QUALITY TESTING OF BT-COTTON SEEDS

An Internship Report submitted

for the partial fulfilment of the Degree of Master of Science

By

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C E R T I F I C A T E

This is to certify that this training report of "QUALITY TESTING OF BT-COTTON SEEDS" was successfully carried out by Mr. Hunaid H Rangwala towards the partial fulfilment of requirements for the degree of Master of Science in Biotechnology of Atmiya University, Rajkot. It is an authentic record of his own work, carried out by him under the guidance of Mr. Dennis Dholariya for a period of 3 months during the academic year of 2022-23. The content of this report, in full or in parts, has not been submitted for the award of any other degree or certificate in this or any other University.

Signature

Dr. Nutan Prakash Vishwakarma

Signature

Mr. Dennis Dholariya

D E C L A R A T I O N

I hereby declare that the work incorporated in the present dissertation report entitled "**Quality testing of Bt-Cotton Seeds**" is my own work and is original. This work (in part or in full) has not been submitted to any University for the award of any Degree or a Diploma.

Date: 7 April 2023 Hunaid H Rangwala

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ABSTRACT

Attaining seedling in cotton is the important objective of researchers and producers. Seed testing is determining the standards of a seed lot, physical purity, moisture, germination thereby enabling the farming community to get quality seeds.

The Seed Testing Laboratory is the hub of seed quality control. Seed testing services are required from time to time to gain information regarding planting value of seed lots. Seed testing is possible for all those who produce, sell and use seeds.

We test for the germination process of seed, also test for the important gene that are present in it or not. We have done the sandwich ELISA test performed for the Cry1Ac- Cry2Ab.there are a different variety of criteria/range for the testing the various types of seed that contain the range of seed that can be acceptable for the better quality. After that testing of germination and Elisa test the seed that pass the quality test will go for the packaging.

Before the packaging the seed will be coated by the polymer for to prevent it from the fungicide and the insecticide.

After the product has been packed and it will be checked for the quality.it will be distributed to the dealers.by performing these test on the cotton seed we will be identifying the quality of the seeds.

INTRODUCTION

Cotton is a soft, fluffy staple fiber that grows in a boll, or protective case, around the seeds of the cotton plants of the genus *Gossypium* in the mallow family Malvaceae. The fiber is almost pure cellulose, and can contain minor percentages of waxes, fats, pectins, and water. Under natural conditions, the cotton bolls will increase the dispersal of the seeds.

Cotton is used to make a number of textile products. These include terrycloth for highly absorbent bath towels and robes; denim for blue jeans; cambric, popularly used in the manufacture of blue work shirts and corduroy, seersucker, and cotton twill. socks, underwear, and most T-shirts are made from cotton. Bed sheets often are made from cotton. It is a preferred material for sheets as it is hypoallergenic, easy to maintain and non-irritant to the skin. In addition to the textile industry, cotton is used in fishing nets, coffee filters, tents, explosives manufacture, cotton paper, and in bookbinding. The cottonseed which remains after the cotton is ginned is used to produce cottonseed oil, which, after refining, can be consumed by humans like any other vegetable oil.

Solar Agrotech Private Limited, Rajkot, one of India's **leading seed producing company** & No. 1 seed producing company in Gujarat, was founded by an agricultural technocrat **Dr. Thobhan L. Dholaria (CEO)**, who has a degree of M.Sc. (Agriculture) & Ph.D. (Plant Breeding and Genetics).

• Dr. Dholaria had a bright academic career and awarded the research fellowships of CSIR as well as ICAR 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 trademark of "**Dr. Seeds®**". Dr. Dholaria having bright academic career, so he established the state-of-the-art **R&D facilities** at Solar Agrotech Private Limited.

"Dr. Seeds" sees good future business using the **Biotechnology** and strengthening R&D company's young Director, Dennis Dholaria (M.Sc. Plant Biotechnology from Australia), for **advancements in biotech crops breedings** to support the breeding work of team headed by company's Director of Research, Dr. J. M. Kamani (Ph.D. in Plant Breeding and Genetics) along with Mr. Rahul Kothiya (M.Sc. Plant Biotechnology) and Dr. M. K. Bhalala (ex-University Registrar) as an advisor to the company in the government matters.

OBJECTIVES

• To understand the **basic principles** of Plant Biotechnology, Molecular Biology and Plant Pathology.

• To understand about the **integration** of two disciplines – Agriculture & Biotechnology.

• To know about the **research opportunities** in the Agricultural Biotechnology field.

• To understand the **concepts & techniques used** to make healthy plants in a safe environment for food, non-food, feed and health applications.

WORK PLAN

Seed Sample Preparation:

Ginning: Cotton & Seeds separation.

Seeds treatment: Acid (Pure H_2SO_4) & Base (Lime) treatment, washing, drying.

Grading of seeds: By Gravity air OR Gradient

Seeds Transport: To the testing facility, Rajkot Laboratory

Firstly we have to separate the seeds from the cotton so the seed can be further processed and the cotton is used in different industries. This processes is called ginning. After ginning, the seeds are treated with pure Hydrochloric Acid to remove the extra cotton which remains on the seed and then it is treated with Lime for neutralization of the seeds. Then this seeds are washed and dried.

After the neutralizing of the seeds, the grading of the seeds is done by gravity air or gradient method in which the unsuitable seeds are removed and the seeds which are useful for further processes is packed and send to the Laboratory which is situated in Rajkot which is a testing facility for the same.

Seeds Physical Purity & Germination Testing

Germination of a seed in a laboratory is the emergence and development of the seedling to a stage where the aspect of its essential structure indicates whether or not it is able to grow further into a satisfactory plant under favourable condition in soil. These essential structure are a well developed and intact root system, hypocotyl, plumule and one or two cotyledons according to the species. It cannot be evaluated in a germination test until these essential structure are clearly identified.

PHYSICAL PURITY ANALYSIS: It is done on a working sample of the prescribed weight (100 gram) drawn from the submitted sample. The working sample is separated into different components like pure seed, other crop seeds, weed seed and inert matter by examination and the details are recorded in the seed analysis card. The composition of different components is expressed either in percentage by weight or as number per kilogram.

GERMINATION TESTING: On cellulose paper, count a 100 seeds and tie this cellulose paper in prescribed pattern. This method is called "Paper method" of seeds germination test.

On samples	On cellulose papers
Variety / Lot No.	Variety
No. of Bags	Lot No.
Weight	

Spray water on the cellulose paper daily twice a day and fungicide one time on them, during the germination incubation period which could lasts upto 15 to 20 days. Room temperature should be maintained during the germination period.

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 Fahrenheit for about seven days in most of the cases.

After completion of the germination test, the seedlings are classified into different categories:

(A) Normal seedling
(B) Abnormal seedling
(C) Ungerminated seedling
(D) Hard seeds
(E) Fresh ungerminated seedlings
(F) Dead 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 10 bags.

The passing criteria for the germination test is 75 to 80% which means out of 100 seeds 75 to 80 seedlings should be normal seedlings.

MOISTURE PERCENTAGE: Moisture percentage calculated using moisture meter should be recorded on the seed analysis card. Moisture percentage should be 6 or less.

SANDWICH ELISA TEST

SAMPLE PREPARATION FOR ELISA TEST

Prepared & Tested **313 seed lots** samples.

1) Take 96 wells (12-horizontal x 8-vertical) box.

2) Add **seeds** in all wells.

3) Add metal balls in all wells.

4) Add 750 µL of 1x Protein Extraction Buffer (Contains Deionized/Distilled Water + Tween-20) in all wells.

5) Crush in **QIAGEN® TissueLyser II** (Fast & High-throughput process of protein extraction from cotton leaves/seeds) for 5 minutes.

6) Mix well & incubate at room temperature for 30-60 minutes or overnight.

7) Allow particles to settle and use only the supernatant (contains crude protein) to do the ELISA test.

Cry1Ac-Cry2A in-vitro ELISA Kit (eurofins Technologies)

Intended use:

• 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 kit is ideally suited for testing large numbers of seed lots for Cry1Ac and Cry2A simultaneously.

Principle of the test (Sandwich ELISA):

• In the test, cotton sample extracts are added to test wells coated with antibodies raised against Cry1Ac and Cry2A proteins.
• Any Cry1Ac or Cry2A protein present in the sample extract binds to the antibodies. This is detected by addition of alkaline phosphatase-labeled Cry2A antibody **OR** horseradish peroxidase-labeled Cry1Ac antibody.

• After simple wash step, the results of the Cry2A assay are visualized via the addition of a pNPP substrate.

• Once the yellow color 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 color which, subsequently, becomes yellow on addition of stop solution.

• Cry2A = AP + pNPP = Yellow

• Cry1Ac = HRP + TMB = Blue

• **Coating:** Anti-Cry1Ac & Cry2A antibody

Procedure:

Step-1: Take 96 wells Anti-Cry1Ac & Anti-Cry2A Combo ELISA Plate (eurofins Technologies)

Step-2: Add 45 µL Enzyme/Antibody conjugate (Cry1Ac & Cry2Ab combined) in all 96 wells

Step-3: Add 50 µL Cry1Ac/Cry2A Positive control (in-house) in C1 well, Cry1Ac/Cry2A Negative control in D1 well & keep A1-B1 as Blank (only extraction buffer)

Step-4: Add 50 µL supernatant of crushed samples in all wells (according to the markings), except wells A1-B1-C1-D1

Step-5: Incubate for 60 minutes at room temperature

Step-6: Wash-1 (2 times) (Wash solution contains deionized/distilled water + Tween-20)

Step-7: Add 70 µL Substrate-1 (pNPP) & incubate in dark for 30 minutes (Yellow color will appear)

Step-8: Absorbance-1 for Cry2A at 405 nm (With 630 nm as secondary filter)

Step-9: Wash-2 (1 time)

Step-10: Add 90 µL Substrate-2 (TMB) & incubate in dark for 30 minutes (Blue color will appear)

Step-11: Add 100 µL Stop solution (27 mL of 98% H_2SO_4 in 973 mL of deionized/distilled water)

Step-12: Absorbnce-2 (Yellow) for Cry1Ac at 450 nm (With 630 nm as secondary filter)

Figure-1: Sample addition (Multichannel Pipette)
Figure-2: ELISA Microplate Washer
Figure-3: ELISA Microplate Reader with 450/620 nm Filter

INTERPRETATIONS OF RESULTS

General test criteria (Absorbance value):

• **Blank:** should not exceed 0.1/0.15

• Absorbance value of blank should be subtracted from the absorbance values of positive control, negative control and samples.

• The coefficient of variance between the positive control, in duplicate, should be less than 15%

• **Positive control:** at least 0.5 (1 or more than 1)

• **Mean negative control:** below 0.1-0.2

Note: If above criteria are not met, the test is invalid and should be repeated. Negative: less than 0.150

Cut-off value: (Mean absorbance of negative control + 0.1)

All negative seeds should give absorbance below 0.2

Negative control should give absorbance below 0.1