Identification and characterisation of cotton boll wall-specific promoters

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Corrections

Page 4, section 1.4.3, line 7: delete "are these".

Page 9, line 11: replace "resistance to the endotoxin" with "genetic resistance to anti-pest treatments".

Page 11, section 1.8, second paragraph, line 13: replace "Arial" with "Aerial".

Page 11, section 1.8, second paragraph, line 9: delete "of".

Page 14, line 3: replace "1997" with "1996".

Page 15, line 1: replace "pupae busting" with "the destruction of pupal burrows".

Page 22, paragraph 3, line 12: replace "While the commercialised variety was successful in preventing insect attack, the high level of transgenic protein in the pollen may have been harmful to the monarch butterfly (*Danaus plexippus*) and the variety was withdrawn from the market." with "While the commercialised variety was successful in preventing insect attack, the high level of transgenic protein in the pollen may have been harmful to the monarch butterfly (*Danaus plexippus*). The variety was withdrawn from the market due to these non-target effects as well as concerns about patent and performance issues."

Page 25, section 2.1.1: replace whole section with:

2.1.1: <u>Plant material</u>

All cotton material was isolated from plants grown from seed provided by either Cotton Seed Distributors (Narrabri, NSW) or the Australian Commonwealth Scientific and Industrial Research Organisation (CSIRO) Cotton Research Unit (Narrabri, NSW) as indicated below. Cotton species and varieties used were:

Cotton Seed Distributors:
G. hirsutum cultivar Siokra 1-4
G. hirsutum cultivar Sicot 189
G. hirsutum cultivar Sicot V-2

CSIRO:
G. raimondii
G. herbaceum variety *africanum*

Page 54, figure legend: replace whole legend with:

Figure 3.7: Northern analysis of boll wall-preferential mRNAs.
The abundance of the six mRNA transcripts in various tissues was assessed by Northern analysis. The tissues used were 0, 5, 10, 15, 20, 25, 30, 40 and 50 DPA boll wall, young leaf, 3 to 6 DPA fibre, white film from 5 to 10 DPA bolls, 0 to 5 DPA bract, 5 to 10 DPA calyx, 0 DPA petal and 0 DPA staminal column. Root, stem and cotyledon tissues were obtained from seedlings of approximately seven centimetres in height. 10µg of each RNA was electrophoresed, transferred to a membrane and hybridised with each of the cDNAs. The bottom panel shows a representative ethidium bromide stained RNA gel used to monitor RNA loading. Marker lane sizes are indicated in nucleotides.

Page 62, second paragraph, line 24: replace "ball" with "boll".

Page 72, second paragraph line 3: replace "The high abundance of SuSy mRNA in the boll wall corresponds with the timing of growth and secondary cell wall synthesis of the cotton fibres." with "The peak abundance of SuSy mRNA in the boll wall corresponds with the growth phase of the fibres, with lower abundances of RNA present during secondary cell wall synthesis."

Page 92, line 1: replace "none" with "non".

Page 106, line 4: replace "cotton fibres" with "cotton ovules and attached fibres".

Pages 165 – 177: italicise all occurrences of "*Bacillus thuringiensis*", "*Trifolium subterraneum*", "*Bt*", "*Viola hederacea*", "*cry1Ab*", "*Gossypium hirsutum*", "*Heliothis*", "*Vitis vinifera*", "*Cajanus cajan*", "*Oryza sativa*", "*Medicago sativa*", "*Citrus sinensis*", "*Arabidopsis thaliana*", "*CaPRP1*", "*Capsicum annuum*", "*Populus tremuloides*", "*Tetrastichus howardi*", "*Secale cereale*", "*Solanum tuberosum*", "*Manihot esculenta*", "*Nicotiana glauca*", "*Camellia sinensis*", "*Manduca* sexta", "*Spodoptera littoralis*", "*Helicoverpa armigera*" and "*Lumbricus terrestris*".

Page 167, References. Insert the following:

Clark, B. W., T. A. Phillips and J. R. Coats. 2005. Environmental fate and effects of *Bacillus thuringiensis* proteins from transgenic crops: a review. Journal of Agricultural and Food Chemistry 53: 4643-4653.

Edge, J. M., J. H. Benedict, J. P. Carroll and H. K. Reding. 2001. Contemporary issues. Bollgard cotton: An assessment of global economic, environmental, and social benefits. The Journal of Cotton Science 5: 121-136.

Page 167, line 30: replace "*gossypium*" with "*Gossypium*".

Page 173, References: Insert the following:

Perlak, F. J., R. L. Fuchs, D. A. Dean, S. L. McPherson and D. A. Fischhoff. 1991. Modification of the coding sequence enhances plant expression of insect control protein genes. Proceedings of the National Academy of Sciences of the United States of America 88: 3324-3328.

Declaration

This work contains no material that has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University library, being available for loan and photocopying.

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Damien Lightfoot

Acknowledgements

I would like to thank my principal supervisor Jeremy Timmis for giving me the opportunity to conduct my honours and PhD research in his lab. His guidance and help throughout the last five and a half years have been invaluable. I would also like to thank my co-supervisor Sharon Orford, particularly for her help with all of those complicated cotton methods!

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Abstract

The cotton boll contains the seeds of the plant to which long, white fibres are attached. The cotton industry takes advantage of these fibres to spin yarns for textile production. A major challenge facing the cotton industry is that of crop loss to insect attack. The primary insect pests of cotton preferentially attack the boll, causing damage to the commercially important fibres. The recent introduction of *Bt*-transgenic varieties, containing genes with anti-pest properties from the soil bacterium *Bacillus thuringiensis*, has had positive impacts on pest control and pesticide usage. These transgenes are under control of constitutive promoters, resulting in endotoxin expression in all parts of the plant. This constant high level transgene expression may have several detrimental effects, such as placing strong selective pressure on pest populations to develop resistance, non-target effects of the transgene on other organisms, a yield penalty to the plant, and the presence of transgenic protein in secondary commercial products. For these reasons, this project aims to identify promoters that could be used for tissue-specific expression of anti-pest molecules in only the boll wall of the plant. A differential screening approach was used to identify several boll wall-specific mRNAs and the temporal and spatial abundance of these transcripts was determined using Northern analysis. The promoters corresponding to these transcripts were identified using Genome Walker® PCR and isolated from genomic DNA by PCR. Transient transformation of various cotton tissues with these promoters driving reporter expression resulted in predominant boll wall expression. The cotton promoters identified here provide an alternative tool to constitutive promoters for use in future transgenic varieties.