



TECHNOLOGIES

DEVELOPED BY

CENTRAL INSTITUTE FOR COTTON RESEARCH

POST BAG NO. 2, SHANKAR NAGAR PO, NAGPUR-440010



CENTRAL INSTITUTE FOR COTTON RESEARCH
Post Bag No. 2, Shankarnagar PO,
Nagpur-440 010



PUBLISH BY
DIRECTOR, CICR, Nagpur

Compiled by
Dr. Punit Mohan,
Dr. G.Balasubramani,
Dr. Sandhya Kranthi

Composed by
Smt. Sangeeta Aurangabadkar

Year of Publication: 2016

An Output of: ITMU Cell



FOREWORD

Indian agriculture has come under the ambit of intellectual property rights with international developments directly influencing agricultural research in developing countries. The Indian Council of Agricultural Research has taken several steps towards developing and strengthening the decentralized three tier intellectual management system comprising of the Agricultural Technologies Management Centre at the top, the Zonal Technology Management and Business Planning and Development Centre in the middle and the Institute technology Management units at the bottom.

Technologies are outputs of good science. Technologies drive civilizations towards progress. Agricultural technologies aim at enhancing farm productivity and farmer prosperity. In the current context of global competitiveness, farm technologies play a vital role in modernizing agriculture thereby ensuring and enhancing food security, especially for agriculture based countries in a developing world. Development of a technology is a highly challenging task, but, taking it to the doorstep of the end-user, through commercialization is even more challenging. The Central Institute for cotton research has been in the forefront of technology development, patenting and commercialization. The support of the ICAR in this endeavor has been immense. The institute has pioneered the development of PCR based disease diagnostic kits and immunological kits to detect new Bt genes such as Cry1Ac, Cry1C and VIP3 in new Bt cotton varieties that being released recently. A new 10-minute test kit was developed to detect genetically modified seeds in quarantine ports. The institute filed six patents during the year for new machinery such as planters and solar powered sprayers, disease diagnostic kits and new tissue culture techniques. The Bt-detection kits have become extremely popular with farmers and seed testing agencies, as evidenced by the fact that kits worth more than Rs 4.00 crores, have been purchased thus far. The Bt-detection kits enabled regulation, streamlining and ensuring Bt-cotton seed quality for farmers in the country. All seed testing laboratories in the country have been using the kits and more than 6000 seed lots have been tested using the kits. 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. 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. The roles of CICR and ICAR in regulating Bt-seed quality in India are now being widely acknowledged across the globe. It is gratifying to note that the ICAR has developed guidelines to ensure that good technologies benefit all stakeholders including scientists, entrepreneurs and farmers.

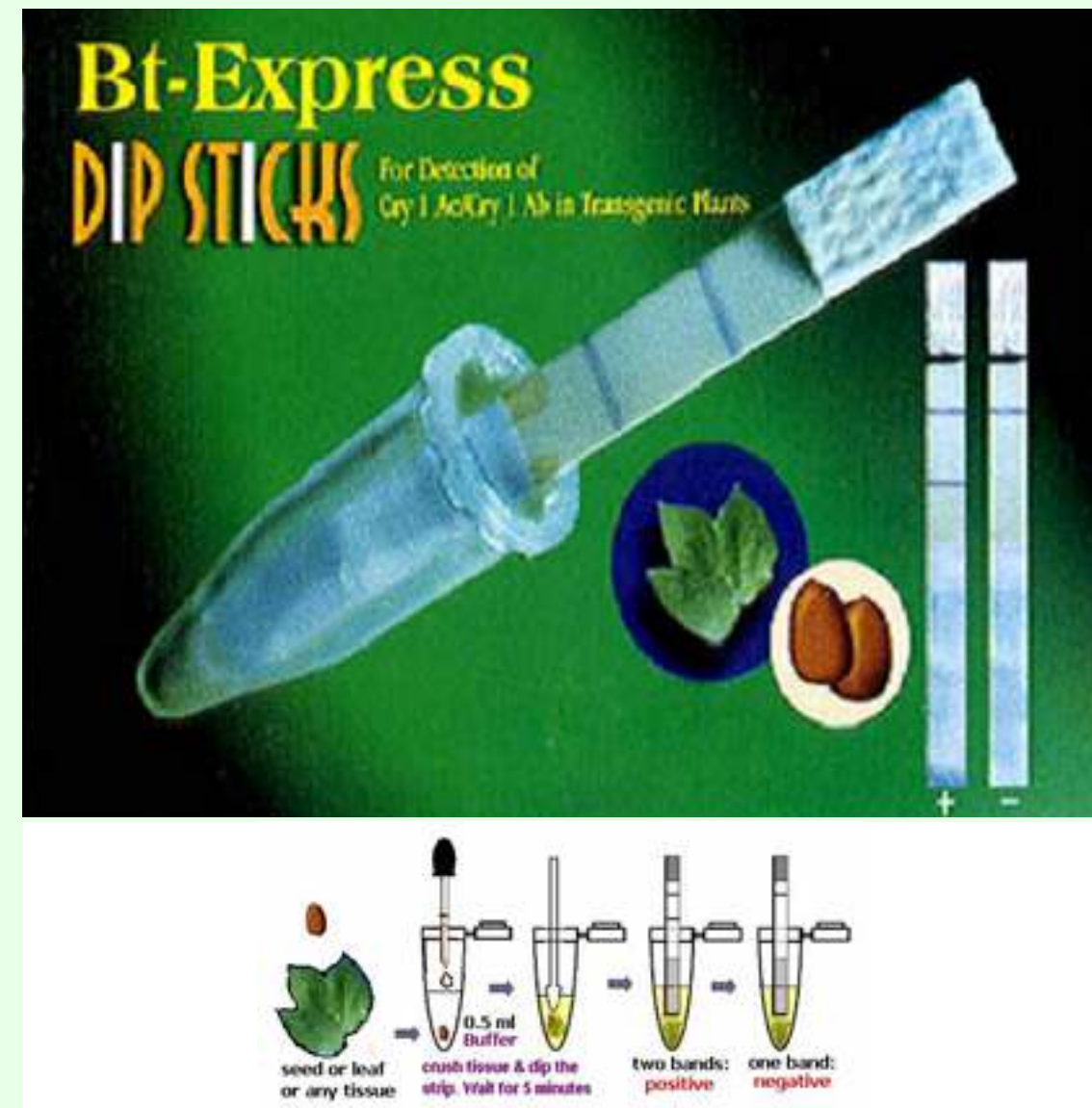
This technical bulletin showcases in brief the technologies that have been patented and those that have either been commercialized or are in the pipeline for commercialization for the benefit of the cotton farmer. We earnestly hope that the information provided in this bulletin will encourage entrepreneurs to explore the possibilities of taking the technologies to end-users through the process of commercialization that can benefit all stakeholders.

(K. R. Kranthi)
Director

Bt Express

(to detect Cry1Ac/Cry1Ab in Bt Cotton)

IPR No. 600/DEL/2002 dated 31-5-02; patents granted in South Africa: Rapid Detection of Bt-Cry Toxin No. IAP 2004-0451; PCT/IN03/00199 filed on May 29, 2007; Patent granted in China: No. FP02228-GB/vcd granted in 2008.



A simple immunological test that can be used directly in the field by even illiterate farmers. The kit is provided with complete material (pestles, plastic vials, buffer etc.) adequate for 50 tests. It can be used for seeds, leaves, squares, flowers or any tissue. The test takes 10 minutes to complete and gives a clear result in detecting the presence/absence of the Bt-toxin in the tissue tested. Highly popular with farmers.

Bt Quant ELISA Kit

(to quantify Cry1Ac/Cry2Ab/Cry1F)

IPR No. 600/DEL/2002 dated 31-5-02; patents granted in South Africa: Rapid Detection of Bt-Cry Toxin No. IAP 2004-0451; PCT/IN03/00199 filed on May 29, 2007; Patent granted in China: No. FP02228-GB/vcd granted in 2008.

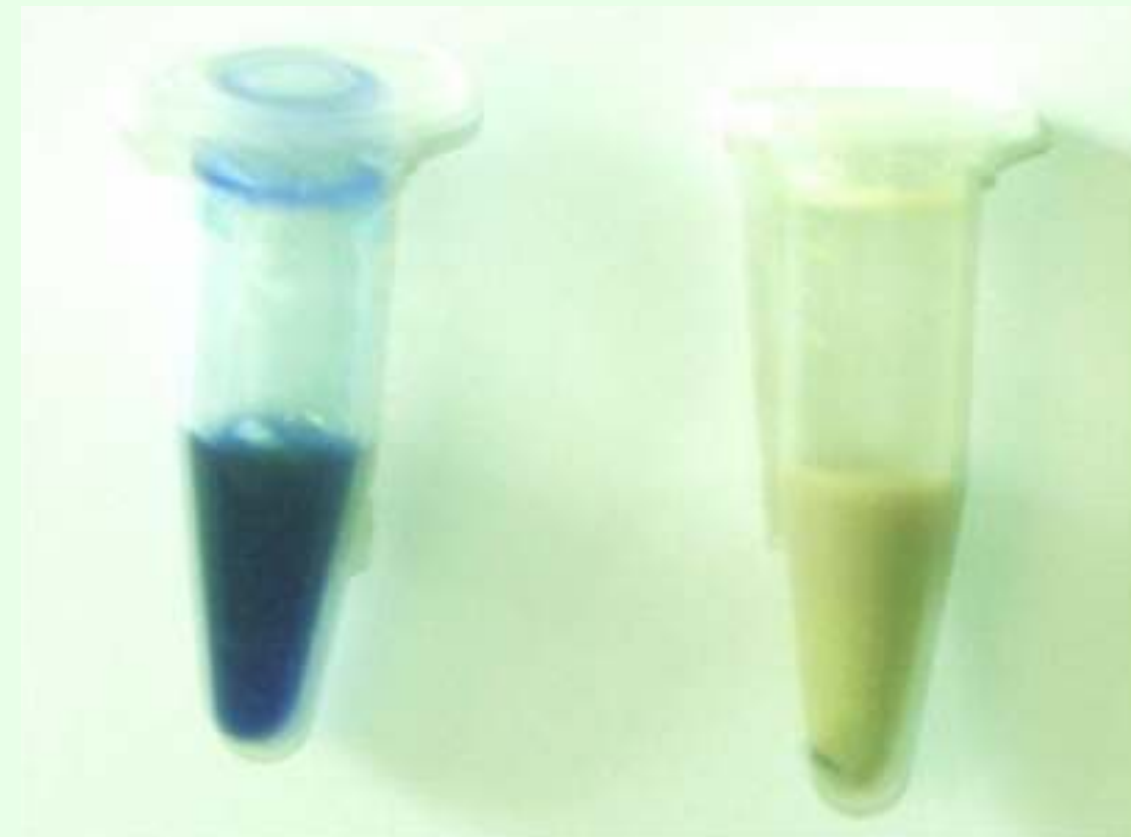


Bt Quant Cry1Ac/Cry1Ab kit
Bt Quant Cry1F kit
Bt Quant Cry2Ab kit

Three ELISA (Enzyme Linked Immunosorbent Assay) kits have been developed for the quantification of Cry1Ac, Cry1Ab, Cry1F and Cry2Ab in Bt Cotton transgenic plants. The kits are provided with IgG coated plates, standards, sample extraction buffer, HRP-conjugate, substrate, and PBST. The complete test takes two hours. ELISA is suited for qualitative as well as quantitative detection proteins and can be used as a high-through-put test for simultaneous handling of a large number of samples in routine testing. Qualitative detection does not require equipment, but quantitative detection needs ELISA reader and plate washer and trained personnel.

Bt GUS

(quick test to detect GUS marker)



The kit was designed to detect glucuronidase (GUS) marker expression in transgenic plants. The GUS marker expresses strongly along with Cry2Ab in Bollgard-II and its presence indicates Cry2Ab. The test is inexpensive and takes 30-40 minutes to complete. Seeds are crushed in 0.5 ml buffer in the 1.5 ml plastic vials provided. Addition of 0.1 ml of the reagent to the homogenate will result in blue colour if the sample is positive for the presence of the transgenic marker Cry2Ab.

Bt Zygoty

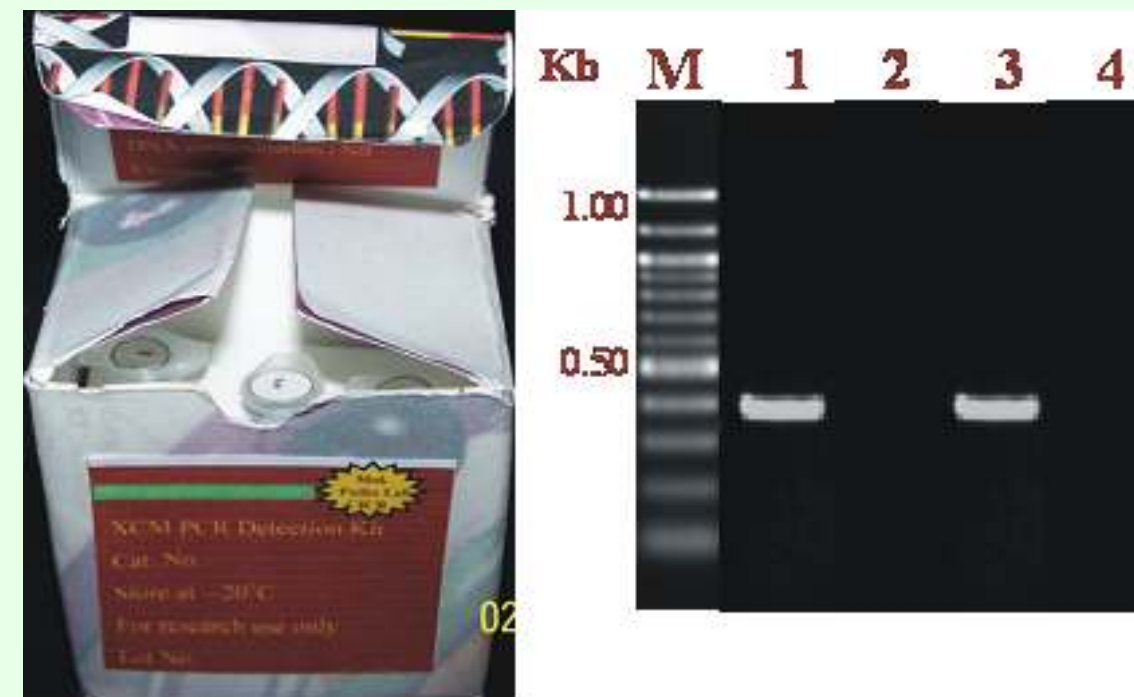
(to detect specific genes & events)



DNA-PCR test kit for 50 samples. Various primer combinations have been developed to detect GM cotton events based on the flanking sequences of the insert. DNA based PCR methods can help in detecting transgenes that may or may not express in the crop and do not constitute the economically important trait. Several promoters, enhancers, reporters and marker genes fall in this category. PCR based kits have been developed to detect the presence of *P-35S* (Cauliflower mosaic virus 35S promoter), *npt-II* (neomycin phospho transferase II) *pat/bar* (phosphinothricin acetyltransferase) *bxn* (bromoxynil nitrilase) *uid A gus* (glucuronidase) *epsps* (5-enoylpyruvyl shikimate-3-phosphate synthase) *Bacillus thuringiensis* toxin genes such as *cry1Aa*, *cry1Ab*, *cry1B*, *cry1C*, *cry1F*, *cry2Aa* and, *vip3A*. Kits are also available to detect the zygosity status for specific events or locus.

Detection of Bacterial Blight

(1558 / MUM / 2009)



A ready-to-use PCR kit for detection of strains of *Xanthomonas axonopodis* pv. *malvacearum* has been developed. Each kit contains ready-mix with all ingredients of PCR, except Taq Polymerase, mixed in proper proportion, sufficient for 50 PCR reactions in 25 ul volumes. A vial containing boiled bacterial cells serves as positive control. Each kit will be accompanied with an instruction leaflet containing all information for effective utilization of the components, including lot number and expiry date. Procedure to process the sample from various sources for use as template in PCR including culture, seeds and infected tissues will be enclosed. In case of positive reaction PCR-mix is able to support amplification of a 0.4 kb diagnostic fragment without any loss in efficacy even after 24 months when stored at -20°C.

Features

1. The kit can be used for the detection of pathogen strains and to differentiate it from morphologically alike yellow colored non-Xanthomonads.
2. Detection of pathogen in infected sources/ seeds/ plant tissues/ cultures.
3. Useful for Plant quarantine stations, Plant Protection agencies, Scientists & Researchers in Agricultural Universities and Private seed Companies.

Detection of Root Rot



The PCR protocol has been formulated using genomic DNA extracted from the fungus as template and will be standardized for detection of the pathogens in soil / tissue. The technology would enable rapid detection of two species of *Rhizoctonia* that causes root rot of cotton, within 1hr 30min time by Polymerase chain Reaction (PCR). A set of oligonucleotide primer specific to a defined region in the genome of the fungus is designed. A standard PCR of the fungal propagule with species-specific DNA primer using a series of cycles in thermal cycler would amplify DNA fragment of 280 or 311 bp, which can be viewed on agarose gel following electrophoresis.

Features

1. The technology can be used for rapid detection of the pathogen in the source.
2. Can help in decision support system for undertaking management options.
3. Useful for Plant Protection Agencies, Scientists and Researchers in Agricultural Universities and Private companies.

Detection of cotton leaf curl virus

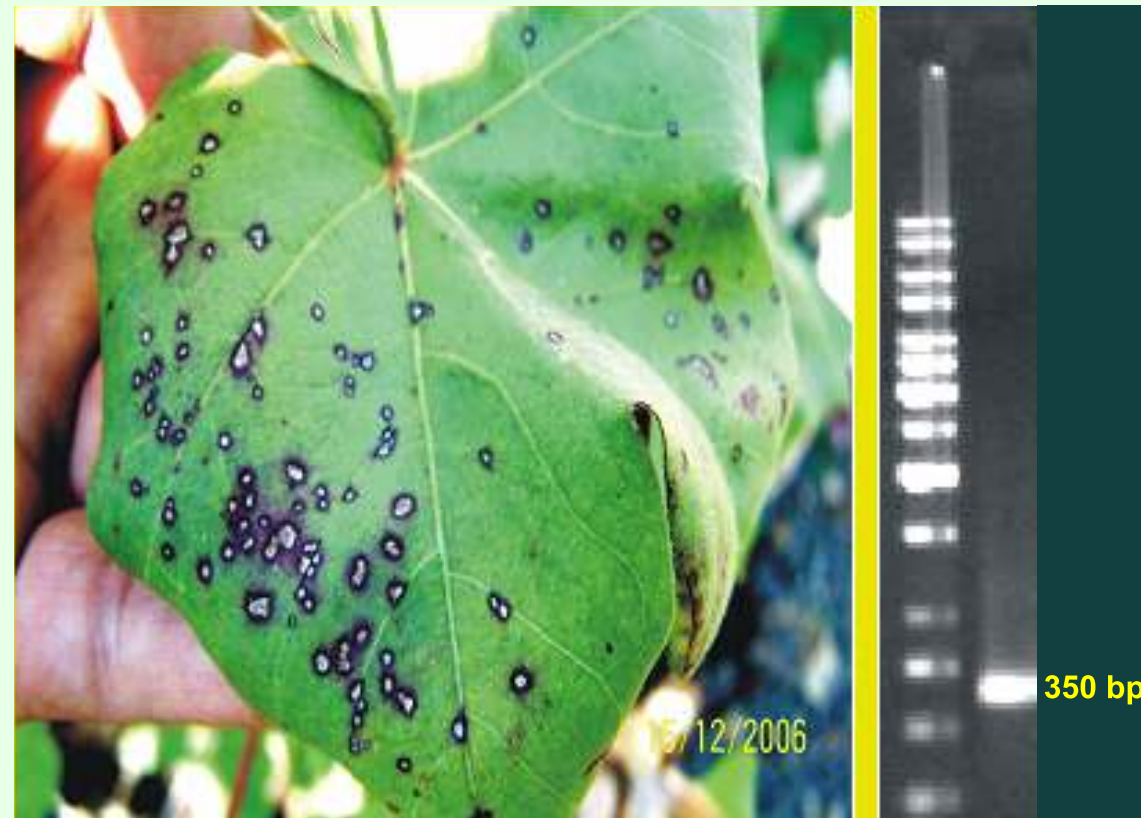


The technology would, enable rapid detection cotton leaf curl virus within 1hr 45min time by Polymerase chain Reaction (PCR). A set of oligonucleotide primer specific to a defined region in the genome of the virus is designed. A standard PCR of the infected host/whitefly vector with virus specific DNA primer using a series of cyclic steps in thermal cycler would amplify DNA fragment of 770 bp, which can be viewed on agarose gel following electrophoresis.

Features

1. The technology can be used for rapid detection of the pathogen in the source.
2. Can help in decision support system for undertaking management options.
3. Useful for Plant Protection authorities, Scientists and Researchers in Agricultural Universities and Private companies.

Detection leaf spot of cotton (*Myrothecium roridum*)



The PCR protocol has been formulated using genomic DNA extracted from the fungus as template and will be standardized for detection of the pathogens in soil/infected tissue. The technology would enable rapid detection of strains *Myrothecium roridum* within 1hr 40min time by Polymerase chain Reaction (PCR). A set of oligonucleotide primer specific to a defined region in the genome of the fungus is designed. A standard PCR of the fungal propagule with species-specific DNA primer using a series of cyclic steps in thermal cyler would amplify DNA fragment of 350 bp which can be viewed on agarose gel, following electrophoresis.

Features

1. The technology can be used for rapid detection of the pathogen in the source.
2. Can help in decision support system for undertaking management options.
3. Will be useful for Plant Protection authorities, Scientists and Researchers in Agricultural Universities and Private companies.

Detection leaf spot of cotton (*Alternaria macrospora*)



The method would enable rapid detection of strains of *Alternaria macrospora* within 1hr 45min time by Polymerase chain Reaction (PCR). A set of oligonucleotide of primer specific to a defined region in the genome of the fungus is designed. A standard PCR of the fungal propagule with species-specific DNA primer using a series of cyclic steps in thermal cyler would amplify DNA fragment of 542 bp which can be viewed on agarose gel following electrophoresis.

Features:

1. The technology can be used for rapid detection of the pathogen in the source.
2. Can help in decision support system for undertaking management options.
3. Will be useful for Plant Protection authorities, Scientists and Researchers in Agricultural Universities and Private Companies.

Detection of Grey mildew disease (*Ramularia areola*)



The technology would enable rapid detection of strains of *Ramularia areola* that cause grey mildew disease in cotton within 1hr 40min time by Polymerase chain Reaction (PCR). A set of oligonucleotide primer specific to a defined region in the genome of the fungus is designed. A standard PCR of the fungal propagule with species-specific DNA primer using a series of cyclic steps in thermal cycler would amplify DNA fragment of 372 bp which can be viewed on agarose gel following electrophoresis.

Features:

1. The technology can be used for rapid detection of the pathogen in the source.
2. Can help in decision support system for undertaking management options.
3. Will be useful for Plant Protection authorities, Scientists and Researchers in Agricultural Universities and Private Companies.

Bullock Drawn Planter

(Patent Filed No. 1560/MUM/2009, Dated 2.07.09)



Planters are used for metering bold seeds maintaining accurate plant to plant spacing within a row. However, these have not been successful as they lacked in accuracy, especially those drawn by bullocks. This was due to the sticking of wet soils (especially vertisols) on the ground drive wheels (increasing its effective diameter) which provides the transmission to seed metering mechanism through chain & sprocket and/or a set of gears. The present innovation on planter is such that the diameter of the ground drive wheel is maintained by constant cleaning of the sticking soils of vertisols, thus maintaining the correct gear ratios and hence ensuring accurate placement of seeds.

Practical applicability : Using this technology the farmer may realize better plant stand and population, which will translate into lesser seed used per unit area and saving in labour.

Commercial Potential : Farm implements manufacturers, private entrepreneurs, agro-industries

Solar powered knapsack

(Patent Filed No. 1559/MUM/2009, dated 2.07.09)



It is a modified battery operated sprayer which can be used in places facing long electricity power cuts, where electrically charging the battery is not possible. This innovation uses solar power to continuously charge the battery and provide a cutoff beyond a range of voltages to minimize the variation in droplet size generation. A novel feature of the sprayer is the tilting arrangement provided so that the solar panel could be given the desired inclination of 15° - 18° N-S so as to maximize the catch of incident sun rays, depending on the direction of travel of the operator.

Practical applicability : Using this technology can result in Saving of costly pesticide, Saving of environment and Better control of insects, ultimately, resulting in reduced cost of cultivation.

Commercial Potential : Sprayer manufacturers, Farm implements manufacturers, private entrepreneurs, agro-industries

Cotton Seed Blower

(Patent Filed No. 1561/MUM/2009, dated 2.07.09)



Seed may contain impurities in the form of trash, broken and unviable seeds which can result in reduced germination percentages, producing gaps which the farmer has to fill manually consuming labour, additional seeds and increasing cost of cultivation. The end user i.e., the farmer at present resorts to manual sorting of clean viable seeds from the trash and broken ones. This technology provides a system for cleaning of the seeds at end user level. The system is simple, so as to be serviced and repaired locally, while being robust and portable.

Practical applicability : The machine could be used by the farmer individually or on a custom hire basis. Cleaner seed free from unviable seeds will translate into better crop stand and population

Commercial Potential : Seed companies, Farm implements manufacturers, private entrepreneurs, agro-industries

Enhancing Cry 1Ac expression

(Patent Filed No. 111/MUM/2009)



An increase in Cry1Ac toxin expression in the terminal leaves of Bt cotton plants is facilitated through the use of a volatile exogenous inducer namely, fragrant formulation. A method has been demonstrated for the use of a fragrant formulation in cotton fields so as to exploit its interplant and intraplant effects in Bt cotton.

Cry1Ac toxin expression can be enhanced by up to 671 percent over control during peak vegetative phase through the use of fragrant formulation on cloudy days.

Cry1Ac toxin expression can be enhanced by up to 260 percent over control 123 DAS through the use of fragrant formulation.

The terminal leaves of Bt cotton plants continue to produce Cry 1Ac above the threshold level of 1ug/g up to 123 DAS in fragrant formulation treated plots.

Diploid cotton

[Patent Filed No. 1557/MUB/2009, DATED 02.07.09]



The regeneration protocol by multiple shoot induction was developed using shoot tip culture technique. The shoot tip explants isolated from the in-vitro germinating seedling were cultured on multiple shoot induction medium. The explants undergo morphogenic changes and produced multiple shoot buds within 30-40 days. The shoot bud mass on transfer to shoot differentiation medium with at least two passages of MS medium, developed into large number of shoots within 20-30 days of culture. Individual shoot was rooted after their separation from the mass of multiple shoot. At least 15-20 shoots/explants can be harvested in 2-3 successive cycle. Rooted plantlet were first hardened in MS liquid medium with filter paper support and allow to grow under high light intensity for first 15 days. Thereafter they were transferred in earthen pot covered with polythene bag for further hardening. The mature plants were fertile and true to the type.

Features: Regenerated plants are true to the types and low somaclonal variation

Uses: The protocol can be used in micro propagation of rare material and development of transgenic plants.