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Research Article

Identification of *Cry1Ac* Protein in Bt Brinjal by ELISA Method

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Abstract

Brinjal fruit and shoot borer resistant Bt brinjal was introduced in Bangladesh in 2013 for commercialization and became a member of GMO world. Bt gene was introgressed in 9 popular varieties of Bangladesh. Four varieties were released in 2013 and three are in pipelines. ELISA based protein detection and PCR based gene detection are widely used for GMO detection. Here, Bt protein was detected through qualitative ELISA in four Bt brinjal varieties and three Bt brinjal lines. Changes of colour of the microtiter plate and the absorbance value indicated the presence *Cy1Ac* protein in Bt brinjal samples.

Keywords: Bt Brinjal; Cry1Ac protein; ELISA

Introduction

Genetically Modified (GM) crops have been expanded rapidly in the world. Adoption rate of biotech crops increased globally and it was about 110-fold during 1996 to 2016 (ISAAA, 2016). Brinjal (Eggplant or Aubergin) is one of the most important and popular vegetable crops grown all over the country throughout the year. The area of brinjal was 49398 ha and the production was 450146 metric tons during 2014-15 in Bangladesh (BBS, 2016). It plays a significant role in Bangladeshis' daily diet, livelihood and farm income. The crop is damaged severely by the notorious insect called brinjal fruit and shoot borer (BFSB) and the damage due to this insect ranges from 30-70%. BFSB resistant Bt brinjal was developed through the introgression of *Cry1Ac* gene (from *Bacillus thuringiensis*) into the brinjal varieties of Bangladesh. Bangladesh has become the first country in South Asia to allow the cultivation of GMO Bt brinjal in 2013. Based on experimental data, Bt brinjal can increase yield by at least 30 % and reduce the number and cost of insecticide applications by 71-90% (Choudhary *et al.*, 2014). The popularity of Bt brinjal among the farmers increasing day by day and the area for Bt brinjal is expanding with the passage of time. Due to increasing of transgenic crops in the market, it has demand for testing GMOs for certifying non GMO (Amiri *et al*., 2013). Within the arena of expanding techniques for identification and quantification of transgenic crops, two major approaches for detecting GMOs are still applicable on large scale (Vidhya *et al*., 2012). First method includes the identification of proteins produced by the introduced trait gene through the detection of its specific antibody, such as by Enzyme Linked Immunosorbent Assay (ELISA) (Ahmed, 2002; Chalam & Khetarpal, 2012), while second method employs the identification of specific DNA sequence used for gene modification by Polymerase Chain Reaction (PCR) (Khetarpal & Kumar, 1996; Gachet *et al*., 1999). ELISA is a relatively efficient detection method, offering simple, fast, and reliable protein determination, and it has been widely used for qualitative and quantitative analyses of Cry proteins in Bt plants (Wang *et al*., 2014; Zhang *et al*., 2011). The antibody-coated micro wells are used where protein is not denatured, but no extra information can be obtained concerning the presence of transgene at the ingredient level in transgenic organism (Chalam & Khetarpal, 2012). Although enzyme-linked-immunosorbent assays (ELISA) are commercially available to detect the expressed Cry-protein(s), no published methods are available to determine if an individual plant contains the Cry1Ac gene.

Materials and Methods

Four Bt brinjal varieties and three lines with their respective counter parts (Table 1) were collected and tested for ELISA at Biotechnology Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, Bangladesh. Qualitative ELISA test kit for *Cry1Ac* was obtained from DesiGen QL 96 ELISA (DGH030), MHYCO, India.

Table 1: Name of the Bt brinjal varieties and its counterparts

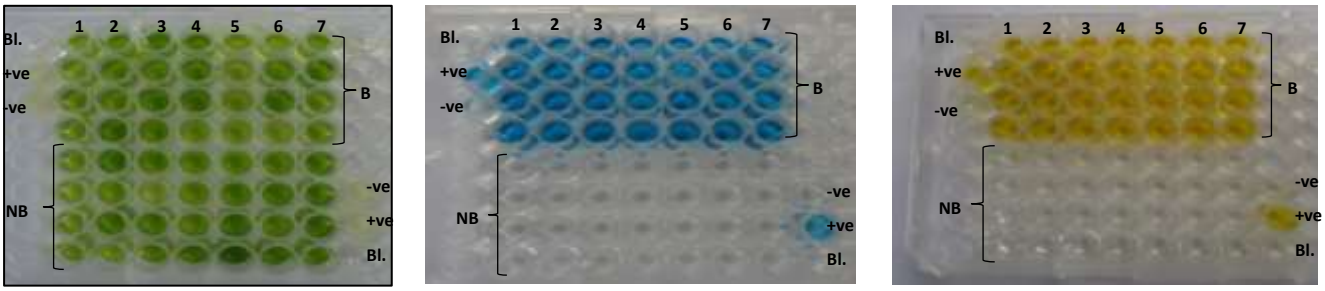
Bt brinjal varieties/lines	Counterparts (non-Bt)
BARI Bt Begun 1	BARI Begun 1 (Uttara)
BARI Bt Begun 2	BARI Begun 4 (Kazla)
BARI Bt Begun 3	BARI Begun 5 (Nayantara)
BARI Bt Begun 4	BARI Begun 6 (ISD006)
Bt Dohazari	BARI Begun 9 (Dohazari)
Bt Khatkhatia	Khatkhatia
Bt Shingnath	BARI Begun 7 (Shingnath)

Seeds of the varieties were sown in the soil and leaves were collected at the age of 3 weeks of the plant. A small piece of leaf was put into the 1.5 ml Eppendorf tube and added 500 µl 1X sample extraction buffer. It was crushed with pestle for 30 seconds and 50 µl of the diluted extract was taken for loading. Precoated microtiter plate with anti-*Cry1Ac* antibodies was taken. On one side, 50 µl of blank, 50 µl of positive control of *Cry1Ac* (available in kit) was added to two wells on opposite corners while on the opposite side, 50 µl of blank, 50 µl negative control of *Cry1Ac* was added to two wells on the opposite corners. Among rest of the wells, 50 µl of each sample (Table 1) was added in individual rows into replicates of four (Fig. 1). Conjugate buffer of 50 µl was added in all the wells and incubated for 45 minutes at room temperature. At completion of incubation period, each well was then washed carefully with 1X buffer (wash buffer) for approximately four times. Excess buffer was removed by blotting paper. After washing, to each well of substrate buffer (ready to use) of 1X 100 µl was added to each well after washing and kept for 15 minutes at room temperature in dark condition. After completion of the incubation, stop solution of 100 µl was added to each well immediately. The absorbance of contents from each well was then measured at 450 nm (using UV/Vis Spectrophotometer, Shimadzu-1800) along with the positive and negative control well. The absorbance of a blank well was subtracted from absorbance values of samples and control. The data were recorded and the mean absorbance was calculated along with standard deviation and standard error of mean using the MS Excel. It is mentioned that precoated microtiter, sample extraction buffer, positive control, negative control, conjugate buffer, wash buffer, stop solution etc. were supplied into the kit.

Result and Discussions

From the Fig. 1, it was observed that when substrate buffer added into the samples, the colour of the wells containing Bt brinjal leaf extract turned into blue but the colour of wells of non-Bt were unchanged (Fig.1.b). Due to the application of stop solution, the blue colour of the Bt brinjal samples turned into golden. The colours of the wells of the non-Bt brinjal sample were remained unchanged. The phenomena of the colour changing indicated the presence of *Cry1Ac* protein in the Bt brinjal samples.

Moreover, the interpretations of results were done based on the mean absorbance data of the individual samples (Table 2). The cut off value was estimated as 0.182. The samples of Bt showed the above of cut off values which confirmed the presence of Cry1Ac protein. On the other hand, the samples of non-Bt (counterparts) showed the absorbance below of the cut off value. Moreover, the range of the mean observance of Bt brinjal varieties/lines was 1.179 to 1.401 which indicated the presence of *Cry1Ac* protein in the samples. On the other hand, the value of mean observance of the counterparts of Bt varieties/lines i.e. non-Bt brinjal ranged from 0.064 to 0.080 which are lower than the negative control demonstrated that the absence of *Cry1Ac* protein in the samples (Table 2). Liu *et al*. (2016) also using the ELISA method, to measure the levels of *Cry* proteins in Bt rice plants. The levels of *Cry* protein in different plant tissues varied significantly, with the highest level in leaves, followed by stems, roots and seeds.



1.a. added of sample 1.b. added substrate buffer 1.c. added stop solution

Fig.1: ELISA test for Cry1AC protein identification
[Bl. = Blank, +ve = positive, -ve = negative, NBt = non-Bt]

Table 2: Mean absorbance at 450 nm, standard deviation and standard error for *Cry1AC* in brinjal

Bt brinjal variety/lines and its counterparts	**Mean Absorbance**	**Standard deviation**	**Standard error**
Positive control	0.822		
Negative control	0.082		
Bt brinjal varieties/lines			
BARI Bt Begun 1	1.379	0.123	0.061
BARI Bt Begun 2	1.179	0.126	0.062
BARI Bt Begun 3	1.401	0.234	0.117
BARI Bt Begun 4	1.209	0.095	0.048
Bt Dohazari	1.244	0.348	0.174
Bt Khatkhatia	1.254	0.099	0.049
Bt Shingnath	1.292	0.063	0.031
Non-Bt brinjal varieties/lines (counterparts)			
BARI Begun 1 (Uttara) (counterpart of BARI Bt Begun 1)	0.066	0.003	0.002
BARI Begun 2 (Kazla) (counterpart of BARI Bt Begun 2)	0.080	0.011	0.006
BARI Begun 5 (Nayantara) (counterpart of BARI Bt Begun 3)	0.072	0.005	0.002
BARI Begun 6 (counterpart of BARI Bt Begun 4)	0.074	0.016	0.008
BARI Begun 9 (counterpart of Bt Dohazari)	0.068	0.016	0.008
Khatkhatia (counterpart of Bt Khatkhatia)	0.064	0.007	0.003
BARI Begun 7 (counterpart of Bt Shingnath)	0.066	0.006	0.003

Conclusion

Bt brijal was introduced in Bangladesh as first GMO crop. Research is being done on other transgenic crops late blight resistant potato, Bt cotton, golden rice, salt tolerant rice, virus resistant tomato etc. Testing for GMO is important for introducing more GMO crops. The technique of ELISA for identification of Cry1Ac is simple and reliable.

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