Codon Optimised *cry1Ah* and *cry2Ah1* confer a High level of Protection against Cotton Bollworm in Tobacco: The Prospective Candidates for Gene Stacking Strategy – Review

Saleem Mohammed[1], Lateefat Hassan B.[1] and Maryam Mohammed[2]

[1]Department of Plant Science, Ahmadu Bello University, Zaria, Nigeria
[2]Department of Biological Sciences, Federal University, Dutse, Nigeria

SUNDAY 5TH – THURSDAY 9TH AUGUST 2018

Introduction

- Insect pests are a major concern to farmers around the world due to the serious economic damage they cause
- The efficacy of chemical measures is now limited by the evolution of resistance in many major pests
- Until recently the Bt transgenic crops engineered to express foreign lethal genes to targeted insects have been efficient in the control of insects
- An alarming level of Bt resistance has been recently observed in some major insects in both field and laboratory
- Discovery of novel Bt genes with different biding sites in the insects' midgut that are suitable for use in gene stacking strategy to delay resistance

Objectives

- Based on the analysis performed on global monitory data collected for over a decade, it reveals that the frequency of Bt resistance alleles has increased substantially in the field population of some major insects. Hence, this necessitates the discovery of novel Cry genes. Presently, only a few Cry genes are cloned and used commercially in Bt crops. This work therefore, aims to review recent literature to identify novel Cry genes;

  - based on plant codon usage bias
  - with different biding properties in the insect's midgut
  - to be used in the development of stacking traits in future Bt crops

Methodology

- Wild-type tobacco (NC89) was used as experimental material in the studies conducted by Shengyan et al., (2018) and Li et al., (2013) for cry2Ah1 and cry1Ah respectively

- The GC contents of the genes were altered to generate different alleles such as; 0%, 34% and 61% for cry2Ah1 and 25%, 50% and 100% for cry1Ah

- The expression vectors containing the alleles generated from both genes, CaMV35S promoter and *nptII* as plant selection marker gene were transformed into the plant using *Agrobacterium tumefaciens* strain EHA105. The infected leaf explants were obtained and selected on MS regeneration medium

- A month old transgenic tobacco plants grown in the greenhouse were used for quantitative RT-PCR analysis, ELISA for quantitative detection of the proteins in fresh leaf tissues and bioassay, where 12 neonate insect larvae were used to determine the level of damage

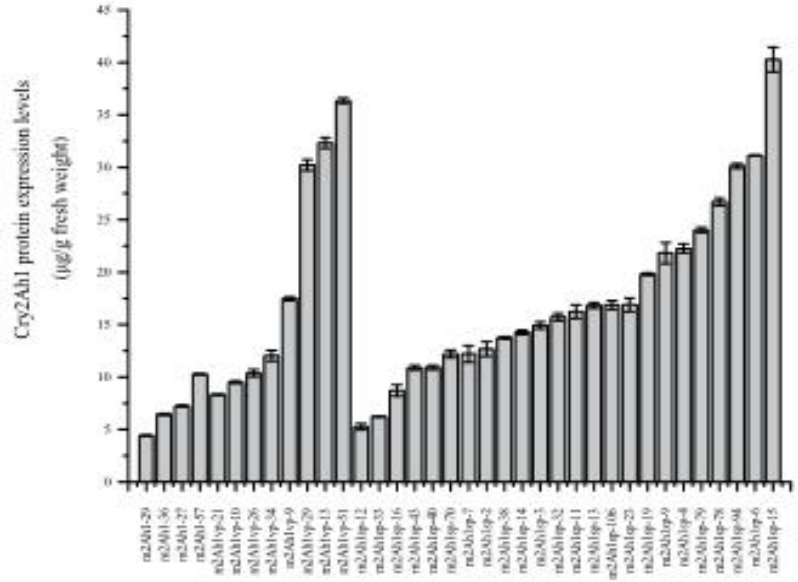
Results and Discussion

- The quantitative RT-PCR results demonstrated that the ctp-m3-cry1Ah plants showed the highest cry1Ah transcript level of the four different transgenic constructs, approximately 12.5 folds compared with the level in m1-cry1Ah plants while the mcry2Ah1 transcript levels of 36 transgenic lines which had a high Cry2Ah1 protein expression

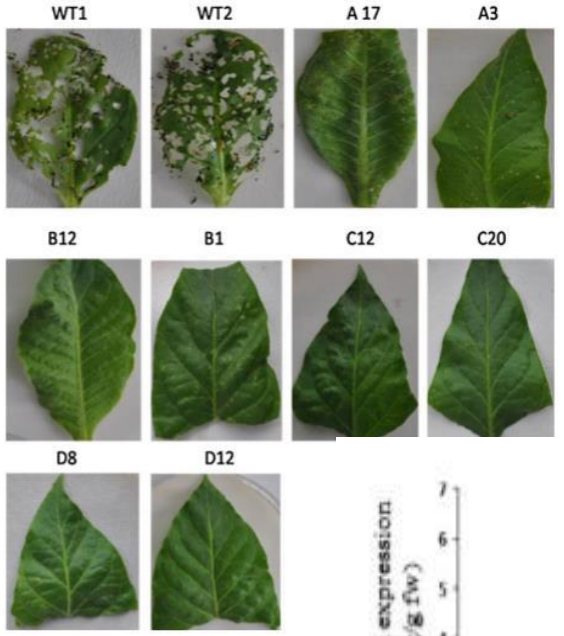
- Leaf damage was estimated through visual inspection after 3 days of infestation, and damage was classified using a severity scale of 1–4 according to the Technical Specification for Evaluating Resistance of Cotton to Disease and Insect Pests (Part 1: Cotton Bollworm; GB/T 22101.1-2008).

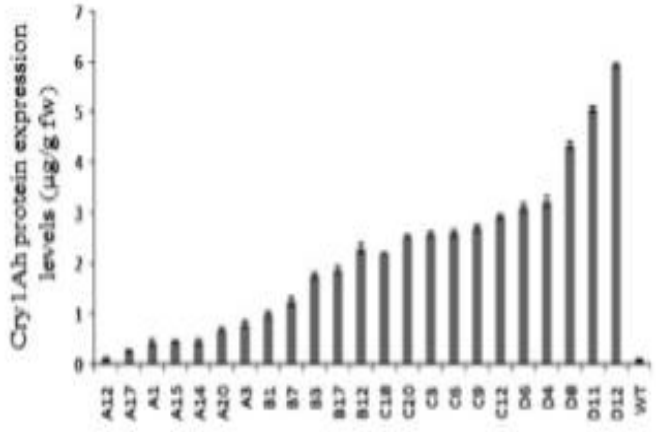
- The level of leaves damage caused by the insect larvae of the wild type appeared severe, compared to the transgenic lines, which appeared affected

Results and Discussion



The appearance of control and transgenic tobacco leaves after insect bioassays with susceptible and Cry1Acresistant cotton bollworm. *Credit: Shengyan et al., 2018*





The appearance of control (WT1 and WT2) and transgenic tobacco leaves after a larval feeding assay with H. armigera. Leaves A3 and A17 were from m1-cry1Ah plants, leaves B1 and B12 were from m2-cry1Ah plants, leaves C12 and C20 were from m3-cry1Ah plants, leaves D8 and D12 were from ctp-m3-cry1Ah plants. *Credit: Li et al., 2013*

Conclusions & Recommendations

- Based on the findings from these studies, it clearly showed that *cry2Ah1* and *cryAh1* were insecticidal genes that showed great efficiency in eliminating *Helicoverpa armigera*. Both genes showed high level of toxicity to the Lepidopteran larvae making them potentially useful for insect biocontrol and especially as candidates for 'pyramid' strategy, as evolution of resistance by pests has become the primary threat to the continued efficacy of Bt crops.

- More cry genes with different binding sites in the insect's midgut should be cloned to broaden the Bt insecticidal efficiency

References

Dively, G. P., Venugobal, P. D., Finkenbinder, C. (2016) Fiel-Evolved Resistance in Corn Earworm to Cry Proteins Expressed by Transgenic Sweet Corn. PLoS ONE 11(12): E0169115. https://doi.org/10.1371/journal.pone.069115pmid:2 8036388

Li, X., Shengyan, L., Zhihong, L., Jie, Z., Li, Z. and Dafang, H. (2013) Chloroplast-targeted expression of the codon-optimized truncated cry1Ah gene in transgenic tobacco confers a high level of protection against insects. Plant Cell Rep. 32, 1299–1308.

Roush, R. T. (1998) Two-toxin strategies for management of insecticidal transgenic crops: can pyramiding succeed where pesticide mixtures have not? Philos. Trans. R. Soc. B Biol. Sci. 353, 1777– 1786.

Shengyan, L., Zeyu W., Yiyao, Z., Changhui, L., Guiping, W., Hai,Wang., Jie, Z., Gemei, L. & Zhihong, L. (2018) Expression of cry2Ah1 and two domain II mutants in transgenic tobacco confers high resistance to susceptible and Cry1Ac-resistant cotton bollworm. Scientific Reports (2018) 8:508 DOI:10.1038/s41598-017-19064-5

Schwember, A. R. (2008) An update on genetically modified crops. Cienc Investig Agrar 35:231–250

Acknowledgements

- The reviewers acknowledge the amazing works of Li et al., 2013 and Shengyan et al., 2018), which provided the basis for this review.

THANK YOU FOR LISTENING