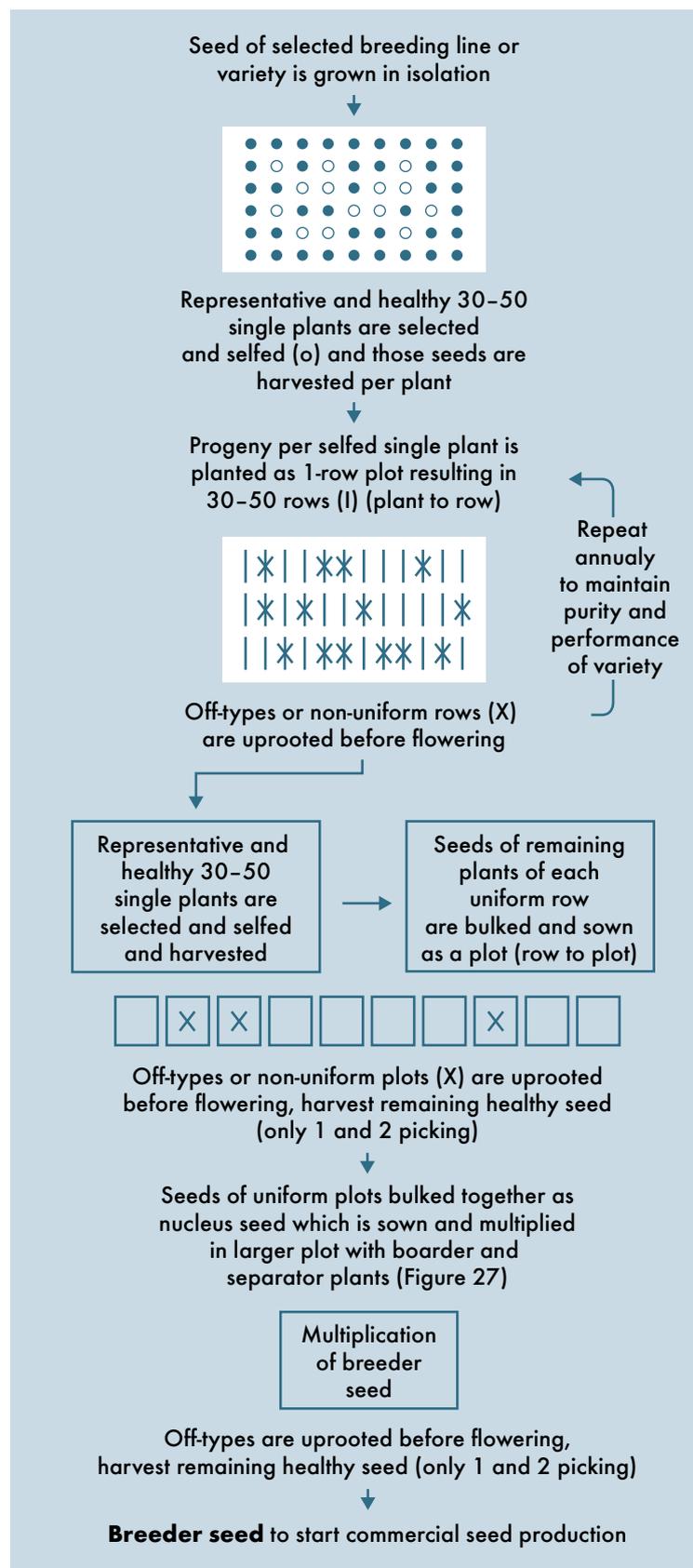


Figure 26. Scheme for nucleus seed production

Selfing of individual plants to produce nucleus seed

The selfing procedure is critical to facilitate 100 percent self-pollination, thereby avoiding contamination from GM fields and other cultivars via cross-pollination. Although this process is labour intensive, it results in pure and non-GM nucleus seed.

For selfing:

- The selfing process should begin each day around 1:00 pm throughout the flowering period.
- Search for all the mature flower buds (candle) on each plant in the multiplication plot.
- Tie all the mature flower buds with a thread both on the stalk and on top, so that the flowers will not open the next morning ensuring 100 percent self-pollination.
- Alternatively, the mature flower buds can also be covered using selfing bags so that opened flowers will remain inside the bags. However, for identification, selfed buds must be tagged with threads.



Unopened bud tied for selfing



Use of selfing bags to ensure self-pollination

5.3.2 Production of breeder seed

The breeder seed is based on the harvest of nucleus seed. Breeder seed should be sown on clean, fertile land, which was not under cotton cultivation in the previous years. The field should be appropriately isolated (refer to Section 6.3.2), and additional border rows should be used for protection (Figure 27).

Best practices for sowing, weed control and harvesting should be applied:

- Sowing the seeds: One by one in rows makes the best use of the limited amount of seed available and facilitates roguing. The row spacing should be large enough to permit the examination of the plants for possible mixture or off-types.
- Roguing: All plants that are not typical of the variety should be pulled out and removed. There should be very few plants to rogue out if the nucleus seed from the previous year was well protected from natural crossing and careful roguing was done, and if there were no impurities during cleaning etc. The roguing should be done before flowering, similar to the production of nucleus seed (see Section 5.4).
- Harvesting the breeder seed: When the breeder seed is harvested and ginned, the equipment used must be scrupulously clean and free from seeds of any other varieties. This cleanliness should be extended to carts and bags as well as to the gin itself. The breeder seed is then ready for multiplication for the production of foundation seed. For security reasons, the breeders should retain a portion of the breeder seed.

For the maintenance of breeder seed of established varieties: If no nucleus seed is available, the breeder seed can be maintained satisfactorily by using one of the following methods:

- a. Raising the crop in isolation: The breeder seed of local varieties can be maintained by growing it in isolated plots and by very rigorous roguing at various stages of crop growth, where the various plant characters are observable. The method of handling the harvest of breeder seed crop is the same as described earlier for breeder seed of newly released varieties.

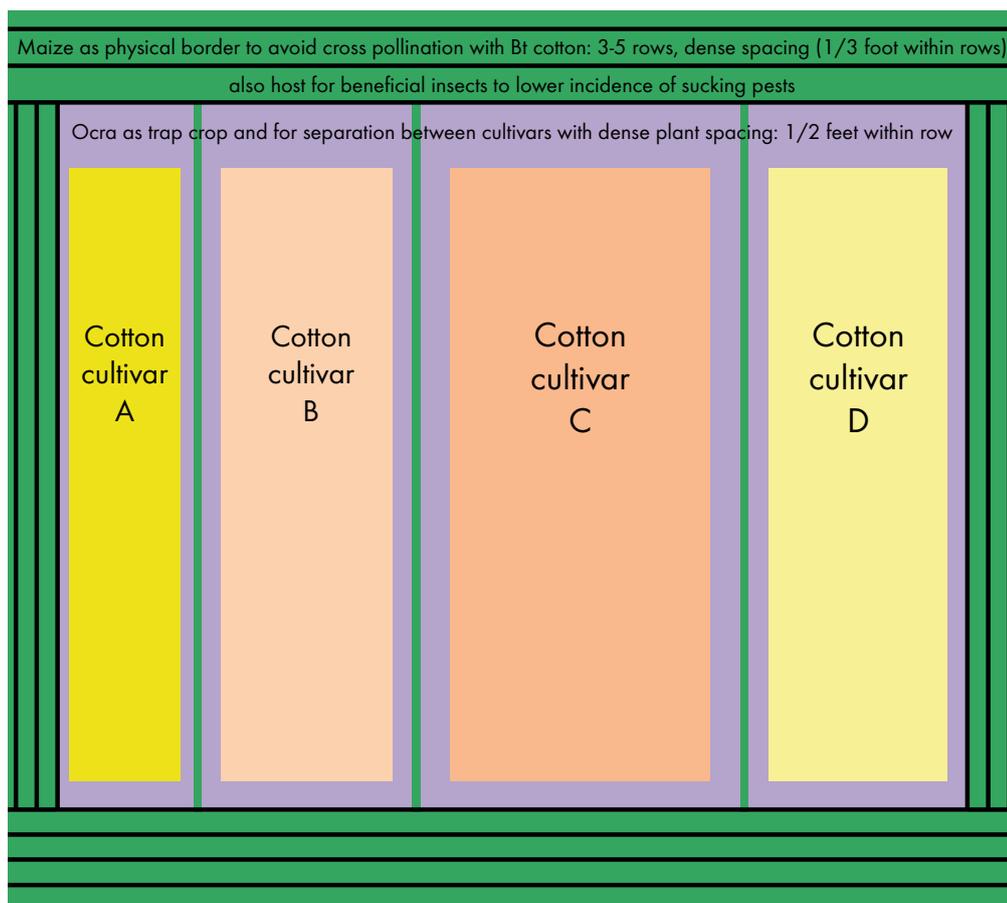


Figure 27. Protection against cross-pollination of breeder seed

- b. Bulk selection: The genetic purity of established varieties can be adequately improved by bulk selection. In this method, typically 2000 to 2500 plants of the variety are separately selected, harvested, and ginned. The seeds and the fibre from each plant are examined, and any obvious off-types are discarded. The remaining seeds are bulked to constitute the breeder seed. The other practices of handling seeds remains same.
- c. Carrying-over seed: The breeder must carry-over at least enough seed to safeguard against the loss of variety if there is a complete failure during the foundation seed multiplication phase. In addition, the breeder should arrange to have a portion of the seed originally released stored under ideal conditions to safeguard the variety further.

5.3.3 Commercial seed multiplication

The bulk of the breeder seed is the basis for commercial seed production by public or private seed production agencies (Figure 24). Breeder seed must be tested for GMO contamination using Bt-strips of 20 individual samples and qPCR test of the bulked sample before it is sown for the production of foundation seed in isolated fields. Off-types are removed before flowering, and only healthy and representative plants are harvested as bulk. qPCR tests using several representative samples of the harvested foundation seeds verify the absence of GMO contamination and must be conducted before the seed is further multiplied (see also Chapter 6). The foundation seed can be multiplied one or two times to directly obtain the certified seed, or first registered seed, or so-called foundation seed stage II, which can be further multiplied to obtain certified seed. It is crucial that only three multiplication steps are conducted from breeder seed in isolation with strict GMO testing to obtain officially certified seed

of released varieties which can be sold to traders or organic farmer organisations (Figure 28). It is also possible to sell non-certified seed as truthfully labelled seed, whereby seed companies are liable for the quality of the seed.

For commercial seed production, it is crucial to have far-sighted planning to ensure timely commencement of nucleus seed production even before releasing the cultivar, to avoid a delayed

market introduction. With a multiplication rate from 10 to 30, it takes at least four to five years from the nucleus seed to become commercial seed, the seed reaching the farmers (Figure 29). The different requirements for official variety registration and commercialisation of truthfully labelled seed are explained in more detail in Chapter 7.

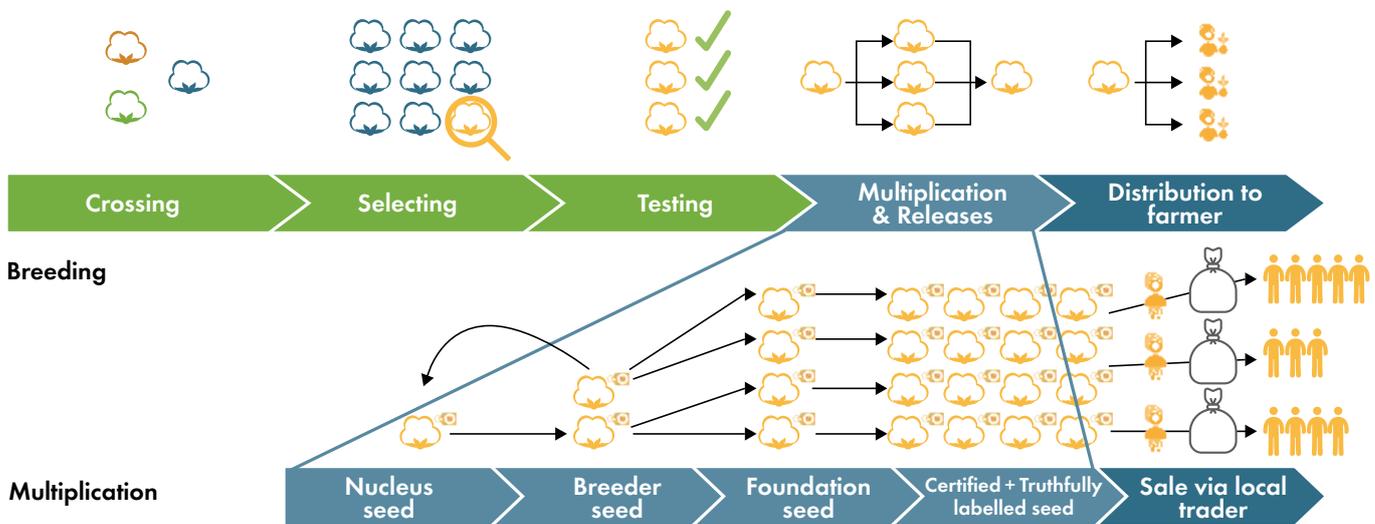
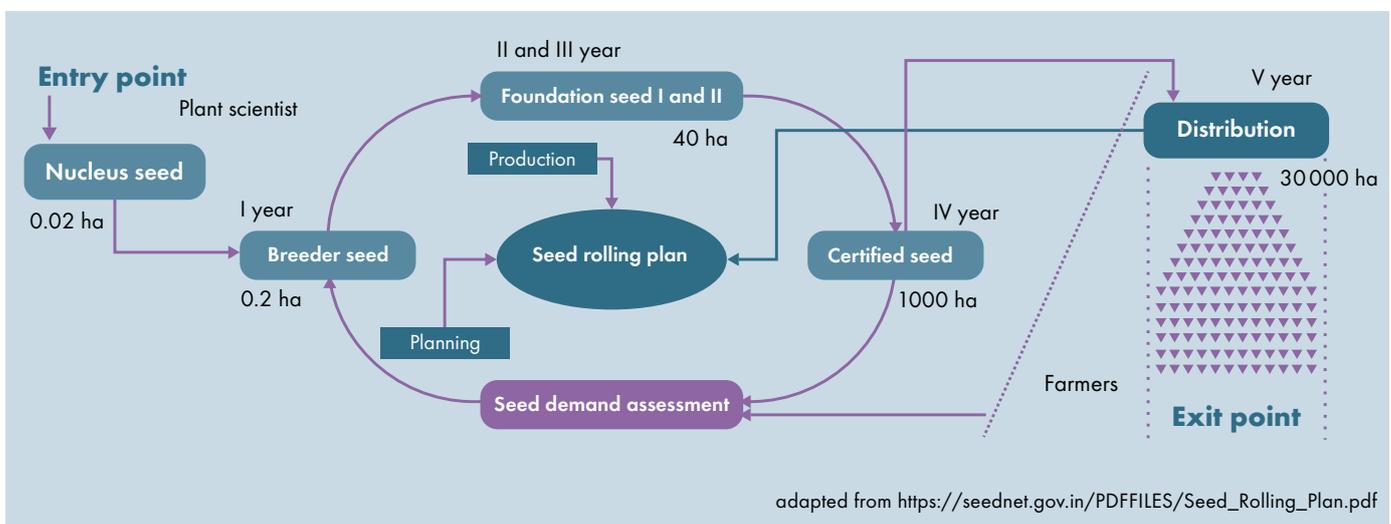


Figure 28. Continuous maintenance breeding including purification and GMO testing in isolation. Maximum of three multiplication steps from nucleus seed to commercial certified seed with an annual renewal of nucleus seed



adapted from https://seednet.gov.in/PDFFILES/Seed_Rolling_Plan.pdf

Figure 29. Seed maintenance and rolling plan

5.4 Training on roguing

5.4.1 Learning objectives

- Understand the importance of roguing to maintain genetic purity.
- Critically examine key characteristics for identification and differentiating between a rogue and specific varieties/cultivars of different cotton species.
- Learn how to perform roguing in organic cotton fields.

5.4.2 What is purification through roguing?

- Roguing is the removal of all off-types or mixtures of plants.
- Roguing in any seed production plot is extremely important to prevent pollen from off-type plants to cross-pollinate, causing irreparable damage to the crop.
- Plants with heterogeneous characters in a seed production plot are off-types.

5.4.3 What is the purpose of roguing?

- Mutations and outcrossing in self-pollinated crops occur at specific, measurable frequencies, generally at low levels. This results in genetically and often phenotypically different plants called off-types.
- Seed producers are responsible for keeping these variants at a minimum level and reporting the type of rogues removed and their incidence levels to the breeder responsible for maintaining the variety.



Different type of rogues

- This exchange of information between seed growers, seed certification agency and breeders is a critical varietal identity control point for maintaining the purity of breeding lines and varieties.

5.4.4 Off-type / Rogue

- Any undesirable plant in a seed crop.
- The simplest rogue type are different cotton species or cultivars.
- The most difficult rogue type are plants that have arisen from genetic change within a variety or from outcrossing including deviation in leaf shape and colour, hairiness of leaf and stem, plant architecture, flower colour, boll shape and/or fibre length.
- Rogues also result from simple mechanical mixtures with other cultivars, crop kinds or weeds that are difficult to separate: mixtures can occur at many stages in the production of pedigreed seed.
- Volunteer growth from last year's cotton crop, diseased plants, and objectionable weeds.

5.4.5 Off-types and characteristics of plants

- A plant of a different species, for example, a *G. hirsutum* plant in a *G. arboreum* multiplication field.
- A plant of a different cultivar, for example, a F1 hybrid of the same cotton species with increased vigour in a varietal line seed multiplication field.
- A plant derived from outcrossing in the previous season.



An off-type plant



Identification of off-type - Reproductive stage

- Plants that are taller, shorter, more compact or bushy than most of the plants of the population with specific characteristics.
- Plants with different leaf types (hairy or smooth/ glabrous) (see Figure 30) or with unusual branching habit (monopodial or sympodial).
- A plant with different coloured petals, pollen or with a striking difference in flower type.
- Presence or absence of pubescence: if the existence of leaf hairiness in a plant is different from the rest of the population, it is an off-type.
- Plants with strikingly early or late boll formation.
- Plants with inconsistent size, shape or colour of bolls: If most of the plants bear big size bolls then those bearing small balls are off-types.
- Bolls with inconsistent fibre length.
- Diseased or insect-damaged plants.

5.4.6 How can rogues be distinguished from the norm of the variety?

- The same characteristics used to distinguish varieties can be used to recognise and describe rogues.
- To find and remove genetic rogues, growers require a sound knowledge of the distinguishing characteristics of the variety being produced.
- Many factors contribute to the distinguishing characteristics which vary individually in the degree of their expression, and that may be influenced and modified by environment, fertility and growth stage.
- Despite these limitations, growers will first identify a rogue in the standing crop by recognising a deviation from the normal range of the variety.
- The suspect plant can then be checked against the norm to determine if it is a rogue.
- Off-types can be identified by their morphological differences from the true to type plants.
- It is essential to know the characteristic features of the variety for easy identification of rogues and efficient rogueing.
- As a primary step, any plant found outside the rows has to be removed as it may be a volunteer plant.
- Remove all the plants that are either taller or shorter than the rest of the variety.
- Off-type plants can also be identified by a different leaf blade size, shape and colour of the leaf sheath, flower colour, or fibre length.



Gossypium hirsutum

G. barbadense

G. arboreum



G. hirsutum

G. barbadense

G. arboreum

Figure 30. Identification traits of different species of cotton

5.4.7 When and how should crops be rogued?

Generally, plants show deviations throughout the growing season as they grow and mature. Many identifying characteristics reach optimum expression at different stages of plant development (see Table 8). Once a crop is established, it must be rogued repeatedly and systematically to take advantage of the differences as they appear at different stages of growth including germination, early vegetative phase, squaring, flowering, boll development, boll opening and fibre quality.

- Floral parts of particular importance should be monitored through the flowering period.
- Hairiness of the leaves or stem is evident in the foliage.
- Roguing in early vegetative growth makes it easier to find and remove volunteer plants.
- For successful roguing, a roguing plan must be established and observed.
- The first apparent rogues that can be removed are other crops, diseased plants and weeds.
- Plants maturing much earlier than the main crop are suspect, and should be removed.
- All rogues should be removed from the field and destroyed.
- Pests and weeds may be managed with appropriate contingency measures, and during crop inspection periods, the organic control measures adopted should be brought to the attention of the breeder.
- Growers should remember that the primary objective of roguing is to maintain genetic or varietal purity and freedom from other kinds.
- The time of day is an essential consideration in roguing. Roguing during mid-day is not as effective as in the morning or afternoon due to the glare of the sun.
- Some rogues are easily spotted when backlit by the evening sun when one bends low over the crop and looks into the sun.
- The plots should be sown in single-spaced rows or subplots to assist roguing. Only one seed per hole should be sown.

5.4.8 Summarising activities

1. Explanation of different categories of seed.
2. Production and maintenance of nucleus and breeder seed.
3. Field visit for carrying out roguing.

Table 8. Stages of crop growth and characters to be examined for roguing

Stages of crop growth	Characters examined
Vegetative	Height, colour of vegetation, leaf size, colour, shape and orientation, stem colour, hairiness of leaf and stem, branching, diseased plants
Flowering	Corolla colour, pollen colour, flower stalks, petal spot, bracts shade, pigmentation
Harvesting	Boll size, shape, colour, beak, texture and pittings, fibre length and micronaire



Roguing at the vegetative growth stage.



Proper spacing and lighting are vital for effective roguing.



6. Organic Seed Production and Seed Quality

6.1 Goals

- Increase the availability of high-quality seeds of GMO-free cotton cultivars.
- Establish an organic seed value chain.
- Empower farmers in organic cottonseed production.

6.2 Role of farmers in organic seed production

The introduction of genetically modified Bt cotton hybrids in India in 2002 has strongly shifted cotton breeding and seed production from public seed programs to commercial seed companies. In 2012, more than 90 percent of the cotton-growing area was covered by Bt cotton. Hence, most seed companies stopped non-GM variety development and seed production, and those who continued are threatened by unintentional GMO contamination. Therefore, organic cotton production has the challenge of ensuring a reliable supply of untreated seed of GMO-free cultivars with good yield potential under organic conditions. Non-GM farmers, which includes all organic farmers, experience increasing difficulties in finding suitable cotton seeds (NEMES 2010). Therefore, it is of great importance that farmers get involved in GMO-free seed production and reorganise the cottonseed value chain.

Participatory Plant Breeding (PPB) presents an opportunity for organic and low input farming systems. PPB can enable farmers to develop locally adapted cultivars, on-farm seed multiplication as well as maintaining and increasing genetic diversity, which will contribute substantially to farmers' non-GM seed security in the future. It also shifts more responsibility toward farmers; thus, farmers will play a central role in re-establishing a GMO-free seed chain in India.

For successful seed multiplication of non-GM cotton seed, the following aspects must be considered:

- The seed source must be free of GMO based on individual qPCR testing.
- A proper field must be chosen for seed multiplication.
- Cross-contamination with Bt and crossing among cultivars must be avoided.



Seed multiplication plot.

- The field must be properly maintained.
- The cotton should be picked for seed multiplication only.
- After harvest, the plants in the field must be thoroughly GMO tested with Bt strips and qPCR.

6.3 Organic seed production of inbred lines

Seed production generally starts from foundation seed, which is multiplied to produce certified or truthfully labelled seed. As certified or truthfully labelled seed can be sold to farmers, at least two representative samples from every seed lot must be tested by qPCR before sowing. Upon a negative report proving the seed to be free of Bt contamination, it should be made available for sale/distribution. Third-party certification should not be considered as an alternative to qPCR testing.

Similar precaution needs to be taken for maintenance breeding and production of nucleus and foundation seed. However, due to a large amount of seed that needs to be produced, it is not possible to follow the mother plant to daughter row principle. Instead of protecting individual flowers from outcrossing through coverage, outcrossing should be minimised by isolating the seed production field from other cotton fields. In general, multiplication of one cultivar per field is recommended. One

should also be mindful about the presence of pollinators close to the cottonseed production fields.

The multiplication rate from foundation seed to certified seed ranges from 20 to 40.

- The field should be as distant as possible from any Bt field (50 to 100 m) or any neighbour who might be cultivating Bt cotton (Table 9).
- For GMO-free cottonseed production, only the central part of the field should be used; field borders can be used for food or seed production of other crops.
- No Bt cotton should have been planted in the field in the last few years.
- The soil should be fertile, and good growing conditions should be created by using sufficient organic fertiliser.
- Water supply should be sufficient and regular using drip irrigation to avoid loss of seed before harvest due to drought (access to water and electricity).

6.3.1 Avoidance of GMO contamination

Cotton is mainly a self-pollinating crop. However, outcrossing can occur to the extent of 2 to 20 percent depending on local conditions. The abundance of various pollinators and their activity can contribute to a higher outcrossing rate and also bears a significant risk of cross-contamination with Bt cotton. It is advisable to conduct organic seed production in areas with no commercial cotton production, to keep seeds GMO-free. This includes plots between isolated hills or close to natural parks.

How to avoid GMO contamination:

- Use only high-quality foundation seed that has no GMO contamination based on qPCR tests.
- Use only one cultivar in a specific field.
- Maintain a distance of 100 m from other cotton fields.
- Sow a tall border crop like pigeon pea or maize with a high plant density.
- Use a broad border of cotton plants that are only harvested for lint but not for seed.
- Remove all off-types, roguing at least 3 to 5 times during the vegetation period as described in Chapter 5.4.
- As soon as the first bolls burst, harvest the seeds of 50 random plants across the whole field and perform individual Bt strip tests. Repeat this procedure every three weeks.

Table 9. Minimum isolation distance from other cotton fields

Seed type	Foundation	Certified
Varieties	100 m	50 m
Hybrids	100 m	50 m



Field selection



Risk of Bt contamination through outcrossing of close-by fields



Pest management using yellow sticky traps.



Pest control using solar-powered light traps.

6.3.2 Optimised pest, disease and weed control

How to proceed:

- Install a fence around the field to protect seed production from goats and cows.
- Use the push-pull method planting a trap crop for bollworms, e.g. okra, on the borders of the field and a few maize plants in the field itself to attract beneficial insects to combat sucking pest. Make sure to harvest and burn the okra plants before the bollwormeggs hatch.
- Remove sick plants and those affected by mealybugs.
- Promote natural enemies of pests by providing suitable habitats by intercropping cotton with flowering plants, applying mulch, setting up bird perches, etc.
- Reduce the development of pest populations by intercropping pulses or other crops in cotton.
- Use pheromone traps to disturb egg-laying of the insects.
- Last season's cotton crop residues should be removed from the field or buried by ploughing the field to reduce the survival of pests in the pupa stage in cotton stalks and seeds.
- If preventive measures are not sufficient to control and retain the pest populations below the economic threshold level, direct control methods may be used to manage the pests:
 - Biological control that involves living organisms or germs to affect the pests (e.g. *Trichogramma*) or,
 - Natural pesticides like neem spray, garlic-onion-pepper extract, pyrethrum, etc.
- Knowledge of the major cotton pests and of the damage they can cause to the crop helps farmers to find the right strategy for managing pests.
- Mass trapping with light, sticky and pheromone traps should be used for reducing and monitoring pest populations.
- Careful and continuous monitoring of the pest levels and disease symptoms in the cotton fields during the entire growth period (from approximately four weeks after sowing up to the second harvest) is essential, as it helps to keep track of the pest population and allows timely use direct control methods, if required.
- Frequent and timely weeding, especially during the vegetative stage of the crop, is most important.

6.3.3 Special harvest and processing instructions for seed production

Picking of cotton from the seed multiplication field

How to proceed:

- Assign workers for picking to one field with one cultivar only, provide correct labels for each bag in the colour of the cultivar and take all measures to avoid any possibility of subsequent confusion.
- Regularly supervise and monitor the picking process to avoid confusion.
- Inform the field workers on the importance of seed quality and the risk of GMO contamination. Making them aware that their wages do not depend on the weight harvested, but on the quality of the harvested seed.
- Pick the cotton after the morning dews have dried, so that the cotton is dry and less prone to fungus during storage.
- Only pick from the centre, not from the border of the field (5 m distance).
- Only pick from mature and healthy plants. Do not pick bolls from sick or bad looking plants.
- Bolls spoiled by rains, damaged by insects, or otherwise damaged, should be picked separately and discarded. Such bolls can be picked 10 to 15 days ahead of the first picking. The damaged bolls should not be picked during normal pickings for seed purposes.
- Timely cotton-picking gives slightly better seeds with higher germination rate.
- Use only pickings of the first and second flush, as they have the best seed quality (i.e. picking until end of November).
- Use the third and fourth picking for fibre harvest, not for seeds.
- Take representative samples from the different harvests and conduct Bt strip tests.
- The picked cotton, once completely dry, should be stored in a dry place and covered, if not ginned immediately.



Harvesting of cotton bolls



Picking of cotton

Processing

- It is vital to avoid contamination and mixing of seed throughout the organic cotton processing chain.
- If possible, separate the seeds of the different cultivars and avoid any risk of mixing with conventional cotton.
- Ginning should be done in separate gins installed at authorised seed processing units or farm gins applying adjusted setting that allows gentle separation of fibre and seed. This should be done under close supervision to ensure purity and avoid GMO contamination and seed damage.
- Check also, if the fibre length is according to the variety description.
- The ginned seeds (fuzzy seeds) are graded by hand sorting.
- Sieve the seeds in two types of mesh to remove small, shrivelled and broken seeds as well as dirt and dust.
- Representative samples should be taken from each harvest to conduct qPCR testing for GMO contamination.
- The dry seeds should be put into bags and properly sealed and labelled with the cultivar name, multiplication field, and the harvest date. The results of GMO and germination testing are added later.



Separate gin for seed production to avoid contamination



Linted and delinted cotton seeds.

6.3.4 Seed storage

- Appropriate storage conditions contribute to maintaining cotton quality and seed viability.
- The GMO-free seeds must be separately stored, away from any GMO cottonseed.
- Proper care should be taken to prevent contamination from dust or chemicals, especially fertilisers, pesticides, and petroleum.
- The storage place needs to be clean, cool and dry.
- The seeds should be stored in a closed room without windows equipped with air-condition to keep the temperature below 25 °C and with a dehumidifier to regulate air humidity.
- Insect, rat and squirrel infestation must be prevented. The seed bags should be put in sealed metal boxes or plastic drums.
- For monitoring of insects, insect traps should be installed. The storage personnel need to be instructed on labelling, sorting, and recording of incoming and dispatched organic seed lots. Inventory of seed storage must be up to date. Different cotton qualities should be marked with signboards, for example, using colour codes.
- Temperature and humidity need to be recorded daily.



Proper seed storage.

6.4 Special case of organic hybrid seed production

The F1 hybrid is the result of the cross between two parental inbred lines to attain higher yield under high input conditions. However, under marginal conditions, hybrids do not always reach their yield potential to justify the lofty seed price. Moreover, in many cases, hybrids and their parental lines are private property and not shared with farmers or universities. Thus, farmers rely entirely on the seed supply of the seed company, hoping that they might not abandon GMO-free seed production. In many cases, commercially produced hybrids and also their parental lines are already contaminated with GMO. If farmers or public institutions have developed their parental material, F1 hybrids might be an option for organic cotton farmers. However, seed production is much more cumbersome and expensive than inbred lines, which can be directly saved from the crop harvest.

Manually cross-pollinating plants produces hybrid seed. The parental lines are chosen from a diverse background so that the hybrids will have the best of both parents. The hybrid vigour contributes to earliness and better performance (Santhy et al. 2008). The seed produced from F1 hybrid crop will not have the same characteristics as the hybrid, as it will segregate like the F2 generation described in Chapter 4, and will not resemble F1 hybrid performance.

Over time, farmers have become dependent on purchasing new hybrid seeds each season, since hybrid seed needs to be produced every year from two parental lines. Understanding the basic crossing technique will enable cotton farmers to produce their own hybrid seed and ultimately make them



Selection of male and female parents.

more independent. But, this is only possible if they get access to the respective parental lines and if they are not contaminated with Bt-cotton.

6.4.1 Multiplication of female and male parental lines

Identification and procurement of nucleus or breeder seed of parent lines of an interesting F1 hybrid is challenging, as they are protected property. The male and female parent lines should be genetically pure and have a fairly long synchronous flowering habit. Both parental lines need to be maintained and multiplied (Chapter 5). While conventional F1 hybrids are derived from hand emasculating and pollination of fully fertile parental lines, the novel F1 hybrids are often derived from Genetically Male Sterile (GMS) female lines to avoid time-consuming hand emasculating.

Selected female and male parents with desired fibre quality (above 28 mm length and micronaire between 3.7 and 4.3) and excellent performance under organic conditions should be tested for GM contamination before sowing. The GMO-free parental line seed should be treated organically (see Section 6.5.5) before sowing to improve seed germination and crop establishment. Off-types, diseased or infected plant and other crop plants from both male and female plots must be removed. Rouging should be done on appropriate crop growth stage considering the variety characteristics. All the fertile plants from GMS female must be removed before the start of the crossing (for further details see Section 5.4).



Hybrid seed production field.

6.4.2 Hybrid seed production with fully fertile or male sterile female plants

For manual hybrid seed production, female and male parents are sown in the ratio of 3 : 1 in separate strips in the same field. Emasculation and pollination must be conducted, as illustrated in chapter 3.3.3.

For hybrid seed production based on GMS, female and male parents are sown in the ratio of 6 : 1 strips in the same field. However, 50 percent of the female plants have to be removed, as only one-half of the plants are genetically sterile. After removal of the fertile female plants, pollination can be conducted without previous time-consuming emasculation. In order to allow better synchronicity for pollination, male parental lines are sown at three different times to lengthen the time of flowering.

Hybrid seed quality needs to be tested as described in chapter 6.5. In addition, 300 F1 hybrid seeds should be sown in the field to test the cross-pollination success rate. In this so-called 'grow out test' farmers can verify if all plants are homogeneous. Smaller, less vigorous plants are observed due to unintended selfing. In F1 hybrid seed only 8.5 percent selfed plants are allowed.

6.5 Workshop on seed production and seed quality

6.5.1 Learning objectives

- Know the opportunities in GMO-free quality seed production of inbred lines.
- Understand the production of hybrid seed process.
- Understand the significance of reliable seed source for the multiplication of healthy, viable and good quality non-GM cottonseed.
- Learn the procedure to test seed for Bt contamination and germination.
- Build a comprehensive understanding of the best package and practices for non-GM cotton seed multiplication.

6.5.2 Why use good seed?

- A higher germination rate reduces the seeding rate.
- Good seed reduces the need for replanting and thinning.
- They create more uniform plant stands, reducing weeding requirements and making harvesting easier.
- Good seed result in more vigorous early plant growth, which helps the plant to compete better with weeds and better resist pests and diseases, or drought.
- Early vigour results in earlier maturity, higher yields and less immature seeds.
- qPCR tested seed reduces the risk of GMO contamination.



Remember: Good seed means good yield!

6.5.3 GMO testing of seeds

- Each seed lot must be tested before sowing to avoid contamination of the breeding material with Bt. This applies also to seed that is certified free from Bt contamination.
- For commercial seed production, foundation seed lot, as well as certified seed or seed from truthful sources need to be tested by qPCR by using services of contract labs or state universities
- Also, it is important to test for GMO contamination at different steps of seed production as described in chapter 6.3. This can be done with Bt strips or ELISA tests. Bt tests have the advantage that they can be applied directly in the field.
- The strip test kits should be stored in cool and dark conditions and not used beyond the expiry date. The manufacturer's instructions should be carefully followed. Always use the recommended dilution of the buffer.

- The Bt strip test is not as sensitive as the qPCR test, but much cheaper and easier to perform. Bt strip tests work very well for detection of Bt contamination above 5 percent. They also work for fresh upper leaves of young plants, but with less accuracy.
- Make sure that no Bt toxins or bacteria are sprayed in seed production field as this will give a positive signal on Bt stripes.
- To test the quality of each batch of Bt stripes, use four seeds of a Bt plant or Bt seed package and four seeds of non-Bt plant or seed package and all combinations as listed in the table below. This will result in five samples (A – E). The four seeds must be ground properly with the device provided and a buffer added in exact dilution.

If Bt is present, the strip will show two clear red lines (Figure 31). Only one top red line indicates the absence of Bt (see Table 10). In sample A, the presence of a stronger red band compared to sample D indicates that the majority of seeds in the lot are Bt contaminated. Sample E should only show one red line (control). Even a faint red line presence below the test line indicates Bt contamination. If no red control line appears on top of the strip, it indicates that the test is not working correctly. In this case, the test must be repeated with new strips.

6.5.4 A seed grower should know what seed viability means

- What is seed viability?
The viability of the seed is a measure of how many seeds are alive and can develop into reproductive plants under appropriate conditions.
- Why do we test seed viability?
It is essential to know that the stored seeds can produce plants. The viability must remain high throughout storage.
- When should the viability be determined?
Viability will need to be determined at the start of storage and at regular intervals during storage to predict the correct time for regeneration of the variety.
- How should viability be determined?
The most accurate method to determine viability is the germination test as described above.
- Why do some seeds fail to germinate in the test?
The main reasons for failed seed germination in suitable conditions are: incomplete maturity or infected or old seed. Very fresh seed may also show low germination due to dormancy.

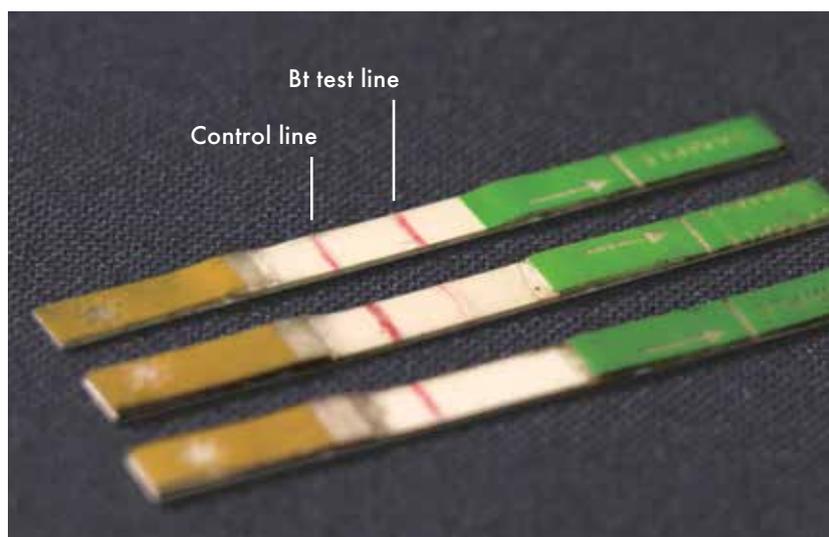


Figure 31. Illustration of Bt strip test results

The strip is placed into the solution, and after the time prescribed by the manufacturer, the strip is interpreted.

Table 10. Result interpretation of the Bt Strip test

Sample	Number of seeds with Bt	Number of seeds without Bt	Results expected (red bands)
A	4	0	2
B	3	1	2
C	2	2	2
D	1	3	2
E	0	4	1

6.5.5 Test viability of seeds

Dead seeds can be identified because they usually soften and rot during the test as a result of an damage from bacteria and fungi, or insects. Immature, poorly filled seeds can be identified by soaking the seeds in a bowl of water. Immature seeds will float, while viable seeds will sink. Viable seeds with no dormancy will germinate within a few days if exposed to water at 25 – 30 °C.

6.5.6 Breaking seed dormancy

Seeds which remain hard or absorb water, but remain firm and in good condition during the germination test are probably dormant. Seed dormancy is common in some varieties straight after harvest (post-harvest dormancy). This dormancy can usually be overcome by storing the seeds in dry conditions for 30 days (Wang et al. 2019). In addition, the seeds are ideally dried in the sun for one or two days before planting.

The seed used for planting should be harvested in October or November and planted in the following May or June. The seeds should be dried thoroughly for 30 days, if possible, and the germination rate should be tested before planting.

6.5.7 Germination test

The germination test is usually the only test a farmer can conduct to test the quality of the seed before planting. The time seeds take to germinate gives an indication of their vigour. The germination test should also be used to determine the germination of seeds distributed in the next season.

For the germination test, a random, but representative seed sample should be cleaned as described in chapter 6.3.3. The test should be conducted one month after seed harvest and post-harvest drying to avoid wrong results due to seed dormancy. The procedure is easy, inexpensive and portable.

1. Samples from different parts of the bag or container should be taken to obtain a random sample for testing.
2. If the seed lot contains more than one bag, samples must be taken from each bag.



Germinating viable seeds.



Preparation of seed for the germination test.



Preparation of the seed in a tray with sand.



Good germination of cottonseeds on a paper towel in a pot.

The germination test can be completed by following the procedure:

1. Place water-absorbent material like paper towel inside waterproof trays or pots. Instead of paper towels, the germination test can also be performed in pots or trays with sand or soil. Take three random samples from each seed lot.
2. Count 100 seeds from each sample and place them on the absorbent paper inside the tray.
3. Carefully saturate the absorbent material with clean water.
4. Verify every day that the absorbent paper is moist and record the number of germinated seeds for ten days.
5. Compute the germination test at five days and ten days.
6. Time to germination is an indicator of seed vigour. Rapid seed germination increases the chance that the seeds establish in the field.

The germination rate is the percentage of seeds that have germinated after five and ten days.

Calculation of the germination rate:

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \times 100$$

Example: 80 seeds germinated in a tray of 100 seeds, the germination rate is: $80/100 * 100 = 80\%$.

6.5.8 Seed treatment

Alternative treatment of seeds can help to reduce damage by pests and diseases caused before and during germination. Some suggested methods are: dipping the seeds in cow urine, coating the seeds with clay and cow dung or treating the seeds with a suspension of beneficial microorganisms (*Trichoderma* or *Bacillus subtilis*). To enhance the uptake of nutrients, some organic farmers treat the seeds with a suspension of azotobacter and phosphorus solubilising bacteria (PSB).

6.5.9 Summarising activities

- Discussion on current management practices followed by the participating farmers and challenges faced.
- GMO testing using Bt strip test kits.
- Identification of good quality seed.
- Seed germination test.



6.6 Training on field monitoring and inspection

6.6.1 Objectives of field inspection

The objective of conducting field inspection is to identify risks which can cause irreversible damage to the genetic purity or seed health. The purpose of field inspection is to check the critical points of

proper seed multiplication for high-quality cottonseed. The inspection can be done by an official seed certification agency or by the internal control system of a farmer organisation.

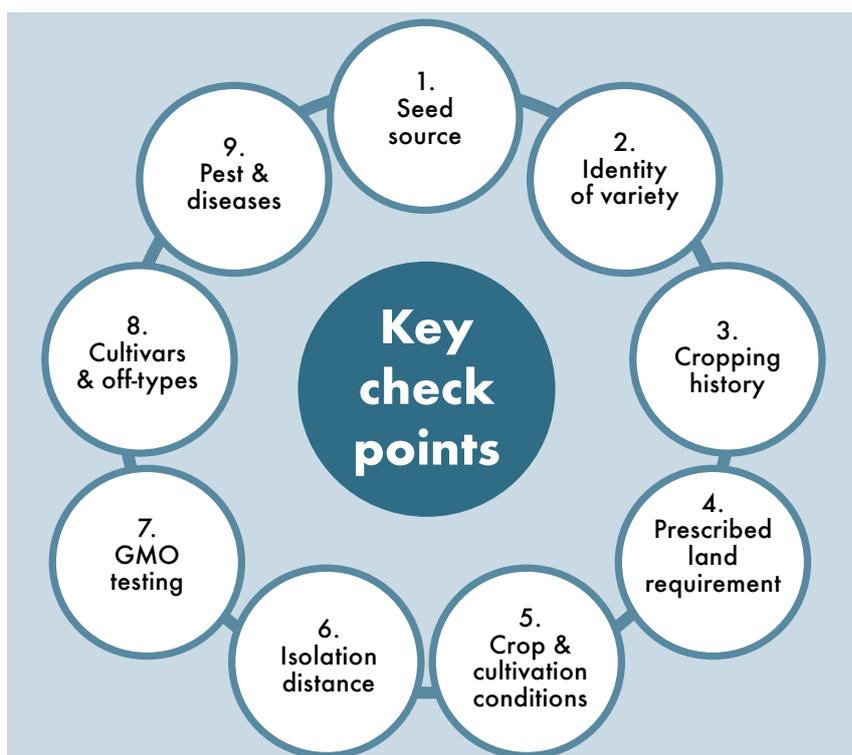


Figure 32. Points to consider during field inspection

6.6.2 Standard procedure for field inspection

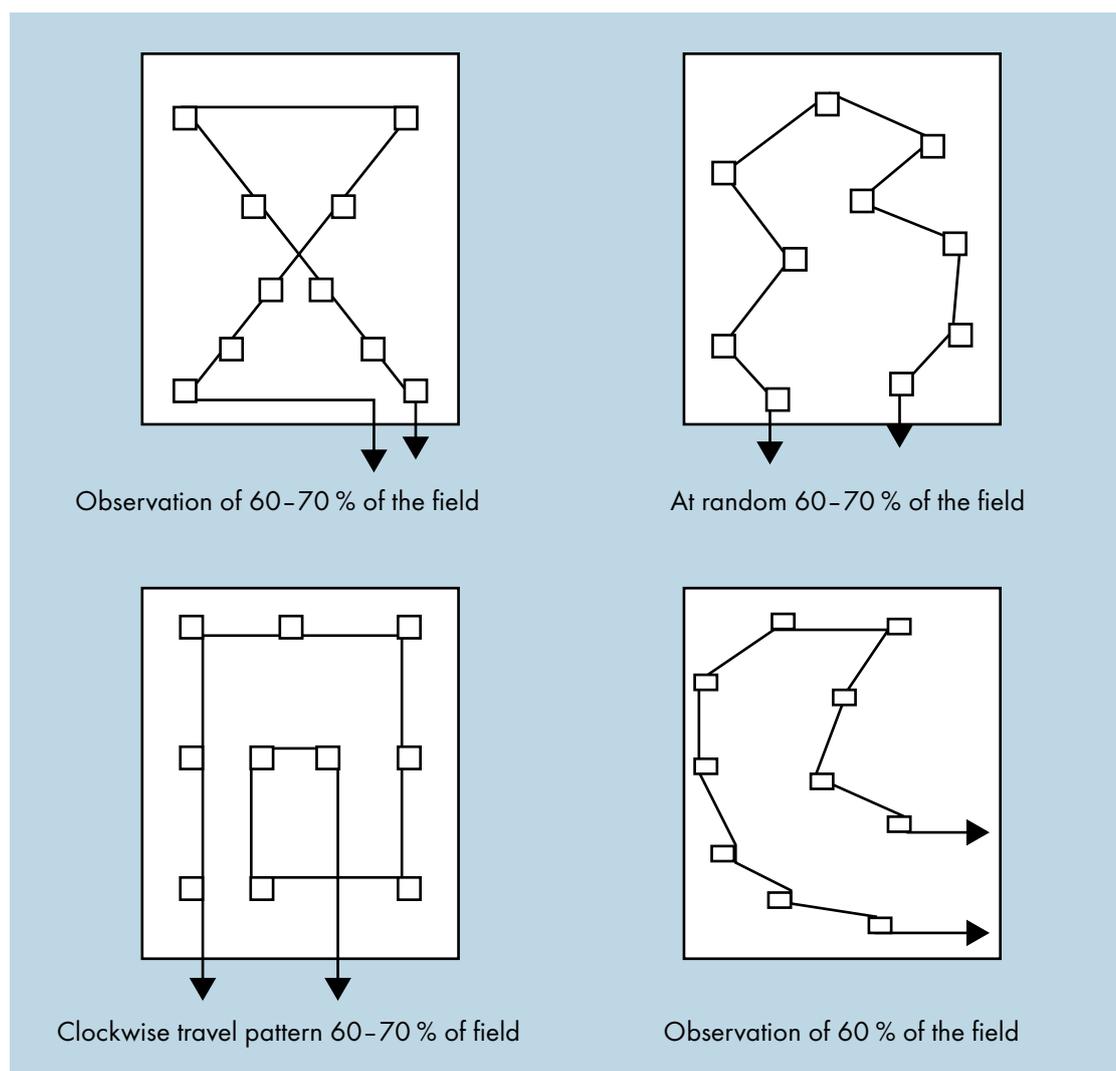
- The inspecting person must know the prerequisites and standards for seed production and must be familiar with the characteristics of the cotton species and the varieties/cultivars being inspected.
- All information about the species, variety, seed origin, qPCR test for GMO contamination, cultivated area, class of seed, and cropping history of the field must be monitored and cross-checked. This also applies to adjacent fields where cotton is grown, as they may contribute to cross-pollination and outcrossing.
- Inspection begins with a tour around the seed multiplication field to check if the isolation distance of 100 m to other cotton crops is met.
- Every field and its boundaries must be clearly marked.
- All parts and rows of the field must be inspected. Special attention should be given to sensitive areas such as the vicinity of farm buildings, threshing areas and paths into or through the field, where seeds of various species and origin may have been dropped for disposal or during transport.

- The field inspection should be carried out schematically so the maximum area can be covered in a systematic manner (Figure 33).
- Every inspection must start at a different point.
- The collected information is recorded on the monitoring information template (Figure 34).
- During field inspection, proper estimates must be made on other varieties, off-types, volunteers, diseases, plants, the general condition of the crop, applied farm practices and estimated yield.
- Estimates of impurities and diseased plants should only be made through actual counts and never on a visual impression.
- Barren rows or long gaps encountered during counts should be skipped and not considered as part of the row steps/walk.



Field monitoring of participatory on-farm breeding trials.

Figure 33. Walking patterns for field monitoring



Adapted from Cereal Seed Technology, FAO Agricultural Development, Paper No. 98

- For short species/cultivars, squat or bend periodically during the inspection so that eye-level observations can be made at the height of the plants.
- Impurities, off-types and diseased plants should be pulled out during monitoring.
- Rogued plants or heads etc. lying on the ground within or on the outskirts of seed fields must be gathered and removed, as they could contaminate the seed crop.
- Samples must be taken for testing GMO contamination with Bt strips.

Field Monitoring Information Sheet

Type of trial

Field N° Report n°

Crop Variety

Date of sowing Date of inspection

Expected duration of picking: From To

1. Name of producer/grower

2. Village Distric State

3. Location of farm

4. Source of seed GPS coordinates

5. Total acreage under production

6. Acreage of the field n° monitored

7. Previous crop Isolation distance

8. Stage of seed crop at this inspection

9. Field counts: Take field counts as directed in the guidelines

N° of plants					
Count n°	Off type	Inseperable other crops	Objectionable weeds	Diseased or Pest Infested Plants	Others
1					
2					
Total					
Percentage					

10. Name(s) of

(a) Pests/insects

(b) Diseases

(c) Inseperable other crop plants

11. Condition of crop at the time of monitoring

14. Quality of seed production work

15. Was the grower or his representative present at inspection time?

16. Remarks

Farmer's Signature

Signature of monitoring staff:
Name:

Figure 34. Sample field monitoring information sheet

6.6.3 Various crop stages of inspection

The inspection of seed multiplication is done at different stages of crop growth to make multiple verifications and estimates of impurities, off-types and diseases, etc.

Inspection at sowing

Farmer training at the time of sowing for the farmers new to seed production programme, or when a new cultivar is introduced, is instrumental to ensure a good seed quality. The growers should be informed about land and isolation requirements to allow them to check whether their seed field meets the requirements relating to the seed bags, planting, planting rates, sowing time, seed treatment, GMO test results, border rows and irrigation facility, etc.

Inspection during pre-flowering/ vegetative stage

At this stage, the growers are trained to recognise the plants that need to be rogued and other corrective measures are suggested, if required, after the following verifications:

- The seed planted was obtained from an approved source and tested to be GM-free.
- The isolation, border and land requirements have been met.
- If Bt-cotton plants are found in isolation distance, the direct distance between Bt-plants and non-GM should be measured to estimate the potential of contamination.
- At this stage of the crop, the importance of completing corrective measures and roguing before flowering to avoid cross-pollination should be explained.
- Leaves of 10 to 20 random plants must be taken to perform Bt strip tests.
- Check and advise on frequent and timely weed control and preventive pest and disease measures.

Inspection during flowering

During flowering, the following features are monitored:

- Have the various corrective measures, if suggested earlier, been carried out?
- Is the isolation distance to Bt cotton fields maintained?
- The potentially contaminating factors are counted as described earlier.
- Are weed, pest and disease control appropriately managed? If necessary, farmers are instructed on the correct procedure. The farmers are also advised to continue roguing all through the flowering season.



Field inspection at the time of sowing



Field inspection at early vegetative growth stage



Inspection at flowering

Inspection after flowering and at the pre-harvest stage

- After flowering, detailed counts should be taken to determine the presence and extent of various contaminants in the seed production field.
- The farmers are trained regarding rogues especially the ones that were not identifiable at earlier stages, to remove them before final inspection.
- The corrective measures suggested earlier may also be checked to ensure whether these have been done.
- Samples of at least 20 random plants are taken for individual Bt strip testing. If any Bt cotton contamination is suspected, the farmers are instructed to increase frequency of Bt strip testing.

Inspection before harvest

This is the last inspection conducted on the crop in the field.

- Seed samples of at least 20 random plants are taken for individual Bt strip testing. If any Bt cotton contamination is suspected, the farmers are instructed to intensify Bt strip testing. If the crop meets the requirements for seed production, the farmers are given the instructions regarding necessary precautions to be taken during picking, storage and ginning.



Inspection of the cotton plants at the time of harvest.



Roguing of off-types, other crop plants, weeds, diseased and infected plants in a cottonseed crop.

6.6.4 Contaminants to be observed during field inspections

Off-types

Plants of the same crop species, differing in the expression of morphological characters such as plant type, branching type, pigmentation, etc. are classified as off-types (see Chapter 5.4). The variety of off-types does not necessarily need to be identified (off-types are off-types regardless of their origin, their characteristics and the stage of growth). If there are too many off-types, the crop cannot be used for seed multiplication. Therefore, the farmers must be instructed to check the crop regularly for off-types and remove them immediately.

Inseparable other crop plants

Inseparable crop plants are plant species found in seed production fields with seeds that are so similar to the cultivar that it is difficult to separate them with reasonable mechanical means (e.g. *G. hirsutum* and *G. arboreum*).

Objectionable weed plants

Objectionable weeds are species with similar seed size and shape as the crop. Objectionable weeds also include weed species whose eradication is difficult when introduced, or that are alternate hosts of crop pests or diseases.

Disease- and pest-infested plants

Plants affected by designated diseases should invariably be counted. The grower should be advised to rogue them out from the seed production fields.

Table 11. Number of plants per count performed at each monitoring

Row spacing	Number of plants
Wide	100
Medium	500
Dense	1,000

6.6.5 Summarising activities

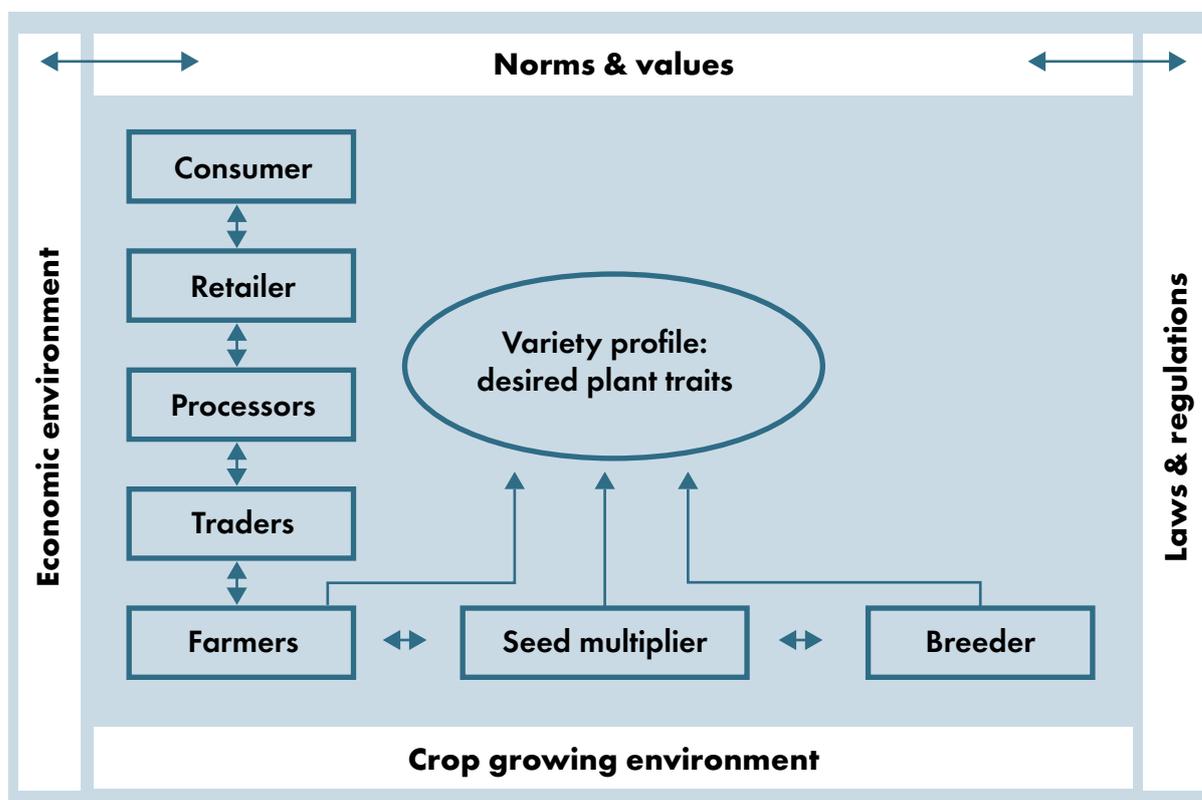
1. Discussion with the participants on the need for monitoring and inspection.
2. Identification and counting of inaccurate practices or errors observed during the inspection.
3. Identification of potential GMO plants with the Bt strip test.



7. Commercialisation of Cultivars

The cottonseed supply chain consists of four major steps before processing and marketing: cultivar development, cultivar release, cultivar maintenance

and seed propagation. The supply chain is embedded in legal frameworks and normative values (Figure 35).



adapted from Osman et al., 2015

Figure 35. Situational context influencing plant breeding and seed production

7.1 Seed distribution systems in India

In India, different seed distribution systems complement each other:

- Farmer to farmer distribution: This is the traditional distribution method, where farmers obtain the seeds from neighbours either on cash payment or on an exchange basis. For this type of distribution, no formal marketing organisation is required.
- Distribution by co-operatives: This system involves the procurement of seeds by co-operatives and the subsequent distribution among its member farmers. The distribution of seeds through co-operatives has often been encouraged by the government with subsidies and guarantees.

- Distribution by agriculture departments: In this system, the seeds are purchased by the governments, with government funds, and are distributed by district agricultural officers and block development officers.
- Distribution of seeds by private commercial seed companies: In this system, the seeds are distributed through a network of seed distributors and seed dealers.

While option a) and b) belong to the informal seed system, options c) and d) belong to the formal seed system. The different interactions between the formal and informal seed systems are shown in Figure 36.

In recent years, the farmers' dependency on the formal seed market has increased drastically. This is especially true for cotton, where more

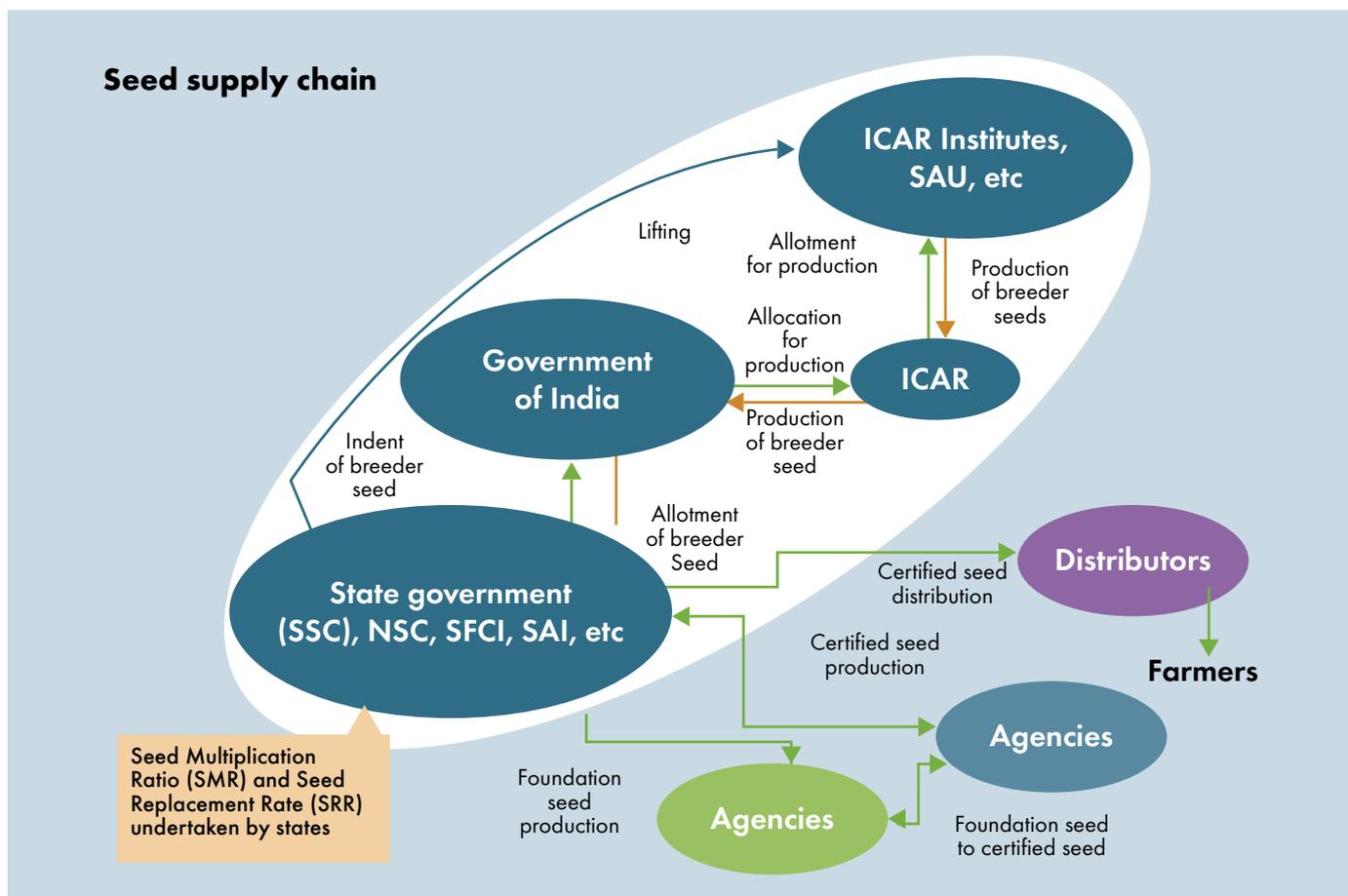


Figure 36. Channels of seed supply

Source: Parimala et al. 2013

organic and conventional farmers grow F1 hybrid seed that needs to be purchased every year. In addition, commercial companies have displaced public cotton breeding and seed multiplication since the introduction of Bt cotton in 2002. Today, private companies have taken over a substantial share of the seed production of maize, sunflower and cotton. Therefore, both the informal and public seed sectors must be strengthened and incentivised to address the needs of farmers for high-quality seed (Manjunatha et al. 2016).

7.2 Formal seed sector

Formal seed sectors follow seed certification procedures and standards to produce seed of a particular variety. The marketing of seed is regulated in India by the Seed Act of 1969 and the Protection of Plant Variety and Farmers Right (PPV&FR) Act of 2001. The new Seed Bill of 2004 restricting the informal seed sector has not yet been implemented in India. The Plant Variety and Farmers Right Act is unique in the world with the inclusion of rights of farmers, breeders, researchers and equity concerns.

The Indian Protection of Plant Variety and Farmers Right Act became operational in 2005 with the aims to (i) establish an effective system for the protection of plant varieties, the rights of farmers and plant breeders, and to encourage the development of new varieties; (ii) to recognise and protect the rights of farmers in respect to their contribution in conserving, improving and making the available plant genetic resources for the development of new plant varieties; (iii) to accelerate agricultural devel-

opment in the country by stimulating investment for research and development both in public and private sectors; and (iv) to facilitate the growth of the seed industry to ensure the availability of quality seeds and planting material to the farmers. Any breeder, university, farmer or farmers' organisation can apply for registration of a new variety (GOI Circular Ref. No. 11-71/88-SDI).

Special emphasis is put on farmers' rights: A farmer who has developed or bred a new variety shall be entitled to registration as a breeder of a variety. The farmer shall be deemed entitled to save, use, sow, re-sow, exchange, share or sell his farm produce, including seed of a variety protected under this act, in the same manner as he was entitled before this act came into force. However, the farmer shall not be entitled to sell branded seed of a variety protected under this act. The farmer's variety shall be entitled to registration (Farmers' Rights, 2016). 'Branded seed' includes any seed put in a package or any other container and labelled in a manner indicating that such seed is of a variety protected under this act. (for further information visit the PPV & FR Authority website www.plantauthority.gov.in).

Plant Breeder's Rights (PBR) are granted by government to the breeder, originator or owner of a variety. These rights empower the breeder to exclude others from producing or commercialising the propagating material of the protected variety of cotton for 15 years. A person holding the PBR title to a variety can authorise other parties to produce and sell the propagating material of the variety. He

or she is expected to set reasonable terms for the transfer of the PBR title or the sale of the propagating materials; otherwise, the government can grant the license of the right in the public interests, called compulsory licensing (Santhy et al. 2009).

Right to adequate availability of registered material: When seeds of protected varieties are unavailable, the authority can grant a compulsory license to any person to produce and distribute the seeds to the public at a reasonable price, provided the expiry period of three years of registration is completed (Santhy et al. 2009). The breeder is required to provide an adequate supply of seeds or material of the variety to the public at a reasonable price. If after three years of registration of the variety, the breeder fails to do so, any person can apply to the authority for a compulsory license. Compulsory license revokes the exclusive right given to the breeder and enable third parties to produce, distribute or sell the registered variety.

Regulation of sale of seeds of notified kinds or varieties: No person shall, himself or by any other person on his behalf, carry on the business of selling, keeping for sale, offering to sell, bartering or otherwise supplying any seed of any notified kind or variety, unless: (a) such seed is identifiable as to its kind or variety; (b) such seed conforms to the minimum limits of germination and purity; (c) the container of such seed bears in the prescribed manner, the mark or label containing the correct particulars thereof (Seed Act, 1996).

Table 10. Differences between the formal and the informal seed sector

Formal seed system	Informal seed system
<ul style="list-style-type: none"> Centrally managed system with mechanised production Homogenous in nature With a quality control system Use of identified and notified varieties Certified and truthfully labelled seeds Seed production by national government agencies, state government agencies, government-assisted and other co-operatives Produced through multi- and transnational co-operatives' domestic private sector and joint ventures Large quantities of seeds marketed through government-owned companies, private companies and state universities 	<ul style="list-style-type: none"> Locally managed system with unmechanised production using local resources No specialisation Heterogeneous in space and time Traditional system of seed processing Use of locally available cultivars Truthfully labelled and other unlabelled seeds Farmer saved seeds, farmer to farmer exchange, farmer co-operatives, community groups, non-government organisations, seed growers associations Small quantities of seeds marketed through community level, highly localised, conventional and unconventional exchange mechanisms

7.2.1 Variety release

Before the Central Sub-Committee can release a variety, it must follow an established procedure of testing the new variety for its Value for Cultivation and Use (VCU) by the All India Coordinated Crop Improvement Projects and identification of the variety for releases by the AICCIP workshop. The breeder can apply for the nationwide release of state-released cultivar. State-released varieties are constrained by a recommendation domain restricted to a single state because they are not accepted in other states with similar agro-ecological conditions.

The AICCIPs of the ICAR, function as national bodies for developing improved crop cultivars and for developing production and protection technologies that will benefit farmers. AICCIPs have been created for all the major crops or crop groups (Paroda, 1992). AICCIP trials follow a uniform testing procedure (Figure 37), and acts as a well-organised and powerful sieve to screen. The AICCIP recommends well-tested and outstanding new varieties. This system for varieties/hybrids is developed and standardised on AICCIP centres located at ICAR research institutes or state agricultural universities. The varieties initiated by or referred to the AICCIP workshops can be recommended to single or more than one state for identification.

Release and notification of a variety follow its identification and recommendation by the AICCIP workshop after a minimum of three years of multi-location trials and assessment for Value for Cultivation and Use (VCU). The variety should be suitable for specified agro-climatic and soil conditions, can withstand typical stress conditions and have tolerance/resistance to pests and diseases. It should also show distinct advantages over the existing equivalent released varieties. Trial data on agronomic performance needs to be provided. For a proper assessment, data on performance against popular varieties on farmers' fields are also required, but the degree to which such data is collected and included in release proposals varies; provision is not mandatory. At the end of the second stage of AVT II, the proposal for identification of a variety is submitted by the concerned breeder on a variety identification proforma specified by the ICAR (Paroda, 1992). This form was standardised in 1992 and is similar for central and state releases. If the central sub-committee accepts the proposal, the variety/hybrid is released for the concerned states (i.e., for more than one state, and often country-wide) it is simultaneously notified for certified seed production purposes, usually for the entire country. Registration can be done under the State Seed Sub-Committees (Tandon et al. 2015).

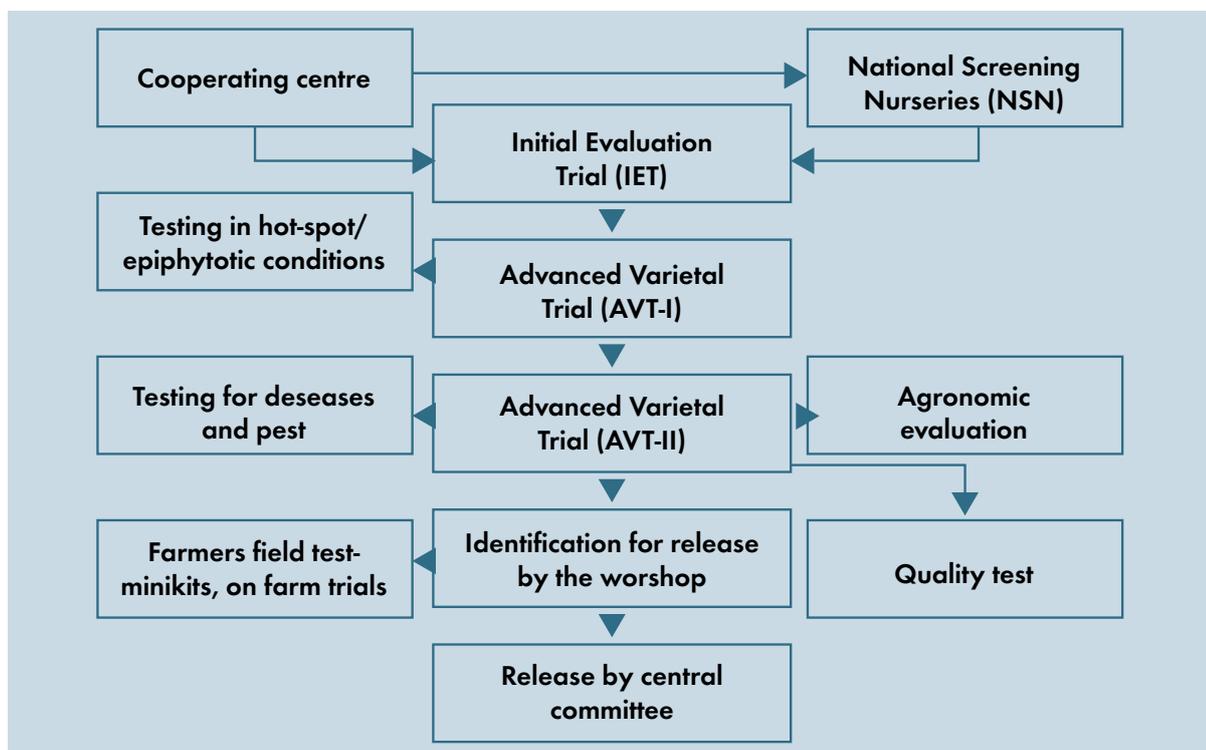


Figure 37. Procedure for varietal testing and release in the AICCIP system

7.3 Informal seed sector (farmer bred and released varieties)

A similar procedure applies to varieties produced by the private sector if they are intended to be officially released (see Figure 38). However, it is not yet mandatory that a variety developed by the private sector be released centrally or by state committees, and private sector participation in the AICCIP trials are still optional to date. A state research institute or a private seed company can attempt to release a variety through the central or the state system. If the variety is widely adapted, and suited to conditions beyond a single state, the breeder may try for central release; in other cases, state release may be more accessible and appropriate. Identification and testing of new varieties are mainly the responsibility of one or two State Agricultural Universities (SAUs).

Under the PPV&FR Act, farmers have the privilege of being completely exempted from payment of any kind of fees or other payments that are normally payable for variety registration; tests for distinctness, uniformity and stability (DUS), and other services rendered by the PPV&FR Authority. Popularisation is the function of the Department of Agriculture and the agricultural universities, acting in collaboration and separately. Seed production, certification and distribution are undertaken by various state seed agencies, universities and private seed companies. To date, NGOs have played only a small part in the whole process. Overall authority lies with the State Seed Sub-Committee. Some farmers multiply seed, maintaining sufficient purity, and sell it as Truthfully Labelled Seed (TLS).

For various reasons, including the considerable procedures involved in getting a variety released, some companies produce Truthfully Labelled Seed under their own trade name (Vrik 1998) (see online at <http://r4d.dfid.gov.uk/PDF/Outputs/RLPSR-bookchap6.pdf>).

7.3.1 Truthfully Labelled Seed (TLS)

The seed certification rules are uniformly applicable to the public and private sector (Figure 38). The private sector takes advantage of selling 'Truthfully Labelled' seed of any variety. TLS is not field certified to assure genetic purity, but the seed standards are not lower than the certified class of seed. Unreleased varieties (private sector or public sector) do not come under the purview of the Seeds Act for certification. For regular notification, an additional two years of testing will be required. TLS shall be the progeny of foundation, certified or labelled seed. Seed and field standards are equivalent to certified seed, and production procedure is the same as certified seed (Parimala et al. 2013). Nevertheless, a Seed Certification Agency is not necessary. Market monitoring is the only quality testing tool for TLS. The producer will declare and guarantee the quality attributes of the TLS. The TLS production is completely free from government certification scheme, and therefore no inspection is needed at field level. All planning, production, post-harvest activities and testing for quality control should be performed by the producers' in TLS production.

7.4 Cottonseed multiplication

In India, more than 95 percent of the total cottonseed market is dominated by genetically modified (GM) Bt cotton. Before the introduction of Bt cotton, cotton breeding and seed multiplication were mainly conducted by the Central Institute for Cotton Research (CICR), state universities, and state seed agencies. At present, the breeding seed production and sale of Bt cotton are primarily under the control of commercial seed companies. These seeds are distributed to local seed traders who sell F1 hybrid seeds directly to the farmers.

The Indian seed system recognises different seed categories: nucleus seed, breeder seed, foundation seed and certified or truthfully labelled seed (see Chapter 5). They need to be reproduced each year to maintain the purity and performance of a cultivar. Thus, only three multiplication cycles (Figure 28) are possible to safeguard the quality in the seed multiplication chain which maintains the purity of the variety, as it flows from the breeder to the farmer.

Currently, mainly F1 hybrid seed of cotton is cultivated. Hybrid seed have to be produced each year by crossing the female with the male line. This is either done by manual emasculation or genetic male sterility and manual pollination (see Chapter 6.4).

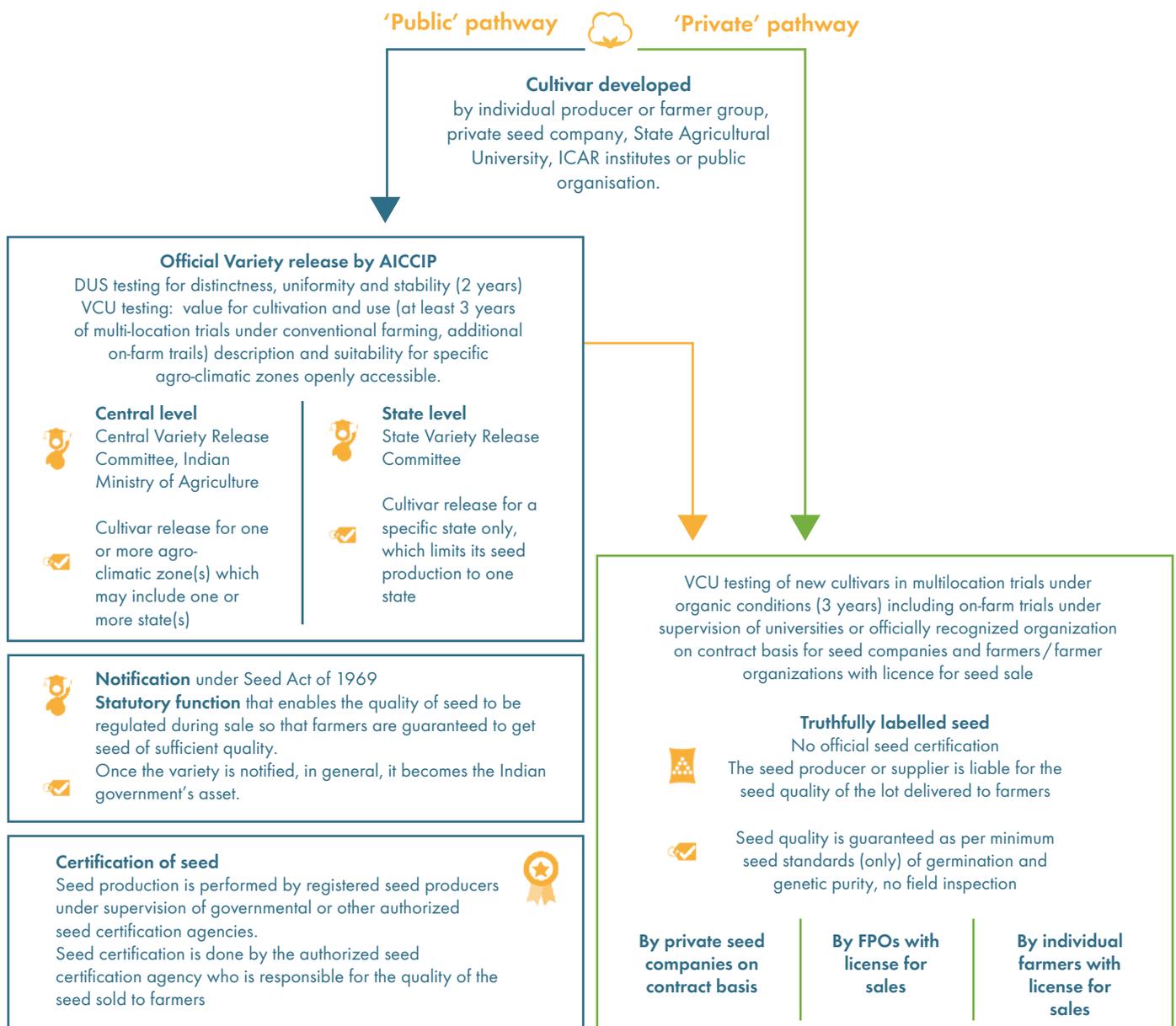


Figure 38. Approval for commercialisation of seeds of new varieties. Schematic comparison of the varietal release processes in the public and private sectors

As a consequence, both parental lines need to be maintained and multiplied in the first year to produce F1 hybrid seed in the second year. In contrast, varietal lines of *G. hirsutum* or *G. arboreum* can be multiplied within one year by allowing self-pollination.

Seed production and marketing of non-GM cottonseed can be conducted either by (i) organic cotton projects or associations or (ii) small scale farmers producing their own seed, either F1 hybrid

seed or varietal lines. However in most cases, these are varietal lines, as few farmers are involved in parental line multiplication and hybrid seed production. Parental lines are in most instances property of commercial companies or public breeders and not (easily) available to farmers. Differences between the two seed producer groups are listed in Table 11.

Table 11. Characteristics of non-GM cottonseed producers

Characteristics	Organic cotton project/association	Individual small-scale farmer
Production conditions	<ul style="list-style-type: none"> • Case specific • Varying soil fertility • Limited irrigation opportunity 	<ul style="list-style-type: none"> • Marginal soil fertility • Resource-poor smallholders • Usually no irrigation • Fertiliser and pesticide applications
Organisation	<ul style="list-style-type: none"> • Organised • Support from the organic organisation, production for export 	<ul style="list-style-type: none"> • Unorganised • Little extension support
Market demand	<ul style="list-style-type: none"> • Demand for cotton cultivars with high lint quality (high staple length and strength) for garment production • Usually <i>G. hirsutum</i> cotton 	<ul style="list-style-type: none"> • Demand for cotton cultivar suiting the difficult growing conditions; quality requirements are secondary. • Usually desi cotton <i>G. arboreum</i>
Seed purchasing	<ul style="list-style-type: none"> • Organised and coordinated by organic cotton organisation, pre-order at seed companies 	<ul style="list-style-type: none"> • By individual farmer at local trader
Organic certification	<ul style="list-style-type: none"> • Certification arranged by organisations • Less bureaucracy and cost for farmers • Less risk of Bt contamination 	<ul style="list-style-type: none"> • High risk of Bt contamination • High costs of Bt testing make it impossible for small farmers to afford lab tests and obtain organic certification.
Farmer involvement	<ul style="list-style-type: none"> • Farmers as organisers, producers and managers of the organisations 	<ul style="list-style-type: none"> • Individual farmer
Types of organisations	<ul style="list-style-type: none"> • Textile manufacturers, NGOs, cotton traders and commercial organic cotton producing organisations 	<ul style="list-style-type: none"> • Individual farmer

7.5 Potential non-GM organic cottonseed supply chain models

As the traditional system of buying non-Bt cottonseed on the local market has completely collapsed, different models to re-establish the non-GM cottonseed supply chain were analysed by Marty (2013). The alternative models are differentiated by the involvement and control of the cotton producer in the seed supply chain and the quantity of seed produced by an individual seed production unit. The organisation of the different models is possible based on one cotton grower unit (individual farmer or organic cotton association) or the integration and organisation of several cotton grower units (several individual farmers together or several organic cotton projects together). Four different pathways are possible to cover the seed demand of small-scale organic farmers (yellow dots) or organic cotton projects (red dots), as shown in Figure 39.

7.5.1 Seed purchase from market

The market for non-GM seeds, produced by seed companies and sold to distributors and dealers has become very small. Only very small quantities of *G. arboreum* hybrids are being produced and sold via seed dealers. Even if there are eventually some non-GM *G. hirsutum* seed offered on the local market, there is a very high risk that this seed is Bt cotton or Bt contaminated.

7.5.2 External seed production on contract basis

As an alternative to buying seeds from the market, non-GM seeds can be pre-ordered with commercial seed companies or public seed producers' agencies. The cotton grower unit will make a contract with the seed producer and pay an indent. In the contract date of delivery, quantity, price and quality requirements (such as genetic purity and no GM contamination) will be fixed. If the seed producer is a producers' organisation, then parental lines or varieties need to be provided by the cotton breed-

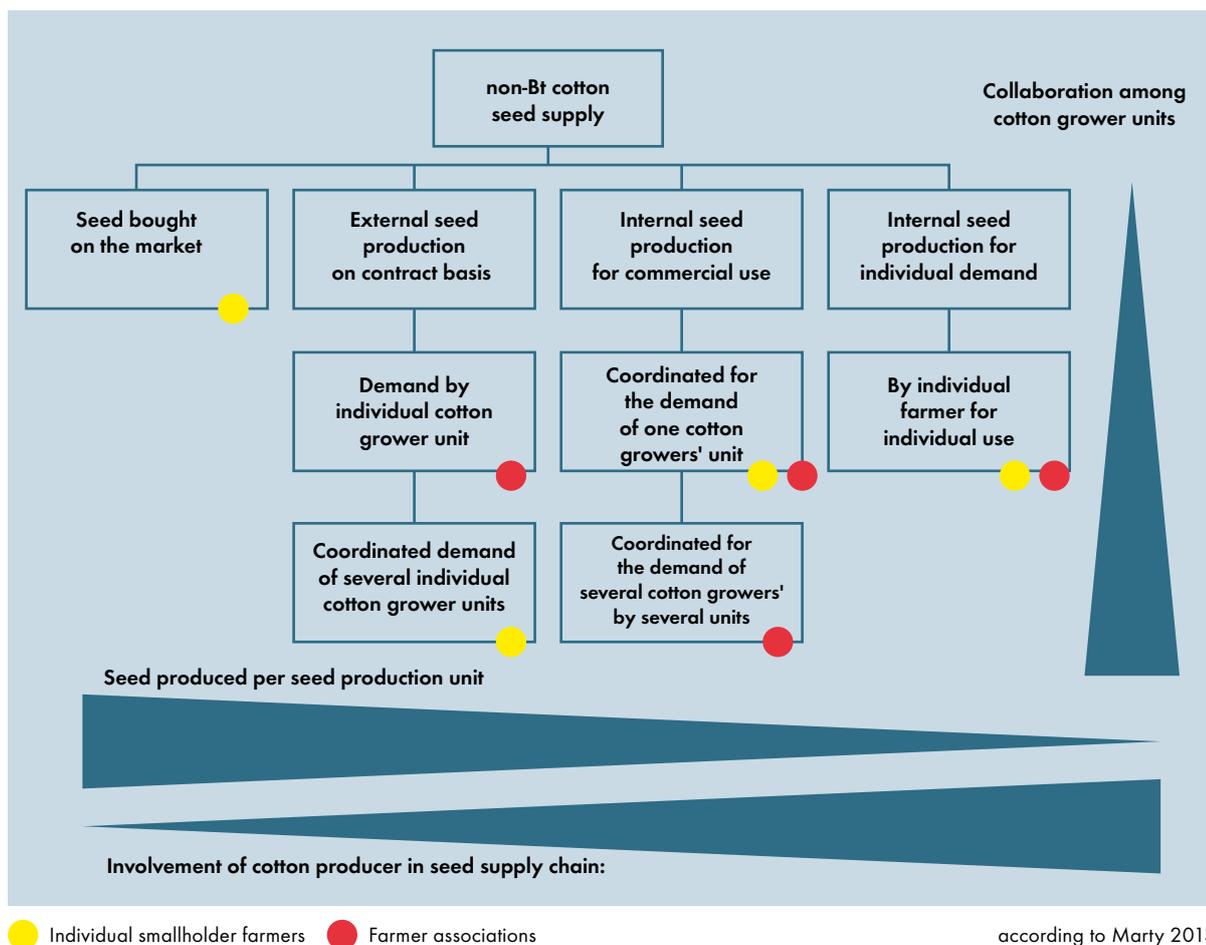


Figure 39. Non-GM organic cottonseed production and distribution models

er. These parental lines or varieties can also be ordered with universities on a contract basis. In the case of a seed company, the company will provide the proprietary parental lines with a strict confidential agreement and encoded labelling. The cotton grower will receive the packaged and ready-to-plant seeds as agreed in the contract, usually with a seed certificate.

7.5.3 Internal seed production for commercial use

To become more independent from external seed producers, there is the option for cotton grower organisations to act as commercial seed producers themselves. In this case, (i) individual farmers or specialised farmers producing seed for the demand of their own organic cotton organisation and (ii) the common seed production coordinated by different organic cotton organisations are distinguished.

7.5.4 Internal seed production for individual demand

Seed production is done by individual farmers to suit their own demand. However, in this case, the risk of Bt contamination is very high, as presently the organic cotton fields are very small (0.5 to 1.0 acre) and surrounded by Bt cotton fields of neighbours. Thus, each seed lot from every field needs to be tested for Bt contamination before the seed can be used in organic production.

The results from the market analysis demonstrated that organic cotton producers have to be engaged in seed production if they want to safeguard non-GM cotton seed in India. They have to actively initiate seed production and carry the production risks (Marty 2013). Hybrid seed production is shown to have a much higher production risk compared to varietal lines, as production costs are a six-fold of the varietal lines. If equally good

varietal cultivars can be made available as shown in previous trials, then there is no point in pursuing a labour intensive and risky hybridisation process. To enable farmers to make an informed cultivar choice, an inventory of varietal lines characterised by their agronomic and fibre quality traits would be very useful. When good cultivars have been developed, it is crucial to maintain purity of newly released varieties and those in advanced stages of testing throughout the production of nucleus and breeder seed (produced in the first generation by the plant breeder) and foundation seed (the next step to increase the amount of seed). This is followed by the production of certified seed which is usually conducted and/or monitored by a governmental seed agency for purity and seed quality and is distributed via local dealers to the farmers. Commercial seed producers need a compulsory registration before engaging in the seed business.

7.6 Summary

The following options can be explored for making non-GM seed available to farmers in India:

Formal seed system, variety released by

- a. University
- b. Farmer breeder
- c. University and farmer breeder

Informal seed system

- a. Farm saved seed of smallholder farmers (many small lots, high risk of Bt contamination)
- b. Collective investment of farmer organisations in seed, and provision of improved seed free of cost (no commercial seed business, but part of a value chain investment) for seed sale organised by farmers organisation

Seed multiplication as certified seed, if notified cultivar or as truthfully labelled seed by

Public seed agencies

- a. Public cultivars
- b. Farmer cultivars

Seed companies on contract basis

- a. Own commercial cultivars
- b. Public cultivars
- c. Farmers cultivars

Selected farmers produce for farmers organisation with a license for seed sale as truthfully labelled seed

- a. Farmers cultivars
- b. Public cultivars

Selected farmers produce for a farmer organisation with planning and GMO testing, distribution of the seed by the farmer organisation (no commercial sale)

- a. Farmers cultivars
- b. Public cultivars

If some public or private cultivars are no longer provided as seed to farmers, farmers have the right to seek rights for permission to multiply.

References

- Aktar, W., Sengupta, D., and Chowdhury, A. (2009). Impact of pesticides use in agriculture: their benefits and hazards. *Interdisciplinary toxicology* 2(1), 1-12. doi:10.2478/v10102-009-0001-7
- Boopathi, N.M., Sathish, S., Dachinamoorthy, P., Kavitha, P., and Ravikesavan, R. (2014). Usefulness and utilisation of Indian cotton germplasm. *World Cotton Germplasm Resources*, 315-323.
- Briefs, I. (2017). Global status of commercialised biotech / GM crops in 2017: Biotech crop adoption surges as economic benefits accumulate in 22 years.
- Ceccarelli, S. and Grando, S. (2019). From participatory to evolutionary plant breeding. In Westengen, O. and Winge, T. (eds.), *Farmer Participation in Plant Breeding: Current Approaches and Perspectives*, Abingdon, UK: Routledge.
- Ceccarelli, S., Guimarães, E.P., and Weltzien, E. (2009). Plant breeding and farmer participation. Food and Agriculture Organisation of the United Nations, Rome, Italy.
- Central Institute for Cotton Research CICR (2010). Vision 2030. Indian Council of Agricultural Research. Available online, pdf: http://www.cicr.org.in/pdf/CICR_VISION_2030.pdf, accessed on 29.03.2012.
- Eyhorn F., Ratter S.G. and Ramakrishnan M. (2005): *Organic Cotton Crop Guide. A manual for practitioners in the tropics*. Research Institute of Organic Agriculture FiBL, Frick, Switzerland.
- Farmers' Rights: Resource pages for decision-makers and practitioners. Available online: www.farmersrights.org, accessed on 06.07.2020.
- Gonsalves, J., T. Becker, A. Braun, D. Campilan, H. De Chavez, E. Fajber, M. Kapiriri, J. Rivaca-Caminade and R. Vernooy (eds.) (2005a). *Participatory Research and Development for Sustainable Agriculture and Natural Resource Management: A Sourcebook. Volume 1: Understanding Participatory Research and Development*. International Potato Center-Users' Perspectives With Agricultural Research and Development, Laguna, Philippines and International Development Research Centre, Ottawa, Canada. <https://www.idrc.ca/en/book/participatory-research-and-development-sustainable-agriculture-and-natural-resource-management?PublicationID=191>
- Gonsalves, J., T. Becker, A. Braun, D. Campilan, H. De Chavez, E. Fajber, M. Kapiriri, J. Rivaca-Caminade and R. Vernooy (eds.) (2005b). *Participatory Research and Development for Sustainable Agriculture and Natural Resource Management: A Sourcebook. Volume 2: Enabling Participatory Research and Development*. International Potato Center-Users' Perspectives With Agricultural Research and Development, Laguna, Philippines and International Development Research Centre, Ottawa, Canada.
- Gonsalves, J., T. Becker, A. Braun, D. Campilan, H. De Chavez, E. Fajber, M. Kapiriri, J. Rivaca-Caminade and R. Vernooy (eds.) (2005c). *Participatory Research and Development for Sustainable Agriculture and Natural Resource Management: A Sourcebook. Volume 3: Doing Participatory Research and Development*. International Potato Center-Users' Perspectives With Agricultural Research and Development, Laguna, Philippines and International Development Research Centre, Ottawa, Canada.
- Gopakumar, K., and Saxena, S. (2005). Seeds Bill 2004: For Whom? *Journal of the India Law Institute* 47(4), 483-501.
- ICAC, I. (2020). *Cotton: World Statistics*. International Cotton Advisory Committee Washington, DC.
- India Seed Bill (2004). Government of India.
- ISAAA. (2017). *Global Status of Commercialised Biotech / GM Crops in 2017: Biotech Crop Adoption Surges as Economic Benefits Accumulate in 22 Years*. ISAAA Brief No. 53. ISAAA: Ithaca, New York.
- Jarvis, D.I., L. Myer, H. Klemick, L. Guarino, M., Smale, A.H.D. Brown, M. Sadiki, B. Sthapit and T. Hodgkin (2000). *A Training Guide for In Situ Conservation On-farm*. Version 1. International Plant Genetic Resources Institute, Rome, Italy.
- Kranthi, K., Venugopalan, M., Sabesh, M., and Yadav, M. (2013). *CICR vision 2030*. Indian Council of Agricultural Research, p. 6.
- Lanting M., Nuijten E., Lammerts van Bueren E. (2015). *Seed availability for non-GM cotton production - An explorative study*. Seed & Soils Task Force. Organic Cotton Round Table (OCRT). Textile Exchange. Publication number 2015-007 LbP.
- Lanting, M. (2015). Close to 96% of respondents believe that seed multiplication programmes are important to ensure the availability of organic cotton seed.
- Lather Virender, S. (2017). *Variety maintenance & quality seed production in pulse crops*. <https://de.slideshare.net/lather/variety-maintenance-seed-production-in-pulses>, accessed on 30.01.2020.
- Manjunatha, S.B., Biradar, D.O., Aladakatti, Y.R. (2016). Nanotechnology and its applications in agriculture: a review. *J. Farm Sci.* 1:1-13.
- Marty, L., Sonneveld, M., Baruah, M.R., Rawal, M.V., and Patil, S. (2013). Socioeconomic evaluation of different models for the establishment of a seed supply-chain for non-GM cotton in India. *FiBL & ETH*.
- Menon, M. (2003). *Organic Cotton: Re-inventing the Wheel*. Deccan Development Society and Kalpavriksh. Available online, pdf: http://www.ddsindia.com/www/PDF/Organiccotton_Cover_Text, accessed on 26.01.2012.
- Messmer, M., Riar, A., Vonzun, S., Shrivastava, Y., Mandloi, L., Birla, M., Patidar, I., Sana, R., Mahapatra, G., Ambatipudi, A., Kencharaddi, H.G., and Patil, S.S. (2017) *Participatory non-GM cotton breeding to safeguard organic cotton production in India*. Poster at: 19th Organic World Congress, New Delhi, India, 9.11.2017. Available online, pdf: https://www.orgprints.org/34849/2/_Messmer%20et%20al%20OWC2017%20science%20track%20proceedings%20in%20TR_54_Vol1_Delhi.pdf.
- Messmer, M., Shivas, Y., Verma, R., Ramprasad, S., Ambatibudi, A., and Patil, S. (2014). *Participatory cotton breeding and cultivar evaluation for organic smallholders in India*. In: Rahmann, G. and Aksoy, U. (eds.) *Building Organic Bridges*, Johann Heinrich von Thünen-Institut, Braunschweig, Germany, 2, Thuenen Report, Nr. 20, S. 671-674. Available online, pdf: https://www.orgprints.org/24285/1/24285_Messmer_cotton%20breeding%20OWC14_MM.pdf.
- Ministry of Environment and Forests (MoEF). *Biology of cotton*. Available online, pdf: http://dbtbiosafety.nic.in/Comments_Invited.htm, accessed on 27.03.2012.
- Manikanda Boopathi, N., Sathish, S., Dachinamoorthy, P., Kavitha, P., and Ravikesavan, R. (2014). Usefulness and Utilisation of Indian Cotton Germplasm. *World Cotton Germplasm Resources*, Ibrokhim Y. Abdurakhmonov, IntechOpen, DOI: 10.5772/58619. Available online from: <https://www.intechopen.com/books/world-cotton-germplasm-resources/usefulness-and-utilization-of-indian-cotton-germplasm>.
- Nemes, N. (2010). *Seed Security among Organic Cotton Farmers in South India*. Master thesis, University of Hohenheim, Germany.
- Organic Cotton Community Worldwide OCCW. (2012). Online available: <http://www.organiccotton.org/oc/index.php>, accessed on 29.03.2012.
- Osei, M., Asante, M., Agyeman, A., Adebayo, M., and Adu-Dapaah, H. (2014). *Plant breeding: a tool for achieving food sufficiency, in Sustainable Horticultural Systems*. Springer, 253-274.
- OSGATA (2014). *Protecting organic seed integrity, the organic*

- farmer's handbook to GE avoidance and testing. Organic Seed Growers and Trade Association (OSGATA). Washington ME.
- Osman, A., Bonthuis, H., Van den Brink, L., Struik, P., Almekinders, C., and Lammers van Bueren, E. (2015). The case of spring wheat value for cultivation and use testing in the Netherlands. *Org Agric* 5: 101–111.
- Parimala, K., Subramanian, K., Mahalinga Kannan, S. and Vijayalakshmi, K. (2013). *A Manual on Seed Production and Certification*, Centre for Indian Knowledge Systems. Chennai Revitalising Rainfed Agriculture Network, Chennai, pp. 1-27.
- Paroda, R.S. (1992). Crop Improvement for sustainable Agriculture, Invited Lecture delivered on 10th July, 1992 at CCSHAU, Hisar.
- Pimbert, M.P. (1991). Participatory Research with Women Farmers. Raman, U., and S.D., Hall (eds.). *Farmers' Practices and Soil and Water Conservation Programs*. Workshop, Patancheru, Andhra Pradesh, India, June 19-21, 1991. Iv+58p. Icrisat (International Crops Research Institute for the Semi-Arid Tropics): Patancheru, Andhra Pradesh, India. Illus. Paper:43-44.
- PPV & FR, Act (2001). GOI Circular Ref. No. 11–71/88-SDI, dated 16 September, on New Policy on Seed Development, Department of Agriculture & Co-operation, Ministry of Agriculture, New Delhi, 1988.
- Prasad, R.S. (2015). The International Certificate Course 'Requisites of seed production, processing, testing and quality assurance' organised by Directorate of Seed Research (DSR), Maunath Bhanjan, Uttar Pradesh, India. <https://krishi.icar.gov.in/jspui/bitstream/123456789/11980/1/Manual%20Volume%201.pdf> assessed 30th Jan 2020.
- Pray, C., Nagarajan, L., Huang J., Hu, R. & Ramaswami, B. (2011). Chapter 4. The Impact of Bt Cotton and the Potential Impact of Biotechnology on Other Crops in China and India. *Frontiers of Economics and Globalization*. 10. 83-114. 10.1108/S1574-8715(2011)0000010009.
- Pray, C.E., Nagarajan, L., Huang, J., Hu, R., and Ramaswami, B. (2011). The impact of Bt cotton and the potential impact of biotechnology on other crops in China and India, in *Genetically modified food and global welfare*. Emerald Group Publishing Limited.
- Roner, T., Messmer, M.M., Finckh, M.R., Forster, D., Verma, R., Baruah, R., et al. (2012). Participatory cotton breeding for organic and low input farming in Central India. M. Sc., Georg August-University of Göttingen, Germany.
- Santhy, V., Khadi, B., Singh, P., Kumari, P., Deshmukh, R., and Vishwanathan, A. (2008). Hybrid seed production in cotton. CICR Technical Bulletin No. 35. Central Institute for Cotton Research Nagpur, 25.
- Santhy, V., Kumari, P.V., Vishwanathan, A., and Deshmukh, R. (2001). *Legislations for Seed Quality Regulation in India*.
- Snapp S. (2002). Quantifying Farmer Evaluation of Technologies: The mother and baby trial design. In: Bellon.
- Tandon J.P., Sharma S.P., Sandhu J.S., Yadava D.K., Prabhu K.V. and Yadav O.P. (2015). *Guidelines for Testing Crop Varieties under the All-India Coordinated Crop Improvement Projects*, Indian Council of Agricultural Research, New Delhi, India.
- Textile Exchange (2019). *Organic Cotton Market Report 2019*. Textile Exchange.
- The Seed Act (1966). *Legislation on Seeds*, National Seed Corporation Publication, New Delhi, 1990, pp. 1–9.
- Trivedi, R., and Gunasekaran, M. (2013). Indian minimum seed certification standards. The Central Seed Certification Board, Department of Agriculture and Cooperation, Ministry of Agriculture, Government of India, New Delhi 2013, 401-402.
- Santhy, V., Khadi, B., Phundan Singh, P., Vijaya Kumari, P.R., Deshmukh R.K., Vishwanathan, A. (2008). Hybrid seed production in cotton. Central Institute for Cotton Research CICR. Technical Bulletin NO: 35.
- Santhy, V., Vijaya Kumari, P.R., Vishwanathan, A., Deshmukh, R.K. (2009). *Legislations for seed quality regulation in India*. CICR Technical Bulletin No: 38.
- Virk, D. (1998). The regulatory framework for varietal testing and release in India. *Seeds of Choice: Making the Most of New Varieties for Small Farmers*, 69-84.
- Vonzun, S., Messmer, M.M., Boller, T., Shrivastava, Y., Patil, S.S., Riar, A. (2019). Extent of Bollworm and Sucking Pest Damage on Modern and Traditional Cotton Species and Potential for Breeding in Organic Cotton. *Sustainability* 2019, 11, 6353.
- Walker, T.S. (2007). *Participatory Varietal Selection, Participatory Plant Breeding, and Varietal Change*. Background paper for the WDR, 2008.
- Weltzien, E., Smith, M.E., Meitzner, L.S., Sperling, L. (2003). Technical and institutional issues in participatory plant breeding from the perspective of formal plant breeding. A global analysis of issues, results, and current experience. PPB Monograph No. 1. PRGA Programme, Cali, Colombia.
- Witcombe, J.R., Virk, D.S., and Farrington, J. (eds.) (1998). *Seeds of Choice: Making the Most of New Varieties for Small Farmers*, pp. 69–84. Intermediate Technology Publications, London, UK.
- Zahumensky, Y. (2014). Evaluation of Participatory Tools. Case Study about Participatory Technology Development (PTD) in Central India, in: *Global Studies Working Papers 29*. Institute of Geography. Universität Tübingen.

Imprint

Published by

Research Institute of Organic Agriculture FiBL
Ackerstrasse 113, Postfach 219
CH-5070 Frick, Switzerland
Tel. +41 62 865 72 72
info.suisse@fibl.org
www.fibl.org

Authors

Amritbir Riar, Tanay Joshi, Monika Messmer (all FiBL)

Contributors

Surendra Deshmukh (PDKV), Frank Eyhorn (former FiBL), Matthias Klais (FiBL), Ashok Kumar (Chetna Organics), Jonathan Locqueville (FiBL), Lokendra S. Mandloi (bioRe Association), Laura Marthi (ETHZ & FiBL), Gian Nicolay (FiBL), Ramesh Patel (ASA), Shivraj Raghuvanshi (Pratibha Syntex), Mahesh Ramakrishnan (affiliated with MoA, India), Saro G. Ratter (Independent Researcher), Tina Roner (Univ. Kassel & FiBL), Ramprasad Sana (CSA), Brian Ssebunya (FiBL) Devendra Shrivastava (RVSKVV), Rajeev Verma (CottonConnect), Seraina Vonzun (Univ. Basel & FiBL), Benjamin Waltner (FiBL), Yvonne Zahumensky (Univ. Tübingen & FiBL)

Editing

Gilles Weidmann (FiBL)

Layout

Kurt Riedi and Sandra Walti (FiBL)

Photo credits

Action for Social Advancement (ASA), Madhya Pradesh (www.asaindia.org)
Chetna Organic, Odisha (www.chetnaorganic.org.in)
CottonConnect, Maharashtra and Madhya Pradesh, Gujarat (www.cottonconnect.org)
Centre for Sustainable Agriculture (CSA), Andhra Pradesh (csa-india.org)
Research Institute of Organic Agriculture FiBL (www.fibl.org)
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola University (PDKV), Maharashtra (www.pdkv.ac.in)
Pratibha Syntex Ltd., Madhya Pradesh (pratibhasyntex.com)
Rajmata Vijayaraje Scindia Krishi Vishwavidyalaya, Gwalior University (RVSKVV), Madhya Pradesh (www.rvskvv.net)

FiBL ordering no. 1185

ISBN PDF 978-3-03736-381-2 Print version 978-3-03736-380-5

1st edition 2020 © FiBL

Disclaimer

All information contained in this guide was produced by the authors to the best of their knowledge, and checked by them and other experts with the utmost care. However, errors cannot be completely excluded. Therefore, all involved people and FiBL do not accept liability for any loss or damage that may be incurred to/by any person, private, government or non-government organisation based on the information provided in this manual, and will not be responsible for any detrimental effects, financial, intrinsic or material losses, intellectual property issues related to transfer of genetic material and benefit sharing nor any GM contamination, if detected, in the seeds procured from third-party trades, market or commercial seed companies.

Recommended citation

Riar, A., Joshi, T. and Messmer, M. (2020). Participatory On-Farm Breeding of Organic Cotton – a Manual. Research Institute of Organic Agriculture FiBL.

This work is licensed under a Creative Commons Attribution-Non Commercial-ShareAlike 4.0 International



Acknowledgements

The manual was developed in the scope of the participatory cotton breeding project Green Cotton (www.greencotton.org) and the successive project Seeding the Green Future (SGF-cotton.org) supported by the Mercator Foundation Switzerland, the Organic Cotton Accelerator and The Giving Circle of Amsterdam Foundation.

GREEN COTTON



STIFTUNG
MERCATOR
SCHWEIZ



