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Testing Seed Quality of Bt Cotton

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The views expressed in this column are his own and not that of Cotton Association of India)

EXPERT's Column

Dr K.R. Kranthi

Bt Cotton

Bt cotton is genetically modified (GM) cotton variety/hybrid that has crystal (Cry) protein toxin producing genes derived from a soil bacteria called Bacillus thuringiensis (Bt). The bacterial species was first discovered by Prof Ishiwata in 1901 in Japan. Many crystal proteins produced by the bacteria are toxic as stomach poison to many species of insects. When insects feed on the toxins, they cause holes in the membrane that lines the insect stomach.

The genetically modified (GM) technology was developed first by Monsanto and released commercially in USA, Mexico and Australia in 1996. Subsequently, the technology was introduced into China (1997), South Africa (1998), Argentina (1998), India (2002), Colombia (2002), Brazil (2005), Costa-Rica (2008), Burkina Faso (2009) and recently in Pakistan and Mynamar in 2010. Thus 13 countries cultivated Bt cotton in 161 lakh hectares in 2012, which accounts for 48% of the global cotton area. Bt Cotton was introduced into India in the year 2002 and became extremely popular to the extent that about 95% of India's cotton area is under Bt cotton hybrids with almost all of the area under Monsanto's Bt technology. Incidentally, cotton is the only GM crop approved for commercial cultivation in India. Bt-cotton hybrids that express Cry1Ac, Cry2Ab, Cry1C and fusion gene (Cry1Ac) have been approved by the GEAC for commercial cultivation in India.

The GEAC approved the following GM Bt cotton events:

1. Monsanto: MON531 (Cry1Ac) event Bollgard;
2. Monsanto: Mon15985 (Cry1Ac+Cry2Ab2) event in Bollgard-II;
3. JK seeds, India: JK Event-1 (Cry1Ac);
4. Chinese Academy of Agricultural Sciences, China: GFM Cry1A (Cry1Ac), introduced by Nath seeds India;
5. NRCPB, New Delhi and UAS Dharwad, India: BNLA601 (Cry1Ac) event;
6. Metahelix, India: Event 9124 (Cry1C).

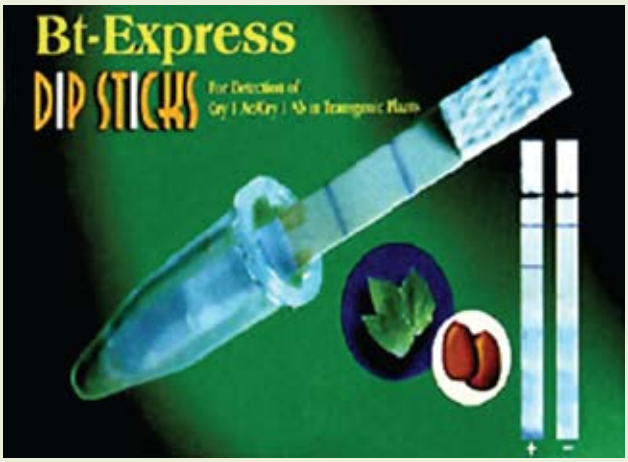
The GEAC has thus far approved the cultivation of 1128 Bt cotton hybrids for commercial cultivation in India. However, about 25 to 30 hybrids developed by 6 to 7 major companies occupy more than 70.0% of the area. It is estimated that about 85% of the current area is under Bollgard-II (Cry1Ac+Cry2Ab) and rest under Bollgard (cry1Ac) being produced and marketed by 44 Indian seed companies.

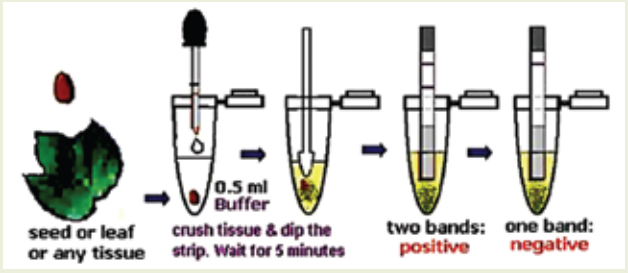
Bt testing kits developed by CICR

Bt detection kits were developed and commercialized by Central Institute for Cotton Research (CICR). Patents for the Bt detection kit were granted in the following countries vide patent numbers (Inventor: Dr K. R. Kranthi: Patents: South Africa, 2007: Patent No. 2004110268. /ZA200410268; China, 2008: Patent No. ZL 03817641.6CN1672049; Mexico, 2008: Patent No. MXPA04011769; Uzbekistan, 2008: Patent No. WO03102208 and South Korea, 2008: Patent No. KR20050026396).

CICR developed simple 'dip-stick' kits and 'ELISA' (Enzyme Linked Immuno-Sorbent Assay) to enable farmers, seed testing officers, researchers, seed companies and regulators detect Bt-seed quality and Bt-toxin expression in plants under field conditions. ELISA test kits have been developed by CICR for detection and quantitative testing of Cry1Ac, Cry2Ab and Cry1C proteins, in the test material such as seeds, leaves or other plant parts. CICR developed PCR tests to detect specific gene and events for five major events based on the data available in public domain. Event specific data was unavailable for Metahelix Event 9124 (Cry1C). CICR developed 'dip-stick-strip test kits' for instantaneous 10-min detection test at 'on-site' conditions for leaves or seeds containing Cry1Ac, Cry2Ab and Cry1C proteins. Additionally another test called "GUS-reporter test" has been developed as a 30-min easy reagent based test to detect the reporter protein/gene associated with Cry2Ab. The dip-stick kits can be used to regulate quality while the ELISA kits are used to quantify the levels of Bt-toxin expression so as to identify the time when Bt-crop shows poor expression of Bt-toxin, when farmers need to take up appropriate control measures.

The Bt-detection kits have been commercialised and became extremely popular with farmers and seed testing agencies, as evidenced by the fact that more than 40,000 kits have been used by stake holders. The Bt-detection kits enabled regulation, streamlining and ensuring Bt-cotton seed quality for farmers in the country. All seed testing laboratories in India have been using the kits and more than 6000 seed lots have been tested using the kits. Legal cases have been filed in courts of several cotton growing states of north, central and south India and are under review. In the absence of the testing kits, illegal Bt-seed would have been rampant and proliferated without any control. It has been widely acknowledged that the kits acted as deterrents for spurious seed traders. It is estimated that the cotton yield losses due to illegal seed trade and Bt-spurious-seed trade would have reached about Rs 250 crores worth each year, if the kits were not available. The kits assisted the technology developers of Bt-cotton to introduce the technology and establish it in the market to an extent of 90% coverage, that resulted in cotton yields doubling to 31.5 million bales (170 kg lint per bale) in just 5 years from a meager 16.5 million bales in 2001. The sub-standard seed samples have now decreased to 5.23% in 2007-08 as compared to 69% in 2003-04 apparently due to the constant vigil and continuous testing. One significant advantage of the simple 'dip-stick test developed by CICR, has been that it empowered farmers, extension workers and seed testing agencies with a rapid test that can be conducted 'on-the-spot' directly in shops or in fields. Therefore it served as a strong deterrent to the manufacturers and traders who would have otherwise continued to produce substandard and spurious seeds which were being





sold either in the name of the GEAC approved brands or most of them as unapproved brands. The role of CICR and ICAR in regulating Bt-seed quality in India has been widely acknowledged.

Seed testing methods of the approved events

Three kinds of tests are used commonly in India to detect the purity of GM seeds.

1. Antibody based tests:
   a. ELISA (Enzyme Linked Immuno-Sorbent Assay) and
   b. Dip-stick-strip immuno-sorbent test
2. PCR (polymerase Chain Reaction) based tests:
   a. Regular PCR
   b. Real-Time qPCR (quantitative PCR)
3. Biochemical tests
   a. GUS (Glucuronidase Assay) to detect the GM reporter enzyme

Gazette notifications for GM seed testing

There are ten main gazette notifications that relate to seed testing of GM crops.

On the 12th November, 2003, S.O. 1300(E).-In exercise of the powers conferred by Sub-section (1) of Section 4 of the Seeds Act, 1966 (54 of 1966), the Central Government declared the laboratory of Central Institute of Cotton Research (CICR), Indian Council of Agricultural Research (ICAR), Nagpur as the Central Seed Laboratory to carry out the functions of ascertaining the presence or absence of cry1Ac gene in Cotton seeds under the said Act with effect from the date of publication for the whole of lndia. 2. In pursuance of clause (c) of rule 5 of the Seeds Rules, 1968, the Central Government also entrusted the

Central Institute of Cotton Research, Indian Council of Agricultural Research, Nagpur to act as a referral laboratory for Bacillus thuringiensis Cotton seeds (Bt. Cotton seeds).

A sample of 25 gm seeds would be drawn from 450 gm seed packet and ten seeds will be tested for the Cry toxin. The minimum limits of quantification were stipulated at 420 ng/gm seed or 420 ng per sq cm of a leaf disc for the sample to be qualified as positive for Bt.

On 5th November 2005 -S.O. 1567(E)-ln exercise of the powers conferred by Section6 of the Seeds Act, 1966 (Act 54 of 1966), the Central Government, after consultation with the Central Seed Committee specified the purity in terms of quantum of gene express of Bacillus thuringienisis (Bt.) Protein (Toxin) as 90 per cent in Bacillus thuringiensis cotton seed lot for labelling of Bacillus thuringienisis Cotton Seed.

On 21st September 2006, six gazette notifications (G.S.R.584 (E) to 589(E) dated September 21, 2006) were issued to empower all the seed Inspectors/ analysts and laboratories notified under Seed Act, also under EPA, 1986.

On 8th May 2008, S.O.1107(E)- In exercise of the powers conferred by Sub-section (1) of section 4 of the Seeds act, 1966 (54 of 1966), the Central Government made the following amendment in the notification of the Government of India, DAC number S,O, 1300(E) dated 12th November 2003 and published in the Gazette of India, Extraordinary, Part II, Section 3, Sub-section (ii) namely: In the said notification, in paragraph 1, in line No 7, for the words and figures 'cry1Ac', the following words shall be substituted, namely –all types of Bacillus thuringiensis.

Methods to test Bt cotton

A working sample of 25 g should be taken in a random manner from the seed packet.

1. ELISA test / dip-stick-strip test: 90 seeds to be tested from working sample size of 25 g drawn from a single packet of 450 g. A minimum number of 81 seeds tested positive for the test protein, Cry1Ac, Cry2Ab, Cry1C and fusion-gene protein Cry1Ab-Cry1Ac, may be taken as the acceptable value for 90% gene purity.

2. PCR test: 30 seeds to be tested from working sample size of 25 g drawn from a single packet of 450 g. A minimum number of 27 seeds tested positive for primers specific to the gene (cry1Ac, cry2Ab, cry1C and fusion cry1Ac gene (cry1Ab+cry1Ac)) may be taken as the acceptable value for 90% gene purity. If only 25-26 seeds are positive, 30 freshly drawn seeds from the same working sample may be re-tested again on PCR. The total number of positive seeds from the two tests should be equivalent to or more than 54 out of the total 60 seeds tested from the working sample.

3. Event specific PCR (only for referral purposes): 10 seeds to be tested from working sample size of 25 g drawn from a single packet of 450 g. A minimum number of 9 seeds tested positive for the event may be taken as the acceptable value for 90% event purity.

4. Gus-Reporter test: 90 seeds to be tested from working sample size of 25 g drawn from a single packet of 450 g. A minimum number of 81 seeds tested positive for the test protein GUS (ß-Glucuronidase) which is the reporter gene for Bollgard-II may be taken as the acceptable value for 90% gene purity of Cry2Ab.

Suggestions:

The current gazette notification does not include essential requirement of 90% purity for each of the Cry toxins in Bollgard-II. It only mentions that the hybrid seeds should contain 90% of Bt toxins, which, in effect are present even in F-2 seeds. This should be rectified. The state seed testing laboratories must be provided with Bt testing fw acilities. Seed analysts should be trained in Bt testing methods. Only Andhra Pradesh has been able to set up such facilities. Gazette notifications must be issued for DNA based PCR testing to include all GM events approved in India. Unless and until seed testing is done rigorously, there is every possibility that the crop suffers in farmers' feilds. Therefore, the seed testing laboratories in all the cotton growing states must develop Bt testing facilities and test for the presence of Bt in random samples to ensure that the best quality seed reaches farmers.