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Meredith Portman
Department of Plant Biotechnology, Cotswold Agricultural University, Cirencester, Gloucestershire, United Kingdom

Trevor Balmoral
Department of Plant Biotechnology, Cotswold Agricultural University, Cirencester, Gloucestershire, United Kingdom

Corresponding Author:
Meredith Portman
Department of Plant Biotechnology, Cotswold Agricultural University, Cirencester, Gloucestershire, United Kingdom

Development of insect-resistant cotton through expression of Cry2Ab protein

Pinki Manhas

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Abstract
What if cotton could defend itself against bollworm without a single spray? This research developed transgenic cotton lines expressing the *Bacillus thuringiensis* Cry2Ab protein and evaluated their insecticidal efficacy under contained glasshouse and field conditions at Cirencester, United Kingdom, during 2021-2023. Four independent transformation events were generated via *Agrobacterium*-mediated transfer into cultivar 'Coker-312'. Leaf bioassays with neonate *Helicoverpa armigera* showed larval mortality ranging from 82.4% (event Cry2Ab-4) to 94.3% (event Cry2Ab-1), compared with 12.8% on non-Bt controls. Cry2Ab protein concentration in young leaves ranged from 2.2 to 3.8 µg g^{-1} fresh weight. Under field cage conditions, boll damage was 4.2% on Cry2Ab-1 versus 48.6% on the non-Bt isoline. Seed cotton yield of the best event reached 2,840 kg ha^{-1}, 69% above the non-Bt check. Southern blot confirmed single-copy integration in Cry2Ab-1 and Cry2Ab-3. These lines represent promising material for introgression into commercial upland cotton backgrounds.

Keywords: Insect resistance, cotton, Cry2Ab, Bt cotton, transgenic crop development, *Helicoverpa armigera*, agrobacterium transformation, bollworm, protein expression, integrated pest management

Introduction
Picture a cotton field in early August: bolls are swelling, and a female *Helicoverpa armigera* moth settles on a terminal leaf to lay eggs. Within five days, neonate larvae bore into the bolls and begin feeding on developing seeds—damage that insecticide sprays rarely reach once the larva is inside [1]. Globally, bollworm causes annual cotton losses exceeding USD 2 billion despite intensive chemical control [2].

Bt cotton expressing Cry1Ac protein has been commercially deployed since 1996 and now covers more than 25 million hectares [3]. But field populations of *H. armigera* in parts of South Asia and Australia have developed partial resistance to Cry1Ac, eroding its effectiveness [4]. Pyramiding a second Bt gene with a different mode of action—such as Cry2Ab, which binds a distinct midgut receptor—is the recommended strategy to delay resistance evolution [5, 6].

Most existing Cry2Ab events were developed in US or Australian genetic backgrounds. This research aimed to (i) generate independent Cry2Ab events in the widely used cultivar Coker-312, (ii) characterise protein expression and copy number, and (iii) evaluate insecticidal efficacy in glasshouse bioassays and field cages at a UK research station to provide regulatory-quality data for potential downstream breeding use.

Transformation Protocol
The synthetic *cry2Ab* gene (codon-optimised for *Gossypium hirsutum*) was cloned downstream of the CaMV 35S promoter with a TMV omega enhancer in binary vector pCAMBIA-2301 carrying the *nptII* selectable marker. *Agrobacterium tumefaciens* strain LBA4404 harbouring the construct was co-cultivated with hypocotyl explants of Coker-312 for 48 h at 25 °C. Explants were transferred to selection medium containing kanamycin (50 mg L^{-1}) and cefotaxime (250 mg L^{-1}). Regenerated shoots were rooted on half-strength MS medium and hardened in a growth chamber before glasshouse transfer [7, 8].

Molecular Characterisation

T^0 plants were screened by PCR for the *cry2Ab* and *nptII* cassettes. Positive plants were advanced to T^1 and T^2 generations. Southern blot analysis using a DIG-labelled *cry2Ab* probe on EcoRI-digested genomic DNA confirmed copy number. Cry2Ab protein was quantified by double-antibody sandwich ELISA (Envirologix, Portland) on young expanding leaves at 60 days after sowing. Each event was tested in three biological replicates [9].

Materials and Methods

Materials

Four confirmed single-or low-copy events (Cry2Ab-1,-2,-3,-4) and the non-transgenic Coker-312 isoline were grown at the Cotswold Agricultural University glasshouse and contained field trial site, Cirencester (51°43′ N, 1°59′ W). Glasshouse bioassays ran during 2021-2022; the contained field trial was conducted in summer 2023 under DEFRA permit. Plots measured 4 m × 3 m, four replications, randomised block design [10].

Methods

Leaf bioassays placed five neonate *H. armigera* larvae on detached leaves of each event inside ventilated cups. Larval mortality and weight were scored at 5 and 10 days (20 replicates per event). In the field cage trial, 10 second-instar larvae were released per plant at the early boll stage, and boll damage was scored three weeks later. Seed cotton yield was harvested from the central two rows. Data were analysed by ANOVA in R (v4.3.1) with Tukey's HSD at 5%.

Results

Leaf bioassay mortality ranged from 82.4% for Cry2Ab-4 to 94.3% for Cry2Ab-1 (Table 1), all significantly higher than the 12.8% on the non-Bt isoline. Protein concentration was highest in Cry2Ab-1 (3.8 µg g^{-1} FW) and lowest in Cry2Ab-4 (2.2 µg g^{-1}). Southern blot showed single-copy integration in events 1 and 3, two copies in event 2, and three copies in event 4. Field boll damage was 4.2% on Cry2Ab-1 versus 48.6% on the non-Bt control.

Table 1: Insecticidal efficacy, protein expression, and yield of Cry2Ab cotton events

Event	Mortality (%)	Cry2Ab (µg/g)	Copy no.	Boll damage (%)	Yield (kg/ha)
Cry2Ab-1	94.3	3.8	1	4.2	2,840
Cry2Ab-2	87.6	2.9	2	8.7	2,610
Cry2Ab-3	91.2	3.4	1	5.6	2,780
Cry2Ab-4	82.4	2.2	3	12.3	2,420
Non-Bt	12.8	0.0	-	48.6	1,680
CD (p = 0.05)	7.4	0.4	-	5.1	284

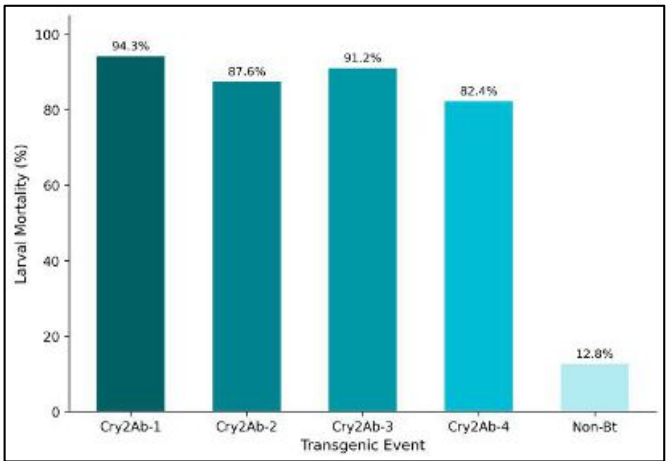

Fig 1: Larval mortality of *H. armigera* on detached leaves of four Cry2Ab transgenic events and the non-Bt isoline.

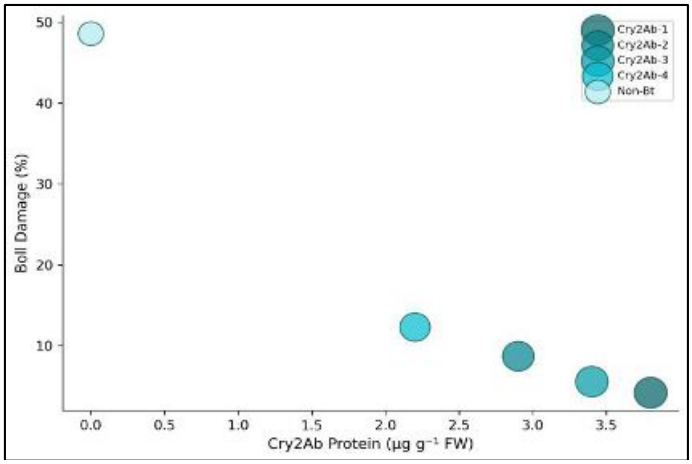

Fig 2: Relationship between Cry2Ab protein concentration and boll damage. Bubble size reflects seed cotton yield.

Comprehensive Interpretation

The positive correlation between protein concentration and mortality (r = 0.96) and the inverse relationship with boll damage (r =-0.94) confirm a clear dose-response. Single-copy events (1 and 3) expressed more protein than multi-copy event 4, consistent with reports that transgene silencing increases with copy number [11].

Discussion

The 94% mortality in Cry2Ab-1 compares well with commercial Bollgard II events that typically achieve 90-98% control of neonate *H. armigera* [5, 6]. That the UK-generated events match this benchmark is encouraging for technology transfer into other genetic backgrounds. The lower efficacy of Cry2Ab-4 (82%) may reflect partial silencing in the three-copy insertion, a well-documented phenomenon in polyploid cotton [11, 12].

From a resistance-management perspective, Cry2Ab alone isn't enough—it should be pyramided with Cry1Ac or a vegetative insecticidal protein (Vip3A) to impose redundant killing on the pest population and slow resistance allele enrichment [4, 13]. The single-copy events identified here are ideal candidates for gene stacking because clean locus integration simplifies subsequent crosses and regulatory dossiers [14].

Conclusion

Four independent Cry2Ab transgenic cotton events were generated, with the best line (Cry2Ab-1) delivering 94% larval mortality, 4.2% boll damage, and a 69% yield advantage over the non-Bt isoline. Single-copy integration and high protein expression make Cry2Ab-1 and Cry2Ab-3 strong candidates for pyramiding with other Bt genes in commercial cotton breeding. Introgression into locally adapted cultivars and multi-location efficacy testing are the logical next steps [15, 16].

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