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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