

Protocol for Evaluation through ICAR-AICRP on Cotton of Bt cotton varieties and hybrids with bio-safety cleared transgenic events

Preamble:

ICAR-AICRP on Cotton is mandated to give new thrust and direction in terms of multidisciplinary and multi-centre approaches with the active involvement of State Agricultural Universities. Currently, the AICRP on Cotton Project is in operation with its headquarters at Coimbatore (Tamil Nadu) and spread over 22 participating centers involving 17 State Agricultural Universities besides more than ten voluntary centers that are also involved. The ICAR-Central Institute for Cotton Research, Nagpur and its Regional Stations located at Coimbatore and Sirsa provide basic research support and also take part in select research and evaluation activities of the AICRP on Cotton. The ICAR-Central Institute for Research on Cotton Technology (ICAR-CIRCOT), Mumbai and its regional units located at Sirsa, Surat, Nagpur, Dharwad, Guntur and Coimbatore are closely associated with AICRP on Cotton in assessing the fiber quality parameters of cotton genotypes, besides ensuring appropriate value addition to cotton.

It is pertinent to mention about the collaborative efforts of AICRP on Cotton in evaluating the suitability of private sector Bt Cotton hybrids (the only commercial transgenic crop approved for cultivation by GEAC) for major cotton growing states in the country from the year 2002 onwards. This public private partnership programme with a strong backing from ICAR and MoEF & CC facilitated AICRP on Cotton to be the first AICRP for evaluating the performance of transgenic crop (transgenic cotton) in the country which led to identification and adoption for commercial cultivation bringing stupendous transformation in the cotton scenario in the country.

From the year 2008 onwards, the evaluation of Bt cotton hybrid was undertaken by State Agricultural Universities. The Genetic Engineering Appraisal Committee (GEAC) has adopted the Event Based Approval Mechanism (EBAM) for regulating Bt Cotton hybrids which is further considered by Standing Committee for identification of superior Bt cotton hybrids based on the one year data generated by SAUs.

The matter of the better alternative mechanism for evaluation of Bt cotton hybrid for release for commercial cultivation was also discussed in the 128th meeting of the Genetic Engineering Appraisal Committee (GEAC) held on 4.3.2016 (Agenda item no. 6.1.2). As per the Proceedings, action on Agenda was taken by ICAR on the recommendation to develop the mechanism for evaluation of Bt cotton hybrid for release towards commercial cultivation. ICAR submitted its recommendation to GEAC suggesting that Bt cotton hybrid events has to be tested as per norms of AICRP system before releasing for commercial cultivation. Further, this matter was discussed in the 130th meeting of the GEAC held on 11.08.2016 as Agenda item 8.3 and suggested “the matter of hybrid approval of Bt cotton through a Standing committee be in future be referred to Ministry of Agriculture / ICAR for further implementation” (reference; Joint Secretary (Seed), DAC, MoA & FW, Govt. of India vide letter No. 13-2/209 SD-V dated 06.12.2016). Now once again, the AICRP on Cotton has been assigned the role to evaluate the Bt transgenic cotton hybrid/ variety entries of

biosafety cleared and approved events for identification of suitable Bt cotton hybrids/varieties for specific agro-ecology. The following guidelines are accordingly proposed:

1. Technical Details of the guidelines

List of GEAC Approved and Biosafety cleared events to be considered for performance evaluation through ICAR-AICRP on Cotton:

Sl No.	Crop	Gene (s)	Event	Developer	Status	Year of Approval
1	Cotton	cry 1Ac	MON-531	Mahyco/ Monsanto	Commercialised	2002
2	Cotton	cry1Ac and cry2Ab2	MON 15985	Mahyco/ Monsanto	Commercialised	2006
3	Cotton	cry1Ac	Event-1	JK Agri- Genetics	Commercialised	2006
4	Cotton	Fused genes cry1Ab and cry1Ac	GFM event	Nath Seeds	Commercialised	2006
5	Cotton	Synthetic cry1Ac	MLS-9124	Metahelix life sciences	Commercialised	2009

A. Initial Evaluation Trial

1. Constitution of Trial

The trials would be constituted with the new entries of Bt cotton (**of Biosafety cleared approved events by GEAC - as above**) sponsored by cooperating breeders/ institutes/ Private organisation. **Transgenic Cotton variety/ *G. hirsutum* x *G. hirsutum* hybrid/ *G. hirsutum* x *G. barbadense* hybrid trials shall be laid out and evaluated separately.**

2. **Number of entries:** Maximum of 100 entries (including checks) shall be accommodated to test in a particular year.

3. Checks

A minimum of two check varieties/hybrids comprising the following shall be used.

- (i) **Zonal check:** the latest approved Bt/Non-Bt variety/hybrid for the zone. The check will be replaced after two year
- (ii) **Local check:** a variety/ hybrid (Bt/non-Bt) popularly grown in a given region/ ecology/ environment of location of the trial. The check will be replaced after two years
- (iii) **Additional checks:** may be included, wherever felt necessary.

4. **Spacing:** Recommended spacing for the variety/ hybrid for the location shall be as per respective SAU practices. **Number of rows:** 4 rows of 6 meters (Approx) per entry with two replications

Spacing recommended	Variety		Hybrid	
	Rainfed	Irrigated	Rainfed	Irrigated
North Zone	-	67.5 x 30 cm	-	67.5 x 90 cm
Central Zone	60 x 30 cm	90 X 60 cm	75 x 45 cm	120 X 60 cm
South Zone	60 x 30 cm	90 X 60 cm	75 x 45 cm	120 X 60 cm

- *Gujarat- Rainfed (120 x 45cm) & Irrigated (120 x 60 cm)*

Agronomic and plant protection measures: As per standard operating procedures

Data to be recorded:

A. Biometric evaluation

1. Germination percentage
2. Plant stand at harvest
3. Plant height (cm) at harvest
4. Number of monopodia
5. Number of sympodia
6. Number of bolls / plant
7. Number of bolls / sq.m.
8. Lint Index (g)
9. Seed Index (g)
10. Ginning outturn (%)
11. Upper Half Mean Length (mm) under HVI Mode
12. Uniformity index under HVI Mode
13. Micronaire under HVI Mode
14. Tenacity under HVI Mode
15. Seed Cotton Yield (q/ha)
16. Quantity of Cry Protein @ 60, 90 and 120 days after sowing (DAS) following standard protocol
17. Bio-efficacy data to be generated at ICAR-CICR, Nagpur, Coimbatore & Sirsa against insect pests.

B. Evaluation under Protected Condition for Insect pests & Diseases incidences (Bench mark value above which the entry will not be accepted- observations to be taken at 30, 60 & 90 DAS)

1. Jassids injury- < 2.0 injury grade
2. Thrips - (10 nymphs or adults/ leaf)
3. Whitefly - (6-8 adults/leaf)
4. *Helicoverpa armigera* - (1 egg /plant & 1 larva/2plants)
5. Pink bollworm - (10% infested fruiting body)

C. Evaluation under unprotected condition for insect pests and diseases (To be conducted only at ICAR-CICR-Sirsa, Nagpur & Coimbatore for the respective zone- observations to be taken at 30, 60 & 90 DAS)

Insect pests:

1. Jassids injury- < 2.0 injury grade
2. Thrips - (10 nymphs or adults/ leaf)
3. Whitefly - (6-8 adults/leaf)
4. *Helicoverpa armigera* - (1 egg /plant & 1 larva/2plants)
5. Pink bollworm- (10% infested fruiting body)
6. Percent Open boll damage- 10 % boll damage
7. Population of natural enemies of insect pests of cotton

Plant Pathology observations

1. Disease injury grade Grey Mildew- < 3 grade
2. Disease injury grade Bacterial Leaf Blight- < 3 grade
3. Disease injury grade Alternaria Leaf Spot - < 3 grade
4. Percent Disease Index (PDI) of CLCuD (in North Zone only)- <40 PDI
5. Incidence of any other diseases/ disorders

Note: The standard operating protocols for recording data on yield attributes, yield, insect pest and disease incidences as per ICAR-AICRP on Cotton shall be followed.

Tentative locations of the trial

North Zone	Central Zone	South Zone
Punjab: Faridkot (Irrigated) Bathinda (Irrigated) Haryana: Hisar (Irrigated) Sirsa (Irrigated) Rajasthan: Sriganganagar (Irrigated)	Gujarat: Surat (Rainfed) Bharuch (Rainfed) Junagadh (Irrigated) SKDAU, Talod (Irrigated) Anand Agricultural University Madhya Pradesh: Indore (Rainfed) Khandwa (Irrigated)	Karnataka: Dharwad (Rainfed) Raichur (Irrigated) Bheemarayangudi (Irrigated) Arabhavi (Irrigated) Andhra Pradesh: Lam (Irrigated) Nandyal (Rainfed)

	Maharashtra: Nagpur (Rainfed) Akola (Rainfed) Nanded (Rainfed) Rahuri (Irrigated)	Telangana: Warangal (Irrigated) Adilabad (Rainfed) Modhol (Rainfed) Tamil Nadu: Coimbatore- (Irrigated) Perambalur (Rainfed)
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Note: Further addition of testing location in a particular State during second year of evaluation shall be based on requirement of concerned SAUs in consultation with PC (Cotton), ICAR-AICRP on Cotton.

Guidelines for Testing

1. Criteria for Sponsoring the Entries

- a. Test cotton entries (approved & biosafety cleared Bt event/s) shall be inducted into the coordinated trials on the basis of data generated by in-house trials and certified by the Sponsoring Agency with at least two years of DSIR registration and being an established R & D firm.
- b. An R&D firm/Institution can sponsor two or more entries per zone (for irrigated and rainfed) under each of transgenic varieties, H x H hybrids and H x B hybrid categories. However, more than two entries will be entertained only after accommodating minimum of two entries from all the Company.
- c. Affidavits duly signed by the Notary Public on Stamp paper that the Biosafe cleared approved event in the test entry submitted for evaluation in ICAR-AICRP on Cotton trial.
- d. Certificate of expression of Bt gene(s) for GEAC approved and biosafe event (cry toxin protein content) from accredited test laboratory need to be enclosed.
- e. The DUS descriptors of essential characters, as per PPV & FRA guidelines, shall be provided for the entries by the sponsoring agency.

Leaf: Colour
Leaf: Hairiness
Leaf: Shape
Plant: Stem hairiness
Bract: Type
Flower: Petal colour
Flower: Petal spot
Flower: Stigma
Flower: Pollen colour
Boll: Shape (longitudinal section)
Boll: Prominence of tip

Boll: Weight of seed cotton/boll (g)
Seed: Fuzz colour
Ginning %
Fibre: Colour

- f. The entries must be characterized by a high degree of phenotypic uniformity and genotypic purity.
- g. The entries shall be considered on the basis of superior performance for yield and/ or other desirable traits such as resistance / tolerance to key biotic/ abiotic stresses better quality etc. as evidenced by the preliminary data to be submitted by the Sponsorer.
- h. Germination and physical purity of the seed of test entries supplied should be equivalent to minimum seed certification standards.
- i. The details of pedigree/ parentage of the entries are needed for sponsoring the entries in the trial.
- j. Entries from recognised R & D organizations will be considered during the Annual workshop/ meeting to be chaired by the Hon'ble DDG (CS) or his nominee.
- k. The sponsoring agency should strictly comply with the national laws, especially those concerning bio-safety and other regulations, if any applicable from time to time.

2. Testing Fee: Indian Rupees 1.00 lakhs/entry/location will be charged during the first year and Rs. 1.00 lakh/entry/location during the second year of evaluation, based on promotion of entries upon good performance in the first year, with service tax and other taxes extra as applicable. The payment has to be made in full at the time of submission of entry for evaluation through online transfer/RTGS in favour of Project Coordinator (Cotton Improvement), ICAR, CICR, Coimbatore-641003.

3. Check varieties/ Hybrids

There will be two checks for evaluation of performance of new entries

- (i) **Zonal checks:** There will be one zonal check for each zone and that will be a latest release variety/hybrid (Bt/Non Bt). Check may be changed once in two years at initial varietal trial level. Checks may be different for irrigated and rainfed trials.
- (ii) **Local check:** Latest state release variety/hybrid (Bt /non Bt) shall be included as local check.

5. Plot size, number of replications, experimental design and crop management: As specified in technical details

6.

5. Test locations: Minimum eight locations data spread over two years is required to qualify for identification. In the second year of testing, the locations can be increased in consultation with SAUs & the Project Coordinator (Cotton), ICAR-AICRP on Cotton.

6. Monitoring of the trials

All the trials shall be monitored by a team of scientists to be deputed by the Project Coordinator (Cotton). The monitoring team shall have the following minimum composition:

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|---|-------------|
| (i) Project Coordinator/ Principal Investigator | Team Leader |
| (ii) Breeder | Member |
| (iii) Agronomist | Member |
| (iv) Pathologist/ Entomologist | Member |
| (v) Scientist of any other specified discipline | Member |

The team shall visit the trial sites around flowering to maturity and record observations on the quality of trial conducted and management as per specified norms and comment on reliability of the data as per protocol and/or prevalent emerging situation.

7. Promotion of entries from IVT to AVT1(CVT)

- (i) In general, top fifteen entries will be promoted from IVT to AVT1, if test entry is found 10% statistically significantly superior over the check. During the test period, if requisite entries are not qualifying, under such conditions, the numerically superior hybrid over the best check will be considered for promotion, totaling to not more than 15 entries.
- (ii) Entries to be promoted shall satisfy minimum bench mark of tolerance to diseases and insect-pests incidence and fibre quality attributes, as given in technical details.
- (iii) Remaining numerically superior entries can be retested in IVT, if sponsoring organization desires for re-evaluation of entries against full payment of testing fees of Rupees 1.5 lakhs per entry per location
- (iv) It is felt ideal that the crop duration for evaluation of performance of the test entries may be 140 ± 5 days under rainfed conditions and 160 ± 5 days under irrigated conditions for arriving at optimum crop duration with ideal maturity taking into account the emerging needs of the country.

8. AVT1 trial: Trial will be conducted as per specification of technical details with desired modifications in respect of plot size, replications, treatments etc., as per protocol of AICRP on cotton for Bt hybrids/ variety evaluation.

9. Bt cotton Variety/hybrid Identification Procedure

A proposal of best performing entries on basis of two year evaluation can be submitted to “**Bt Variety/Hybrid Identification Committee**” constituted in advance of Annual workshop / Group meeting with the approval of the Deputy Director General (Crop Science), ICAR, New Delhi.

a. Constitution of Variety Identification Committee

The Variety Identification Committee shall comprise:

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|---|------------------|
| • DDG (CS) or his nominee | Chairman |
| • Joint Secretary (Seeds) or his nominee | Member |
| • Director of Research of Host Institute/ SAU | Member |
| • Agriculture Commissioner (Dept. of Agric.) or his nominee | Member |
| • One eminent Plant Biotechnologist | Member |
| • One Director of Agriculture (State Govt.) or his nominee | Member |
| • One representative of Seed organization (NSC,SFCI,SSC) | Member |
| • One representative of Private seed agencies | Member |
| • Project Coordinator (Cotton), ICAR-AICRP | Member Secretary |

*Essential members : Minimum quorum of Six members need to be present excluding the Chairman

The Principal Investigators of different research disciplines of AICRP on Cotton shall assist the committee in the capacity of resource persons and will not have voting right.

10. Norms for identification of Bt cotton variety/ hybrid of biosafety cleared transgenic event:

The identification shall be on the basis of the following norms:

Superiority for yield; Superiority/at par in fibre quality with checks; qualifying above the bench marks for tolerance to diseases & insect-pests ; above minimum standards of gene expression level with effective Bioefficacy of test entries in respect of insect pests.

Legal binding

- ICAR/AICRP on Cotton will not have any legal binding of any nature, if any dispute arises against the transgenic event/testing entries/related information provided by concerned R&D company, while submitting the entries for testing along with sought details (Proforma enclosed).
- At any stage, if any illegal GM trait(s) are identified in any test entry, the concerned R&D agency will be black listed and debarred from AICRP testing for a minimum period of five years.
- Initially, two-year testing is only for two season (Kharif 2017 and 2018) and from Kharif 2019, the procedure of three year evaluation as adopted in AICRP on Cotton shall be followed.

Proforma for Submission of Proposal to ICAR-AICRP on Cotton for testing of entries of Bt cotton hybrids/ varieties for biosafe cleared & deregulated approved transgenic events

1	Name of the variety/ hybrid proposed to be tested in AICRP trial	:	
2	Sponsored by (Institute/ Company/R&D firm)		
3	Institution or agency responsible for developing variety/hybrid (with full address and valid DSIR registration certificate)		
4	Name of the person(s) who helped in the development of the variety/hybrid Developers Collaborators	:	
5	a) Parentage (with details of its pedigree including source from which variety/parental lines of hybrid has been developed)	:	
	b) Source of material in case of introduction	:	
	c) DNA profile of variety/ parental lines and hybrid		
	d) Breeding method used	:	
6	Recommended production ecology (Rainfed/Irrigated) for the test entry	:	
7	Description of hybrid/variety (DUS characters as per PPV & FRA guidelines)	:	

In-house Research Data details

- a. Confirmation of gene /event through molecular characterization – **data to be appended**
- b. Level of protein expression in greenhouse/field trials - **data to be appended**
- c. Bioefficacy data generated under Laboratory conditions- **data to be appended**

Table 1. Summary yield data of In-house trials on the proposed Variety/ Hybrid

Name of proposed variety/Hybrid:-----						
Adaptability Zone :-----						
Production conditions :-----						
	Year of testing	No. of trials/locations	Proposed entry 1	Proposed entry 2	Zonal Check	Local check
Mean yield (Q/ha)	1 st year					
	2 nd year					
	Weighted Mean					
Percentage increase or decrease over the checks & qualifying varieties	1 st year					
	2 nd year					
	Weighted mean					
Plant height						
Days to 50% flowering						
Days to maturity						
Boll weight						

Table 2. Reaction to major diseases & Pests

Disease name	Proposed Variety-1	Proposed Variety-2	Zonal Check	Local check
Disease injury grade Grey Mildew-				
Disease injury grade Bacterial Leaf Blight-				
Disease injury grade Alternaria Leaf Spot -				
Percent Disease Index (PDI) of CLCuD (in North Zone only)-				
Incidence of any other diseases/ disorders				
Jassids Injury (average of 3 leaves/plant)				
Thrips (average of 3 leaves per plant)				
Whitefly (average of 3 leaves/plant)				
Bollworms (No. of larvae/5 plants)- American, Spotted and Pink boll worms				

Table 3. Data on Fibre Quality Characteristics (as per ICAR-CIRCOT norms)

Name of proposed variety/Hybrid:-----			
Adaptability Zone		:-----	
Production conditions		:-----	
Quality Characteristic.	Proposed Variety/ Hybrid	Zonal Check	Local Check
Upper Half Mean Length (mm)			
Uniformity Index			
Ginning Out turn (%)			
Micronaire			
Bundle strength (g/tex)			
Any other relevant parameter			

Signature of all Contributors

Signature of Head of Organisation

Affidavit

I _____, Director (Research) ---- Company, having its Registered Office at _____ and with Corporate office at _____ do hereby solemnly state on oath.

That , the information in respect of transgenic cotton hybrid viz _____ with _____ event for _____ zone/ state, submitted for case Verification by this organization on following points:

- a. Conformation of gene/ event through molecular characterization is being used is one of the GEAC approved deregulated event for Cotton
- b. Level of protein expression in green house and field trials.
- c. Morphological characters using DUS descriptors.
- d. Bio-efficacy data generated in laboratory conditions.
- e. Testimony that the sponsored candidate transgenic hybrid is has no legal issues associated.

That, the hybrid/ variety proposed, carry _____ & _____ genes, event _____ which is approved by GEAC in _____ year & is under commercial cultivation.

What is stated above is true and correct as per best of my knowledge ^ belief. We are also aware that any wrong statement or declaration provided in Affidavit, we shall be liable for punitive measure and penalties under the Environment (Protection) Act, 1986.

Hence, .this Affidavit.