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Molecular Analysis of Genes
of White Clover
(*Trifolium repens* L.)

A thesis presented in partial fulfilment of
the requirements for the degree of
Doctor of Philosophy in Biotechnology
at Massey University, Palmerston North,
New Zealand

Nicholas Ellison

1990

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Dedicated to
Kay, Jessica, Anna and Catherine

Abstract

The expression of the genes for the small subunit (SSU) of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), alcohol dehydrogenase (Adh) and lectin in the white clover plant was investigated by Northern analysis, using heterologous plant probes. SSU was shown to be expressed in the leaves/stems, Adh in the roots and lectin in both the leaves/stems and the roots of the mature plant.

A series of independent, white clover, leaf/stem and root cDNA libraries was constructed in the lambda vector λ gt10 from polyadenylated messenger RNA isolated from mature plants. A number of SSU and Adh cDNA clones was isolated from these libraries and the inserts from these clones were characterized by restriction enzyme mapping and DNA sequence analysis. These clones included a partial SSU cDNA clone from a leaf/stem library and a full length Adh clone from a root library. Two uncharacterized lectin cDNA clones were also isolated from each of the leaf/stem and root cDNA libraries.

A fully-representative genomic library was constructed in the lambda vector λ EMBL3 from total white clover DNA. This library was screened with the previously isolated white clover SSU and Adh cDNA clones. One SSU and three Adh genomic clones were isolated and the inserts from these clones were characterized by restriction enzyme mapping and Southern blotting. Restriction fragments, to which the corresponding cDNA probe hybridized, were subcloned and characterized by additional restriction enzyme mapping and DNA sequence analysis.

The one SSU genomic clone represented a functional white clover *rbcS* gene, corresponding to the white clover SSU cDNA clone, and was complete with 5' and 3' non-transcribed regions. Conserved sequences were identified in this gene that have been implicated in the regulation of plant gene expression in general and the regulation of *rbcS* genes in

particular. The three *Adh* genomic clones represented different, non-functional, white clover *Adh* pseudogenes, each with regions of strong homology to only limited regions of the white clover *Adh* cDNA clone.

List of Publications

Data from this work have been published in part in the following papers:

Ellison, N.W., Yu, P.L., and White, D.W.R. (1990a).
Nucleotide sequence of a white clover ribulose
biphosphate carboxylase small subunit gene. Nucl. Acids
Res. 18, 4914.

Ellison, N.W., Yu, P.L., and White, D.W.R. (1990b).
Nucleotide sequence of a white clover alcohol
dehydrogenase cDNA. Nucl. Acids Res. 18, 4913.

Acknowledgements

I wish to thank my supervisors, Dr P.L. Yu and Dr D.W.R. White, for their continued support throughout the duration of this study.

I am indebted to Dr W.D. Sutton for my introduction to plant molecular biology and to Dr J.P. Kerr, Director, Plant Physiology Division, and Mr J.A. Lancashire, Director, Grasslands Division, DSIR, for allowing that brief introduction to develop.

Thanks are also due to my fellow Molecular Biologists in the Palmerston North research community, and in particular to Dr G. Limsowtin, Dairy Research Institute, and Dr B. Mansfield, Massey University, for their helpful suggestions and technical advice on many aspects of molecular biology throughout the course of this study.

The assistance of Mr P. Spring (DSIR) with the photography, Mr H. Coenders (DSIR) with the growth of white clover plants in the glasshouse, and Mr C Tunnicliffe (DSIR) with keeping various items of equipment going, is gratefully acknowledged.

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Abbreviations

The following abbreviations have been used in this thesis:

A	absorbance
Adh	alcohol dehydrogenase
ASCII	American Standard Code for Information Interchange
bp	base pair
BSA	bovine serum albumin
cDNA	complementary DNA
ddNTP	dideoxynucleoside triphosphate
dNTP	deoxynucleoside triphosphate
DTT	dithiothreitol
kb	kilobases
LB	Luria-Bertani medium
mw	molecular weight
PEG	polyethylene glycol
pfu	plaque forming units
rpm	revolutions per minute
Rubisco	ribulose-1,5-bisphosphate carboxylase/oxygenase
SSU	small subunit of Rubisco
U	units

Abbreviations not included in this list are either defined where appropriate in the text or are in accordance with "Instructions to Authors", *Biochem. J.* 241, 1-24, (1987).