Tille: Development of farmer friendly diagnostic kits for transgenic event seed purity.'

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Name of the PI and Lead centre	Dr. R. K. Khetarpal NBPGR, New Delhi
Name of the CCPI and centre	Dr. K. Kranthi CICR, Nagpur

INTRODUCTION

Six genes (Cry1 Aa, Cry1 Ab, Cry1 Ac, Cry1 F, Cry1 B, Cry2Ab) and vip-3A from *Bacillus thuringiensis* have been utilised for insect resistance in various crops including cotton. It is important to develop simple, cost effective methods to assist farmers in the detection of transgenic purity before they use the seed for sowing. Designing a common lateral flow strip to detect any of the most commonly cultivated GMOs will also be useful at port of entry quarantine purposes.

OBJECTIVES

1. To develop farmer usable 'on-the-spot' rapid immuno-diagnostic kits (5-min test) to detect specific transgenic products/events released for commercial cultivation in India.
2. To develop a ubiquitous test kit for the detection of any GMOs/ LMOs at port of entry
3. To develop standard quantifiable parameters for regulatory testing purposes.
4. To develop methodology for detecting the presence of transgenes in a bulk sample

Name of the Lead Center: NBPGR, New Delhi

Co-operating Centers: CICR, Nagpur,

ACTIVITIES

1. Database on markers, promoters & genes. Short-listing of candidates for detection kits
2. Purification of candidate antigen proteins. Immunization & antiserum development.. Development of ELISA, Dip-sticks kits
4. Commercialization and making kits available for use by training farmers and stake holders.

SALIENT FINDINGS

The project incorporated objectives to develop farmer usable 'on-the-spot' rapid immuno-diagnostic kits (5-min test) to detect specific transgenic products/events released for commercial cultivation in India and also to develop standard quantifiable parameters for specificity,' sensitivity,' repeatability,' reproducibility,' limits-of-detection, overall accuracy and robustness (includes product stability,' and user friendliness) of transgenic detection kits for validated regulatory testing purposes. The technical programme included purification of candidate antigen proteins, immunization & antiserum development & development of ELISA, Dip-sticks & Dot-blot kits, creation of a database on markers, promoters & genes, short-listing of candidates for detection kits, primer designing, PCR testing, locus identification and validation and establishing detection limits, accuracy & repeatability,'

Antigens (Cry1 C, VIP3A, Cry1 F & Cry2Ab2 & NPT-II) were purified. Six genes have been shortlisted based on AgBios database for the development of kits. Antisera was raised and ELISA developed for Cry1 C. The Cry2Ab ELISA was developed as quantification Bt-Express-2 commercial kits and were sent to AP state seed testing laboratories for spot validation. The kits were found to be accurate, sensitive and robust.. The shelf life of the Cry2Ab2 and Cry1 C detection kits were estimated for 6 months at 4° C and found to be sturdy, with no deterioration in the quality of the kits. Primer sets were developed and validated for 6 genes (Cry1 F, Cry1 C, Cry1 C, VIP3A, Cry1 Aa and Round-up Ready).