QUALITY TESTING OF BT-COTTON SEEDS

An industrial training report submitted for the partial

fulfilment of the degree of master of science by,

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Under the guidance of

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DECLARATION

I hereby declare that, the project entitled is an outcome of my own efforts under the guidance of **Dr. Denish Dholariya** in **Solar Agrotech Pvt. Ltd.** The report is submitted to Atmiya University Rajkot. For the partial fulfillment of the Master of Science 2021-2022.

I also declare that this report has not been previously submitted to any other University.

DEVEN M. VARA

Place: RAJKOT

Date: 05/04/2023

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Abstract

This internship focuses on the detection of the Cry gene in Bt cotton seeds using sandwich ELISA and the evaluation of seed germination using cellulose paper. The main purpose is to determine whether the Bt cotton seed contains the Cry gene, which encodes the production of an antibiotic that protects the plant from pests, and to evaluate the potency of the seed using germination techniques. This method includes the preparation of seed extracts for sandwich ELISA and germination testing using cellulose paper. The extracted proteins are subjected to sandwich ELISA, which involves the use of specific antibodies to detect the presence of Cry proteins in the sample. Germination tests were performed using water-moistened cellulose paper and were used to measure seed viability based on seedling emergence.

In the ELISA sandwich test, samples are prepared by grinding the seeds into a fine powder and extracting the proteins in a buffered solution. Protein concentrations were then determined using the Bradford assay. The sample is incubated with an antibody specific to the Cry protein followed by a second antibody conjugated to the enzyme. When substrate is added, the enzyme catalyzes a colorimetric reaction, allowing Cry proteins to be detected in the sample. The assay was optimized for sensitivity and specificity, and the results were analyzed to determine the presence of the Cry gene in Bt cotton seeds.

In the germination test, cellulose paper is placed in a Petri dish with a layer of filter paper on top. The filter paper was moistened with water and the seeds were evenly spread on the filter paper. The Petri dish is covered with a plastic sheet to retain moisture and the seeds are allowed to germinate for 7 days. The occurrence of genes is observed and calculated for the evaluation of gene strength. The internship led to several major discoveries, including the successful detection of Cry proteins in Bt cotton seeds using a sandwich ELISA.

The results showed that the seeds had different Cry protein levels depending on the variety of Bt cotton. In addition, germination tests showed increased growth of Bt cotton seeds, indicating their full viability. These findings have important implications for the development of better farming techniques and for ensuring the quality of cotton seeds. Confirming the presence and potential of the Cry gene in Bt cotton seeds by germination tests, this study adds to current knowledge about the properties and potential of Bt cotton as a crop. Overall, this internship provides a valuable opportunity to gain experience in molecular biology and biotechnology and to contribute to ongoing research to improve plant protection strategy.

Introduction of Company

Solar Agrotech Private Limited was founded by an agricultural technocrat Dr. Thobhan Dholaria, has a degree in M.Sc. (Agri) & Ph.D. degree in Plant Breeding and Genetics. Dr Dholaria had a bright academic carrier and awarded the research fellowships of Council of Scientific & Industrial Research as well as Indian Council of Agricultural Research during his postgraduate and doctorate study.

The company was incorporated in 1994 aiming to fulfil the needs of farming community to provide the High-tech and High Yielding Quality Seeds of various crops under the registered trade brand of "DOCTOR SEEDS".

Dr. Dholaria having bright Academy career, so he established the state of the art Research and Development facilities at Solar Agrotech Private Limited. The company had earned very good reputation in large farming community and won the confidence of trade partners in last 25 years and Dr. Dholaria has earned the trust of farmer as The Trusted Seedsman.

Dr. Dholaria provides needful information for increasing crop production and protect the crop under varying climatic condition through Dr Dholaria Agricultural Foundation which was established for providing service to the farming community.

Doctor Seeds sees is good future business using the biotechnology and strengthening R&D company's young Director, Denish Dholaria, who is MSc. degreed in Plant biotechnology for advancement in biotech crop breeding to support the breeding work of team headed by company's Director of Research, Dr. J. M. Kamani, who is having PhD in Plant Breeding & Genetics along with Mr Rahul Kothiya, MSc. Plant Biotechnology and Dr. M. K. Bhalala (ex-University Registrar) as an advisor to the company in the of government matters.

Further, in order to provide the farmers with good quality pesticides, Dr. Dholaria went into the business of pesticides to fulfil the farmers needs of good quality low-cost pesticides. The pesticides manufacturing company name is Cistronics Technovations Private Limited and marketed pesticides under Cistronics Pesticides LLP.

Introduction

1. Germination Testing

Germination steps – seedling On cellulose paper, count 100 seeds and band this cellulose paper in prescribed pattern. This method is called “Paper method” of seeds germination test.

Spray water (daily, twice in a day) and fungicide (one-time) on these cellulose papers during the germination incubation period (15-20 days). Incubation temperature should be room temperature.

The most common test is a warm germination test because it is required by seed laws to appear on the label. Usually 400 seed from each seed lot are placed under moist conditions on blotters, rolled towels, or sand and maintained about 75 to 85 degrees F for about seven days in most of the cases.

At the end of this period the seedlings are categorized normal, abnormal, or diseased, and dead or hard seeds.

The percentage germination is calculated from the number of normal seedlings from the total number of seeds evaluated. The method of testing germination is discussed below.

To obtain a random sample for testing it is always best to take samples from different parts of the bag or container. If the seed to be tested is from a seed lot that contains more than one bag, samples must be taken from several bags. A good rule of thumb for determining how many bags to sample is to take samples from a number of bags that represents the square root of the lot size. For example, if the lot contains 100 bags, the sample at least three bags. If the lot contains 100 bags, the sample at least 10bags.

2. Sandwich ELISA

We had used Bollgard II combination kit for sandwich Elisa.

The Bollgard II combination kit is used for simultaneous qualitative detection of Cry1Ac and Cry2A protein in cotton leaf or seed samples with both analytes, measured in the same well of the assay plate.

The format helps the laboratory to cut down work load by half thereby increasing productivity The kit is ideally suited for testing large numbers of seed lots for Cry1Ac and Cry2A simultaneously.

Principle of the test

In the test, cotton sample extracts are added to test wells coated with antibodies raised against Cry¹Ac and Cry2A proteins. Any Cry Ac or Cry2A protein present in the sample extract binds to the antibodies. This is detected by addition of alkaline phosphatase labelled Cry2A antibody or horseradish peroxidase-labelled Cry1Ac-antibody After a simple wash step, the results of the cry2A assay are visualized via the addition of a PNPP substrate Once the yellow colour develops and is read, the wash step is repeated, and TMB substrate is added The Cry1Ac results are visualized via the development of the resulting blue colour which, subsequently, becomes yellow on addition of stop solution.

Precautions

The Cry 1 Ac-Cry2A ELISA Kit is intended for in vitro use only. The reagents contain Thimerosal as a preservative Prevent direct skin and eye contact with kit components Seek medical attention in case of accidental ingestion of kit components.

Kits are provided in 10 plates or 50 plates format.

Contents of 10-plate kit:

1. Ten plates of 96 wells coated with Anti-Cry 1Ac & Cry2A antibody, each packed individually in a laminate bag with silica gel

2. Ac & Cry2A combined conjugate One ready-to-use bottle of 55 ml

3.. Extraction buffer concentrate One packet of powder with a 25ml vial of Tween-20

4. Wash solution concentrate. One packet of powder with a 2.5 ml vial of Tween-20

5. Substrate #1 (NPP): One ready-to-use bottle of 110 ml

6. Substrate #2 (TMB) One ready-to-use bottle of 110 ml

7. Cry 1Ac/Cry2A Positive control. One ready-to-use vial of 10 mi

8. Cry¹Ac & Cry2A Negative control: One ready-to-use vial of 10 mi

Material and equipment required but not provided

1.Pipette with disposable plastic tips

2.Multichannel pipette with disposable pipette tips

3.Deionized or distilled water

4.Graduated cylinders of one litre capacity

5.Reagent troughs

6.ELISA microplate washer or wash bottle ELISA microplate reader with 450/620 nm filter

7.Tabletop centrifuge

8.Marking pen, pipette tips, timer and paper towels

Procedure

Test protocol for individual seed or leaf samples. Estimated procedure time in two hours (60-30-30 minutes)

1. Add 50 µl Cry1Ac-Cry2A enzyme conjugate in each well. Add 50 µl of working extraction buffer in blank well.

2. Add 50 µl of Cry1Ac-Cry2A positive control in two separate wells Add 50 µl of Cry 1Ac-Cry2A negative control in two separate wells Add 50 µl of each sample to rest of the wells.

3. Incubate for 60 minutes at room temperature.

4. Remove content of the wells by decanting into a sink or a waste container. Add 300 u/wet wash solution to all wells and then empty wells by inverting the plate Repeat washing procedure three more times Alternately, perform four washes by using microtiter plate washer. After last wash, tap the inverted microbiter plate on paper towel to remove as much liquid as possible

5. Add 100ul Substrate #1. PNPP

6. Incubate for 30 minutes at room temperature.

7. Take absorbance at 405 nm with 630 nm as secondary filter. Presence of yellow colour indicates that samples the same manner as indicated in step 4 above are positive for Cry2A

8. Wash the plate.

9. Add 100 µl Substrate #2 TMB

10. Incubate for 30 minutes at room temperature. Add 100 µl stop solution. Take absorbance at 450 nm with 630 nm as secondary filter. Presence of yellow colour indicates that sample are positive for Cry1A.