BARLEY CELLULOSE SYNTHASES INVOLVED IN SECONDARY CELL WALL FORMATION AND STEM STRENGTH: GENERATION OF cDNA CONSTRUCTS FOR FUNCTIONAL ANALYSIS

by

Partha Varanashi

A thesis submitted for the partial fulfillment of the requirements of the Masters of Biotechnology (Plant Biotechnology)

The University of Adelaide

Faculty of Sciences

School of Agriculture, Food and Wine

Waite Campus 2008

DECLARATION

I declare that this thesis is a record of original work and contains no material which has been accepted for the award of any other degree or diploma in any university. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text.

Partha Varanashi

Associate Professor Maria Hrmova

PREFACE

This research was performed over 10 months as part of a Masters in Biotechnology (Plant Biotechnology). The literature review was previously assessed in accordance with the correction suggested by the examiners. The main focus of the project remains very similar to that of the research proposal. However the goals were not achieved according to the time deadline stated in the research proposal. Hence protein purification was could not be carried out.

Although the research manuscript contained herein will provide the first draft of a future publication to be submitted to Plant journal, due to time constraint, all data relevant to that publication has not been collected. However, additional data which was not conclusive was collected and this is provided within the appendices. The research manuscript outlines stages involved in the construction and heterologus expression of barley CesA4 cDNA. While the appendices contain additional data from HvCesA4 protein structure prediction, media recipes, in-silico representation of the HvCesA4 constructs with respective vectors.

Title: BARLEY CELLULOSE SYNTHASES INVOLVED IN SECONDARY CELL WALL FORMATION AND STEM STRENGTH: GENERATION OF cDNA CONSTRUCTS FOR FUNCTIONAL ANALYSIS

Author:

Partha Varanashi

The University of Adelaide

Plant Genomics Centre

Glen Osmond 5064, Australia

Email partha.varanashi@student.adelaide.edu.au

Corresponding author:

Dr Maria Hrmova

Associate Professor

The University of Adelaide

Plant Genomics Centre

Glen Osmond 5064, Australia

T: +61 8 8303 7280

F: +61 8 8303 7102

Email: maria.hrmova@adelaide.edu.au

Table of Contents

PART I- LITERATURE REVIEW & RESEARCH PLAN

Introduction	
Literature Review	
Introduction	
Cellulose	8
Cellulose Biosynthesis	9
Cellulose synthase gene family	10
Conclusions	12
The Research Plan Overview	
Bioinformatics	13
Design of HvCesA4 constructs	14
Primer design	16
Cloning into pPICZ and pPICZα vectors:	17
Transformation into Pichia pastoris	18
Pichia pastoris expression system	18
Time Table	19
Conclusions	20
Appendix	21
References	
Acknowledgements	
SummaryIntroductionIntroduction	
Results	
Prediction of membrane helices and catalytic module of HvCesA4	27
Cloning into Entry Vector Systems	28
Cloning into destination vector system	29
Protein expression	29
Discussion	30
Experimental Procedures	
Bioinformatics and predictions of membrane helices	33
Molecular cloning and primers for HvCesA4 constructs	34
DNA sequencing	37

Transformation into Pichia pastoris	38
Protein expression	
Protein extraction, dot blot, Western blot and SDS-PAGE analyses	39
Acknowledgements	39
References	
Figures Legends	
Supplementary data	
Appendices	