‘Analysis of Key Glycosyltransferase (GT) Families in Barley’

by

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

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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.

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28 November 2008

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28 November 2008

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28 November 2008

PREFACE

This research was performed over 10 months as part of a Masters in Biotechnology (Plant Biotechnology). This thesis was previously assessed and updated in accordance with the corrections suggested by the examiners. The main focus of the research is essentially the same as proposed initially in the literature review, although slight modifications have been made in the methodology and the focus of the study has been further narrowed. Only the *E. coli* expression system was used to express the proteins instead of both *Pichia pastoris* and E.*coli*. Also, one glycosylsyltransferase (GT) family, GT43 and one clade of GT47 family were characterized instead of three. Thus, the title in the manuscript is narrower as opposed to the title of the thesis.

Although the research manuscript contained herein will provide the first draft of a future publication to be submitted to Plant Physiology, due to time constraints, all the data required for the publication has not been finalised. Further experiments are needed to verify and obtain more comprehensive data for the study. However, data which was collected but is not included in the manuscript due to space constraints is provided within the appendices such as the transcript profiling of glycosyltransferase family GT61 genes. Protein expression of genes in this family could not be achieved as amplification of the GT61 cDNA was not successful.

The research manuscript begins by outlining the significance of heteroxylans in plant cell wall biology and in the utilization of cereals and grasses, and pointed to glycosyltransferases that had been implicated in their synthesis. The results of the study consisted of phylogenetic and bioinformatic analysis of barley GT43 family and one clade of barley GT47 family, transcript profiles of the GT43 and GT47 genes in a series of barley tissues, heterologous expression and the purification of two proteins of interest, and finally an assay of the purified proteins. In addition, the appendices contain data collected for family GT61, nucleotide and protein sequences for all the GT genes studied, buffer list, Plant Physiology's "instructions for authors" acknowledgements, and a dedication.

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