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Development of an Indigenous Quick Lateral Flow Strip (Dipsticks) Detection Method for the Analysis of Bt Crops

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Abstract. Generation of genetically modified (GM) crops is quickly expanding around the world. The quality assessment of these harvests is a vital issue and this droves of the administrative specialists to execute an arrangement for the endorsement to develop and use GM to create and produced an interest in scientific techniques equipped for identifying GM crops. The GM crops have added to the effective fuse of various attributes by presenting transgenes, *e.g*. *Bacillus thuringiensis* (Bt) insecticidal qualities, in various yield species. Dependable recognition strategies are vital for the identification of un-approved GMOs. According to James in (2018), the planting area of global genetically modified (GM) crops has reached 1.971 billion hectares, a 100-fold increase from the 1.7 million hectares in 1996. GM crops give financial, natural, well-being, social advantages to both little and huge agriculturists in creating and mechanical motions. A quick and simple immuno-chromatographic (ICS) test was set up which is modest compact and simple to utilize. The ICS is a semi-quantitative method for screening and semi measurement new proteins presented through hereditary change of plants. Strip assay developed first time in Asia and used it for assessment of several Bt transformed crop plants for insecticidal quality.

The cultivation of GM crops is important and more traits are emerging and more area is being planted with GM varieties. The release of GM crops products in the markets worldwide has increased regulatory need to monitor, verify the presence and amount of GM varieties in crops and products. In 2017, global GM area reached to 189 million hectares, which indicates high adoption rate of GM crops by farmers and reflects its consistent performance. More than 8 million farmers are benefiting from it. About 90% of the beneficiary farmers are resource-poor farmers from developing countries, whose increased incomes from biotech crops contributed to alleviation of poverty. So, developed the quick strip assay, utilized it for assessment of Bt-ICP crop plants.

Keywords: Immuno-chromatography, lateral flow strips, detection assay, genetically modified, trait-specific

Introduction

The total populace is growing rapidly. Assessments of populace development recommend that nourishment necessities are probably going to rise significantly in the coming 20 years. More than 800 million individuals in creating nations, including 33% of the number of inhabitants in sub-saharan Africa are under nourished (www.fao.org). More than 90% of these are enduring long term malnourishment and micronutrient insufficiency. Hereditary modifications can possibly take care of these issues. A hereditarily changed life form generally modified organism (GMO) is a life form whose hereditary synthesis has been modified by methods for recombinant DNA innovation. This innovation adjusts or embeds at least one qualities into a life form through quality change. GMOs hold extraordinary potential to build trim yield, enhance sustenance quality, decrease input costs and enhance creativity. To date, insect protection and herbicide resilient are the primary business attributes utilized as a part of maize, cotton and soybean (Dunwell, 1998). These qualities are giving monetary advantages to the agrochemical business, seed makers and agriculturists because of improved profitability. They additionally conceivably advantage the earth because of a lessening in the utilization of chemicals or a move to the utilization of all the more naturally agreeable chemicals.

The development of hereditarily adjusted yields is winding up progressively essential, more qualities are rising and an increased number of sections of land than any time in recent memory are being planted with GM assortments. The arrival of GM harvests and items in

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the business sectors worldwide has expanded the administrative need to screen and check the nearness and the measure of GM assortments in yields and items. The worldwide region of GM crops expanded 47-fold, from 1.7 million hectares in 1996 to 81 million hectares in 2004, with an expanding extent developed by creating nations. More than 8 million ranchers are profiting from this innovation (James, 2004). Around 90% of the recipient ranchers are asset poor agriculturists from creating nations, whose expanded earnings from biotech crops added to the lightening of destitution.

The administrative need to screen and confirm the nearness and the measure of GM assortments in harvests and items has expanded with the development of the development of the GM crops (Tripathi, 2005). Effective screening of GM items must be accomplished with the improvement of proper techniques for location.

GM crops and their items can be distinguished by identifying the embedded hereditary material at DNA level, the subsequent protein or phenotype. A few expository techniques, for example, strategies in view of the polymerase chain response (PCR) for identifying the transgene, immunological measures for distinguishing the subsequent protein or utilizing bioassays to recognize the resultant phenotype have been produced. Western blotting, ELISA (Enzyme-Linked Immunosorbent Assay) and parallel stream sticks are common protein based test techniques (Brett *et al*., 1999). A few other diagnostic advances that can give answers for current specialized issues in GM test examination are rising. These techniques incorporate mass spectrometry, chromatography, close infrared spectroscopy, miniaturized scale manufactured gadgets and, specifically, DNA chip innovation (microarrays).

Mostly, screening tests that may distinguish scope of GMOs. This can be trailed by a particular test to recognize the sort of GMO display in the sample and now and again this test is additionally intended to measure the quantity of a particular GMO. The major proportion of protein recognition strategies depends on immunoassays, for discovery and evaluation of new foreign proteins presented through the hereditary change of plants. Immunoassay depends on the particular official between an antigen and a counteracting agent. Protein location strategies for the GMO testing shift from those that are generally modest and simple to perform to more refined measures requiring respectably costly instrumentation. Protein recognition techniques are exceptionally appropriate for observing particular GM qualities. Protein discovery strategies can be utilized to recognize GM attributes in some prepared nourishments (Stave *et al*., 2000). GMO testing has turned into a vital and essential piece of food production to ensure compliance with labeling regulations, to confirm IP frameworks and secure customers by approving "non-GMO" item publicizing claims (Spiegelhalter *et al*., 2000).

Binding assays are in wide use in laboratories for the detection and measurement of analytes in samples. For biological samples such as urine, whole blood, plasma, serum and other biological fluids, assays are often performed in hospitals and clinical laboratories. These binding assays can likewise be performed in natural, horticultural veterinary mechanical athletic lawful/ criminological settings and furthermore snappy discovery of irresistible sicknesses in serological testing of people and creatures.

The principles involved in such assays are well known by those skilled in the art. Many such devices have been described and are available commercially. Immunological binding assay is the sandwich assay. However, in clinical laboratories the use of solid phase chromatographic binding assay devices has become popular for their relative ease of use, economy and reproducibility. Typically, these chromatographic assay devices are comprised of a porous chromatographic medium which acts as the matrix for the binding assay. The sample of interest is added directly or indirectly to one end of the medium, and is chromatographically transported to a detection reagent with which it reacts to form a labeled product, which is then transported to a test zone containing an immobilized capture reagent such as a capture antibody, in which the presence, absence or quantity of an analyte of interest can be determined.

The strategies (ELISA, western blotting etc) are not reasonable for on-spot testing but rather dipstick test has been discovered faster, less difficult, more affordable and discovered appropriate for on-location testing by untrained individuals. Dipstick groups have been utilized prompt discovery of transgenic proteins in leaves, seeds and grains of GM crops/nourishments. It gives a quick (on location is/no outcomes in 10 min) solid and practical screening in the generation, confirmation of the immaculateness of GMO and non-GMO material. It can be utilized to segregate amongst approved and un-approved GM material or utilization of material, to recognize the

safe or possibly dangerous material. Dipstick that is less demanding to perform than different systems. For a long time, the dipstick design was exceptionally prevalent and was executed in different tests. This creation depends on Immuno-chromatography and sidelong stream measures. It identifies with immuno-measure dipsticks and especially to those test gadgets used to lead immunological and serological restricting examines. These highlights make strip tests perfect for applications, for example, home testing, quick purpose of care testing and testing in natural field.

The immuno-chromatographic test strip showed lower sensitivity but higher specificity than ELISA, which mainly for the differences existed in the assay formats (Dong *et al*., 2019). This new strip examine is low in cost, quick, monetary, convenient and less laborious. It can be utilized to recognize subjectively or semi-quantitatively the nearness or non-attendance of analytes of enthusiasm for natural specimens, in spite of the fact that it isn't so constrained. The development identifies with techniques for utilizing such dipsticks for the location of GMOs and irresistible sicknesses.

GMOs hold the awesome potential to expand crop yield, enhance nourishment quality, lessen input costs and enhance creativity. To date, insect and herbicide resistance are the principal business attributes utilized as a part of maize, cotton and soybean. The worldwide territory of GM crops expanded 47 fold, from 1.7 million hectares in 1996 to 81 million hectares in 2004, with an expanding extent developed by creating nations (James, 2004). GMO testing has turned into an indispensable and essential piece of food production to ensure compliance with labeling regulations. Protein-based methods, for example, ELISA and strip tests are viable for natural items yet rely upon the accessibility of business units and are not appropriate for prepared items because of protein degradation (Spiegelhalter *et al*., 2000). Parallel stream systems are subjective or semi quantitative (Tripathi, 2005).

In this study, a few GM cotton (Cry1Ac) and rice plants were examined by lateral flow strip test, immunoassays, and insect biotoxicity measures.

Material and Methods

All the organic and inorganic chemicals used were from Sigma Chemical Company (USA). Nitrocellulose membrane reagents were from Millipore Company (Canada).

Sampling. Picked developing leaves from cotton (transgenic and control) plants and protected in liquid nitrogen around then and for long time safeguarding, stored at -70 °C.

Protein extraction. Cotton leaves spared in liquid nitrogen, were grinded in the pestle mortar. Took approximately 33% of grinded powder leaf alongwith 600 uL protein extraction buffer [0.5 mol EDTA, Glycerol, 5 mol NaCl, 2 mol Tris-Cl, NH_4Cl, PMSF, DTT (dithiothreitol)] was taken and placed at room temperature for 1 h, centrifuge at 13000 rpm for 25 min, Supernatant was taken in an new 1.5 mL Eppendorf tube for insecticidal protein examination.

Immunoassay. Immunoblot examination of Bt proteins were performed. The specificity Cry proteins were assessed by dot blotting. After protein extraction, proteins spotted on nitrocellulose layer and incubated for one hour in 3% skimmed milk. The dot blots were prepared as western blots as depicted by (Towbin *et al*., 1979). Anti-serums (Monoclonal) against refined insecticidal crystal proteins (ICP) of Bt, utilized as an essential counteracting agent. HR peroxidase conjugated goat hostile to mouse Immunoglobulin G (IgG) was utilized as an optional neutralizing agent.

Cotton leaf bioassays. Five leaves from upper, center and lower part of the plant were chosen and pre-starved *Heliothis armigera* were fed in petri plates. Following 2-3 days mortality rate was noted near to 70-90%. Stayed alive hatchlings were slow and inert to be destructive to the plant.

Colloidal gold conjugation. Dialyzed the proteins against a 2 mM sodium borate support, pH 9.0, for 4 h. The dialysis step equilibrated protein to a low molarity cushion with the required pH, yet wiped out low atomic weight contaminants (additives that contain sulphur or mercury and tie firmly to gold colloids). The centralization of immune response arrangement was 0.2 mg/mL. Emptied the gold colloid into a receptacle of fitting size, blended quickly, include the immune response arrangement dropwise and mixed for another 10 min. Include BSA from a sifted 10% (w/v) stock answer for the last centralization of 1% mixed delicately for another 10 min. Centrifuged the conjugate at 16,000 × g for 45 min at 4 °C. Discard supernatant and re-suspended the pellet in buffer (TBS with 1% BSA and 0.02% sodium azide). Filtered the conjugate through a 0.2 µm cellulose acetic acid derivation film and stored the conjugate at 4 °C.

Dipsticks preparation. Cry1Ac antibodies were joined as the test line (T line) on strips (nitrocellulose film with plastic help). The control line has species particular against immunoglobulin neutralizing agent, particularly for the conjugate antibodies on the microspheres. Conjugated protein connected to the conjugate pads and settled the pads on the strips.

Sample preparation. Collected the Bt-Cry1Ac cotton, rice leaves and seeds respectively, grinding them independently and re-suspended in protein extraction buffer. Centrifuge after 30 min and save the supernatant for tests.

Sample testing. To play out the test, the test strip is sub-merged in the extracted sample of Cry1Ab transformed cotton plant seeds and leave and Cry1Ac transformed rice plant seeds and leave to recognize the Cry1Ac expression. The fluid sample moved inside the permeable bearer to the course of the dry end, while moving in the permeable transporter, the sample mobilizes a labeled reagent that has been reversibly immobilized in the porous carrier. At the point when analyte is available in the fluid example, a "sandwich" in the form of mobilized labeled reagent: analyte: immobilized reagent is shaped and the resulting concentration of the labeled reagent leads to a noticeable line showing up in the detection zone, which is demonstrative of a positive result or the signal reagent in a conjugate pad is solubilized and ties to the antigen in the specimen and moves through the membrane by capillary action. In the event that analyte is available, the signal reagent ties to it, and a second antibody or antigen, immobilized as a line in the strip, at that point catches the complex. In case of positive sample, a coloured line creates. In conclusion, remaining specimen fluid, together with rest of the labeled reagent additionally moves to a control zone, where a second line shows up indicating that sample has progressed through the detection and control zones and that the assay has provided the valid test result. Results can usually be read in 5 to 10 min.

Table 1. Results of positive and ND cotton samples

Total plant samples	Presence of protein	Amount of protein
188	++++	3-5 ppb & above
338	+++	2-3 ppb
667	++	1-1.5 ppb
522	+	Below 1 pp
447	ND	Non detectable

ppb = parts per billion; + = positive.

Results and Discussion

Immunoassay included recognition of Bt protein from leaves utilizing antibodies. Leaves were ground and protein was separated by including extraction buffer. Antigen antibody reaction was performed by recognizing the extracted protein on nitrocellulose layer (Hybond C). Positive and negative test controls were spotted alongside analyte tests. Subsequent to treating the smears with essential and auxiliary antibodies, the protein was recognized by including the substrate. The outcomes were translated by utilizing picture examination programming lab works 4.2. Several cotton and rice plants were checked. Results were given in Table 1.

Biotoxicity assays and % mortality of larvae was determined by following formula:

$$\% \text{ mortality} = \frac{\text{no. of dead larvae}}{\text{total no. of larvae}} \times 100$$

The results of larval are shown in Table 2.

Transgenic plants with the Cry genes will express insect protection in the plant and seed. The dipstick distinguishes the Cry protein located in the seed and other plant parts. For transgenic crops examination, the test

Table 2. Result of larval (*Heliothis armigera* : 2nd Instar) mortality feeding on the leave of transgenic plant

Plant #	Protein level	% Larval mortality	Plant #	Protein level	% Larval mortality
28	++++	100	52	+++	100
30	++++	100	55	+++	80
47	++	100	26	+	100
57	++	100	42	+	100
50	ND	100	CIM-482	Non Bt	0

ND = Non detectable.

strip is immersed in the extracted sample of Cry1Ab transformed cotton plant leaf. Cry1Ab antigen reacts with coloured antibody colloidal gold conjugate to shape antigen antibody complexes. The mixture then moves chromatographically upward on the membrane to the immobilized mouse monoclonal anti-Cry1Ab antibody at the test region. If Cry1Ab antigen is available in the sample, a coloured sandwich of antibody/Cry1Ab antigen/gold conjugate antibody agent is formed on the test line (T line). Non-appearance of a coloured line in the test region indicated a negative sample. Despite the presence of Cry1Ab antigen, the extracted mixture keeps on moving laterally upward on the test strip menbrane to the control line (C line) region. A control line at the control region should always appear. The presence of this coloured line serves as verification that adequate volume has been included and proper flow was obtained. Line intensity may change from test to test. A positive outcome demonstrates the specimen contains Cry1A antigen (Fig. 1).

The precision of the test relies upon the nature of the specimen. Surfactants (saponins), phenolic mixes in concentrates of plant tissues hinders the antigen-antibody binding. A negative outcome might be acquired because of low antigen concentration. The pink control line is an internal control and the test strip must retain the correct measure of test and the test strip must work appropriately for the pink control line to show up. For the test strip to work legitimately fine stream must occur.

Immunoassays can be broadly executed on a business scale for the recognition of novel or transgenic proteins in crude items. In a perfect world, an antibody must be chosen that will particularly tie to the state of the protein of intrigue, not with standing when there are many different proteins present, each with their one of kind shapes. Once a particular neutralizer has been produced, it can be joined into an immunoassay by naming with fluorescent or shaded colours or connection to a surface where it is utilized to 'catch' the protein of intrigue. Immunoassay advances are perfect for the subjective and quantitative discovery of many sorts of proteins and pathogens in complex grids (Ahmed, 2002; Brett *et al*., 1999).

Effective checking of GM items must be accomplished with the improvement of proper strategies for recognition. Molecular strategies are for the most part in view of the recognition of the novel proteins or DNA (Matsuoka *et al*., 2002).

Approval of strategies is the way toward demonstrating that the joined methods of test extraction, arrangement, and investigation will yield acceptably precise and reproducible results for a given examination. For approval of a scientific strategy, the testing objective must be characterized and execution qualities. Execution qualities incorporate exactness, extraction proficiency,

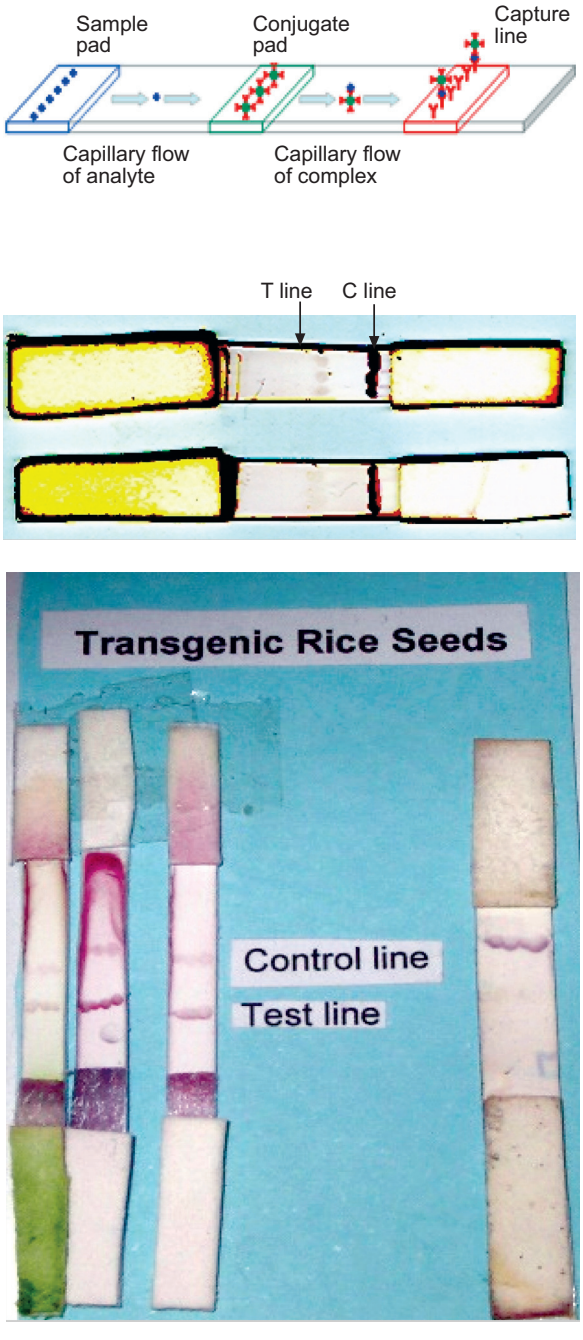

Fig. 1. A new (ICS) test, for quick, easy detection of Cry1Ab transformed cotton leaf sample.

accuracy, reproducibility, affectability, specificity and strength. The utilization of approved strategies is essential for reliable results delivered by diagnostic research facilities (Lipp *et al*., 2000). The major part of protein location techniques depends on immunoassays. Protein location techniques can possibly recognize the presence of a particular GM quality and to give the total measurement of the level of GMO exhibit. Protein identification strategies are not worthy for checking GM. Among several GMO detection strategies, mainly based on DNA, the quantitative PCR (qPCR) is the method of choice for the enforcement laboratories in GMO routine analysis due to its numerous advantages. However, given the increasing number and diversity of GMO developed and put on the market around the world, some technical hurdles could be encountered with the qPCR technology, mainly owing to its inherent properties. Alternative GMO detection methods have been developed to address these challenges, allowing faster detections of single GM target (e.g., loop mediated isothermal amplification), simultaneous detections of multiple GM targets (e.g., PCR capillary gel electrophoresis, microarray and Luminex), more accurate quantification of GM targets (e.g., digital PCR) or characterization of partially known (e.g., DNA walking and next generation sequencing (NGS)) or unknown (e.g., NGS) GMO (Fraiture *et al*., 2015).

A lateral flow immunoassay (LFT) was developed and used to detect Bt-GM crops for the expression of insecticidal crystal protein (ICP) of *Bacillus thuringiensis*. One-step lateral flow tests, which are also called immuno-chromatographic strips (ICS) or dipstick tests, have been a popular platform for qualitative rapid visual tests, use colloidal gold conjugate to generate signal.

In previous methodologies, Quickstix lateral flow test devices employ the same immuno assay principles as the plate format but coat the antibodies and other reagents on a nitrocellulose membrane rather than on the inside of test wells or tubes. Nitrocellulose (NC) membranes that have been the first choice of device manufacturers for over 20 years. A test strip assay device, in which a mobile conjugate labeled with colloidal labels such as gold, can be deposited on a chromatographic medium and after reaction with an analyte, thus transported with the solvent to a test zone. The labeled mobilizable detection reagent reacts with an analyte and the resulting product migrates with the liquid sample as the sample progress to the test zone. During manufacture, after the un-labeled binding agent is added to and immobilized in the test zone, the remainder of the test strip material is treated with blocking agents to block any remaining binding sites. The zone where the mobilizable labeled reagent is located is often referred to as the "labeling zone" but can be referred to as the "reversible immobilization zone" or "mobilization zone", while analyte is reacting with the mobilized labeled reagent, the liquid sample and mobilized labeled reagent migrates further within the porous carrier to the detection zone, where reagent that binds the same analyte is fixed or immobilized, usually in the form of a line. The important aspects of antibody pairs include steric separation of epitopes, adequate titer of stocks, increased affinity, improved specificity, amplified avidity and purity.

In this study, strip assay developed in 2008, while an easy to use field-deployable methodology was developed for onsite detection of pesticidal crystal protein Cry2Ab from transgenic cotton crops to reduce seed adulteration (Kanagasubbulakshmi and Kadirvelu, 2021).

The benefits of immuno-chromatographic tests includes the user-friendly format, quick, efficacy over a wide range of climates and relatively in expensive to make. These features make strip tests ideal for applications such as home testing, rapid point of care testing and testing in the field for various environmental and agricultural analytes. Furthermore, the achievable sensitivity is a factor of about 10 to 100 poorer than an instrumented laboratory immunoassay, restricting the technology's utility to relatively high abundance analytes only. Some of the more common lateral flow tests currently on the market are tests for pregnancy, Strep throat (Edwards *et al*., 1982), chlamydia and human brucellosis (Smits *et al*., 2003). Lateral flow assays have been used extensively as diagnostic tools for monitoring toxins. Globally, the sensitivity of the rapid Detection Tests (RDTs) examined was good (higher than 95%) and the specificity was excellent >99% (Angheben *et al*., 2019). Immunoassays, being the preferred method for detection of Cry toxins. Owing to limitations of traditional colourimetric enzyme-linked immunosorbent assay, the trend of detection strategies shifts to modified immunoassays based on nanomaterials, which provide ultrasensitive detection capacity (Faheem *et al*., 2021).

Conclusion

In conclusion the ICS is a semiquantitative method for indicative screening and semi-measurement of new remote proteins presented through hereditary change

of plants. The strip is the easiest method for the assessment of several Bt crop plants for insecticidal quality. The developed approach may provide insights into the development of ICS for analyzing simultaneously multiple components in genetically modified crops (Zeng *et al*., 2021).

Conflict of Interest. The authors declare that they have no conflict of interest.

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