Egyptian Journal of Biological Pest Control

RESEARCH

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Cry toxin expression in different plant parts of *Bt* cotton at different phenological stages

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Abstract

Background Compared to Bollgard-I, the utilization of Cry2Ab protein in Bollgard-II cotton cultivars enhances insect control. Field and laboratory studies demonstrated reduction in the numbers of bollworm-infested terminals, squares, and bolls in Bollgard-II cotton lines when compared to both Bollgard-I and non-Bollgard cotton cultivars. This indicates that the combination of Cry2Ab with Cry1Ac increased the overall expression of proteins. The expression of Cry protein varied across different plant parts, such as leaves, bracts, squares, and bolls. As the season progresses, the expression of Cry protein decreased in these plant parts. Leaves exhibited the highest levels of Cry protein expression, followed by squares, flowers, and bolls. Variation in the expression levels of delta endotoxins in different plant parts was one of the contributing factors to the survival of pest populations on *Bt* cotton.

Results Using a commercially available QL 96 ELISA plate kit, the concentration of delta endotoxin in various plant parts at different phenological stages was determined in twelve BG-II cotton hybrids, namely Ajeet-155, JKCH-2245, RCH-3863, NCS-866, MRC-7373, JKCH-99, MRC-7387, NCEH-21, ANKR-3324, NCSI-1904, and NCHB 9902. Cry1Ac and Cry2Ab protein levels were determined from samples of flowers and fruiting parts (Rind, locule, seed, locule wall and seed) at 40, 75, 100 and 125 days after sowing (DAS) over two consecutive years 2018–2019 and 2019–2020. Cry1Ac protein content and expression was the highest at 100DAS in locule, seed and rind; followed by rind and locule wall and seed in green bolls at 125 DAS; followed by seed, locule, rind and flowers at 75 DAS; followed by flowers at 40 DAS in all the tested twelve Bollgard-II hybrids. Cry2Ab protein content and expression was the highest at 125DAS in locule wall and seed and rind, followed by seed, locule and rind in green bolls at 100 DAS, followed by seed, locule, rind and flowers at 75 DAS, then by flowers at 40 DAS in all the tested twelve Bollgard-II hybrids. Cry1Ac protein expression was less in comparison with Cry2Ab.

Conclusions The research findings indicate that the locule of BG-II cotton plants exhibited the highest expression levels of Cry1Ac and Cry2Ab proteins, followed by the seeds, rind, locule wall, and flowers. These results provide valuable insights into the distribution of Cry protein expression in different plant parts, which can contribute to a better understanding of insect control in *Bt* cotton cultivars.

Keywords Cry1Ac, Cry2Ab, Toxin expression, ELISA, *Bt* cotton hybrids, Phenological stages

Background

Bacillus thuringiensis (*Bt*) is a gram-positive, spore-forming, facultative, aerobic soil bacterium (Bacillaceae: Eubacteriales) from the silkworm *Bombyx mori* (Linnaeus L). The bacterium produces crystalline inclusion bodies in the course of sporulation and releases spores, Cry (Crystal) toxins or δ-endotoxins. Using the molecular biology tools, scientists have introduced genes from *B. thuringiensis* bacterium into cotton plants. These

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genotypes are referred to as transgenic *Bt*-cotton. The transgenic cotton containing Cry genes responsible for crystalline *δ*-endotoxin production in soil bacterium, B. *thuringiensis* var. *kurstaki* (Berliner) were transferred to cotton via *Agrobacterium* with CaMV 35S promoter. On ingestion in the midgut (having the alkaline condition) of susceptible insects Cry protein is activated by insect gut proteases, bind to receptors and get inserted into the microvillar brush-border membranes leading to disruption of osmotic balance, lysis of epithelial cells, starvation and ultimate death of insect (http://www.lifesci.sussex.ac.uk/Home/NeilCrickmore/Bt/). In India, the cultivation of *Bt* cotton expressing the Cry1Ac gene (BG I) has resulted in the effective control of all bollworms, including *Helicoverpa armigera* (Hubner), *Erias vitella* (Hubner) and *Pectinophora gossypiella* (Saunders) since 2002 (Bambawale et al. 2004). To maintain *Bt* cotton susceptibility and delay bollworm resistance to BGI, second-generation *Bt* cotton (BGII) expressing two Cry toxins (Cry1 Ac+Cry2Ab) was commercialized in the USA (2003) and India (2006), replacing more than 95 per cent of traditional cotton cultivation in India. For a long time (nearly ten years), *Bt* cotton was able to shield the crop from bollworms, reducing pesticide use from 46 per cent to less than 21 per cent. Pink bollworm has been more aggressive in the last three to four years since it has developed insecticide resistance (Kranthi 2016). However, for the *Bt*-transgenic cotton technology to be viable, the toxin expression levels must be present in sufficient amounts in appropriate plant parts at the appropriate time of the season to provide protection against major target insect pests, especially bollworms. The variance in overall Cry1Ac expression levels among Bollgards has been linked to the survival of various lepidopteran pests, suggesting that cultivars do not have the same degree of control during the crop season. Those *Bt* cotton hybrids which were being used by farmers contain adequate *Bt* toxin or not, period [at different phenological stages of cotton plant, *i.e.,* (on leaf, square, bolls)] of *Bt* toxin expression is the major question. Considering the above objective, the present research aimed to study Cry toxin expression in different plant parts of *Bt* cotton at different phenological stages.

Methods

Cry1Ac and Cry2Ab protein was estimated from replicated samples of flowers and fruiting parts (Rind, locule, seed, locule wall and seed) at 40, 75, 100 and 125 days after sowing (DAS) in two consecutive years 2018–2019 and 2019–2020 from plant samples collected from different BG II cultivars from different districts of Vidarbha (research farms of DR.PDKV, Akola).

Methodology adopted

The qualitative estimation of Cry protein in Bollgard-II hybrids was performed at Biotechnology center, Dr PDKV, Akola and Mahabeej, Akola using commercially available QL 96 enzyme-linked immunosorbent assay (ELISA) plate kits (Table 1) of Design, Agrisure Diagnostics LLP, Jalna-Aurangabad as per manufacturer's protocol.

Samples of 50 mg were collected in a 1.5-ml microfuge tube. One ml of ice-cold sample extraction buffer was added and the mixture was macerated at 4000 rpm for 5 min at room temperature with a motor-driven pestle. It was then chilled on ice for 10 min before being macerated for 30 s. Finally, the sample was centrifuged for 10 min at 10,000 rpm. The supernatant was then extracted and stored at 4 °C.

The positive and negative standards were diluted with standard buffer before loading the sample extracts onto the plate. After that, the respective antibody-coated plates were opened. In each well of the ELISA plate, 50 µl of conjugate is added. Then, in each plate, 50 µl of negative and positive control were added. Then, in each ELISA plate, 50 µl of supernatant sample was added. After loading, the plates were incubated at 37 °C for 1.5 h in a humid environment and in

Table 1 Calibrators used for qualitative ELISA

S. no.	Cry1Ac calibrators (ready to use)	Cry2Ab calibrators (ready to use)
1	Cry1Ac mAb-coated 96-well ELISA plate	Cry2Ab mAb-coated 96-well ELISA plate
2	Anti-Cry1Ac conjugate	Anti-Cry2Ab conjugate
3	Cry1Ac positive control	Cry2Ab positive control
4	Negative control	Negative control
5	10×Extraction buffer	10×Extraction buffer
6	10×Wash buffer	10×Wash buffer
7	TMB substrate (HRP)	TMB substrate (HRP)
8	Stop solution	Stop solution

the dark, after which the samples were discarded and the plates were washed twice with 200 µl wash buffer to remove traces of wash buffer. Plates were dried on paper towels before 100 µl of substrate solution (dark reaction) was added and plates were gently vibrated for 2–3 min. Plates were incubated in the dark for 15 min at 37 °C. Following the incubation period, 100 µl of stop solution was added. For color development, the plate was incubated at room temperature for 30 min. The plates were then run through an ELISA reader.

Data analysis

The qualitative analysis of Cry protein was estimated using the Gen5 3.04 EPOCH/2 program based on absorbance values of the plate at 405 nm,were depicted and the results were reported as optical density values. Optical density (OD) values were used to determine the presence or absence of a specific antigen or antibody in a sample. The OD of the wells is then measured using a microplate reader at a specific wavelength, typically 450 nm for most ELISAs. The OD value is a measure of the intensity of the color developed in each well.

Results

Cry protein (Cry1Ac) content in BG-II cotton hybrids (optical density) (2018–2019 and 2019–2020 pooled)

Pooled results on Cry protein (Cry1Ac) content in different BG-II cotton hybrids for seasons 2018–2019 and 2019–2020 are depicted in Table 2.

Flowers

The Cry1Ac protein content was estimated in flowers at 40 and 75 DAS during study period. The Cry1 Ac protein in flowers at 40 DAS in descending order was 2.795, 2.77, 2.747, 2.673, 2.449, 2.359, 2.312, 2.212, 2.122, 2.1065, 2.105 and 2.048 (optical density) in NCHB-9902, ANKR-3324, NCEH-21, MRC-7387, ANKR-3066, NCS-866, NCSI-1904, JKCH-99, MRC-7373, RCH-3863, JKCH-2245 and Ajeet-155, respectively. Among all the hybrids, NCHB-9902 recorded highest Cry1Ac protein content and Ajeet-155 had the lowest Cry1Ac protein content. The Cry1Ac protein in flowers at 75 DAS in descending order was 2.43, 2.269, 2.201, 2.2005, 2.191, 2.153, 2.117, 2.1105, 2.109, 2.0645, 2.06 and 2.040 (optical density) in MRC-7373, NCS-866, JKCH-2245, NCEH-21, RCH-3863, ANKR-3066, JKCH-99,NCSI-1904, MRC-7387, NCHB-9902 and ANKR-3324, respectively. Among all the hybrids, Ajeet-155 recorded the highest Cry1Ac protein content and ANKR-3324 had the lowest Cry1Ac protein content. Among all hybrids Cry protein level was the highest in Ajeet-155 cultivar at 75 DAS (2.43 OD). The lowest Cry1Ac protein concentration was recorded in Ajeet-155 (2.048 OD) at 40 DAS. The Cry protein content in flowers remained uncertain with advance in the age of the crop from 40 to 75 DAS in twelve BG-II cotton hybrids.

Rind

The Cry1Ac protein content was estimated in rinds at 75, 100 and 125DAS. The Cry1Ac protein in rind at 75 DAS in descending order was 2.886, 2.802, 2.91, 2.269, 2.237,

Table 2 Cry protein (Cry1Ac) content in BG-II cotton hybrids (optical density) (2018–2019 and 2019–2020 pooled)

S. no.	Variety	Cry protein (Cry1Ac) content (optical density) in different plant parts at different phenological stages of BG II cotton hybrids									
		40DAS	75DAS				100DAS			125DAS	
		Flowers	Flowers	Rind	Locule	Seed	Rind	Locule	Seed	Rind	Locule wall and seed
1	Ajeet-155	2.048	2.43	2.1065	2.741	3.736	3.146	3.819	3.063	3.587	3.432
2	JKCH-2245	2.105	2.2005	2.269	3.975	3.995	3.07	3.096	3.65	2.846	3.938
3	RCH-3863	2.1065	2.153	2.094	2.182	2.79	3.394	3.892	3.671	2.821	3.559
4	NCS-866	2.359	2.201	2.114	2.139	3.73	3.236	3.091	3.633	2.702	3.682
5	MRC-7373	2.122	2.269	2.237	3.631	2.985	3.044	3.44	3.884	2.631	3.006
6	JKCH-99	2.212	2.1105	2.207	3.465	3.743	2.981	3.041	4.152	3.1	3.188
7	MRC-7387	2.673	2.0645	2.291	2.161	3.118	2.831	2.728	3.152	3.790	3.751
8	NCEH-21	2.747	2.191	2.196	4.235	3.633	3.53	3.723	3.124	3.351	3.683
9	ANKR-3324	2.77	2.040	2.156	3.751	3.724	3.21	3.253	2.757	2.967	3.420
10	NCSI-1904	2.312	2.109	2.147	2.614	2.828	3.023	3.445	3.502	2.896	3.748
11	NCHB-9902	2.795	2.06	2.802	2.615	3.346	3.834	3.542	2.867	3.59	3.308
12	ANKR-3066	2.449	2.117	2.886	3.55	3.413	2.58	2.886	2.943	2.870	3.65

**DAS* days after sowing

2.207, 2.196, 2.156, 2.147, 2.114, 2.1065 and 2.094 OD in ANKR-3066, NCHB-9902, MRC-7387, JKCH-2245, MRC-7373, JKCH-99, NCEH-21, ANKR-3324, NCSI-1904, NCS-866, Ajeet-155 and RCH-3863, respectively. Among all the hybrids, ANKR-3066 recorded the highest Cry1Ac protein content and RCH-3863 had the lowest Cry1Ac protein content. The Cry1Ac protein in rind at 100 DAS in descending order was 3.834, 3.53, 3.394, 3.236, 3.21, 3.146, 3.07, 3.044, 3.023, 2.981, 2.831and 2.58 OD in NCHB-9902, NCEH-21, RCH-3863, NCS-866, ANKR-3324, Ajeet-155, JKCH-2245, MRC-7373, NCSI-1904, JKCH-99, MRC-7387 and ANKR-3066, respectively. Among all the hybrids, NCHB-9902 recorded the highest Cry1Ac protein content and ANKR-3066 had the lowest Cry1Ac protein content.

The Cry1Ac protein in rind at 125 DAS in descending order was 3.790, 3.59, 3.587, 3.351, 3.1, 2.967, 2.896, 2.870, 2.846, 2.821, 2.702 and 2.631 OD in, MRC-7387, NCHB-9902, Ajeet-155, NCEH-21, JKCH-99, ANKR-3324, NCSI-1904, ANKR-3066, JKCH-2245, RCH-3863, NCS-866, and MRC-7373, respectively. Among all hybrids MRC-7387 recorded the highest Cry1Ac protein content and MRC-7373 had the lowest Cry1Ac protein content. Among all the hybrids, Cry protein level was the highest in MRC-7387 cultivar at 125 DAS (3.790 OD). The lowest Cry1Ac protein concentration was recorded in RCH-3863 (2.094 OD) at 75 DAS. The Cry protein content in rind remained increased from 75 to 100 DAS and decreased from 100 to 125 DAS in twelve BG-II cotton hybrids.

Locule

The Cry1Ac protein content was estimated in locule at 75 and 100DAS. The Cry1Ac protein in locule at 75 DAS in descending order is 4.235, 3.995, 3.751, 3.631, 3.55, 3.465, 2.741, 2.615, 2.614, 2.182, 2.161 and 2.139 OD in NCEH-21, JKCH-2245, ANKR-3324, MRC-7373, ANKR-3066, JKCH-99, Ajeet-155, NCHB-9902, NCSI-1904, RCH-3863, MRC-7387 and NCS-866, respectively. Among all the hybrids, NCEH-21 recorded the highest Cry1Ac protein content and NCS-866 had the lowest Cry1Ac protein content. The Cry1Ac protein in locule at 100 DAS in descending order is 3.892, 3.819, 3.723, 3.542, 3.445, 3.44, 3.253, 3.096, 3.091, 3.041, 2.886 and 2.728 OD in RCH-3863, Ajeet-155, NCEH-21, NCHB-9902, NCSI-1904, MRC-7373, ANKR-3324, JKCH-2245, NCS-866, JKCH-99, ANKR-3066 and MRC-7387, respectively. Among all the hybrids, RCH-3863 recorded the highest Cry1Ac protein content and MRC-7387 had the lowest Cry1Ac protein content. The lowest Cry1Ac protein concentration was recorded in NCS-866 cultivar (2.139 OD) at 75 DAS. The Cry1Ac protein content in locule decreased from 75 to 100 DAS in seven BG-II cotton hybrids and increased from 75 to 100 DAS in five BG-II cotton hybrids.

Seed

The Cry1Ac protein content was estimated in seed at 75 and 100DAS The Cry1Ac protein in seed at 75 DAS in descending order is 3.995, 3.743, 3.736, 3.73, 3.724, 3.633, 3.413, 3.346, 3.118, 2.985, 2.828 and 2.79 OD in JKCH-2245, JKCH-99, Ajeet-155, NCS-866, ANKR-3324, NCEH-21, ANKR-3066, NCHB-9902, MRC-7387, MRC-7373, NCSI-1904 and RCH-3863, respectively. Among all the hybrids, JKCH-2245 recorded the highest Cry1Ac protein content and RCH-3863 had the lowest Cry1Ac protein content. The Cry1Ac protein in seed at 100 DAS in descending order was 4.152, 3.884, 3.671, 3.65, 3.633, 3.502, 3.152, 3.124, 3.063, 2.943, 2.867 and 2.757 OD in JKCH-99, MRC-7373, RCH-3863, JKCH-2245, NCS-866, NCSI-1904, MRC-7387, NCEH-21, Ajeet-155, ANKR-3066, NCHB-9902 and ANKR-3324, respectively. Among all the hybrids, JKCH-99 recorded the highest Cry1Ac protein content and ANKR-3324 had the lowest Cry1Ac protein content. Among all the hybrids, the Cry protein level was the highest in JKCH-99 cultivar at 100 DAS (4.152 OD). The lowest Cry1Ac protein concentration was recorded in ANKR-3324 cultivar (2.757 OD) at 100 DAS. The Cry1Ac protein content in seed remained uncertain (increased in 6 BG-II cultivars and decreased in other 6 BG-II cultivars) from 75 to 100 DAS in twelve BG-II cotton hybrids.

Locule wall and seed

The Cry1Ac protein in locule wall and seed at 125 DAS in descending order was 3.938, 3.751, 3.748, 3.683, 3.682, 3.65, 3.559, 3.432, 3.420, 3.308, 3.188 and 3.006 OD in JKCH-2245, MRC-7387, NCSI-1904, NCEH-21, NCS-866, ANKR-3066, RCH-3863, Ajeet-155, ANKR-3324, NCHB-9902, JKCH-99 and MRC-7373, respectively. Among all the hybrids, JKCH-2245 recorded the highest Cry1Ac protein content and MRC-7373 had lowest Cry1Ac protein content.

Cry protein (Cry2Ab) content in BG-II cotton hybrids (optical density) (2018–2019 and 2019–2020 pooled)

Pooled results on Cry protein (Cry2Ab) content in different BG-II cotton hybrids for seasons 2018–2019 and 2019–2020 have been depicted in Table 3.

Flowers

Cry2Ab protein content was estimated in flowers at 40 and 75DAS. The Cry2Ab protein in flowers at 40 DAS in descending order was 2.364, 2.287, 2.216, 2.2, 2.165, 2.15, 2.143, 2.123, 2.105, 2.076, 2.057 and 2.022 OD in NCEH-21, MRC-7373, JKCH-2245, NCHB-9902, ANKR-3324,

Table 3 Cry protein (Cry2Ab) content in BG-II cotton hybrids (optical density) (2018–2019 and 2019–2020 pooled)

S. no.	Variety	Cry protein (Cry2Ab) content (optical density) in different plant parts at different phenological stages of BG II cotton hybrids									
		40DAS	75DAS				100DAS			125DAS	
		Flowers	Flowers	Rind	Locule	Seed	Rind	Locule	Seed	Rind	Locule wall and seed
1	Ajeet-155	2.057	2.438	2.080	2.128	3.145	2.691	2.691	3.241	3.645	3.341
2	JKCH-2245	2.216	2.197	2.341	2.204	2.399	2.452	2.452	2.900	3.889	3.306
3	RCH-3863	2.123	2.023	2.02	2.131	3.256	2.911	2.911	2.88	3.627	3.749
4	NCS-866	2.143	2.013	2.138	2.103	2.446	2.94	2.94	2.792	3.417	3.339
5	MRC-7373	2.287	2.11	2.444	2.535	2.439	2.368	2.368	2.941	3.634	3.694
6	JKCH-99	2.022	2.350	2.074	3.002	3.514	3.174	3.174	3.114	3.846	3.204
7	MRC-7387	2.105	2.094	2.322	2.947	2.544	2.975	2.975	3.327	3.565	3.414
8	NCEH-21	2.364	2.194	2.372	2.817	2.805	2.573	2.573	2.457	3.558	3.601
9	ANKR-3324	2.165	2.083	2.180	2.617	2.165	2.912	2.912	3.361	3.611	3.329
10	NCSI-1904	2.15	2.154	2.119	2.690	2.739	3.0885	3.088	3.204	3.874	3.583
11	NCHB-9902	2.2	2.49	2.284	2.828	2.947	2.746	2.746	3.417	3.676	3.214
12	ANKR-3066	2.076	2.159	2.694	2.720	2.923	3.943	3.943	3.253	3.788	3.592

**DAS* days after sowing

NCSI-1904, NCS-866, RCH-3863, MRC-7387, ANKR-3066, Ajeet-155 and JKCH-99, respectively. Among all the hybrids, NCEH-21 recorded the highest Cry2Ab protein content and had JKCH-99 the lowest Cry2Ab protein content. Cry2Ab protein in flowers at 75 DAS in descending order is 2.49, 2.438, 2.350, 2.197, 2.194, 2.159, 2.154, 2.11, 2.094, 2.083, 2.023 and 2.013 OD in NCHB-9902, Ajeet-155, JKCH-99, JKCH-2245, NCEH-21, ANKR-3066, NCSI-1904, MRC-7373, MRC-7387, ANKR-3324, RCH-3863 and NCS-866, respectively. Among all the hybrids, NCHB-9902 recorded the highest Cry2Ab protein content and NCS-866 had the lowest Cry2Ab protein content. There was no much difference in Cry2Ab expression levels at 40 and 75 DAS. Among all the hybrids, Cry2Ab protein level was the highest in NCHB-9902 cultivar at 75 DAS (2.49 OD). The lowest Cry2Ab protein concentration was recorded in NCS-866 (2.013 OD) at 75 DAS. The Cry2Ab protein content in flowers remained uncertain with advance in the age of the crop from 40 to 75 DAS in twelve BG-II cotton hybrids.

Rind

The Cry2Ab protein content was estimated in rind at 75, 100 and 125DAS. The Cry2Ab protein in rind at 75 DAS in descending order was 2.694, 2.444, 2.372, 2.341, 2.322, 2.284, 2.180, 2.138, 2.119, 2.080, 2.074 and 2.02 OD in ANKR-3066, MRC-7373, NCEH-21, JKCH-2245, MRC-7387, NCHB-9902, ANKR-3324, NCS-866, NCSI-1904, Ajeet-155, JKCH-99 and RCH-3863, respectively. Among all the hybrids, ANKR-3066 recorded the highest Cry2Ab protein content and RCH-3863 had the lowest Cry2Ab protein content. The Cry2Ab protein in rind at 100 DAS in descending order was 3.943, 3.174, 3.088, 2.975, 2.94, 2.912, 2.911, 2.746, 2.691, 2.573, 2.452 and 2.368 OD in ANKR-3066, JKCH-99, NCSI-1904, MRC-7387, NCS-866, ANKR-3324, RCH-3863, NCHB-9902, Ajeet-155, NCEH-21, JKCH-2245 and MRC-7373, respectively. Among all the hybrids, ANKR-3066 recorded the highest Cry2Ab protein content and MRC-7373 had the lowest Cry2Ab protein content. The Cry2Ab protein in rind at 125 DAS in descending order was 3.889, 3.874, 3.846, 3.788, 3.676, 3.645, 3.634, 3.627, 3.611, 3.565, 3.558 and 3.417 OD in JKCH-2245, NCSI-1904, JKCH-99, ANKR-3066, NCHB-9902, Ajeet-155, MRC-7373, RCH-3863, ANKR-3324, MRC-7387, NCEH-21 and NCS-866, respectively. Among all the hybrids, JKCH-2245 recorded the highest Cry2Ab protein content and NCS-866 had the lowest Cry2Ab protein content. The Cry2Ab levels in rind increased from 100 to 125 DAS in all twelve test BG-II cotton hybrids.

Among all the hybrids, the Cry protein level was highest in ANKR-3066 cultivar at 100 DAS (3.943 OD). The lowest Cry2Ab protein concentration was recorded in NCS-866 (2.138 OD) at 75 DAS. The Cry2Ab protein content in rind remained slight increase from 75 to 100 DAS and increased from 100 to 125 DAS in twelve BG-II cotton hybrids.

Locule

The Cry2Ab protein content was estimated in locule at 75 and 100DAS. The Cry2Ab protein in locule at

75 DAS in descending order was 3.002, 2.947, 2.828, 2.817, 2.720, 2.690, 2.617, 2.535, 2.204, 2.131, 2.128 and 2.103 OD in JKCH-99, MRC-7387, NCHB-9902, NCEH-21, ANKR-3066, NCSI-1904, ANKR-3324, MRC-7373, JKCH-2245, RCH-3863, Ajeet-155 and NCS-866, respectively. Among all the hybrids, JKCH-99 recorded highest Cry2Ab protein content and NCS-866 had lowest Cry2Ab protein content. The Cry2Ab protein in locule at 100 DAS in descending order was 3.943, 3.174, 3.008, 2.975, 2.94, 2.912, 2.911, 2.746, 2.691, 2.573, 2.452 and 2.368 OD in ANKR-3066, JKCH-99, NCSI-1904, MRC-7387, NCS-866, ANKR-3324, RCH-3863, NCHB-9902, Ajeet-155, NCEH-21, JKCH-2245 and MRC-7373, respectively. Among all the hybrids, ANKR-3066 recorded the highest Cry2Ab protein content and MRC-7373 had the lowest Cry2Ab protein content. Among all the hybrids, the Cry2Ab protein level was the highest in ANKR-3066 cultivar at 100 DAS (3.943 OD). The lowest Cry2Ab protein concentration was recorded in NCS-866 cultivar (2.103 OD) at 75 DAS. The Cry2Ab protein content in locule increased in from 75 to 100 DAS in seven BG-II cotton hybrids and decreased in five BG-II cotton hybrids from 75 to 100 DAS.

Seed

The Cry2Ab protein content was estimated in seed at 75 and 100DAS. The Cry2Ab protein in seed at 75 DAS in descending order was 3.514, 3.256, 3.145, 2.947, 2.923, 2.805, 2.739, 2.544, 2.446, 2.439, 2.399 and 2.165 (OD) in JKCH-99, RCH-3863, Ajeet-155, NCHB-9902, ANKR-3066, NCEH-21, NCSI-1904, MRC-7387, NCS-866, MRC-7373, JKCH-2245 and ANKR-3324, respectively. Among all the hybrids, JKCH-99 recorded the highest Cry2Ab protein content and ANKR-3324 had lowest Cry2Ab protein content. The Cry2Ab protein in seed at 100 DAS in descending order is 3.417, 3.361, 3.327, 3.253, 3.241, 3.204, 3.114, 2.941, 2.900, 2.88, 2.792 and 2.457 (OD) in NCHB-9902, ANKR-3324, MRC-7387, ANKR-3066, Ajeet-155, NCSI-1904, JKCH-99, MRC-7373, JKCH-2245, RCH-3863, NCS-866 and NCEH-21, respectively. Among all the hybrids, NCHB-9902 recorded the highest Cry2Ab protein content and NCEH-21 had the lowest Cry2Ab protein content. Among all the hybrids, the Cry protein level was the highest in JKCH-99 cultivar at 75 DAS (3.514 OD). The lowest Cry2Ab protein content was recorded in MRC-7387 cultivar (2.165 OD) at 75 DAS. The Cry2Ab protein content in seed remained uncertain (increased in 6 BG-II cultivars and decreased in 6 BG-II cultivars) from 75 to 100 DAS in twelve BG-II cotton hybrids.

Locule wall and seed

The Cry2Ab protein in locule wall and seed at 125 DAS in descending order was 3.749, 3.694, 3.601, 3.592, 3.583, 3.414, 3.341, 3.339, 3.329, 3.306, 3.214 and 3.204 (OD) in RCH-3863, MRC-7373, NCEH-21, ANKR-3066, NCSI-1904, MRC-7387, Ajeet-155, NCS-866, ANKR-3324, JKCH-2245, NCHB-9902 and JKCH-99, respectively. Among all the hybrids, RCH-3863 recorded the highest Cry2Ab protein content and JKCH-99 had the lowest Cry2Ab protein content.

Discussion

In the present study, Cry protein concentrations were the highest at 100 DAS, followed by 75,125 and 40 DAS. Sagar et al. (2011) also observed the highest concentration of Cry1Ac toxins in seeds as compared to locule, square and boll rind during initial phases of crop (100 DAS) and subsequent decline in concentration with the least content of Cry toxin at 160DAS. Through ELISA quantification was studied. Naik et al. (2013) reported that protein expression was significantly low in the square bract, square bud, seed, green boll and floral parts of all *Bt* hybrids as season progressed. Toxin expression was significantly high in all plant parts at 60 DAS, but gradually decreased to extremely low levels by 150 DAS in various transgenic *Bt* cotton hybrids. According to Mahalakshmi and Prasad (2013), the level of Cry1Ac in squares decreased at the start of the sampling period before increasing the level in locule higher than other plant parts (seed, rind, locule wall). The current findings are consistent with the findings of Mahon et al. (2004) who found a good evidence for variable expression of *Cry* genes not only for one gene but also for two genes in a pyramid case, as expression of Cry2Ab in Bollgard-II were the highest in squares, followed by leaves and then the entire plant. Suji et al. (2013) quantified the Cry1Ac toxin during different stages of the cotton crop and discovered a typical pattern of toxin expression with maximum expression at the beginning of the vegetative phase, a decrease in concentration at the beginning of the reproductive stage, a gain in the middle of the reproductive stage and a decrease at the end of crop. Ramanjali et al. (2015) discovered the highest level of Cry1Ac toxins at 75 days after sowing, followed by decreasing toxins at 60 and 90 DAS. However, Zaman et al. (2015) discovered an increasing trend in Cry1Ac concentration from 60 to 90 DAS, with a peak value at 90 DAS. Cry1Ac toxin showed a decreasing trend after 90 days, with the lowest value at 150 DAS. Cry protein content was significantly high in locules of all BG-II cotton hybrids. The current findings were consistent with Srinivasa and Arjuna (2008) who reported higher toxin content in locules, rind and

seeds of bolls than flower (anthers and pollen), square bracts and square buds. Cheema et al. (2015) reported that a variety of internal and external factors reduced transgene expression at both the transcriptional and translational levels. The same type of event was observed in all genotypes, with significant variation in toxin level, emphasizing the role of genetic background in transgene expression. The Cry toxin protein was abundant in early vegetative and mid reproductive stage of the crop and then decreased as the crop aged. The decline in Cryprotein expression in *Bt* cotton was caused by the parental background and a reduction in mRNA production levels, Apart from the reduction in Cry2Ab toxicity, the interference of condensed tannins with Cry1Ac toxicity was especially pronounced (Olsen and Daly 2000).Toxin expression was significantly high in all plant parts at 90 DAS and gradually declined to very low levels by 125 DAS in different hybrids of transgenic *Bt* cotton. Soujanya (2008) reported that the high toxin content at early to mid-reproductive stages of crop growth compared to later stages.

Conclusion

The highest levels of Cry1Ac and Cry2Ab expression were found in the locule of BG-II cotton plants, followed by the seeds, rind, locule wall and flowers.

Abbreviations

QL 96 ELISA	Qualitative 96 enzyme linked immuno sorbent assay
rpm	Revolutions per minute
TMB	Tetramethylbenzidine
DAS	Days after sowing

Acknowledgements

The first author expresses gratitude to Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, and Mahabeej, Akola, for their financial support, encouragement, and continuous assistance throughout the duration of the study. The first author is also indebted to the Jawaharlal Nehru Memorial Fund, New Delhi, for awarding a scholarship during the course of the study.

Author contributions

Current work is a crucial component of PL's Ph.D. research project. The experimental paradigm was collaboratively formulated and developed by DBU, USK, AVK and MPM. PL conducted field sampling, executed laboratory procedures, and interpreted the resulting data. Additionally, PL was responsible for drafting and editing the manuscript. All contributors thoroughly reviewed and approved the final version of the manuscript.

Funding

The present research work was supported and funded by Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India-444001.

Availability of data and materials

All results obtained during this research are reported in this article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 24 May 2023 Accepted: 21 September 2023
Published online: 29 September 2023

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