FINAL REPORT
TEMPLATE

CRDC ID: UNE1605

Project Title: PhD “Characterisation of brassinosteroid effects and brassinosteroid - responsive genes in cotton for growth and stress tolerance enhancement"

Confidential or for public release? Choose an item.

Part 1 – Contact Details & Submission Checklist

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Submission checklist.

Please ensure all documentation has been completed and included with this final report:

☐ Final report template (this document)

☐ Final Technical Report (see Part 3)

☐ Final Schedule 2: IP register

☐ Final financial report

☐ PDF of all journal articles (for CRDC's records)

Signature of Research Provider Representative: ________________________

Date submitted: ____________

Part 2 - Monitoring & Evaluation

This data forms part of CRDC's M&E data collection. Please complete all fields and add additional rows into each table if required.

Achievement against milestones in the Full Research Proposal

Milestone	Achieved/ Partially Achieved/ Not Achieved	Explanation
To study the effects of exogenous BR on cotton plant growth and tolerance to water, salt and pathogen stresses	Achieved	The exogenous application of EBR at low concentrations of 0.1 and 0.2 µM had no positive effect on seedling growth under 100 mM salt. In addition, EBR at a higher concentration (0.5 µM) or with the surfactant Tween 20 caused toxic effects under salt stress. Similarly, there were no positive effect of exogenous application of EBR at 0.2 μ M on seedlings growth in response to *Verticillium dahliae*. No positive effect of exogenous EBR at 1 μ M and 2 μ M on seedlings growth after withholding water for 8 days followed by re-watering for five days as compared to the control.
To characterise in detail CBP60 related genes in cotton for gene structure, phylogenetic relationships, their transcriptional responses to exogenous BR, drought, salinity and pathogen challenge, as well as their tissue/organ-specific expression. -The transcriptional responses of *GhCBP60s* to salt.	Achieved -Achieved	Bioinformatics approaches revealed the presence of GhCBP60 orthologues of AtCBP60. Phylogenetic analysis indicated that CBP60a, CBP60g and CBP60h from Arabidopsis each have four co-orthologues in cotton. AtCBP60f has two co-orthologues, whereas CBP60b/c/d have nine co-orthologues. Multiple amino acid sequence alignments indicate that the DNA-binding and CaM-binding domains of AtCBP60 are highly conserved in GhCBP60, suggesting similar protein structures to AtCBP60. Prediction of subcellular localisation suggested that all GhCBP60 proteins contain a nuclear localisation signal. This, together with the highly conserved putative DNA binding region, suggests that all GhCBP60 are transcription factors. The overall findings from the microarray meta-analysis suggested that the following genes were most likely to be involved in a stress response: *GhCBP60a-12D*, *GhCBP60g-8A*, *GhCBP60f-8A* and *GhCBP60f-8D*. - The results of qRT-PCR demonstrated that EBR treatment of cotton up-regulated the expression of *GhCBP60a/f/g*. On the other hand, salt down-regulated the expression of *GhCBP60a* but up-regulated the expression of *GhCBP60f/g* genes. Interestingly, treatment with EBR in the absence of salt restored the

- The transcriptional responses of *GhCBP60s* to drought and pathogen.	-Not achieved	expression of *GhCBP60a* to levels similar to the control tissue. - Because there is no positive effect of the exogenous application of EBR on seedling growth and development are obtained
To identify genes and pathways most affected by BR under conditions where positive effects of BR are obtained	-not Achieved	-Because there is no positive effect of EBR on seedlings growth were observed under all stresses.
To test functional equivalence of a cotton CBP60 gene in Arabidopsis through expression in Arabidopsis knockout mutants.	Not achieved	-There isn't enough time to complete this work.

Outputs produced *(Please refer to examples document to assist in completing this section).*

Output	Description
Presentation	*Presentation of PhD research findings at the University of Queensland*
Scholarships, travel, and learning programs	*I attended the conference "Tropical Agriculture 2015 "that was hosted in Brisbane Convention & Exhibition Centre, Brisbane, Australia*

Outcomes from project outputs *(Refer to examples document).*

Outcome	Description
Publications	*Two Research articles will be submitted for two publications*
Adoption for research findings by industry and government	*The findings of this research will be useful for the Australian cotton industry* and the respective stakeholders.

Part 3 – Technical Report

Projects may require different approaches to the structure of the Technical report. A detailed technical report should normally include the following items:

- *Table of contents (if necessary – depends on the length and complexity of your report);*
- *Executive summary*
- *Introduction*
- *Materials and methods*

- *Results*
- *Discussion*
- *Conclusions*
- *Key word index and*
- *A full list of industry and scientific publications, presentations, extension activities and other outputs.*

Please contact your R&D manager if you would like to adopt a different approach.

Part 4 – Summary for public release

This summary will be published on Inside Cotton, CRDC's digital repository, along with the full final report (if suitable for public release). It is designed to provide a short overview of the project for all interested parties. Please complete all fields, ensuring that this exceeds no more than two pages.

Project title:	**Type Title here**	
Project details:	CRDC project ID:	1605
	CRDC goal:	*2. Improve cotton farming sustainability and value chain competitiveness*
	CRDC key focus area:	*1.3 Protection from biotic threats and environmental stresses*
	Principal researcher:	*Anahid Al-Amery* PhD candidate *School of Environmental and Rural Sciences*
	Organisation:	University of New England
	Start date:	*01/09/2015*
	End date:	*22/04/2020*
Objectives	*1)* to study the effects of exogenous BR on cotton growth and tolerance to water, salt and pathogen stresses; 2) to characterise in detail CBP60-related genes in cotton for gene structure, phylogenetic relationships, their transcriptional responses to exogenous BR, drought, salinity and pathogen challenge, as well as their tissue/organ-specific expression;	
Background	Brassinosteroids (BRs) are plant steroidal hormones that play a versatile role in modulating plant growth and development. They are also known for their involvement in mediating tolerance to abiotic and biotic stresses. Studies on the manipulation of genes involve in BR biosynthesis or signalling reveal the essential role of BRs on plant development (Bishop and Yokota, 2001). Loss-of-function mutation of these genes usually leads to multiple developmental defects, male sterility, altering stomatal distribution, delay flowering and dwarfism (Clouse, 2011). Studies of the BR signaling pathway and BR gene-regulation indicate that BR act independently or dependently through interaction with other plants hormones to mediate stress response in plants. A group of calmodulin binding proteins, known as CBP60s also involved in mediating the response of plants to stress. Many CBP60s have been identified at the whole genome level in several plant species including Arabidopsis, rice and maize (Subbaiah et al., 1994a, 1994b, 1996, Boonburapong and Buaboocha, 2007). Taken together these observations suggest that BR and CBP60s genes can be used as strong targets for increasing plant growth and development under various stimuli. This will provide valuable information for understanding whether there is a possible connection between BR signalling and CBP60 transcription factors in mediating abiotic stress response in cotton.	

Research activities	Brassinosteroids (BRs) are plant steroid hormones that not only play vital roles in plant growth and development but also in mediating stress response. A group of calmodulin binding proteins, known as CBP60s are also involved in mediating the response of plants to stress. The aims of the present study were: (1) to investigate the effect of exogenous 24-epibrassinolide (EBR) on the phenotype of cotton *Gossypium. hirsutum* seedlings under mild to moderate biotic and abiotic stresses, (2) to find and characterise cotton CBP60-encoding genes, orthologous to Arabidopsis CBP60s with known involvement in stress responses, and to investigate whether EBR may act by modulating expression of GhCBP60 genes in cotton leaf tissues under salt stress. Experiments were designed to demonstrate the effects of EBR application from 0.1 to 2 µM on the phenotypic responses of cotton seedlings to mild to moderate salt, drought and pathogen (*Verticillium dahlia*) stresses. Results show that the exogenous application of EBR at low concentrations of 0.1 and 0.2 µM had no positive effect on seedling growth under all stresses. In addition, EBR at a higher concentration (0.5 µM) or with the surfactant Tween 20 caused toxic effects. Bioinformatics approaches revealed the presence of GhCBP60 orthologues of AtCBP60. Phylogenetic analysis indicated that CBP60a, CBP60g and CBP60h from Arabidopsis each have four co-orthologues in cotton. AtCBP60f has two co-orthologues, whereas CBP60b/c/d have nine co-orthologues. Multiple amino acid sequence alignments indicate that the DNA-binding and CaM-binding domains of AtCBP60 are highly conserved in GhCBP60, suggesting similar protein structures to AtCBP60. Prediction of subcellular localisation suggested that all GhCBP60 proteins contain a nuclear localisation signal. This, together with the highly conserved putative DNA binding region, suggests that all GhCBP60 are transcription factors. The results of qRT-PCR demonstrated that EBR treatment of cotton up-regulated the expression of *GhCBP60a/f/g*. On the other hand, salt down-regulated the expression of *GhCBP60a* but up-regulated the expression of GhCBP60f/g. Interestingly, treatment with EBR in the absence of salt restored the expression of GhCBP60a to levels similar to the control tissue. Analysis of promoters of *GhCBP60* genes for putative BR-related transcription factor binding motifs indicated the presence of CANNTG and GGTCC elements. However, these were not significantly enriched in stress-regulated genes. Furthermore, higher stringency BR-signalling-related elements: BRRE (CGTGTG/CGTGCG), G-box (CACGTG) and transcription factors TGA 1/TGA 4 (TGACG) sense strand were absent in stress-responsive genes GhCBP60a/f/g/h as compared to other groups. In the light of these results, we concluded that BR positively regulates the expression of novel *GhCBP60* genes suggesting a possible connection between BR signalling and *GhCBP60* transcription factors in mediating abiotic stress responses in cotton. However, the results from the cis-element search suggest that this connection is likely to be indirect rather than via a direct interaction with the BR signal transduction pathway.
Outputs	*- The exogenous application of EBR on seedling growth under salt, drought and drought stresses showed no positive effect* *- Bioinformatics approaches revealed the presence of GhCBP60 orthologues of AtCBP60 with highly conserved CaM and DNA binding regulatory domains that contain nuclear localisation signal suggesting similar function to AtCBP60 in other plants.*

	- EBR treatment up- regulates the expression of novel *GhCBP60a/f/g* genes. Similarly, salt up regulates the expression of *GhCBP60f/g* but down regulate the expression of *GhCBP60a* in the absence of EBR. Interestingly, treatment with EBR in the absence of salt restored the expression of GhCBP60a to similar level with the control tissue. However, the analysis of promoters of *GhCBP60* genes for putative BR-related transcription suggest that there is indirect link between the *GhCBP60* and BR-regulated genes.
Impacts	-The discovery of *GhCBP60a/f/g* can be used as a molecular tool for plant breeding and subsequently will produce breakthroughs in understanding the stress signalling mechanisms and adaptation in cotton. -The new insights obtained will be considered as a foundation for future studies to illustrate the mechanism of GhCBP60 proteins and their relation to BR signal transduction pathways in cotton.
Key publications	-The following two chapters will be published in two journals. 1-The Structure, Phylogeny and Prediction of Subcellular Localisation of Calmodulin-binding Protein 60 (CBP60) in Cotton *G hirsutum*. Expression profiling of *GhCBP60* in cotton seedlings treated with brassinosteroids and salt, and analysis of cis-regulatory elements.

References

Clouse, S. D. (2011). Brassinosteroid signal transduction: from receptor kinase activation to transcriptional networks regulating plant development. *Plant Cell* 23(4): 1219-1230.

Subbaiah, C. C., Bush, D. S., & Sachs, M. M. (1994a). Elevation of cytosolic calcium precedes anoxic gene expression in maize suspension-cultured cells. *The Plant Cell*, 6(12), 1747-1762.

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Boonburapong, B., & Buaboocha, T. (2007). Genome-wide identification and analyses of the rice calmodulin and related potential calcium sensor proteins. *BMC Plant Biology*, 7(1), 4.

Bishop, G.J., and Yokota, T. (2001). Plants steroid hormones, brassinosteroids: Current highlights of molecular aspects on their synthesis/metabolism, transport, perception and response. *Plant Cell Physiol.* 42, 114–120.