FACTS ABOUT Bt COTTON

BCIL
Biotech Consortium India Limited
New Delhi

सत्यमेव जयते
Department of Agriculture & Cooperation
Ministry of Agriculture
Govt. of India

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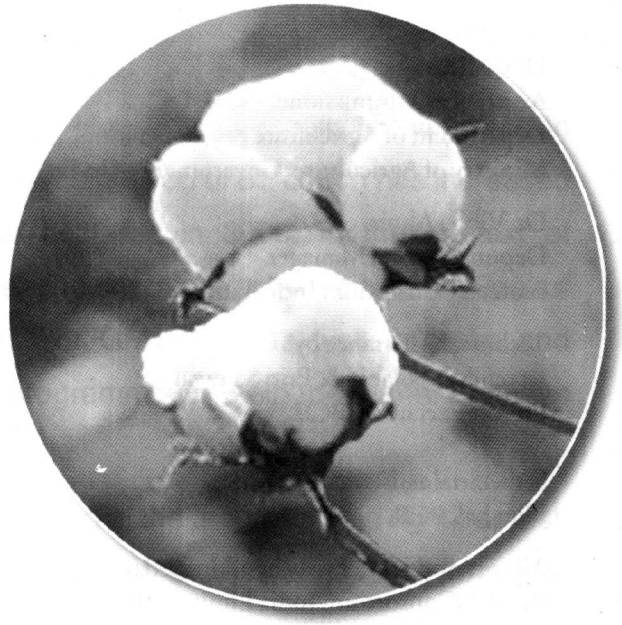

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Edited by
D.S. Mishra
Assistance Commissioner (Seeds)
Department of Agriculture & Cooperation,
Ministry of Agriculture, Government of India

Dr. Vibha Ahuja
Deputy General Manager
Biotech Consortium India Limited

Technical Assistance by
Jeetendera Solanki & Priti Semwal
Project Executive, BCIL

Secretarial assistance by
Hira Lal, BCIL

All correspondence may be addressed to
Biotech Consortium India Limited (BCIL)
Anuvrat Bhawan, 5th Floor
210, Deen Dayal Upadhyaya Marg
New Delhi - 110 002
Tel. No. ++91-11-23219064-67
Fax No. ++91-11-23219063
Email: biotechdelhi@vsnl.com
bcildelhi@vsnl.com
Website: www.biotech.co.in

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We thankfully acknowledge the contribution of:

- Dr. O.P. Govila
  Former Head,
  Department of Genetics,
  Indian Agricultural Research
  Institute, New Delhi.
- Dr. K.K. Tripathi
  Member-Secretary,
  (Review Committee on
  Genetics Manipulation) &
  Advisor, Department of Biotechnology,
  Ministry of Science & Technology
  Government of India.
- Dr. Ranjini Warrier
  Member-Secretary,
  (Genetic Engineering Approval
  Committee) & Director, Ministry of
  Environment and Forests,
  Government of India

S. L. BHAT
संयुक्त सचिव
भारत सरकार
कृषि मंत्रालय
(कृषि एवं सहकारिता विभाग)
कृषि भवन, नई दिल्ली–110001

सत्यमेव जयते

Joint secretary
Government of India
Ministry of Agriculture
Department of Agriculture
& Cooperation)
Krishi Bhawan, New Delhi-110001

e-mail: sl.bhat@nic.in
Telefax: 91-11-2338 9348

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FOREWORD

India is one of the major countries which have released Genetically Modified cotton for commercial cultivation. The area under the cultivation of Bt. cotton has increased rapidly to reach the figure of 3.8 million hectares in 2006. In the context of developments, it is necessary that there should be effective dissemination of information and awareness about benefits of GM crops as well as risks and constraints, in a transparent manner. This programme will help the farmers to have right perspective about the GM crops.

I am glad that the BCIL has taken up the execution of the programme in right earnest. The training programmes are being conducted at District and Tehsil levels in association with State Agriculture Department. I would like to compliment the BCIL for bringing out useful and comprehensive material for the benefit of the trainees in their vernacular languages.

(S. L. Bhat)

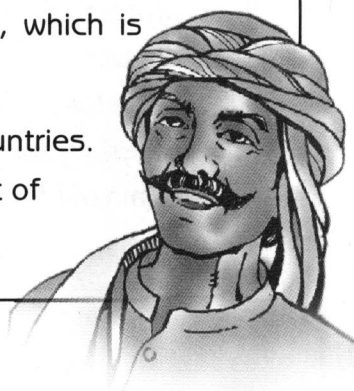
FACTS ABOUT Bt COTTON

INTRODUCTION

Some organisms provide natural protection for plants as they can control target pests but do not harm humans, animals, fish, birds or beneficial insects. Using recombinant technology, the gene that makes these organisms resistant to target insects can be transferred into the plants on which that insect feeds. The plant that once was a food source for the insect now does not allow it to grow on it, thus reducing the need for chemical pesticides to control infestation. *Bacillus thuringiensis* (Bt) is one such bacterium that produces insecticidal proteins. Bt is easily cultured by fermentation and Bt based insecticides in the form of sprays and powders have been in use worldwide for many years and are considered safe for mammals, birds, and for non-target insects. However, the effect of application as sprays/powder is quite limited as the insect does not often come in contact with insecticide because the larvae are found on the underside of leaves or have already penetrated the plant.

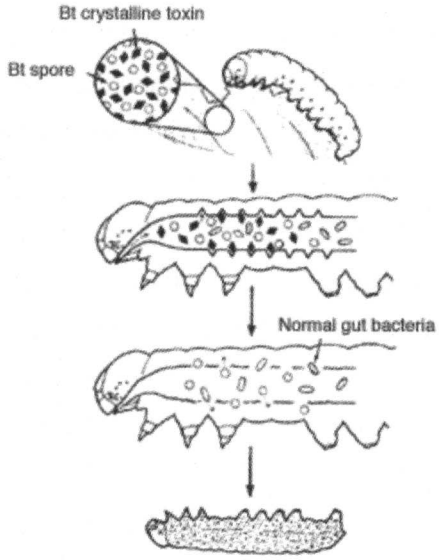
To overcome this problem, genetically modified (GM) cotton has been developed by introducing the insecticidal gene from the bacterium *Bacillus thuringiensis* in the cotton plant, referred to as Bt cotton, which is effective against the Bollworms, the major pests on cotton.

Bt cotton has been cultivated since 1996 in various countries. The use of Bt varieties has dramatically reduced the amount of chemical pesticides applied to these crops.



MECHANISM OF ACTION

Bt protein binds to the receptors in the gut wall and creates pores in the intestinal lining of lepidopteran pests such as bollworms, resulting in breakdown of the digestive wall, and consequent death. As the bacterial *cry* gene encoding for Bt insecticidal protein has now been incorporated into the plants DNA itself, the plant's cells produce this protein themselves. When the insect feeds on a leaf, or boll of Bt gene containing plant, it ingests the Bt protein and dies as depicted below:



1. Caterpillar consumes foliage with Bt protein
2. Protein binds to receptors in the gut wall
3. Gut wall breaks down, leading to leaching of ingested material
4. Caterpillar dies in 1-2 days

Bt proteins are highly specific to receptors in insect gut wall. Bt toxicity depends on recognizing these receptors and damage to the gut by the protein occurs upon binding to that receptor. Each species possesses different types of receptors that will match only certain toxin proteins, like a lock to a key. In view of this specificity, beneficial insects or any other organisms are not harmed by proteins produced by a particular strain of Bt.

ADVANTAGES OF BT COTTON

As Bt cotton plants have a built in mechanism of protection against targeted pests, the protein produced by the plants does not get washed away nor is destroyed by sunlight unlike externally applied pesticides. The plant is thus protected from the bollworm round the clock and throughout its life. The advantages of Bt cotton with genes integrated in the plant are as follows:

- Active protein provides moderate to high dose control that allows fair to excellent control of selected bollworms.
- Active protein expressed in all plant parts
- Active protein expressed throughout the season, hence timing of insecticide applications in relation to an infestation is not an issue
- Wash off of insecticide during rain, and degradation in sunlight are not issues as they are with spray formulations
- Less farmer exposure to insecticide
- Labor saving technology, due to elimination or reduction of insecticide sprays
- Decreases production risks
- Contributes to, and provides the foundation for an integrated pest management (IPM) strategy.

GLOBAL STATUS

Bt cotton is grown in most of the major cotton growing countries, including USA, China, India, Argentina, Brazil, Colombia, South Africa, Australia and Mexico. The total area under Bt cotton cultivation has been estimated to be 13.4 million Hectares in 2006 amounting to 38% of the global area under cotton.

Several Bt genes for pest resistance have been identified and commercialized world over. Monsanto Company developed and deployed the cry1Ac gene from the isolate B. thuringiensis, subsp kurstaki into Coker 312 cotton designated MON 531 and later named Bollgard cotton. This has been followed by development of a second generation of Bt technology called Bollgard II containing two different genes i.e. cry2Ab2 and *cry1Ac*. The Bollgard II (event 15985) is expected to provide broader control over a wide variety of insects such as Spodoptera and leaf roller in addition to common tree Bollworms. Chinese Academy of Agricultural Sciences (CAAS) has developed a modified fusion gene, *cry1Ab/cry1Ac* which has been incorporated in several cotton varieties being grown over large areas in China. The fusion gene has also been incorporated in cotton hybrids in India. Syngenta Seeds has commercialized COT102 containing vip3A (a) gene that imparts resistance among others, to cotton bollworms.

INDIAN STATUS

In India, Maharashtra Hybrids Seed Company (MAHYCO) first imported parental cotton cultivar Coker 312 containing *cry1Ac* gene (MON 531) from Monsanto and carried out a contained breeding programme to incorporate the same into their elite cotton inbred lines.

Bt cotton (MON531) contains the following three genes inserted via genetic engineering techniques:

- The *cry1Ac* gene, which encodes for an insecticidal protein, *cry1Ac*, derived from the common soil microbe *Bacillus thuringiensis* subsp. kurstaki (B.t.k.).
- The *nptII* gene, which encodes the selectable marker enzyme neomycin phosphotransferase II (NPTII).
- The *aad* gene which encodes the bacterial selectable marker enzyme 3"(9)-O- amino glycoside adenyltransferase (AAD).

Both NPT II and AAD proteins are used as selectable markers encoded by respected genes and have no pesticidal activity and are not known to be toxic to any species. The Bt transgene in the converted Indian inbred lines behaves as a single dominant Mendelian factor and is a stably integrated in the plant genome.

After the transfer of Bt trait into Indian cotton germplasm, the biosafety of the *cry1Ac* Bt gene was assessed in these hybrids and field-testing undertaken through systematic trials under All India Coordinated Cotton Improvement Programe under ICAR (AICCIP-ICAR) for two years during 2000 and 2001. M/s MAHYCO undertook around 400 field trials throughout India under different agro climatic conditions.

The chronology of Bt cotton development by MAHYCO is as follows:

1996
- Import of Bt cotton seed of Coker 312 from Monsanto, USA.
- Limited field trial (1 Location) to assess pollen escape
- Back crossing breeding for transfer of Bt gene into elite parental lines in green house.

1997
- Limited field trials (5 locations) to assess pollen escape

1998
- Toxicological (Ruminant goat model) and Allergenicity (BNR model) studies
- Multi-centric research trials (15+25 locations) to assess efficacy of Bt gene in Indian elite germplasm.

1999
Multi-centric research trials (11 locations) to assess efficacy of Bt gene in Indian elite germplasm.

2000
- Large-scale trials (100ha)
- Hybrid seed production (150 ha)
- Various biosafety studies.
- ICAR trials at 6 locations

2001-02
- Large-scale trials (100ha)
- Hybrid seed production (300 ha)
- Biosafety studies
- ICAR Trials at 11 locations

April 2002 Commercial approval of three hybrids in Six States (Andhra Pradesh, Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Tamil Nadu) with the following conditions:
- Valid for three years

- Provide same non Bt seed to meet refuge requirements
- Conduct studies to monitor resistance development
- Provide information to government on distribution of the seed through its dealers and agents
- Labeling requirements such as GEAC number, etc.,
- Develop Bt based IPM program
- Undertake education and awareness program

Subsequently one more hybrid of Rasi seeds was given approval in 2004. In 2005, hybrids were approved for cultivation in three northern cotton growing states i.e., Punjab, Haryana and Rajasthan. Several companies have taken sub-license from Mahyco Monsanto Biotech India Limited, a commercial arm of MAHYCO and Monsanto to incorporate Bt character into their hybrids by back crossing, undertaken field trials and are marketing their own Bt cotton hybrids. Taking into consideration the need for introducing diversity in the gene as well as germplasm as a tool to contain the development of insect resistance, hybrids containing three new Bt cotton genes/events have been approved in 2006. These are:

i. *cry1Ac* gene (event 1) by M/s. J.K. Agri Seeds Ltd.

ii. Fusion genes *(cry1Ab+cry1Ac)* GFM by M/s Nath Seeds

iii. Stacked genes *cry1Ac* and *cry2Ab2* by M/s MAHYCO

As of now 62 hybrids of all the four events are approved for commercial cultivation in India.

Bt cotton hybrids approved till 2006 for commercial cultivation in India

Zone	Company	Hybrid
North	Ankur Seeds Ltd	Ankur 2534 Bt
	J.K. Agri Genetics Seeds Ltd.	JKCH 1947 Bt
	MAHYCO	MRC 6304 Bt, MRC 6029 Bt., MRC-6025 Bt.
	Nath Seeds Ltd.	NCEH-6R
	Nuziveedu Seeds Ltd	NCS 138 Bt.
	Rasi Seeds Ltd	RCH 134 Bt, RCH 308 Bt., RCH 317 Bt, RCH 314 Bt.
Central & North	Ankur Seeds Ltd	Ankur 651 Bt
	MAHYCO	MRC 6301 Bt
Central	Ajeet Seeds Ltd.	ACH-11-2 BG II
	Ankur Seeds Ltd	Ankur 09
	Ganga Kaveri Seeds Pvt. Ltd.	GK 205 Bt., GK 204 Bt.
	J.K.Agri Genetics Seeds Ltd.	JK Varun Bt.
	Krishidhan Seeds Pvt. Ltd.	KDCHH 9821 Bt., KDCHH-441 BG II
	MAHYCO	MECH 12 Bt *, MRC 7301 BG II, MRC-7326 BG II, MRC-7347 BG II
	Nath Seeds Ltd.	NCEH-2R
	Pravardhan Seeds Ltd.	PRCH-102 Bt.
	Rasi Seeds	RCH 377 Bt., RCH 144 Bt, RCH 118 Bt, RCH - 138 Bt
	Vikki Agrotech Pvt. Ltd.	VCH-111 Bt.
Central & South	Ajeet Seeds Ltd.	ACH-33-1 Bt., ACH-155-1
	Emergent Genetics	Brahma Bt.
	Krishidhan Seeds Pvt. Ltd.	KDCHH 9810 Bt., KDCHH 9632 Bt.
	MAHYCO	MECH 162 Bt* , MECH 184 Bt*
	Nuziveedu Seeds Ltd.	NCS 207 Mallika, NCS 145 Bunny
	Prabhat Seeds Ltd.	NPH 2171 Bt.
	Rasi Seeds Ltd	RCH 2 Bt
	Tulasi Seeds Pvt. Ltd.	Tulasi 4 Bt., Tulasi 117 Bt.
	Vikram Seeds Pvt. Ltd.	VICH 5 Bt., VICH 9 Bt.
North/ Central/ South	Nuziveedu Seeds Ltd	NCS-913 Bt.
South	Ganga Kaveri	GK-209 Bt., GK-207 Bt.
	JK Agri Genetics Ltd.	JK Durga Bt, JKCH-99 Bt
	MAHYCO	MRC 6322 Bt, MRC 6918 Bt, MRC-7351 BG II, MRC 7201 BG II
	Nath Seeds Ltd.	NCEH-3 R
	Prabhat Seeds Ltd.	PCH-2270 Bt.
	Rasi Seeds Ltd	RCH 20 Bt, RCH 368 Bt, RCH 111 BG I, RCH-371 BG I, RCHB-708 BG I

*Approval not renewed for Andhra Pradesh. **North zone:** Haryana, Punjab & Haryana **Central zone:** Gujarat Madhya Pradesh and Maharashtra **South Zone:** Andhra Pradesh, Karnataka and Tamil Nadu

AREA UNDER BT COTTON CULTIVATION IN INDIA

Bt cotton has been grown in six states i.e. Madhya Pradesh, Gujarat, Maharashtra, Andhra Pradesh, Karnataka and Tamil Nadu since 2002 and three northern states i.e. Punjab, Haryana and Rajasthan since 2005. India grew approximately 38,000 hectares of Bt cotton hybrids for the first time in 2002, and doubled its Bt cotton area to approximately 100,000 hectares in 2003. The Bt cotton area increased again four-fold in 2004 to reach over half a million hectares. In 2005, the area planted to Bt cotton in India continued to climb reaching 1.3 million hectares, an increase of 160% over 2004. In 2006, the record increase in adoption in India continued with almost a tripling of area of Bt cotton to 3.8 million hectares of which 34% was under irrigation and 66% rainfed. The distribution of Bt cotton in the major growing states in 2004, 2005 and 2006 is shown in the following table:

Adoption of Bt Cotton in India, by Major State, in 2004, 2005, and 2006 ('000 hectares)

State	2004	2005	2006
Maharashtra	200	607	1,840
Andhra Pradesh	75	280	830
Gujarat	122	150	470
Madhya Pradesh	80	146	310
Northern Zone*	–	60	215
Karnataka	18	30	85
Tamil Nadu	5	27	45
Other	–	–	5
Total	500	1,300	3,800

* Punjab, Haryana, Rajasthan
Source: International Service for the Acquisition of Agri-biotech Applications (http://www.isaaa.org)

SAFETY STUDIES

Prior to its approval for commercial cultivation, Bt cotton was subjected to biosafety assessment studies carried out in the laboratories and experimental fields designated by regulatory authorities as summarized below:

1. STUDIES ON ENVIRONMENTAL SAFETY

- **Pollen escape/out crossing:** As the cotton pollen is heavy and sticky, the range of pollen transfer is limited. This was confirmed by multi location experiments. Also there is essentially no chance that the Bt gene will transfer from cultivated tetraploid species such as the present Bt hybrids to traditionally cultivated diploid species.
- **Aggressiveness and Weediness:** The results demonstrated that there are no differences between Bt and non-Bt cotton for germination, vigor, weediness and aggressiveness potential
- **Effect on non-target organisms:** Studies conducted during the multi-location field trials revealed that the Bt cotton hybrids do not have any toxic effects on the non-target species, namely sucking pests (aphids, jassids, whitefly and mites). The beneficial insects such as lady bird beetle and spiders remained active in both Bt and non Bt varieties.
- **Soil Studies:** Bt protein was not detected in soil samples indicating that Bt protein is rapidly degraded in the soil on which Bt cotton is grown. No difference was found in population of microbes and soil invertebrates (like earthworms and nematodes) between Bt and non Bt samples

2. STUDIES TO EVALUATE THE FOOD SAFETY

- **Compositional analysis:** The studies revealed that there is no significant change in the composition of Bt and non Bt seeds, with respect to proteins, carbohydrates, fats, ash content and calorific value.
- **Allergenicity studies:** There was no significant change in endogenous allergens of Bt seed compared to non Bt cotton seed
- **Toxicological study:** No significant differences were found between goats fed with Bt and non Bt cottonseed. The gross as well as histopathology showed no significant diffrence.
- **Absence of *cry1AC* gene and Bt protein in Bt cottonseed oil:** Studies confirmed that *cry 1AC* gene and Bt protein are not found in refined oil obtained from Bt cottonseeds.
- **Feeding studies on cows, buffaloes, fish and chicken:** Results indicated that Bt cotton seed meal is nutritionally equivalent, wholesome and safe as the non-Bt cottonseed meal.

PEST MANAGEMENT IN BT COTTON

Simple insertion of Bt gene is not sufficient for pest management and constant monitoring of crop for the presence of bollworm is essential. The spray schedules for bollworm have to be on based on Economic Threshold Limit (ETL) that needs to be established by scouting twice a week in morning hours. Twenty plants should be counted at random for presence of live bollworm larvae. If count exceeds 20 live bollworm larvae, then spraying needs to be adopted. The three-step process for scouting is as follows:

Scouting: Three step process

Step-1
Select at random 20 plants in 1 acre of cotton field.

Step-2
Count the number of living (1/4th inch or large) bollworm larvae on each of these plants.

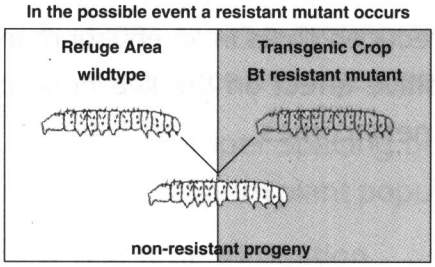
Step-3
If total number of living bollworm larvae on 20 plants are 20 or more than 20 then we need to spray.

Similar spray schedules may be required for pest management of other pests such as jassids, whitefly, aphids, thrips, mites etc. as undertaken in case of non-Bt cotton crops.

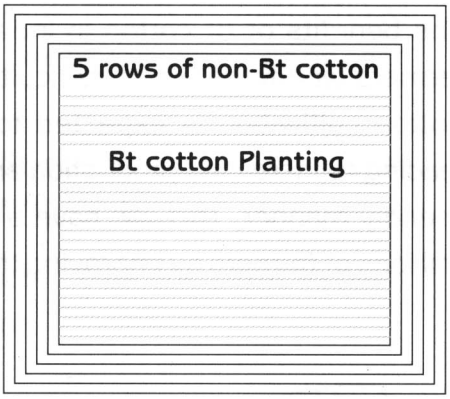
INSECT RESISTANCE MANAGEMENT

Pest populations exposed to Bt-crops continuously for several years may develop resistance to Cry proteins. Resistance is not unique to Bt-crops. In view of this, proactive Insect Resistance Management (IRM) strategies have been developed and are in place. A key element of these plans is that growers should plant sufficient acreage of non-Bt crops to serve as a refuge for producing Bt-sensitive insects. The refuge strategy is designed to ensure that Bt-sensitive insects will be available to mate with Bt-resistance insects, if they arise. The offspring of these matings will be Bt-sensitive, thus mitigating the spread of resistance in the population. Gene pyramiding, optimum dose and deployment of Bt-crops as one of the components of integrated pest management (IPM) are the other options for IRM.

Growing refuge has been made as one of the conditions while giving approval for Bt cotton in India. In India, *Helicoverpa armigera* (American Bollworm), besides cotton, has a large number of alternative hosts like chickpea, pigeonpea, tomato, sunflower, maize and sorghum that are grown in the same area at the same time as cotton. These also serve as natural refugia, thereby helping IRM.



Refuge Strategy



A sample refuge planting

IMPACT OF BT COTTON

Cotton is a very important commercial crop in India with nearly 9 million hectares of land being used for cotton cultivation under diverse agroclimatic conditions. Cotton contributes to nearly 30% of the gross agricultural domestic product and provides a livelihood to more than 60 million people by way of support in agriculture, processing and use of cotton in textiles.

With the introduction of Bt technology, there has been a significant change in the cotton cultivation scenario in the country. Within a period of five years after the approval of Bt cotton with *cry1Ac* (Mon 531 event), 62 hybrids have been approved for commercial cultivation and about 121 Bt cotton hybrids are under various stages of field trials. The area under Bt cotton in India has increased from 38000 hectares in 2002 to 3.8 million hectares in 2006. In addition to Bt hybrids containing the *cry1Ac* gene (MON 531 event), hybrids with three new gene/event namely Bt hybrids expressing fusion genes *(cry 1Ab+cry1Ac)* "GFM *cry1A*" developed by M/s Nath Seeds, Bt hybrids expressing *cry1Ac* gene (Event-1) by M/s JK Seeds Ltd and Bt hybrids expressing stacked genes *cry1 Ac* and *cry2Ab* (MON 15985 event)BG-II by M/s Mahyco have been approved.

The benefits of Bt cotton in India are in line with those enjoyed by farmers worldwide who have cultivated Bt cotton. The area under Bt cotton cultivation is expected to increase further in coming years leading to increased production and reduced costs in an environment friendly manner. This will have a positive effect on the livelihood of millions of farmers by improving their net incomes.

DO'S & DON'TS

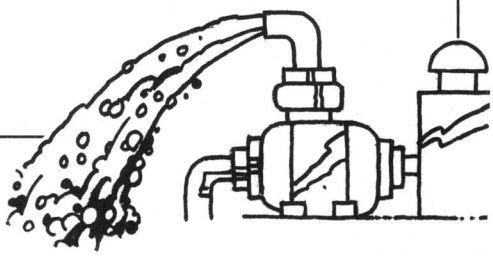
- Purchase Bt-cotton seeds of approved hybrids only from authorized dealers/distributors and ensure government approval seal on each packet.

- Never buy loose seeds

- Storage, distribution and sale of unapproved Bt cotton seed is an offence under Environment Protection Act.
- Always insist for receipt/Bill while purchasing Bt cotton seeds

- Receipt/cash memo must show Lot no., name of the Hybrid & other relevant details
- Have full information about practices & procedures of cultivation

- Spray pesticides for pests (other than bollworms) such as sucking pests i.e. aphids, white flies, thrips, jassids, etc. as normal practice.
- In case of heavy infestation of bollworms, Bt cotton may need spray of pesticides to control the same. Decision on this should be based on Economic Threshold Level (ETL) as recommended i.e. if average one larva is observed on each plant as described
- Plant refugia around the Bt cotton as part of insect resistance management (IRM) strategies
- Have proper spacing between rows and plant to plant for optimum plant population
- Do Timely irrigation



NOTES




2007

January

1- Id-ul-Zuha, 13- Lohri
23- Basant Pancami,
26- Republic Day, 30- Moharram

Sun	Mon	Tue	Wed	Thu	Fri	Sat
	1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	16	17	18	19	20
21	22	23	24	25	26	27
28	29	30	31			

February

16- Maha Shivratri

Sun	Mon	Tue	Wed	Thu	Fri	Sat
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11	12	13	14	15	16	17
18	19	20	21	22	23	24
25	26	27	28			

March

3- Holi, 4- Dhulendi, 27- Ram Navami
31- Mahaveer Jayanti

Sun	Mon	Tue	Wed	Thu	Fri	Sat
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April

1- Id-Ul-Milad, 6- Good Friday
14- Baisakhi, 14- Ambedkar Jayanti

Sun	Mon	Tue	Wed	Thu	Fri	Sat
1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30					

May

2- Budh Purnima

Sun	Mon	Tue	Wed	Thu	Fri	Sat
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July

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29	30	31				

August

15- Independence Day
28- Raksha Bandhan

Sun	Mon	Tue	Wed	Thu	Fri	Sat
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12	13	14	15	16	17	18
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September

4- Janmashtmi, 15- Ganesh Chaturthi, 25- Anant Chaudas

Sun	Mon	Tue	Wed	Thu	Fri	Sat
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9	10	11	12	13	14	15
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October

2- Gandhi Jayanti
14- Id-Ul-Fitar, 21- Dushera

Sun	Mon	Tue	Wed	Thu	Fri	Sat
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14	15	16	17	18	19	20
21	22	23	24	25	26	27
28	29	30	31			

November

9- Diwali, 11- Bhaiya Dooj
24- Gurunanak Birthday

Sun	Mon	Tue	Wed	Thu	Fri	Sat
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11	12	13	14	15	16	17
18	19	20	21	22	23	24
25	26	27	28	29	30	

December

21- Id-Ul-Zuha
25- Christmas Day

Sun	Mon	Tue	Wed	Thu	Fri	Sat
30	31					1
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9	10	11	12	13	14	15
16	17	18	19	20	21	22
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