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Studies on Abscission Cell Differentiation in
Sambucus nigra and *Phaseolus vulgaris*

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Abstract

This thesis examines aspects of abscission cell differentiation in *Sambucus nigra* and *Phaseolus vulgaris*. The experimentation is divided into two sections; an *in vivo* study examining the cell wall proteins from the leaf rachis abscission zones of *S. nigra*, to identify proteins that denote the abscission zone as a fully differentiated cell type, and an *in vitro* study examining aspects of secondary or adventitious abscission zone formation in petiole explants of *P. vulgaris*.

As an initial approach to identify abscission cell-specific proteins, a survey of the total cell wall bound proteins in four tissues, leaf mid-rachis (MR), ethylene-treated leaf mid-rachis (MRE), 0 h, or freshly excised leaf rachis abscission zone (OZ) and ethylene-treated abscission zone (ZONE) was undertaken. The study also involved surveying these tissues over the vegetative seasons (spring, summer, autumn). Separation of these protein extracts using SDS-PAGE revealed proteins that were putatively uniquely expressed in each of the tissues. Moreover, the expression of some proteins changed from spring through to autumn. Further fractionation of the extracts using hydrophobic interaction chromatography (HIC), and separation of the fractions using SDS-PAGE, illustrated there were many more proteins that had not been resolved in the initial survey of wall extracts.

In total, four proteins of ca. 10, 28, 38 and 43 kDa were identified in the OZ tissue only and six proteins (ca. 10, 34, 36, 40, 74 and 75 kDa) were detectable in the OZ and ZONE tissues. Three of the putative OZ-specific proteins (designated OZ10, OZ28 and OZ43) were trypsin-digested and some initial amino acid sequence data obtained. The OZ10 tryptic fragment had closest identity to a lipid transfer protein (LTP) from spinach, and the OZ43 fragment had closest identity to an aldose-1-epimerase-like protein expressed in tobacco. Two peptides were sequenced from the OZ28 protein; one had highest identity to a superoxide dismutase and the second had identity to a ribonuclease. Two of these, OZ10 and OZ43 were characterised further. Antibodies raised to LTPs protein from *Daucus carota* and *Arabidopsis thaliana* recognized a protein of 10 kDa that was expressed in both the rachis and abscission zone tissues of *S. nigra* before and after ethylene treatment. Moreover, the LTP antibodies detected a ca. 10 kDa protein in freshly excised and ethylene-treated distal pulvinus, primary

abscission zone and petiole tissues of *P. vulgaris* with highest expression in ethylene-treated petiole tissue. The second protein, to be characterised further was most similar in sequence to a nuclear pore membrane protein identified in tobacco suspension cells and designated gp40. This protein appears to be an aldose-1-epimerase-like enzyme (otherwise known as mutarotase) from its homology to bacterial forms of mutarotase. An antibody to gp40 recognized a ca. 43kDa protein in the non-ethylene treated rachis and zone cell wall extracts of *S. nigra*, the putative OZ43. This same antibody did not recognize any proteins in the protein extract from porcine and lamb kidney, tissues that have mutarotase activity. A coupled enzyme assay was developed to measure the mutarotase activity in the plant samples. Although mutarotase activity was measured in both the soluble and cell wall bound fractions of the rachis cells, purification of the OZ43 protein using column chromatography or through cell fractionation revealed that the ca. 43 kDa protein recognised by the gp40 antibody did not appear to be responsible for this activity.

For the second part of this thesis, the *in vitro* study, the aim was to measure the levels of IAA, ethylene and ACC oxidase enzyme activity in bean petioles explants during IAA-induced secondary abscission zone formation. In the bean explant system, the secondary zone forms at a site along the petiole which is removed from the primary zone and governed by the concentration of IAA added. The petiole tissue that links the primary zone with the secondary zone (the distal segment) remains green and, in this thesis, is designated as G1. The petiole tissue proximal to the zone senesces and yellows, and is divided into Y2 (immediately proximal to the secondary zone), Y3 (mid way) and Y4 (the most proximal petiole tissue).

To measure changes in IAA concentration during secondary zone formation, an immunoassay (ELISA) was developed, initially using polyclonal antibodies to IAA, but the titre of these antibodies was not sufficient and so monoclonal antibodies were used. During secondary zone formation, the concentration of free IAA in the petiole tissue changed dramatically, with measurements ranging between 6 and 2608 pmol/g fresh weight (FW) of tissue. The IAA concentration in the petioles at separation at the primary zone before IAA was added was lower in the G1 and Y2 sections (ca. 30 pmol/g FW) when compared with the Y3 and Y4 sections (166 and 271 pmol/g FW respectively).

At 6 h after the application of IAA, the concentration of IAA had increased to 146 pmol/g FW in the G1 section, remained the same in the Y2 section and increased to 208 and 423 pmol/g FW in the Y3 and Y4 sections, respectively. At 26 h after the application of IAA, and approximately the time of initiation of differentiation of the secondary zone, the IAA concentration was similar to the petioles after 6 h (179 and 21 pmol/g FW for G1 and Y2 respectively) and significantly lower in the Y3 and Y4 sections (35 and 69 pmol/g FW respectively). At the first point at which the green:yellow tissue can be ascertained (at 52 h) the IAA concentration was dramatically higher in the G1 and Y2 tissues (1125 and 1090 pmol/g FW respectively) compared to measurements in the Y3 and Y4 sections at 52 h of 17 and 107 pmol/g FW respectively. At separation at the secondary abscission zone, the IAA measurements in the G1, Y2 and Y4 sections were 405, 315 and 1198 pmol/g FW respectively.

The ethylene produced from freshly excised pulvinus and petiole tissue was ca. 0.20 nmol/h/g FW and increased to 1.7 nmol/h/g FW in the pulvinus, 0.39 nmol/h/g FW in the G1 petiole section and 0.67 nmol/h/g FW in the Y2/Y3/Y4 pooled petiole sections at separation at the primary zone. At separation of the secondary zone, ethylene evolution measurements of 1.73 nmol/h/g FW in G1 and 4.37 nmol/h/g FW in the Y2/Y3/Y4 tissue were observed. However, the activity and expression of ACC oxidase was higher in the fresh tissues and non-senescent petiole region (G1), but was lowest in the senescent (Y2, Y3 and Y4) tissue at the formation of the secondary zone.

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Table of Contents

Abstract.....	ii
Acknowledgements.....	v
List of Figures.....	xiii
List of Tables.....	xx
1. Introduction.....	1
1.1 Abscission zone cells: A model for studying plant cell differentiation.....	1
1.2 Overview of the abscission process.....	1
1.2.1 Cell-cell separation during plant growth and development.....	2
1.2.2 Differentiation and cell-cell separation of abscission zone cells.....	3
1.3 The plant cell wall.....	5
1.3.1 The structural proteins of plant cell walls.....	6
1.3.2 Defence-related proteins in plant cell walls.....	8
1.4 Cell wall enzymes associated with cell-cell separation of abscission zone cells.....	9
1.4.1 Hydrolases.....	9
1.4.1.1 Endo β -1, 4-glucanhydrolase.....	9
1.4.1.2 Polygalacturonase.....	11
1.4.2 Pectin methylesterase.....	12
1.4.3 Peroxidase.....	12
1.4.4 Pathogen-related proteins inducing during abscission zone cell-cell separation.....	13
1.5 The study of abscission cell differentiation.....	13
1.5.1 Mutant studies and the control of differentiation.....	13
1.5.2 Introduction of the <i>in vivo</i> approach to study abscission and abscission cell differentiation	15
1.5.3 Examination of cell wall proteins in <i>S. nigra</i>	15
1.5.4 Identifying antigenic protein determinants in the cell wall.....	16

1.6 Control of abscission cell differentiation: Introduction of the <i>in vitro</i> approach to study abscission cell differentiation.....	18
1.6.1 Overview of secondary abscission zones and their formation.....	18
1.6.2 The hormonal control of the abscission process.....	19
1.6.2.1 Ethylene.....	20
1.6.2.2 ACC oxidase.....	21
1.6.2.3 Auxin (IAA).....	22
1.6.3 Secondary abscission formation is a transdifferentiation event.....	24
1.6.4 Control of secondary abscission zone formation in explants of <i>P. vulgaris</i>	26
1.7 Thesis Aims.....	29
2. Materials and Methods.....	30
2.1 Growth and explant preparation of <i>Phaseolus vulgaris</i>	30
2.1.1 Cultivar and growing media.....	30
2.1.2 Harvest and explant preparation.....	30
2.1.3 Ethylene and IAA treatment of explants.....	32
2.2 Collection and preparation of <i>Sambucus nigra</i>	34
2.2.1 Gathering of <i>S. nigra</i> plant material.....	34
2.2.2 Preparation of the tissue samples of <i>S. nigra</i>	34
2.3 Biochemical and chemical methods.....	38
2.3.1 Protein extraction and fractionation using column chromatography.....	38
2.3.1.1 Extraction of the soluble protein fraction in plant tissues.....	38
2.3.1.2 Extraction of cell wall-associated proteins.....	38
2.3.1.3 Fractionation of cells using ultracentrifugation.....	39
2.3.1.4 Extraction of proteins from lamb kidney.....	39
2.3.1.5 Concentrating protein, solution exchange and dialysis.....	40
2.3.1.6 Separation of proteins using hydrophobic interaction chromatography and Fast Protein Liquid Chromatograph, (FPLC).....	40
2.3.1.7 Ion exchange column chromatography.....	41
2.3.1.8 Gel filtration chromatography.....	42
2.3.2 The Bradford protein assay.....	44

2.3.3 Sodium Dodecyl Sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).....	45
2.3.3.1 Preparing and running SDS-PAGE mini-gels.....	45
2.3.4 Two-dimensional gel electrophoresis.....	48
2.3.4.1 First dimension isoelectric focusing.....	48
2.3.4.1.1 <i>Rehydration of polyacrylamide gel strips</i>	48
2.3.4.1.2 <i>Isoelectric focusing of the polyacrylamide gel strips</i>	49
2.3.4.2 Second Dimension SDS-PAGE gel.....	51
2.3.4.3 Staining the acrylamide gels from the second dimension.....	52
2.3.5 Western analysis of SDS-PAGE gels.....	54
2.3.5.1 Immunodevelopment of electroblotted proteins.....	55
2.3.5.1.1 <i>Immunodevelopment of the electroblotted membranes</i>	55
2.3.5.2 Amino acid sequencing of internal peptide sequences.....	55
2.3.6 ACC oxidase activity measurements in petiole tissues.....	56
2.3.6.1 Extraction of ACC from petiole tissue.....	56
2.3.6.2 Preparation of samples for ACC oxidase assay using Sephadex G-25 spin columns.....	57
2.3.6.3 ACC oxidase assay.....	57
2.3.7 Measurement of ethylene production by intact petiole sections.....	58
2.3.7.1 Measurement of ethylene using gas chromatography.....	58
2.3.7.2 Calculation of ethylene concentrations.....	59
2.3.8 Measurement of aldose-1-epimerase activity.....	59
2.3.8.1 Preparation of plant extracts for the activity assay.....	59
2.3.9 Preliminary activity assays.....	60
2.3.9.1 Optimisation of the coupled enzyme assay.....	60
2.3.10 Calculation of the standard errors.....	61
2.4 Immunological methods.....	62
2.4.1 Production of polyclonal antibodies to indole-3-acetic acid (IAA).....	62
2.4.1.1 Conjugation of IAA to BSA.....	62
2.4.1.2 Immunisation of rabbits with the BSA-IAA conjugate.....	64

2.4.1.3 Antisera preparation and IgG purification.....	64
2.4.2 The Enzyme Linked Immuno-Sorbant Assay (ELISA) for the determination of IAA content in plant tissues.....	65
2.4.2.1 Extraction and methylation of IAA	65
2.4.2.1.1 <i>Preparation of diazomethane</i>	65
2.4.2.1.2 <i>Extraction and methylation of IAA in bean tissues</i>	67
2.4.2.1.3 <i>Methylation of IAA in a stock solution</i>	68
2.4.2.2 Synthesis of the IAA-alkaline phosphatase conjugate (tracer).....	68
2.4.2.3 Optimising the volume of the IAA-alkaline phosphatase tracer for use in the ELISA	69
2.4.2.4 Antibody immobilised ELISA.....	70
2.4.3 Affinity purification of antisera raised to a glutathione S-transferase (GST)-fusion protein to remove the GST epitopes.....	71
3. Results: Abscission cell-specific proteins that denote the differentiated state	
3.1 Identification of proteins in the rachis and leaflet abscission zones of <i>Sambucus nigra</i>	73
3.1.1 Introduction.....	73
3.1.2 Changes in the cell wall protein profile from MR, MRE, OZ and ZONE samples collected in spring, summer and autumn.....	74
3.1.3 Fractionation of cell wall extracts to discover unique or preferentially- expressed proteins in the ethylene and non-ethylene treated tissues.....	78
3.1.4 Identification of proteins revealed by HIC by amino acid sequencing.....	85
3.2 Characterisation of the OZ43 protein.....	89
3.2.1 Initial identification of OZ43 as an aldose-1-epimerase-like protein.....	89
3.2.2 Biochemical characterisation of OZ43.....	93
3.2.2.1 Preliminary mutarotase activity assays.....	93
3.2.2.1.1 <i>Examination of the α-D-glucose and β-D-glucose anomeric equilibrium</i>	93
3.2.2.1.2 The coupled aldose-1-epimerase enzyme assay.....	97
3.2.2.1.3 Investigating the sensitivity of the coupled enzyme assay using porcine kidney mutarotase.....	102
3.2.2.2 Immunological identification of OZ43 in cell wall fractions using an antibody raised to the gp40 protein.....	104

3.2.2.3 Mutarotase activity assays of cell wall fractions containing the gp40 antibody-recognised OZ43	107
3.2.2.4 Mutarotase activity assays of soluble and insoluble components of plant tissues, and immunological identification using gp40 antibodies.....	109
3.2.3 Determination of the immunological relationship of OZ43 with gp40 and mammalian mutarotase.....	114
3.2.4 The effect of ethylene on the OZ43 protein and mutarotase activity.....	116
3.3 Examination of OZ10 as a lipid transfer protein	120
3.4 Identification of other cell wall proteins using specific antibodies.....	126
4. Results: Changes in indole-3-acetic acid (IAA) and ethylene biosynthesis during the time course of secondary abscission zone formation in bean petiole explants.....	129
4.1 Introduction.....	129
4.1.1 Secondary abscission zone formation in bean petiole explants.....	129
4.2 Development of an ELISA for IAA measurement using polyclonal antibodies.....	132
4.2.1 Polyclonal antibody production.....	132
4.2.1.1 Titres of partially purified polyclonal IAA antisera.....	132
4.3 IAA ELISA using the monoclonal antibody.....	136
4.3.1 Cross-reaction test to support the accuracy of using the monoclonal antibody for measurement of IAA samples.....	139
4.3.2 Measurement of IAA in bean tissues.....	141
4.3.3 Measurement of IAA during the time course to secondary zone formation.....	143
4.4 Ethylene evolution from petiole sections from fresh to secondary abscission.....	146
4.5 ACC oxidase activity and expression in petiole tissue from fresh to secondary abscission	149
5. Discussion.....	153
5.1 The <i>in vivo</i> approach to study abscission and abscission cell differentiation.....	153
5.1.1 Introduction.....	153
5.1.2 The survey of cell wall proteins in <i>S.nigra</i> tissue.....	155

5.1.3 Separation of cell wall extracts using SDS-PAGE.....	155
5.1.4 Separation of cell wall HIC fractionated extracts using one and two-dimensional SDS-PAGE.....	156
5.1.5 The major group of cell wall proteins in the crude and fractionated cell wall extracts.....	156
5.1.6 Identification of cell-specific proteins	158
5.1.7 Superoxide dismutase in the abscission zone cell walls of <i>S. nigra</i>	159
5.1.8 Ribonuclease in the abscission zone cell walls of <i>S. nigra</i>	161
5.1.9 Characterisation of a ca. 43 kDa abscission zone cell wall-associated protein from <i>S. nigra</i>	161
5.1.10 The identification of a putative lipid transfer protein in abscission zone cell walls of <i>S. nigra</i>	167
5.1.11 Cellulase in the zone cell wall of <i>S. nigra</i>	169
5.2 The <i>in vitro</i> approach to study abscission zone cell differentiation.....	170
5.2.1 The role of auxin in the determination of abscission zones.....	170
5.2.2 The role of ethylene in the differentiation of abscission zones.....	178
5.3 Summary.....	186
5.3.1 Abscission and abscission cell differentiation: The <i>in vivo</i> approach.....	186
5.3.2 Abscission and abscission cell differentiation: The <i>in vitro</i> approach.....	188
5.4 Future directions.....	191
Appendix A.....	192
Appendix B.....	193
Bibliography.....	194

List of Figures

Figure 2.1. Primary leaf of <i>Phaseolus vulgaris</i> approximately 14 days after germination. The explants were excised from the plant at this stage of development.	33
Figure 2.2. Freshly excised 15 mm petiole explant with the pulvinus attached.....	33
Figure 2.3. A Glass dish used for the ethylene treatment of the petiole explants containing petiole racks set into agar.....	33
Figure 2.4. Excision of petiole segments during the formation, or at separation of the secondary zones. For IAA measurements, the G1, Y2, Y3 and Y4 segments were excised as separate tissues. For ethylene evolution and ACC oxidase activity measurements the Y2, Y3 and Y4 segments were pooled. For all measurements, the shaded areas were discarded.....	35
Figure 2.5. A shrub of <i>Sambucus nigra</i> (elderberry) in flower.....	36
Figure 2.6. A compound leaf of <i>Sambucus nigra</i>	36
Figure 2.7. The segmented <i>S. nigra</i> rachis explant (explant shaded green).....	37
Figure 2.8. The leaflet abscission zones from <i>S. nigra</i> after 36 h ethylene treatment of the rachis explant shown in Figure 2.7. The arrows indicate the separated ZONE tissue and the boxed area represents the MRE tissue.....	37
Figure 2.9. Assembly of the Western blotting apparatus.....	54
Figure 2.10a. Elution of BSA from the Phenyl Superose HR 1/1 hydrophobic interaction column (section 2.3.1.6).....	63
Figure 2.10b. Elution of the BSA-IAA conjugate from the Phenyl Superose HR 1/1 hydrophobic interaction column (section 2.3.1.6).....	63
Figure 2.11. The diazomethane (CH_2N_2) forming reaction.....	66
Figure 3.1a. Separation of cell wall protein extracts of MR, MRE, OZ and ZONE tissues collected in the spring using SDS-PAGE through a 12 % polyacrylamide gel. Proteins are visualised with Coomassie blue R-250 staining.....	76
Figure 3.1b. Separation of cell wall protein extracts of MR, MRE, OZ and ZONE tissues collected in the summer using SDS-PAGE through a 12 % polyacrylamide gel. Proteins are visualised with Coomassie blue R-250 staining.....	76
Figure 3.1c. Separation of cell wall protein extracts of MR, MRE, OZ and ZONE tissues collected in the autumn using SDS-PAGE through a 12 % polyacrylamide gel. Proteins are visualised with Coomassie blue R-250 staining.....	76

Figure 3.2a. Separation of a MR cell wall sample through a hydrophobic interaction column (Phenyl Superose HR 1/1).....	80
Figure 3.2b. Separation, using SDS-PAGE, of HIC fractions D (eluted with 60-65 % buffer B) and E (eluted with 65-70 % buffer B) obtained from the Phenyl Superose column chromatography separation of spring-collected MR, MRE, OZ and ZONE tissue. Proteins are visualised with Coomassie blue R-250 staining. The arrows indicate proteins of interest summarised in Table 3.2.....	81
Figure 3.2c. Separation, using SDS-PAGE, of HIC fractions F (eluted with 70-75 % buffer B) and G (eluted with 75-80 % buffer B) obtained from the Phenyl Superose column chromatography separation of summer-collected MR, MRE, OZ and ZONE tissue. Proteins are visualised with Coomassie blue R-250 staining. The arrows indicate proteins of interest summarised in Table 3.2.....	81
Figure 3.2d. Separation, using SDS-PAGE, of HIC fractions H (eluted with 80-85 % buffer B) and I (eluted with 85-90 % buffer B) obtained from the Phenyl Superose column chromatography separation of autumn-collected MR, MRE, OZ and ZONE tissue. Proteins are visualised with Coomassie blue R-250 staining. The arrows indicate proteins of interest summarised in Table 3.2.....	82
Figure 3.2e. Separation, using SDS-PAGE, of HIC fractions J (eluted with 90-95 % buffer B) and K (eluted with 95-100 %+ buffer B) obtained from the Phenyl Superose column chromatography separation of spring-collected MR, MRE, OZ and ZONE tissue. Proteins are visualised with Coomassie blue R-250 staining. The arrows indicate proteins of interest summarised in Table 3.2.....	82
Figure 3.2f. Two-dimensional separation of OZ HIC fraction I (eluting at 85-90 % buffer B). The proteins were separated using isoelectric focusing in the first dimension and SDS-PAGE in the second dimension. Protein spots are visualised using Coomassie blue G-250.....	84
Figure 3.2g. Two-dimensional separation of MR HIC fraction I (eluting at 85-90 % buffer B). The proteins were separated using isoelectric focusing in the first dimension and SDS-PAGE in the second dimension. Protein spots are visualised using Coomassie blue G-250.....	84
Figure 3.3a. SDS-PAGE separation of fraction E from a Phenyl Superose column chromatography separation of autumn-collected tissue. The proteins arrowed (OZ10, OZ28(a), OZ28(b) and OZ43) were then subjected to tryptic digestion and selected tryptic fragments from each protein were sequenced.....	87
Figure 3.3b. Sequences from a tryptic fragments obtained from the protein bands excised from the SDS-PAGE separation shown in Figure 3.3a.....	87
Figure 3.3c. Amino acid sequence alignment of the internal peptide from OZ28(a) with a ribonuclease from <i>A. thaliana</i> . Gen-bank accession number AAA51406.....	88
Figure 3.3d. Amino acid sequence alignment of the internal peptide from OZ28(b) with a superoxide dismutase from <i>A. thaliana</i> .	

Gen-bank accession number AAF01529.....	88
Figure 3.3e. Amino acid sequence alignment of the internal peptide from OZ43 with gp40 from <i>N. tabacum</i> . Gen-bank accession no. TO1933.....	88
Figure 3.3f. Amino acid sequence alignment of the internal peptide from OZ10 with a lipid transfer protein from spinach. Gen-bank accession no. AAA34032.....	88
Figure 3.4a. Alignment of the amino acid sequence from a tryptic peptide of OZ43 to amino acid sequences of probable and putative and aldose-1-epimerase proteins and aldose-1-epimerase-like proteins from a range of species. The boxed regions contain the amino acids that are the most highly conserved throughout the species examined (present in this alignment in at least 80 % of those plant species compared).....	91
Figure 3.4b. Western analysis, using a monospecific gp40 antibody, of ethylene and non-ethylene treated tissues eluted from the HIC column and separated using SDS-PAGE. Antibody recognition was detected using an alkaline-phosphatase conjugated secondary antibody.....	92
Figure 3.5a. The spontaneous anomeric transformation of α -D-glucose to β -D-glucose measured as the change in absorbance at 340 nm over time (minutes).....	96
Figure 3.5b. The spontaneous anomeric transformation of β -D-glucose to α -D-glucose measured as the change in absorbance at 340 nm over time (minutes).....	96
Figure 3.6a. Reaction scheme for aldose-1-epimerase assay coupled to the formation of D-glucono δ -lactone by glucose dehydrogenase at pH 7.6.....	100
Figure 3.6b. Changes in D-glucono δ -lactone formation (measure at 340 nm) from the glucose dehydrogenase catalysed conversion of β -D-glucose to D-glucono δ -lactone over the range of β -D-glucose concentrations indicated.....	101
Figure 3.6c. Changes in D-glucono δ -lactone formation (measured at 340 nm) from the glucose dehydrogenase catalysed conversion of β -D-glucose to D-glucono δ -lactone over the range of α -D-glucose concentrations indicated.....	101
Figure 3.7a. Increase in formation of D-glucono δ -lactone (measured at 340 nm) in response to an increase in added porcine kidney mutarotase enzyme unit as shown.....	103
Figure 3.7b. The rate of formation of D-glucono δ -lactone (measured as change in absorbance at 340 nm with time) over a range of porcine kidney mutarotase enzyme units as shown.....	103
Figure 3.8a. Western analysis using the gp40 antiserum, of specific fractions	

from the chromatographic columns with positive identification of a 43 kDa protein. Antibody recognition was determined using an alkaline-phosphatase conjugated secondary antibody.....	105
Figure 3.8b. Protein staining after SDS-PAGE separation of the OZ43 positive fractions (as identified by Western analysis with the gp40 antiserum) from successive purification columns using FPLC.....	105
Figure 3.8c. The calibration curve of protein standards generated from elution volumes of each protein from the gel filtration column. Each labelled point represents a protein standard where a = 12.5 kDa, b = 29 kDa, c = 66 kDa, d = 150 kDa and e = 22 kDa. The elution volume of the gel filtration fraction containing OZ43 corresponded to a protein between 29 and 66 kDa.....	106
Figure 3.9a. Mutarotase activity rates in fractions from the chromatographic columns indicated that had been determined to contain the OZ43 protein recognised by the gp40 antiserum.....	108
Figure 3.9b. Expression of mutarotase specific activity rates determined by comparing the activity measurements of the partially purified (gp40 antibody-recognised) OZ43 protein (Figure 3.9a) to the activity of the porcine mutarotase (Figure 3.7b).....	108
Figure 3.10a. Diagrammatic representation of the fractionation of rachis cell extracts. The bold borders denote those samples that were analysed for gp40 antibody recognition and aldose-1- epimerase enzyme activity.....	111
Figure 3.10b. (a) Western analysis, using the gp40 antiserum, of the sub-cellular fractions of MR cell wall tissue as indicated in Figure 3.10a, after separation using SDS-PAGE. Antibody recognition was determined using an alkaline-phosphatase conjugated secondary antibody. (b) Protein staining after SDS-PAGE separation of the sub-cellular fractions of MR cell wall tissue subjected to Western analysis in (a).....	112
Figure 3.10c. Mutarotase activity in the sub-cellular fractions of MR cell wall tissue as indicated in Figure 3.10a and examined with SDS-PAGE Figure 3.10b(a) and Western analysis using the gp40 antiserum in Figure 3.10b(b).....	113
Figure 3.11. Mutarotase activity, measured using the coupled enzyme assay, in the HIC fractions from the lamb kidney membrane protein extract.....	115
Figure 3.12a. Western analysis, using the gp40 antiserum, of (a) mid-rachis (MR), (b) ethylene treated mid-rachis (MRE) and (c) ethylene and 1-MCP treated mid-rachis (MRE-MCP) cell wall extract fractions 11 to 17 (as indicated) from the HIC column after separation using SDS-PAGE. Antibody recognition was determined using an alkaline-phosphatase conjugated secondary antibody.....	118
Figure 3.12b. Mutarotase activity in fraction 14 of the HIC separation of MR, MRE and MRE-MCP tissues, as indicated.....	119

Figure 3.13a. Partial internal sequence of OZ10 aligned with the corresponding fragments of lipid transfer sequences from other plant species (from Kader 1997,1996 and Sterk <i>et al</i> 1991).....	122
Figure 3.13b. Western analysis, using the carrot lipid transfer protein antibody, of the cell wall protein extracts of MR, MRE, OZ and ZONE tissues of <i>S. nigra</i> collected in the summer and separated using SDS-PAGE. Antibody recognition was determined using an alkaline-phosphatase conjugated secondary antibody.....	123
Figure 3.13c. A representation of a leaf of <i>P. vulgaris</i> with the pulvinus, abscission zone and petiole indicated.....	124
Figure 3.13d. Western analysis, using the carrot lipid transfer protein antibody, of the cell wall protein extracts from the ethylene and non-ethylene treated bean petiole tissues. The tissues at 0 h are shown as pulvinus0, 1 ^o zone0 and pulvinus0 (as shown in Figure 3.12c), and these three tissues at 36 h of ethylene treatment are indicated as pulvinus36, 1 ^o zone36 and petiole36. Antibody recognition was determined using an alkaline-phosphatase conjugated secondary antibody.....	124
Figure 3.13e. Western analysis, using the <i>A. thaliana</i> lipid transfer protein antibody, of the cell wall protein extracts from the ethylene and non-ethylene treated bean petiole tissues. The tissues at 0 h are shown as pulvinus0, 1 ^o zone0 and pulvinus0 (as shown in Figure 3.12c), and these three tissues at 36 h of ethylene treatment are indicated as pulvinus36, 1 ^o zone36 and petiole36. Antibody recognition was determined using an alkaline-phosphatase conjugated secondary antibody.....	124
Figure 3.13 f. Western analysis, using the carrot lipid transfer antibody, of a cell wall extract from the MR tissue of <i>S. nigra</i> and the petiole tissue of <i>P. vulgaris</i> after separation using SDS-PAGE. Antibody recognition was determined using an alkaline-phosphatase conjugated secondary antibody.....	125
Figure 3.14a. Western analysis, using the BAC antibody, of cell wall extracts of MR, OZ, MRE and ZONE after separation using SDS-PAGE. Antibody recognition was determined using an alkaline-phosphatase conjugated secondary antibody.....	128
Figure 3.14b. Western analysis, using the BAC antibody, of ethylene and non-ethylene treated bean petiole tissues as indicated in Figure 3.13d. after separation using SDS-PAGE. Antibody recognition was determined using an alkaline-phosphatase conjugated secondary antibody.....	128
Figure 4.1a. A freshly excised explant.....	131
Figure 4.1b. Day 0 bean petiole (at abscission at the primary zone with the pulvinus attached).....	131
Figure 4.1c. Day 3 bean petiole (52 h).....	131
Figure 4.1d. Day 5 bean petiole (98 h).....	131

Figure 4.2a. A schematic representation of the reactions involved in the antibody immobilised ELISA using the polyclonal IAA antibody.....	134
Figure 4.2b. The optimised IAA standard curve for the polyclonal IAA antibodies generated. The polyclonal IAA-CH ₃ antisera displaying the highest titres (from bleed three) were used to generate the curve. *%B refers to the percentage binding of the alkaline-phosphatase conjugate tracer to the immobilised IAA antibody.....	135
Figure 4.2c. The maximum difference in absorbance measured with antibody immobilised ELISA between IAA concentrations of 0 and 1000 pmole/0.1mL using the polyclonal antisera generated from the two rabbits, as indicated, against IAA-CH ₃	135
Figure 4.3a. The absorbance difference between 0% and 100% binding of the IAA-alkaline phosphatase conjugate to IAA antibodies, as indicated, using ELISA. The rabbit antisera used are those in Figure 4.2b for comparison with the monoclonal antibody.....	137
Figure 4.3b. The IAA standard curve using the polyclonal IAA IgG (from Figure 4.2a) compared to the optimised standard curve generated using the monoclonal antibody to IAA. *%B refers to the percentage binding of the alkaline-phosphatase conjugate to the immobilised IAA antibody.....	138
Figure 4.4. Cross-reactivity curves used to validate the measurements of IAA in bean extracts using ELISA.....	140
Figure 4.5. Concentration of IAA in pmol/g fresh weight in extracts of fresh tissues (as indicated) excised from 14-day-old bean plants. The numbers refer to values of IAA as pmol/g fresh weight for each tissue.....	142
Figure 4.6. Changes in endogenous free IAA concentrations in bean petioles from separation at the primary zone to separation at the secondary zone. Values listed in the table at displayed on the adjacent graphs.....	145
Figure 4.7a. Ethylene evolution from wounded fresh petiole and 98 h petiole explants.....	148
Figure 4.7b. Ethylene evolution from petiole segments (G1) and pooled sections (Y2, Y3 and Y4) of explants that are freshly excised (fresh), at abscission of the pulvinus at the primary zone (primary abscission) and at formation of the secondary zone (secondary abscission).....	148
Figure 4.8a. Ethylene evolved in the ACC oxidase activity assay from petiole segments (G1) and pooled sections (Y2, Y3 and Y4) of explants that are freshly excised (fresh), at abscission of the pulvinus at the primary zone (primary abscission) and at formation of the secondary zone (secondary abscission).....	151

- Figure 4.8b.** Western analysis, using the ACC oxidase antibody, from petiole segments (G1) and pooled sections (Y2, Y3 and Y4) of explants freshly excised (fresh), at abscission of the pulvinus at the primary zone (primary abscission) and at formation of the secondary zone (secondary abscission)..... 151
- Figure 4.8c.** A comparison of ethylene evolution and ACC oxidase activity from petiole segments (G1) and pooled sections (Y2, Y3 and Y4) of explants that are freshly excised (fresh), at abscission of the pulvinus at the primary zone (primary abscission) and at formation of the secondary zone (secondary abscission)..... 152
- Figure A.** Optimised standard curve generated using the monoclonal antibody. *%B refers to the percentage binding of the alkaline-phosphatase conjugate to the IAA antibody..... 192

List of Tables

Table 2.1. Names and addresses of the manufactures of reagents and specialised equipment.....	30
Table 2.2. Protein standards used for determination of the molecular weight of proteins eluting from the Separose 12 gel filtration column.....	42
Table 2.3. Formulations of buffers and acrylamide gels solutions for SDS-PAGE mini-gels.....	46
Table 2.4. Formulations for solutions used in polyacrylamide gel strip rehydration for isoelectric focusing.....	49
Table 2.5. Running program for isoelectric focusing of polyacrylamide gel strips.....	49
Table 2.6. Components for the second dimension polyacrylamide gels.....	52
Table 3.1. Summary of proteins designated as unique or preferentially-expressed in the cell walls of the tissues indicated, and collected in Spring ((a); from Figure 3.1a) Summer ((b); from Figure 3.1 b) and Autumn ((c); from Figure 3.1c).....	79
Table 3.2. Summary of proteins designated as unique or preferentially-expressed in the tissues indicated after fractionation by HIC and separation using SDS-PAGE (data from Figures 3.2 b, 3.2 c, 3.2 d, 3.2 c).....	85
Table 5.1. The reported IAA concentrations within the tissues of various angiosperms. The IAA units vary between studies so have been converted to pmol/gram FW so they can be compared to the IAA data collected in this thesis.....	177
Table 5.2. Nucleotide homology percentage values between the seven putative ACO clones isolated from fresh petiole tissue by RT-PCR.....	185
Table 5.3. ACC oxidase mRNA sequences in the NCB1 database from plant with the closest similarities to clones 1 to 6 and clone 7 from <i>Phaseolus vulgaris</i> isolated from fresh petiole tissue by RT-PCR.....	185