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Detection and characterization of Cry 1 Ac in Bt cotton

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ABSTRACT

A field experiment was conducted during the 2024–25 season at the Biotechnology lab Daftari Agro Pvt. Ltd. Agricultural Researchin Seloo, distWardha, to evaluate Cry1Ac protein expression in Bt (Bacillus thuringiensis*)* cotton hybrids. The study focused on the effect of various nutrient management strategies and yield targets on protein levels as plant tissues matured. The leaf discs were incubated in the dark following the ELISA method with economically available kits. Using qualitative ELISA techniques, the research identified substantially higher Cry1Ac concentrations in leaves at 60, 90, and 120 days after sowing (DAS). Specifically, the leading hybrids recorded protein levels of 3.491, 3.281, and 3.395 µg g^{-1} fresh weight, respectively, outperforming other tested varieties. Using ELISA kits at the Seloo worked great. It shows these tests are a reliable way to make sure the commercial Bt seeds farmers are buying actually have the genetic quality and traits they're supposed to have. The development of genetically modified (Bt) cotton, which expresses the *Bacillus thuringiensis* (Cry1Ac) poison, has revolutionized pest management in agriculture.

Key words:Bt cotton, ELISA, Cry 1Ac protein

INTRODUCTION

Cotton is one of the major fibre crops of global significance. Cotton (Gossypium spp.) is an important fibre crop of India contributing 85 %of raw materials to textile industry. Transgenic cotton constitutes approximately 64% to over 80% of the total cotton area in the world. Cotton is an important agricultural crop. The term "Bt" refers to Bacillus thuringiensis, a naturally occurring gram-positive soil bacterium. This bacterium is capable of forming spores.. While primarily grown for fiber, cotton is also categorized as a major oilseed crop and a critical source of high-protein feed for the livestock industry. Although cotton is primarily cultivated for its fibre, it also serves as an important oilseed crop.

The ELISA (Enzyme- linked Immunosorbent Assay)method detects proteins produced by the transgene using antibody-specific recognition. To quantify Cry1Ac protein levels, leaf samples were collected at 3 distinct intervals—60, 90, and 120 days after sowing (DAS)—throughout the duration of the study. Quantification of Cry1Ac the experiments was done using economicallyavailable Quan-T ELISA 96 well plate kits Desi-Gen, Jalna, Maharashtra. The introduction of Bt cotton has provided growers with a new tool for managing bollworms in cotton. About 20 mg of each sample was trimmed off and separated for further analysis. This study investigated how different nutrient management strategies affect the amount of Cry protein produced by Bt cotton. The research focused on cotton grown in medium-deep black soils in areas that receive reliable rainfall. Bt cotton is a genetically modified (GM) pest-resistant cotton variety. Using Bt cotton made it possible to solve the issue of

bollworm attacks on cotton crops. Beyond providing a biological defense against bollworms, Bt cotton plays a pivotal role in enhancing crop yields and significantly increasing the profit margins for agricultural producers.

MATERIALS AND METHODS

In the present investigation an attempt has been made to study ELISA (Enzyme- linked Immunosorbent Assay)Bt cotton in Cry1Ac. Experiments were conducted to study the assessment of Cry 1Ac . The details of the material used and techniques adopted for the collection, data are described here under.

Experimental Location

The present investigation on ELISA (Enzyme- linked Immunosorbent Assay)Bt cotton in Cry1Ac was carried out at biotechnology lab, Dafftari Agro pvt. Ltd. Rehaki Road, Seloo.

Indended use

Cry1Ac ELISA (Enzyme- linked Immunosorbent Assay) test kit is intended to be used for the qualitative detection of Cry1 Ac protein in Bollgard, Bollgard II, or WideStrike sepratecotton leaf sample. It can also be used for detection of Cry 1 Ac protein in cotton leaf tissue. It can also be used to detect Cry 1 Ac in leaf samples :

The total incubation time of the assay is 60 minutes.

Principal of the test

An antibody specific to the Cry 1 Ac molecule is conjugated wish horse radish peroxidase (HRP). When the sample extracts are added to the microtiter wells , the Cry 1 Ac from sample binds to the antibody immobilized in the well. This binding is subsequently detected by addition of enzyme labeled antibody. After a washing step, substrate is added. The enzymatic reaction and development of color is proportional to the amount of Cry 1 Ac present in the sample. The reaction is terminated by addition of stopping solution. Absorbance is then measured on a plate reader. Light colourshow lower concentration and dark colourshow higher concentration of Cry 1 Ac protein in the sample.

Precautions:

The Cry 1Ac ELISA (Enzyme- linked Immunosorbent Assay) test in intended for in vitro use only. The reagents contain Thimerosal as preservative. Prevent direct skin and eye contact with kit components. Seek medical attention in case of accidental ingestion of kit components.

Storage of the kit

The kit should be stored at 2-8 °C. The unopened kit is stable till the expiry date printed on the kit label. The expiry da of each unopened component is printed on the label of the component.

Material and equipment required

- Pipette with disposable plastic tips
- Multi channel pipette with disposable tips
- Distilled water

- Graduated cylinders with one liter capacity
- Reagent troughs, Plate ELISA washer
- Plate ELISA reader with 450/620 nm filter
- Table top centrifuge
- Parafilm, Pipette tips, Timer and Paper towels.

Sample preparation:

Individual leaf: Weigh 20 mg(about two leaf punch) and put it in a microtube. Crush leaf with pestle in 0.5 ml working extraction buffer. Mix well and incubate for 30-60 minutes at RT. Allow particles to settle and use only the supernatant to do the test. Avoid cross contamination between samples.

Procedure

Select the leaf sample and place it into the extraction plate. Add 500µl of Extraction Buffer and two metal grinding balls to each well. Secure the plate in the crushing machine and process until the tissue is fully homogenized. Balance the plate and centrifuge at 4,000 rpm for 2 minutes to pellet the debris. The clear liquid at the top (supernatant) will be used for the assay. Allow the Cry1Ac antibody-coated plate to reach room temperature (approximately 4–5 minutes) before opening. Dispense 50µl of Enzyme Conjugate into the required wells of the Cry1Ac plate. Add 50µl of the leaf (or seed) supernatant to the conjugate in the wells. Incubate the plate in a dark environment for 45 to 60 minutes. Discard the well contents. Wash the plate three times using 200µl of Wash Buffer per well, ensuring the plate is tapped dry between washes. Add 100µl of Substrate Solution to each well. Incubate in the dark for 15 minutes to allow the color reaction to develop. Add 100µl of Stop Solution to each well to halt the reaction. The plate is now ready to be processed by the ELISA plate reader for results.

RESULTS AND DISCUSSION

Analysis of Cry1AC protein by ELISA (Enzyme-Linked Immunosorbent Assay)

The interpretation of results were done based on the mean absorbance data of the individual samples. The cutoff value for the presence and absence of Cry1Ac protein in the sample was 1.0. Samples of absorbance 1 were considered to be as positive for the presence of Cry1Ac protein. The ELISA plates were prepared with blank, positive and negative controls as shown in Figure 1.

Analysis of Cry 1Ac Protein from Bt cotton leaf sample

The cry protein content in the leaves of Bt cotton varied substantially at all growth stages. The data indicated that substantially higher Cry 1Ac protein was recorded with Bt at 60, 90 and 120 DAS (Cry 1Ac: 3.491, 3.281 and 3.395 µg g-1 fresh weight respectively. (table 1and 2)Bacillus thuringiensis produces insecticidal proteins during its sporulation at the stationary phase. Poorer nutrition is of benefit to maximize the formation of spores and crystal protein.

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	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.001	3.396	3.312	1.678	3.491	3.296	3.464	3.341	3.077	3.519	3.272	3.39	Delta OD Cry1Ac
B	1.855	3.39	3.367	2.929	3.449	3.25	3.121	3.314	3.612	3.111	3.555	3.35	Delta OD Cry1Ac
C	0.018	3.472	3.401	1.487	3.371	3.378	3.244	3.483	3.284	3.134	3.365	3.405	Delta OD Cry1Ac
D	3.32	3.245	3.627	1.66	3.382	3.069	3.404	3.313	3.151	3.176	3.181	3.435	Delta OD Cry1Ac
E	3.268	3.167	3.293	1.294	3.253	1.601	3.519	3.337	3.17	3.16	3.44	3.395	Delta OD Cry1Ac
F	3.295	3.145	2.99	1.569	3.453	2.982	3.291	3.425	3.023	3.203	3.411	-0.001	Delta OD Cry1Ac
G	3.248	3.225	3.274	1.617	3.616	3.235	3.419	3.281	3.299	3.167	3.153	1.573	Delta OD Cry1Ac
H	3.283	3.169	3.193	2.857	3.059	3.393	3.353	3.269	3.178	3.177	3.105	0.016	Delta OD Cry1Ac

Table 1. Delta OD of Cry 1 Ac all samples are positive. Mention blank, positive, negative control.

	1	2	3	4	5	6	7	8	9	10	11	12	
A	NEG	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	Symbols (Delta OD Cry1Ac)
B	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	Symbols (Delta OD Cry1Ac)
C	NEG	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	Symbols (Delta OD Cry1Ac)
D	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	Symbols (Delta OD Cry1Ac)
E	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	Symbols (Delta OD Cry1Ac)
F	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	Symbols (Delta OD Cry1Ac)
G	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	Symbols (Delta OD Cry1Ac)
H	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	Symbols (Delta OD Cry1Ac)

Table 2. Symbols of delta OD Cry 1Ac all samples are positive. Mention blank , positive, negative control.



Figure 1. Detection of Cry1Ac proteins in cotton leaf samples through qualitative ELISA

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Interpretation of results:

General test criteria:

1) Absorbance of the blank well should not exceed 0.1

2) Absorbance value of blank should be subtracted from positive control, negative control and samples.

3) The coefficient of variance between the duplicate positive controls should be less than 15%

4) Mean positive control should have absorbance of at least 0.5

5) Mean negative control should have absorbance below 0.1

CONCLUSION

Based on the results, it could be concluded that the cry protein content in the leaves of different Bt cotton hybrids is dependent on the application of nutrients based on target yield. In this study it was confirmed that a Bt testing ELISA method could be more readily for the detection of transgenic Bt cotton using a selected strains of Bt. Studies have shown that ELISA accurately identifies specific Bt proteins, such as Cry1Ac and it is successful method.

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