Annals of Botany **86**: 223–235, 2000
doi:10.1006/anbo.2000.1203, available online at http://www.idealibrary.com on IDEAL®

REVIEW

Cell Separation Processes in Plants—Models, Mechanisms and Manipulation

JEREMY A. ROBERTS*†, CATHERINE A. WHITELAW‡, ZINNIA H. GONZALEZ-CARRANZA† and MICHAEL T. McMANUS§

†Division of Plant Science, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leics. LE12 5RD, UK, ‡USDA, Building 006, Rm 208 BARC-West, 10300 Baltimore Avenue, Beltsville, MD 20705-2350, USA and §Institute of Molecular BioSciences, Massey University, Private Bag 11222, Palmerston North, New Zealand

Received: 1 November 1999 Returned for revision: 21 December 1999 Accepted: 21 March 2000

Abscission and dehiscence are developmental processes that involve the co-ordinated breakdown of the cell wall matrix at discrete sites and at specific stages during the life cycle of a plant. In this review we examine the events that influence the differentiation of abscission and dehiscence zone cells and the changes that are associated with wall degradation. There is convincing evidence to believe that ethylene and auxin co-ordinate the timing of leaf, flower and fruit abscission but the events that regulate dehiscence and seed abscission are unclear. The use of transgenic plants and model systems such as *Arabidopsis* is assisting our understanding of the mechanisms that regulate abscission and dehiscence and the application of this information will advance our understanding of cell separation processes in general. Armed with this knowledge it should be possible to either delay or accelerate abscission and dehiscence, and this could have major benefits for the agricultural and horticultural industries.

© 2000 Annals of Botany Company

Key words: Abscission, dehiscence, cell separation, wall degradation, gene expression, polygalacturonase, β-1,4-glucanase, pathogenesis-related proteins, ethylene.

INTRODUCTION

Cell division is a critical activity during the growth of a plant. It provides the building blocks for the differentiation of tissues and organs and contributes to the overall size of the individual. The consequence of the division process is that the two daughter cells are joined together by a cellulose wall that provides strength but also restricts relative movement. For many cells the structural significance predominates and the cells remain attached throughout their life cycle. For others it is crucial that separation from neighbouring cells is achieved and this event may be triggered by environmental or hormonal signals or by a switch when a cell has reached a specific developmental stage. In this review we will examine the mechanisms by which cell separation takes place at specific sites during plant differentiation and how the process may be co-ordinated.

CELL SEPARATION PROCESSES IN PLANTS

Cell separation is an important event during such developmental phenomena as abscission and dehiscence. In these instances the evidence suggests that cells that comprise the abscission or dehiscence zones are pre-programmed in a different way from adjacent non-separating cells. This hypothesis has gained support because the cells that separate make up a discrete group or layer that are morphologically distinct prior to wall degradation. Cells of ripening fruit undergo a similar series of changes but in addition embark on a process of differentiation during fruit development. In an expanding leaf, where intercellular air spaces develop so enabling gaseous diffusion to take place, cell separation may not be restricted to just a few cells but seems to occur at discontinuous locations at the junctions between groups of neighbouring individuals (Dale and Milthorpe, 1981). Certain cells within the leaf exhibit no separation e.g. the majority of epidermal cells. However, not all epidermal cells fall into this category and during the differentiation of the stomatal complex a specific cell-to-cell interface must be degraded to enable the formation of the pore framed by the guard cells (Stevens and Martin, 1978; Sack, 1987). Another site where cell separation has been observed to take place is the outer layers of the root cap where the peripheral cells are sloughed off and release mucilage so easing the passage of the root through the soil (Hawes and Lin, 1990). It is likely that cell separation is also associated with the emergence of the lateral root tip as it makes its way through the cortical tissues of the primary root (Peretto *et al*., 1992). In addition to cell separation occurring as part of the life cycle of the plant it may also be induced in response to specific environmental conditions. For instance, the development of aerenchyma is precipitated by cell separation and collapse in roots of maize and rice as a consequence of waterlogging or anaerobiosis, and the formation of these cavities facilitates gaseous exchange with the aerial tissues (Drew *et al*., 2000).

* For correpondence. Fax +44 (0) 115 951 6334, e-mail jeremy.roberts@nottingham.ac.uk

0305-7364/00/080223 + 13 $35.00/00 © 2000 Annals of Botany Company

Of the processes outlined most of the research into the regulation of cell separation has been carried out on abscission, dehiscence or ripening systems. This may be in part because they are easiest to study in a laboratory environment as the cell separation events can be synchronized and the changes that lead to wall breakdown can be correlated with the loss of adhesion between cells. In addition, an understanding of all three processes has significance to the agricultural and horticultural industries. In this review we intend to limit our focus to abscission and dehiscence but also consider how our knowledge in these areas might be applied in the broader context of cell separation processes in plants.

MODELS

Abscission

A range of model systems has been used for the study of abscission and the favoured species has changed as new techniques have developed to facilitate the study of the process at the biochemical and molecular level. One of the most extensively documented is the abscission zone located within the primary leaf of *Phaseolus vulgaris*. Two sites of cell separation take place within the petiole tissue. One is located at the junction between the petiole and pulvinus and the other between the petiole and the stem. The distal zone is clearly delineated prior to the advent of lamina shedding and consists of perhaps only a single layer of cells (Wright and Osborne, 1974). The limited number of layers that comprise this abscission zone has led workers to search for other systems where the changes associated with cell separation may be co-ordinated over a greater area. The leaflet abscission zone of *Sambucus nigra* has proved a valuable system in this regard being composed of in excess of 20–30 layers of cells (Osborne and Sargent, 1976). This zone is actually a composite of three sites where cell separation takes place; at the base of each leaflet and between the leaflet insertions. The size of the zone has enabled enzymes and nucleic acids to be extracted from cells during the course of separation and some analysis of the solubilizing cell wall to take place (Taylor *et al*., 1993). The drawback of the *S. nigra* system is that no transformation system is available and this limits the nature of the experimental work that can be undertaken. As a consequence there has been a growing interest in studying tomato leaf and flower abscission (Roberts *et al*., 1984; Taylor *et al*., 1990) as the crop has commercial significance and material can be readily transformed. Furthermore tomato mutants have been characterized that fail to undergo abscission or exhibit organ shedding at a delayed rate (Tucker *et al*., 1984). As yet little work on abscission has been undertaken on the model system of contemporary choice, *Arabidopsis* (Bleecker and Patterson, 1997). The primary explanation for this is that although senescence of the leaves takes place in this species they are not shed but undergo a process of desiccation followed by attrition of the dehydrated tissues. Abscission does take place at both the base of the petals and at the seed: funiculus junction, but the amount of zone tissue that could be extracted from these sites for biochemical or molecular analysis is minute. One strategy that might circumvent this limitation is the use of the close relative and important crop plant *Brassica napus* as a means for the identification of abscission-related genes, followed by the isolation of the *Arabidopsis* homologues and their manipulation in the latter species. Leaf abscission of *B. napus* can be readily studied under laboratory conditions and a number of abscission-related genes have already been identified in *Arabidopsis* using this approach (Gonzalez-Carranza unpubl. res.).

Dehiscence

The process of dehiscence shares many features in common with abscission in that cell separation occurs at a discrete site. A considerable body of work was carried out in the 1970s on dehiscence of fruit such as cotton and pecan (Lipe and Morgan, 1972) as manipulating this phenomenon could improve the quality and quantity of the crop produced. Over the last 10 years, studies of dehiscence have centred on *B. napus* as the losses in yield of seed as a consequence of pod shatter can be as great as 50 % in unfavourable years (Macleod, 1981). Pods of *B. napus* reach their full-length by approximately 20 d after anthesis (DAA) and at this time the site at which dehiscence will take place cannot be distinguished (Meakin and Roberts, 1990*a*). However, during the following 20 d the cells in this region undergo extensive lignification and by 40 DAA only a single layer of cells remains unlignified on either side of the silique vascular bundle. At the time of pod dehiscence cell wall degradation takes place at this discrete site (Meakin and Roberts, 1990*a*; Petersen *et al*., 1996). This row of cells is termed the dehiscence zone. A similar series of events takes place, albeit at an accelerated rate, during the development of pods of *Arabidopsis* (Spence *et al*., 1996).

Dehiscence is not restricted to pods but also takes place during the final stages of development of anthers. In many ways this is analagous to the events that occur during pod shatter and isolated pockets of cell separation have been reported in the stomium region of the anther (Keijzer, 1987). Recent work has shown that not only are pod and anther dehiscence analagous processes but they may also be regulated by a similar mechanism (Jenkins *et al*., 1999).

DIFFERENTIATION OF THE ZONE OF SEPARATION

The differentiation of abscission zone cells in vivo

The concept that abscission zones comprise positionally differentiated, functionally specialized cell types is now widely accepted. These cells have been described as Type II target cells being characterized by elevated cell growth in response to ethylene and auxin (indoleacetic acid—IAA) (Osborne, 1989). However, it is also widely appreciated that the abscission zone comprises subclasses of cell types. Addicott (1982) clearly identifies the separation layer as part of the abscission zone, and describes it as commonly comprising a single layer of cells. As described in this

review, advances in the identification of genes which are expressed during the cell separation process highlight two major classes—one class is involved in the mechanism of cell-to-cell separation while the second class comprises genes which are more likely to be concerned with cellular protection. Expression of these protectant genes may be localized in both the separating and non-separating cells of the abscission zone, both proximal and distal to the separation layer and these proteins may protect the proximal tissues from microbial invasion until the connections between cells are severed. In many species, once cell wall loosening is initiated, an anatomically distinct protective layer is differentiated immediately proximal to the site of separation. This can originate from deposition of suberin or lignin on the newly exposed cells of the separation layer, but is often associated with additional cell division. This periderm-like layer offers protection through the deposition of physical barriers, principally suberin, and often becomes continuous with the periderm layer of the stem. However, of pertinence to this review is that any consideration of the control of the differentiation of the abscission zone must include the notion that the zone comprises cells with different functional specializations.

Before evocation of the cell-to-cell separation process, particularly in leaf tissue, the abscission zone in many species is morphologically distinguishable. The distinctive nature of these cells has been described in detail elsewhere (Addicott, 1982; Sexton and Roberts, 1982; Osborne, 1989), but the major features are small, iso-diametric cells with dense cytoplasm and many plasmadesmatal connections. A widely held view is that these cells differentiate early within the time span of the organ to which they are associated and remain in this state of arrested development (Addicott, 1982) while neighbouring cells enlarge, mature and vacuolate. However, it is clear that abscission cells once differentiated early in development are competent to respond to the appropriate stimulus. For example, in newly germinated seedlings of *P. vulgaris*, ethylene can induce the separation of the unexpanded leaves from the petiole and the subtending petiole from the stem (Osborne, 1989).

In addition to a distinctive anatomy, abscission cells often display a series of other distinctive features which 'mark' their differentiation status. Although considered arrested in development and thus maintaining a meristematic cell appearance, it is clear that these cells have undergone specific changes to confer unique functional characteristics. In terms of cell wall modifications, abscission zones, particularly those from woody species, often display an absence of secondary thickening. However, the abscission zone is not an area of weakness, and in the foliar abscission zone of *Impatiens sultani* the cortex of the zone comprises collenchyma cells (Sexton, 1976). Such secondary modifications to the cell wall may be an important event in the differentiation of the site at which cell separation takes place.

Cytological features have also been reported as features that mark the specific differentiation status of the abscission zone. At the base of the ovary of female flowers of *Ecballium elaterium*, those cells in the abscission zone that will enlarge in response to ethylene can be identified by virtue of their capacity to undergo endoreduplication of their nuclear DNA (Wong and Osborne, 1978). Only once this endoreduplication event to 8C has occurred are these cells competent to enlarge in response to ethylene. Although there is no evidence that endoreduplicated DNA is part of the separation mechanism *per se*, it does serve to mark these cells as competent to separate.

Abscission cells have also been shown to contain specific protein determinants. In *P. vulgaris*, immunostaining with a monoclonal antibody that recognizes xylose/fucose-containing complex-type N-linked glycan structures revealed several peptides that are expressed preferentially in the unevoked leaf petiole : pulvinus abscission zone (McManus and Osborne, 1990). In *S. nigra* an approx. 34 kD cell-wall-associated protein has been shown to be preferentially expressed in abscission zone cells (McManus and Osborne, 1991). While the identity and function of abscission cell-associated proteins have yet to be determined, the identification of protein markers lends itself to the production of antibodies that specifically recognize abscission cells. However, in another approach, the use of developmental mutants with impaired abscission cell differentiation may also yield clues as to the control of abscission cell differentiation. In tomato, mutations at two loci, designated jointless (*j*) and *j-2*, have been identified which do not develop the normal abscission zone in the pedicel of tomato flowers and fruits, and a map-based cloning technique is currently being employed to clone the recessive *jointless* gene (Wing *et al*., 1994). Another tomato gene that affects the development of the pedicel abscission zone is *Lateral suppressor* (*Ls*). This gene has now been cloned and shown to encode a VHID protein that is member of a family of putative transcriptional regulators (Schumacher *et al*., 1999). The function of these peptides is unclear, however their homology with proteins such as GA1, RGA, Rht-1 and d8, that have been shown to be involved in gibberellin (GA) signal transduction (Peng *et al*., 1999), suggests that the Ls protein could modulate localized GA responsiveness.

In an elegant series of experiments, Szymkowiak and Irish (1999) made periclinal chimeras of *jointless* and a wild type tomato genotype and determined that the genotype of the L3 layer determined whether a functional pedicel abscission zone differentiated. However, only chimeras with a wild-type L3 (the layer which gives rise to the vascular tissue and internal tissues) exhibited a morphologically normal and functional abscission zone. If such wild-type L3 chimeras comprised jointless L1 (from which the epidermis is derived) and L2 (which gives rise to the sub-epidermal cell layers), the epidermal and sub-epidermal layers at the abscission zone were normal morphologically. That is, the L1-derived epidermal cells were non-elongated and lacked trichomes and the L2-derived sub-epidermal layers lacked chloroplasts and airspaces; morphological features which are evident in wild type pedicel abscission zones of tomato. Moreover, the authors deduced that it is the outermost L3-derived tissue that plays an important role in the induction of abscission zone formation through the co-ordination of the L1 and L2-derived cells. As the authors conclude, these experiments do not indicate when these cell layers receive the inductive signals to

differentiate—in the meristem or during development of the pedicel. However, our increased understanding of gene expression during the abscission process reveals an increasing complexity of the cells that comprise the abscission zone. It may be, therefore, that these cells receive a series of developmental inputs during their differentiation, which together confer morphological, functional and positional information.

Differentiation of secondary abscission cells in vitro

The observation that certain cells in the mature plant body retain the ability to be converted into cells with the capability to differentiate further into abscission cells has long been known. Secondary, or adventitious zones (Addicott, 1982) are defined as functional abscission zones formed where one would not normally develop, and have been shown to form in stem, petiole, pedicel or phyllomorph tissue. For stem abscission, Lloyd (1914, see Addicott, 1982) observed that if an *Impatiens sultani* shoot is decapitated through an internode, an abscission zone develops just above the next node with subtending leaf and axillary bud and the distal internode segment is shed. Beal and Whiting (1945) also observed the differentiation of an abscission zone at the base of the first internode in plants of *Mirabilis jalapa* that had been decapitated, and demonstrated that the application of IAA to the cut surface prevented abscission zone formation. In further studies of secondary abscission zone formation in stem tissue, excised tissue explants have been used. Using excised cotyledonary nodes of cotton, Bornman *et al.* (1968) observed that the application of gibberellic acid to the cut surface of the internode induced the formation of a stem abscission zone at the base of the internode and also accelerated petiole abscission. While abscission at the petiole occurs during the normal course of plant development, these workers claimed that the stem abscission zone represented a 'latent abscission zone'. Webster and Leopold (1972), using nodal explants of *P. vulgaris* observed the spontaneous formation of an abscission zone in the internode tissue proximal to the node. These workers also investigated the hormonal control of these zones and determined that continuous ethylene treatment increased the frequency of zone formation, but also altered the site of abscission zone formation to a point just above the node (at the internode base).

Such experiments on the hormonal influences of the formation of these zones introduces perhaps the most significant property of these induced cell types—the position of the induced secondary abscission zone can be manipulated by specific hormonal cues. This hormonal regulation of the position of the induced zone was observed in pear pedicels by Pierik (1980) who showed that applied auxin influenced the eventual site of secondary zone formation. However, the same hormone did not change the site of zone formation in apple pedicels (Pierik, 1977). Warren Wilson *et al.* (1986) reported that the position of the secondary zone in excised internodal segments of *Impatiens sultani* was affected by the concentration of basal auxin applied. Interestingly, these workers also examined the influence of applied auxin on the formation of secondary abscission zones in nodal explants (Warren Wilson *et al.*, 1987). In these explants, an abscission zone forms at the base of the leaf petiole above the node, while a second zone, in common with internode segments, forms below the node. The site of formation of the zone below the node is altered by the concentration of auxin added in the same way as demonstrated in internodal segments. However, the zone above the node was not influenced by applied auxin and separation occurred in the identical position irrespective of the concentration of applied auxin. This zone is a secondary abscission zone as it is distinct from the naturally occurring abscission zone at the leaf base and there is no evidence that the zone was differentiated before explant excision. These workers proposed that these secondary zones develop within a morphogenic field generated by the auxin gradient although, as the authors observed, what specific aspect of the gradient is critical to the differentiation process was not determined. That is, the gradient itself or a threshold concentration of auxin or the gradient of auxin in concert with another morphogen may be the critical factor.

The impact of auxin and ethylene has been examined in detail during the differentiation of the secondary abscission zone cells in the cortex of bean petioles (McManus *et al.*, 1998). In this explant system, the presence of ethylene was important for differentiation, and applied IAA seemed to provide information about where the zone would be positioned. Investigations into this phenomenon in bean petioles have also demonstrated that secondary zones share some of the biochemical and ultrastructural changes observed during cell-to-cell separation at the primary zone. Similarities between secondary and primary zones include the activation of dictyosomes in the abscission zone while these structures remain quiescent in cells removed from the separation layer, and the induction of the abscission cell-associated pI 9·5 isoform of β-1,4-glucanase specifically in these cells when compared with neighbouring tissues. A significant further observation from these experiments was that the petiole cells retained an inherent polarity with respect to their developmental fate. In explants where auxin was added to the exposed abscission zone, the petiole tissue distal to the site of secondary zone formation remained green while the proximal tissue yellowed. However, secondary zone formation will also occur in abscission explants to which no IAA has been added. In these explants, the polarity of green : yellow petiole tissue was reversed. Nevertheless, the secondary abscission zone always formed at the site of the green : yellow tissue junction and it was always the cells on the green side of the junction that enlarged and separated from their neighbouring cells. This developmental memory has also been shown in secondary abscission studies using *Agathis australis* (Wilson *et al.*, 1998). Nodal explants excised from the tips of horizontal branches which normally undergo cladoptosis demonstrated the formation of a secondary abscission zone and proximal protection layer and the terminal 3 mm segment is shed. If similar explants are excised from horizontal tissue that is older than one growth flush or from the orthotropic axis (which does not display cladoptosis in the field), then secondary zone formation does not occur.

An important aspect of secondary zone formation is whether the differentiation of these cells from non-abscission tissue involves cell division. The formation of secondary zones in the stem of excised GA-treated cotyledonary nodes of cotton (Bornman *et al*., 1968) and *P. vulgaris* (Webster and Leopold, 1972) is accompanied by cell division. Cell division is also associated with the differentiation of the abscission zone in the phyllomorph of *Streptocarpus molweniensis* (Noel and Van Staden, 1975). However, in secondary zone formation in the petiole of *P. vulgaris*, the conversion of the cortical cells to abscission cells is clearly a transdifferentiation event.

This demonstration that the cortical cells of the leaf petiole can be converted into biochemically functional abscission zone cells in response to specific hormonal cues suggests that the attainment of the competence to undergo cell-to-cell separation, while apparently pre-programmed in the normally formed zone, can be induced in other cell types. As mentioned in this review, cell-to-cell separation is not confined to organ abscission in the mature plant body. Thus the essence of the control of abscission cell differentiation may be the unique inputs, most probably in the meristematic tissues, which confer the precise positional information to these cells and their neighbours.

Differentiation of dehiscence zone cells in vivo

Recently a number of genes have been identified in *Arabidopsis* that contribute to the development of the dehiscence zone within the silique (Gu *et al*., 1998; Ferrandiz *et al*., 1999). Two of these are the MADS-box family members *agamous-like* 1 (*AGL 1*) and *AGL 5* that encode peptides with 85 % identity at the amino acid level. While *agl1* and *agl5* single mutants exhibit no detectable difference in phenotype from the wild type, siliques of the double mutant fail to shatter due to aberrant development of the dehiscence zone (Liljegren *et al*., 1998). Another MADS-box gene termed *FRUITFULL* (*AGL8*) also appears to play a role in the development of the dehiscence zone as overexpression of the gene product inhibits pod shatter (Liljegren *et al*., 1998). It is not yet clear whether *AGL1* and *AGL5* interact with *AGL8*, however one possibility is that the two peptides have an antagonistic association and this defines the site of differentiation of the dehiscence zone cells.

CO-ORDINATION OF CELL SEPARATION

For the processes of abscission and dehiscence to be effective it is necessary that all cells that constitute the zone of separation undergo wall breakdown at approximately the same time. This implies that some event must trigger the onset of the separation programme and that the same or possibly another signal may co-ordinate the time course of the response of the layer of cells. Although such environmental factors as photoperiod may regulate the onset of leaf abscission, there is good reason to believe that the timing of the process is the consequence of elevated ethylene production by cells distal to the site of separation (Jackson and Osborne, 1970). Increased ethylene biosynthesis may not in itself be a sufficient signal, but this coupled with a decline in IAA flux through the abscission zone cells may provide the information that initiates the cell separation programme. It has been proposed that in *P. vulgaris* a crucial signal required for cell separation emanates from within the vascular tissue of the petiole although the identity of this compound is unknown (Thompson and Osborne, 1994). Flower and fruit abscission may also be co-ordinated by ethylene although there is some doubt that the gas acts to trigger the process (Bleecker and Patterson, 1997). An abscission zone also exists at the base of many seeds. This zone at the seed : funiculus junction is triggered once the seed filling stage is complete and the seed has undergone desiccation. The co-ordinating signal in this instance is less clear cut and in wild oat it has been proposed that abscisic acid (ABA) may act to bring about abscission (Sargent *et al*., 1984). A role for ABA in seed shedding is not yet proven, however the contribution of this hormone to seed maturation is well established and this event is co-ordinated with the abscission process.

The induction of pod and anther dehiscence has been less well studied and few programmes have been carried out to ascertain which environmental factors or hormonal ligands might be responsible for initiating cell separation. The dehiscence of the pod is co-ordinated with the abscission of the seed although it can also be triggered prematurely in *B. napus* by infection with pod midge (Meakin and Roberts, 1991). Adults of this insect deposit their eggs through the silique wall into the developing seeds. On emergence the larva consumes the seed and becomes trapped inside the young pod. It achieves its escape by triggering the natural dehiscence process perhaps as early as 30 DAA (Meakin and Roberts, 1991). There is little evidence that the natural regulator of either pod or anther dehiscence is ethylene and *Arabidopsis* mutants displaying non-functional ethylene receptors such as *etr* and *ein4* are fully fertile and exhibit a normal time course of silique opening. Child *et al*. (1998) reported that cell separation in the dehiscence zone of parthenocarpic pods of *B. napus* was delayed by 3–4 d compared to seeded pods and correlated this with a reduced production of ethylene. However, the peak of ethylene production in pathenocarpic pods was approximately half that of seeded pods and occurred 20 d later suggesting that if ethylene does play a role in pod dehiscence only a small amount of the gas is necessary to initiate the process. Another plant hormone that has been proposed to contribute to the timing of pod shatter is IAA although in contrast to its role in abscission it has been suggested that the auxin needs to be above a critical threshold concentration for dehiscence to take place (Chauvaux *et al*., 1997).

CHANGES IN ENZYME ACTIVITY DURING CELL SEPARATION

Studies on changes in enzyme activity have primarily focused on enzymes that might contribute to wall dissolution. An increase in β-1,4-glucanase (cellulase) activity has been reported during the abscission of leaves, flowers and fruit (Roberts *et al*., 1984; Bonghi *et al*., 1992; Webb *et al*., 1993). This rise in activity is primarily restricted to the

abscission zone cells, although in *P. vulgaris* some elevation has also been found in adjacent non-separating regions such as the stele. In general the increase is detected at about the same time as cell wall breakdown is observed. The β-1,4-glucanase that increases during *P. vulgaris* leaf abscission has been purified and found to have a pI of 9·5 (Durbin *et al.*, 1981). Polyclonal antibodies raised against the protein recognize a signal within the abscission zone and injection of them into the zone has been shown to delay abscission (Sexton *et al.*, 1980). An increase in β-1,4-glucanase activity has also been reported during *B. napus* pod dehiscence and this increase precedes shatter and is restricted to the dehiscence zone (DZ) cells (Meakin and Roberts, 1990*b*). Further evidence to support a role for this enzyme in dehiscence comes from the observation that the activity also increases in pod midge infected plants immediately adjacent to the site of premature splitting of the siliques (Meakin and Roberts, 1991). However, whether this increase in enzyme activity originates from the plant or the insect is presently unknown.

Anatomical studies of cell separation within both abscission and dehiscence zones has revealed that the primary site of wall breakdown is the middle lamella (Peterson *et al.*, 1996). This observation has prompted the search for changes in activity of enzymes that might degrade pectin. The principal enzyme that has been analysed is polygalacturonase (PG). Increases in the activity of PG have now been reported in abscission zones of tomato leaves, flowers and fruit (Tucker *et al.*, 1984; Taylor *et al.*, 1990), peach leaves and fruit (Bonghi *et al.*, 1992) and leaflets of *S. nigra* (Taylor *et al.*, 1993). Interestingly, the only study of the enzyme in *P. vulgaris* failed to detect a change in enzyme activity during primary leaf abscission (Berger and Reid, 1979). In general the increases reported in PG activity coincide with the loss of breakstrength of the zone and are restricted to the sites where cell separation takes place. No abscission zone PG has yet been purified to determine its specific biochemical properties, however preliminary characterization of the enzyme from *S. nigra* leaflets has shown that more than one isoform may be involved and that the PG has the properties of an endoacting enzyme (Taylor *et al.*, 1993). Further evidence to support a role for PG during abscission is an analysis of the polyuronides that can be extracted from abscission zone tissues prior to and after wall degradation. After ethylene treatment, the size of the extractable polyuronides from *S. nigra* leaflet abscission zones is much more heterodisperse being composed of a mixture of large and small fragments (Taylor *et al.*, 1993). This is what would be predicted after the action of an endo-acting PG enzyme on the pectin-rich material of the cell wall.

An increase in PG activity has also been observed during pod development in *B. napus* (Petersen *et al.*, 1996). These authors showed that an extract from the dehiscence zone of pods, immediately prior to shatter, was able to degrade polygalacturonic acid and that only a limited amount of free galacturonic acid was released indicating that the enzyme acted in an endo fashion. Although no rise in PG has been reported during anther dehiscence, a proposal was made over 75 years ago that such an enzyme might contribute to the dissolution of the wall of the pectin-rich collenchyma cells that contribute to the release of the pollen (Woycicki, 1924).

CHANGES IN GENE EXPRESSION DURING CELL SEPARATION

β-1,4-glucanase

The first abscission-related gene to be cloned encoded a β-1,4-glucanase from *P. vulgaris* abscission zones. This was identified from an abscission cDNA library using a probe isolated from ripening avocado fruit (Tucker *et al.*, 1988). Expression of the β-1,4-glucanase increased during ethylene-promoted abscission, was repressed by the application of IAA, and was restricted to the abscission zone tissue. An increase in expression of β-1,4-glucanase has subsequently been reported during abscission of *S. nigra* leaflets (Taylor *et al.*, 1994), tomato flower (Lashbrook *et al.*, 1994) and flowers and leaves of pepper (Ferrarese *et al.*, 1995). In tomato at least seven cellulases have been cloned (Cel1–Cel7). During pedicel abscission increases in the expression of three members of the gene family have been reported (Cel1, Cel2 and Cel5) (del Campillo and Bennett, 1996). Cel1 and Cel2 share low nucleotide homology (55 %) and down-regulation of these individual enzymes has been achieved using an antisense RNA strategy. Reduced expression of either of these genes (Lashbrook *et al.*, 1998; Brummel *et al.*, 1999) leads to an increase in the force required to bring about abscission, and these data indicate that the enzymes may have a complementary role to play during cell separation. Interestingly, both Cel1 and Cel2 accumulate during ripening but antisense fruit do not exhibit delayed or reduced softening (Brummell *et al.*, 1999).

Although an increase in cellulase activity has been observed during pod dehiscence in *B. napus* there have been no reports of an increase in expression of genes encoding this enzyme. A number of β-1,4-glucanases mRNA fragments have been amplified by PCR from the dehiscence zone tissue of *B. napus* pods, however it has proved difficult to ascertain their expression patterns (Roberts unpubl. res.).

Polygalacturonase

Increases in expression of *PG*s have been well documented to take place during fruit ripening. Taylor *et al.* (1990) reported that tomatoes transformed with a construct that down-regulated a fruit *PG* exhibited reduced pectin depolymerization in the fruit but underwent leaf and flower abscission at the same time as controls. Moreover, the antisense material did not exhibit a reduced activity of PG within the leaf abscission zone and the antibody raised against the fruit PG was not able to co-precipitate the abscission enzyme. The authors concluded that the tomato fruit PG and the leaf abscission PG were different, and some years later their assertion was proved to be correct (Kalaitzis *et al.*, 1995). Three abscission-related PGs (TAPG1, TAPG2 and TAPG4) have been isolated from

tomato and although they have close homology to each other (80–90 % at the nucleotide level) they exhibit less than 50 % identity with the fruit PG (Kalaitzis *et al*., 1997). In addition they encode a much smaller transcript (1·5 kB) than the fruit enzyme (1·9 kB). The expression pattern of these *PG*s varies with *TAPG4* being expressed in the flower abscission zone within 6 h of exposure to ethylene while *TAPG1* mRNA does not accumulate until 6 h later. A small family of *PG* genes may also be responsible for abscission of *S. nigra* leaflets as two *PG* mRNAs have been shown to accumulate within 36 h of ethylene treatment specifically in the zone tissue (Roberts *et al*., 1997). An up-regulation of *PG* mRNA has also been reported in melon (Hadfield *et al*., 1998) and peach leaf and fruit abscission zones (Bonghi *et al*., 1992).

Using a degenerate RT-PCR approach a *PG* mRNA (*SAC66*) has been cloned from developing pods of *B. napus* (Jenkins *et al*., 1996; Peterson *et al*., 1996). The full length *PG* cDNA exhibits close homology to fruit PGs. The expression of this *PG* increases specifically in the dehiscence zone cells from 30 DAA and reaches a plateau by 60 DAA. The homologous gene from *Arabidopsis* (*SAC70*) has been isolated and characterized and the promoter fused to β-glucuronidase (GUS). Transformation of *B. napus* with this reporter gene construct has revealed that the *PG* promoter is operative at only three sites. The first of these is the pod dehiscence zone where GUS activity can be detected from about 20 DAA beginning at the ends of the pod and working towards the centre (Jenkins *et al*., 1999). The second site is at the junction between the seed and the funiculus. Few studies have been undertaken on seed abscission however this is a critical site at which cell separation takes place, as it is through the funiculus that assimilates flow into the developing seed. Once the seed filling stage is complete then desiccation ensues and abscission takes place. The observation that pod dehiscence and seed abscission are regulated in the same way seems reasonable since the opening of the pod and release of the seed should be co-ordinated. The third site at which the pod *DZ PG* is expressed is within the stomium region of the anther. This is the site where small groups of cells dissociate from one another to allow anther opening to take place (Keijzer, 1987). The locations of the *PG* expression strongly support a role for the enzyme in cell separation. Further support for this hypothesis comes from the use of the *PG* promoter to drive the cell specific expression of a ribonuclease in transgenic *B. napus*. The resultant plants proved to be male sterile as a consequence of the anthers failing to undergo dehiscence (Jenkins *et al*., 1999). The female components of the transgenic plants were unaffected and pollination could be achieved either by applying pollen from untransformed material or extracted from non-dehiscing anthers. Pods generated by either of these mechanisms exhibited a reduced susceptibility to undergo shatter as determined in the laboratory (Jenkins *et al*., 1999). These observations have now been reproduced in transgenic *Arabidopsis* demonstrating that analogous transcriptional regulation systems are operative in this species and *B. napus* (Fig. 1). The availability of *DZ PG:GUS Arabidopsis* material provides an approach by which the events that regulate expression of this gene can be dissected. The generation and characterization of mutants that fail to express GUS at one or more of the three sites where cell separation takes place should lead to the identification of the genetic lesion that attenuates the tissue specific expression. Previous attempts to identify *Arabidopsis* mutants that exhibit a reduced capacity to undergo pod shatter have not been successful. There are two possible explanations for this. The first is because susceptibility to shatter is such a difficult parameter to quantify and as a consequence mutations that affect the time course of the process might have gone undetected. Secondly the close association between the regulation of pod and anther dehiscence may mean that any mutation that leads to a complete failure of the former mechanism may also render the plant sterile.

The *DZ PG* mRNA from *B. napus* accumulates at the site where seed shedding takes place but not at either the leaf or flower abscission zones (Jenkins *et al*., 1999). A *PG* that is expressed at the latter two positions (abscission zone polygalacturonase—*AZ PG*) has now been cloned and this has 72 % homology at the amino acid level with the DZ PG (Gonzalez-Carranza, unpubl. res.). This *AZ PG* gene (*PGAZBRAN*) is not expressed at the site of dehiscence of either the pod or the anther. The equivalent gene from *Arabidopsis* (*PGAZAT*) has also been isolated. A comparison of the *AZ* and *DZ PG* genes from *B. napus* and their homologues in *Arabidopsis* shows a number of interesting features. Firstly the gross structure of the four genes is very similar being comprised of nine exons and eight introns. Moreover the relative size of the introns is comparable. The putative proteins that the genes encode also have close homology even though they are expressed at different sites. Analysis of the promoters of these four genes also reveals sequence similarity but this is limited primarily to the first 250 bp upstream from the site of translation. However, the promoters of the two AZ PGs are highly homologous and over a distance of 1·5 kB exhibit 62·5 % nucleotide homology. This type of *in silica* study is valuable as by comparing and contrasting the four promoters it may be possible to identify domains that have importance for regulating *PG* expression at specific sites and in response to critical signals.

As outlined previously, cell separation occurs at many sites during the life cycle of a plant. The coincident expression of *PG* during abscission and dehiscence strongly implicates this enzyme in the events that lead to wall dissolution. An examination of the *Arabidopsis* EST/Sequencing database reveals that a family of putative PGs can be identified on the basis of amino acid homology. Some of these show close homology to the family of exo-acting PGs that have previously been characterized in pollen. About 50 family members have so far been identified that may encode endo-acting PGs and on the basis of primary sequence these can be classified into a phylogenetic tree (Fig. 2). The DZ and AZ PGs exhibit closer homology to each other than to other members and certain other PGs also can be placed into small groups (Torki *et al*., 2000). The exo-acting PGs are more distant relatives. One approach that might prove valuable for determining a possible role of these different PGs in cell separation is to

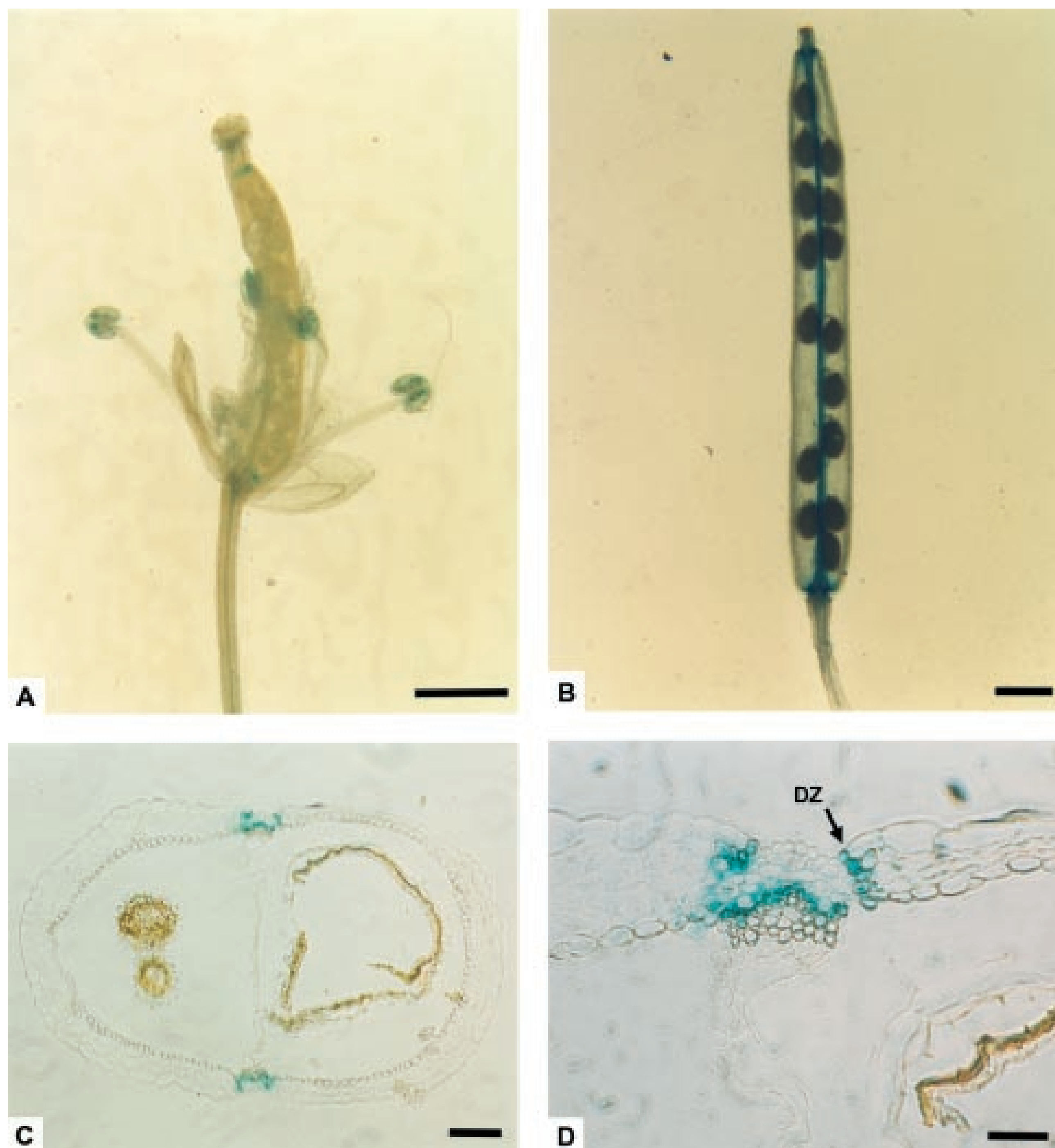

FIG. 1. GUS histochemical analysis of *A. thaliana* PG promoter (1·408 kb) linked to GUS reporter gene and transformed into *A. thaliana*. A, Mature flower; B, mature silique; C, TS of mature silique; D, TS of mature silique showing dehiscence zone. Bars = 1 mm (A and B), 100 µm (C) and 20 µm (D). Photograph courtesy of S. Gattolin.

attach the promoters of representative members of these gene groups to a reporter such as GUS or Green Fluorescent Protein and then to analyse their expression patterns. An attempt has already been made to quantify the expression of some of these genes using a RT-PCR approach (Torki *et al.*, 1999), however, this does not provide information about the cellular sites of expression. It would be hypothesized that these *PG*s would be up-regulated at other positions where cell separation has been observed or has been proposed to occur.

Other genes encoding wall loosening enzymes

It seems likely that cell separation involves a cocktail of enzymes in addition to β-1,4-glucanase and PG that bring about the co-ordinated breakdown of the middle lamella

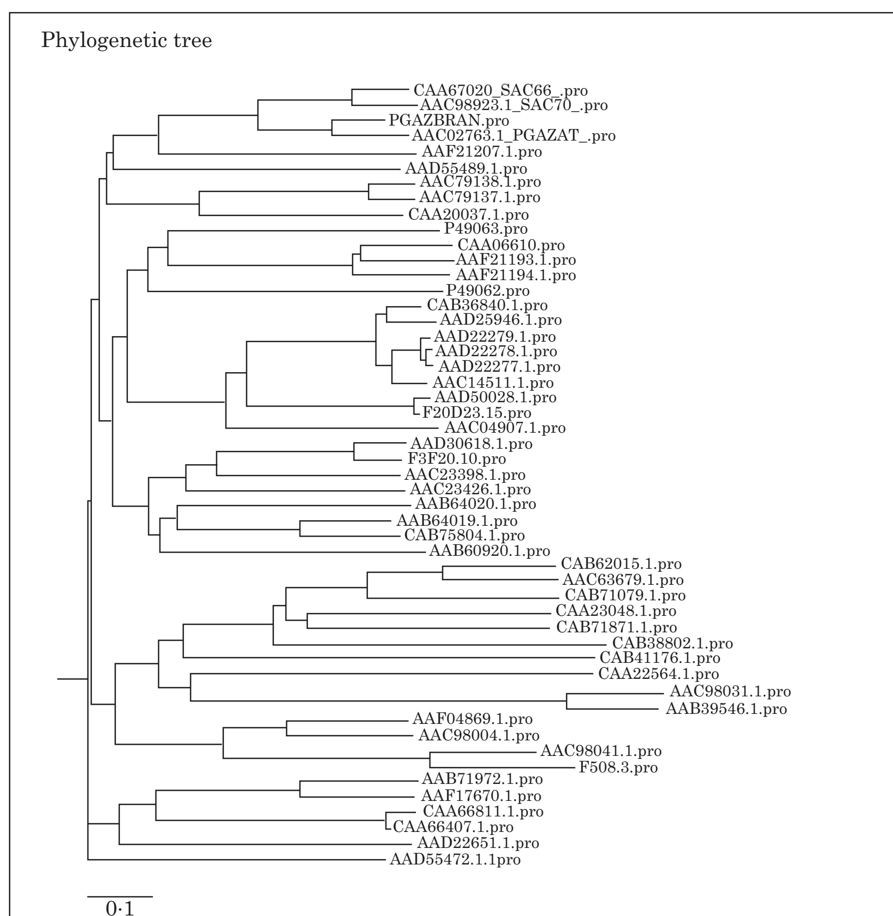

FIG. 2. Phylogenetic tree of *Arabidopsis* putative polygalacturonases and abscission zone (PGAZBRAN) and dehiscence zone (SAC66) polygalacturonases from *B. napus*. Accession numbers are shown for reference purposes.

and perhaps some of the cellulose microfibrils. Another potential candidate for wall degradation is the enzyme expansin that has now been documented to play a role in developmental processes such as growth (McQueen-Mason and Cosgrove, 1995) and fruit ripening (Rose *et al*., 1997). Recent work has shown that the enzyme increases in activity specifically in the abscission zone of ethylene-treated *S. nigra* leaflets (McQueen-Mason, pers. comm.). At least three different cDNAs have been cloned from this tissue and the expression of one of these has been shown to increase specifically in the zone tissue (Belfield, pers. comm.). It has been reported that expansins share a similar secondary structure to Group 1 pollen allergens (Cosgrove *et al*., 1997) and a recent report has revealed that an allergen-like mRNA is up-regulated during abscission in *S. nigra* (Ruperti *et al*., 1999). However, the peptide encoded by the abscission-related mRNA exhibits homology to the Olee1 group of allergens and there is no evidence that these proteins have expansin-like activity. However, the carboxy terminus of this peptide resembles extensin-like proteins (PELPs) (de Goldman *et al*., 1992) and since extensins have been proposed to form part of the wall matrix it is possible that in some way these proteins may influence the integrity of the cell wall. Another enzyme that has been proposed to contribute to wall loosening is xyloglucan endotransglycosylase (XET) (Fry *et al*., 1992). No reports of an association between the activity of XET and abscission have appeared although it has been shown that an XET is specifically up-regulated in the dehiscence zone of *B. napus* pods at the final stages (70 DAA) of pod opening (Whitelaw, unpubl. res.).

Other genes up-regulated during cell separation

The search for abscission-related genes has largely been the consequence of a targeted approach employing either heterologous probes from other species or tissues, or RT-PCR amplification based on degenerate nucleotide sequences. Alternative strategies such as differential screening or differential display RT-PCR make no predictions about the nature of the gene products that are being sought. Moreover, both have proved effective in the quest to identify gene products that are associated with cell separation.

Differential screening is a relatively insensitive strategy and to be effective requires the two populations of mRNAs that are under examination to come from distinct tissues. Excision of most abscission zones would primarily result in a substantial contamination by adjacent non-separating cells. However, the size of the leaflet abscission zone in

S. nigra means that separating cells can be isolated in a relatively pure state. Coupe *et al.* (1997) used this system successfully to isolate a spectrum of gene products that were up-regulated during ethylene-promoted cell separation. In general the abscission-related proteins were found to encode pathogenesis-related (PR) proteins. These included a polyphenoloxidase (PPO), PR-1 and PR-4 type proteins, acidic and basic chitinases, and a protease inhibitor. In addition a metallothionein (MT)-like protein was also found to be up-regulated during abscission (Coupe *et al.*, 1995). The expression of MT-like proteins has been shown to be stimulated by ethylene and to increase during such events as fruit ripening (Ledger and Gardner, 1994) and senescence (Hanfrey *et al.*, 1996). The increase in expression of PR-proteins during abscission is not an unexpected observation since the consequence of cell separation of the epidermal cells would be to allow access of the body of the tissue to bacterial and fungal pathogens. It would be predicted that other sites where cell separation pierces the outer tissues, such as during lateral root emergence, might also be associated with an increase in expression of PR-like proteins.

A number of interesting observations have come from this study of PR-protein expression in *S. nigra*. Firstly, the accumulation of these abscission-related mRNAs is not always a consequence of the presence of ethylene. For instance, the PR-4 mRNA accumulates in abscission cells aged for 24 h to the same degree whether the material is exposed to ethylene or not (Coupe *et al.*, 1995). Other genes seem to have an absolute requirement for ethylene (PPO and class IV chitinase). This would suggest that abscission-related genes fall into more than a single category in terms of their regulation of expression (Gonzalez-Carranza *et al.*, 1998). What is perhaps even more significant is that in contrast to the mRNAs encoding β-1,4-glucanase and PG, the mRNAs of the PR proteins are not degraded when examined by northern analysis (Taylor *et al.*, 1994). There are two possible explanations for this. The mRNAs encoding cell wall degrading enzymes could be specifically targeted for breakdown. The alternative is that the two populations of mRNAs are expressed in different cells and in one group the nucleic acids are being broken down by the degenerative processes associated with cell separation. Certainly the cells exposed at the fracture surface swiftly undergo desiccation and death. If this hypothesis were correct then at least two types of abscission zone cell exist. One secretes the wall hydrolysing enzymes that bring about cell separation and the other produces proteins that protect the cells immediately adjacent to the fracture surface from pathogenic attack (Roberts, 2000).

Recently, a receptor like protein kinase (HAESA) has been shown to be expressed specifically at the site of floral organ abscission in *Arabidopsis* (Jinn *et al.*, 2000). Down-regulation of expression using an antisense strategy significantly delayed flower abscission and the severity of the response was directly correlated to the amount of HAESA protein that could be detected. The function of the peptide is unclear although it is more likely to be involved in co-ordinating the phenomenon of cell separation rather than bringing about wall loosening directly.

Differential screening has also proved effective at isolating dehiscence-related genes. Two genes were isolated by Coupe *et al.* (1993, 1994) that exhibit a pattern of expression that is closely related to pod development. The first is a proline-rich gene that has homology to a range of peptides in the SWISSPROT database. Many of these are hybrid peptides that appear to be secreted and have been shown to be up-regulated in response to a range of environmental and developmental stimuli (Goodwin *et al.*, 1996; Yasuda *et al.*, 1997). The other dehiscence-related gene encodes a peptide that currently has an unknown function although it shares some homology with dehydrogenases.

THE MANIPULATION OF CELL SEPARATION

The regulation of cell separation is not only of inherent scientific interest but it also has considerable commercial significance. Thus an understanding of the events that bring about abscission or dehiscence has considerable value to both the agricultural and horticultural industries. Indeed, the ability to either accelerate or delay the abscission process could have benefits for the grower. For instance, if abscission was prevented losses associated with premature flower or fruit drop might be reduced. Similarly an extension of the longevity of a leaf could contribute to assimilate production as long as the lamina was still acting as a significant source and not as a drain on other organs. A delay in the abscission process might be achieved either by manipulating the expression of key wall hydrolases such as PG and β-1,4-glucanase or perhaps by attenuating the sensitivity of the abscission zone cells to ethylene or IAA. Little is known about the phenomenon of seed abscission, however, a delay in the separation of the seed from the funiculus might alter the balance of storage reserves and could have major consequences on seed yield or seed composition. Impeding the differentiation of abscission zone cells by ectopically expressing a gene such as *Lateral suppressor* (*Ls*) might provide a mechanism whereby abscission was prevented. Such a strategy could have value in controlling seed spread of weed species such as *Avena fatua* which, when shed, may remain dormant within the soil for many years.

The accelerated loosening of ripe fruit would have significant advantages for successful harvesting and this would be particularly beneficial if fruit could be loosened without the associated shedding of leaves. Currently this is achieved by the application of ethylene releasing agents, however it is common for both young and old fruit to be affected to a similar degree and even leaves may be shed as a consequence of the application of chemicals such as ethephon. If different cell wall degrading enzymes are responsible for wall breakdown at the site of fruit and leaf abscission then this might provide a mechanism for the manipulation of the process at one site and not the other. An additional advantage of accelerated shedding might be towards the end of the growing season when remaining flowers or young fruit may not reach maturity but primarily be a site for pathogen attack.

Pod shatter or dehiscence is a major factor limiting the yield of an oilseed rape crop. In addition to seed being lost from the pods and falling to the ground (Macleod, 1981), growers experience the additional problem of weed oilseed rape plants establishing themselves in subsequent years. If the susceptibility of pods to undergo pod shatter could be reduced or eliminated there would be significant benefits to the farmer, the processing industry, and the consumer. For instance, harvesting costs would be reduced as the application of compounds to desiccate the crop and its subsequent swathing would not be necessary. Increased seed recovery would mean that the area of land required to achieve sustainable yields would be reduced and a smaller quantity of herbicides would need to be applied to control volunteer plants in subsequent growing seasons. Inhibition of pod opening might also reduce infestations due to such pests as pod midge. Other crops where seeds are released from pods might also benefit from this type of manipulation including soybean and lentils.

One strategy that has been shown to be effective in this regard is the ablation of those cells that secrete the cell wall degrading enzymes that precipitate shatter. Unfortunately the activity of the *PG* promoter in the stomium region of the pod also brought about sterility. This unfortunate side effect means that additional work needs to be undertaken to determine whether different regions of the *PG* promoter may have the capacity to regulate expression in the anther and the pod. The generation of male sterile plants using this approach is in itself also of potential value especially as a means of restricting the release of transgenic pollen. This might have some value for crops grown for their vegetative rather than fruit or seed components such as leafy vegetables. The problem that has to be overcome before this might be put into practice is the bulking up of seeds prior to sale to the grower. Alternative ways by which dehiscence might be manipulated is through regulating the differentiation of the dehiscence zone or via the ligand that co-ordinates the cell separation process.

CONCLUSIONS

Considerable progress has been made over the last 10 years in our understanding of some of the events associated with cell separation processes in plants. In particular the development of molecular biological techniques and the use of transgenic plants have enabled us to ask more probing questions about the role of enzymes such as β-1,4-glucanase and polygalacturonase during such processes as abscission and dehiscence. The strategies are available by which we can examine the regulation of wall degradation during other developmental phenomena and the use of model organisms such as *Arabidopsis* and tomato will further assist us in achieving this goal.

ACKNOWLEDGEMENTS

The authors would like to thank many colleagues for providing prepublication information that has been included in this review. JAR would also like to acknowledge the tireless work of postgraduate students and postdoctoral workers who have contributed to the story over the last 10 years. BBSRC and BIOGEMMA UK have provided financial support for much of this work.

LITERATURE CITED

Addicott FT. 1982. *Abscission*. Berkeley, Los Angeles, London: University of California Press.

Beal JM, Whiting AG. 1945. Effect of indoleacetic acid in inhibiting stem abscission in *Mirabilis jalapa*. *Botanical Gazette* **106**: 420–431.

Berger R, Reid P. 1979. Role of polygalacturonase in bean leaf abscission. *Plant Physiology* **63**: 1133–1137.

Bleecker AB, Patterson SE. 1997. Last exit: senescence, abscission and meristem arrest in *Arabidopsis*. *The Plant Cell* **9**: 1169–1179.

Bonghi CN, Rascio A, Ramina A, Casadoro G. 1992. Cellulase and polygalacturonase involvement in the abscission of leaf and fruit explants of peach. *Plant Molecular Biology* **20**: 839–848.

Bornmann CH, Addicott FT, Lyon JL, Smith DE. 1968. Anatomy of gibberellin-induced stem abscission in cotton. *American Journal of Botany* **55**: 369–375.

Brummell DA, Hall BD, Bennett A. 1999. Antisense suppression of tomato endo-1,4-β-glucanase Cel2 mRNA accumulation increases the force required to break fruit abscission zones but does not affect fruit softening. *Plant Molecular Biology* **40**: 615–622.

Chauvaux N, Child R, John K, Ulvskov P, Borkhardt B, Prinsen E, Van Onckelen HA. 1997. The role of auxin in cell separation in the dehiscence zone of oilseed rape pods. *Journal of Experimental Botany* **48**: 1423–1429.

Child R, Chauvaux N, John K, Ulvskov P, Van Onckelen HA. 1998. Ethylene biosynthesis in oilseed rape pods in relation to pod shatter. *Journal of Experimental Botany* **49**: 829–838.

Cosgrove DJ, Bedinger P, Durachko DM. 1997. Group I allergens of grass pollen as cell wall loosening agents. *Proceedings of the National Academy of Sciences USA* **94**: 6559–6564.

Coupe SA, Taylor JE, Roberts JA. 1995. Characterisation of an mRNA encoding a metallothionein-like protein that accumulates during ethylene-promoted abscission of *Sambucus nigra* L. leaflets. *Planta* **197**: 442–447.

Coupe SA, Taylor JE, Roberts JA. 1997. Temporal and spatial expression of mRNAs encoding pathogenesis-related proteins during ethylene-promoted leaflet abscission in *Sambucus nigra*. *Plant Cell and Environment* **20**: 1517–1524.

Coupe SA, Taylor JE, Isaac PG, Roberts JA. 1993. Identification and characterisation of a proline-rich mRNA that accumulates during pod development in oilseed rape (*Brassica napus* L.). *Plant Molecular Biology* **23**: 1223–1232.

Coupe SA, Taylor JE, Isaac PG, Roberts JA. 1994. Characterization of a mRNA that accumulates during development of oilseed rape pods. *Plant Molecular Biology* **24**: 223–227.

Dale JE, Milthorpe FL. 1981. General features of the production and growth of leaves. In: Dale JE, Milthorpe FL, eds. *The growth and functioning of leaves*. Cambridge: Cambridge University Press, 151–178.

del Campillo E, Bennett AB. 1996. Pedicel breakstrength and cellulase gene expression during tomato flower abscission. *Plant Physiology* **111**: 813–820.

de Goldman ME, Pezzotti M, Seurinck J, Mariani C. 1992. Developmental expression of tobacco pistil-specific genes encoding novel extensin-like proteins. *The Plant Cell* **4**: 1041–1051.

Drew MC, He C-J, Morgan PW. 2000. Ethylene-triggered cell death during aerenchyma formation in roots. In: Bryant JA, Hughes SG, Garland JM, eds. *Programmed cell death in animals and plants*. Oxford: BIOS Scientific Publishers Ltd., 183–192.

Durbin M, Sexton R, Lewis LN. 1981. The use of immunological methods to study the activity of cellulase isozymes (β 1:4 glucan 4-glucan hydrolase) in bean leaf abscission. *Plant Cell and Environment* **4**: 67–73.

Ferrandiz C, Pelaz S, Yanofsky MF. 1999. Control of carpel development. *Annual Reviews of Biochemistry* **68**: 321–354.

Ferrarese L, Trainotti L, Moretto P, Polverino de Laureto P, Rascio N, Casadoro G. 1995. Differential ethylene-inducible expression of cellulase in pepper plants. *Plant Molecular Biology* **29**: 735–747.

Fry SC, Smith RC, Renwick KF, Martin DJ, Hodge SK, Matthews KG. 1992. Xyloglucan endotransglycosylase, a new wall-loosening enzyme activity from plants. *Biochemical Journal* **282**: 821–828.

Gonzalez-Carranza ZH, Lozoya-Gloria E, Roberts JA. 1998. Recent developments in abscission: shedding light on the shedding process. *Trends in Plant Science* **3**: 10–14.

Goodwin W, Palls JA, Jenkins GI. 1996. Transcripts of a gene encoding a putative cell wall-plasma membrane linker protein are specifically cold-induced in *Brassica napus*. *Plant Molecular Biology* **31**: 771–781.

Gu Q, Ferrandiz C, Yanofsky M, Martienssen R. 1998. The FRUITFULL MADS-box gene mediates cell differentiation during *Arabidopsis* fruit development. *Development* **125**: 1509–1517.

Hadfield KA, Rose JKC, Yaver DS, Berka RM, Bennett AB. 1998. Polygalacturonase gene expression in ripe melon fruit supports a role for polygalacturonase in ripening-associated pectin disassembly. *Plant Physiology* **117**: 363–373.

Hanfrey C, Fife M, Buchanan-Wollaston V. 1996. Leaf senescence in *Brassica napus*: expression of genes encoding pathogenesis-related proteins. *Plant Molecular Biology* **30**: 597–609.

Hawes MC, Lin H-J. 1990. Correlation of pectolytic enzyme activity with the programmed release of cells from root caps of pea (*Pisum sativum*). *Plant Physiology* **94**: 1855–1859.

Jackson MB, Osborne DJ. 1970. Ethylene, the natural regulator of leaf abscission. *Nature* **225**: 1019–1022.

Jenkins ES, Paul W, Coupe SA, Bell SJ, Davies EC, Roberts JA. 1996. Characterization of an mRNA encoding a polygalacturonase expressed during pod development in oilseed rape (*Brassica napus* L.). *Journal of Experimental Botany* **47**: 111–115.

Jenkins ES, Paul W, Craze M, Whitelaw CA, Weigand A, Roberts JA. 1999. Dehiscence-related expression of an *Arabidopsis thaliana* gene encoding a polygalacturonase in transgenic plants of *Brassica napus*. *Plant Cell and Environment* **22**: 159–167.

Jinn T-L, Stone JM, Walker JC. 2000. *HAESA*, an *Arabidopsis* leucine-rich repeat receptor Chinese, controls floral organ abscission. *Genes and Development* **14**: 108–117.

Kalaitzis P, Koehler SM, Tucker ML. 1995. Cloning of tomato polygalacturonase expressed in abscission. *Plant Molecular Biology* **28**: 647–656.

Kalaitzis P, Solomos T, Tucker ML. 1997. Three different polygalacturonases are expressed in tomato leaf and flower abscission, each with a different temporal expression pattern. *Plant Physiology* **113**: 1303–1308.

Keijzer CJ. 1987. The process of anther dehiscence and pollen dispersal. I. The opening mechanism of longitudinally dehiscing anthers. *New Phytologist* **105**: 487–498.

Lashbrook CC, Gonzalez-Boch C, Bennett AB. 1994. Two divergent endo-β-1,4-glucanase genes exhibit overlapping expression in ripening fruit and abscising flowers. *Plant Cell* **6**: 1485–1493.

Lashbrook CC, Giovannoni JJ, Hall BD, Fischer RL, Bennett AB. 1998. Transgenic analysis of tomato endo-β-1,4-glucanase gene function. Role of *cel1* in floral abscission. *Plant Journal* **13**: 303–310.

Ledger SE, Gardner RC. 1994. Cloning and characterization of five cDNAs for genes differentially expressed during fruit development of kiwifruit (*Actinidia deliciosa* var. *deliciosa*). *Plant Molecular Biology* **25**: 877–886.

Liljegren SJ, Ferrandiz C, Alvarez-Buylla ER, Pelaz S, Yanofsky MF. 1998. *Arabidopsis* MADS-box genes involved in fruit dehiscence. *Flowering Newsletter* **25**: 9–19.

Lipe JA, Morgan PW. 1972. Ethylene: role in fruit abscission and dehiscence processes. *Plant Physiology* **50**: 759–764.

Macleod J. 1981. Harvesting. *Oilseed rape book*. Cambridge: Cambridge Agricultural Publications, 107–119.

McManus MT, Osborne DJ. 1990. Evidence for the preferential expression of particular polypeptides in leaf abscission zones of the bean *Phaseolus vulgaris*. *Journal of Plant Physiology* **136**: 391–397.

McManus MT, Osborne DJ. 1991. Identification and characterisation of an ionically-bound cell wall glycoprotein expressed preferentially in the leaf rachis abscission zone of *Sambucus nigra* L.. *Journal of Plant Physiology* **138**: 63–67.

McManus MT, Thompson DS, Merriman C, Lyne L, Osborne DJ. 1998. Transdifferentiation of mature cortical cells to functional abscission cells in bean. *Plant Physiology* **116**: 891–899.

McQueen-Mason SJ, Cosgrove DJ. 1995. Expansin mode of action on cell walls. Analysis of wall hydrolysis, stress relaxation, and binding. *Plant Physiology* **107**: 87–100.

Meakin PJ, Roberts JA. 1990*a*. Dehiscence of fruit in oilseed rape. 1. Anatomy of pod dehiscence. *Journal of Experimental Botany* **41**: 995–1002.

Meakin PJ, Roberts JA. 1990*b*. Dehiscence of fruit in oilseed rape. 2. The role of cell wall degrading enzymes. *Journal of Experimental Botany* **41**: 1003–1011.

Meakin PJ, Roberts JA. 1991. Induction of oilseed rape pod dehiscence by *Dasineura brassica*. *Annals of Botany* **67**: 193–197.

Noel ARA, Van Staden J. 1975. Phyllomorph senescence in *Streptocarpus molweniensis*. *Annals of Botany* **39**: 921–929.

Osborne DJ. 1989. Abscission. *Critical Reviews in Plant Sciences* **8**: 103–129.

Osborne DJ, Sargent J. 1976. The positional differentiation of ethylene-responsive cells in rachis abscission zones in leaves of *Sambucus nigra* and their growth and ultrastructural changes at senescence and separation. *Planta* **130**: 203–210.

Peng J, Richards DE, Hartley NM, Murphy GP, Devos KM, Flintham JE, Beales J, Fish LJ, Worland AJ, Peica F, Sudhakar D, Christou P, Snape JW, Gale MD, Harberd NP. 1999. 'Green revolution' genes encode mutant gibberellin response modulators. *Nature* **400**: 256–261.

Peretto R, Favaron F, Bettini V, De Lorenzo G, Marini S, Alghisi P, Cervone F, Bonfante P. 1992. Expression and localization of polygalacturonase during the outgrowth of lateral roots in *Allium porrum* L. *Planta* **188**: 164–172.

Peterson M, Sander L, Child R, Van Onckelen H, Ulvskov P, Borkhardt B. 1996. Isolation and characterization of a pod dehiscence zone-specific polygalacturonase from *Brassica napus*. *Plant Molecular Biology* **31**: 517–527.

Pierik RLM. 1977. Induction of secondary abscission in apple pedicels *in vitro*. *Physiologia Plantarum* **39**: 271–274.

Pierik RLM. 1980. Hormonal regulation of secondary abscission in pear pedicels *in vitro*. *Physiologia Plantarum* **48**: 5–8.

Roberts JA. 2000. Abscission and dehiscence. In: Bryant JA, Hughes SG, Garland JM, eds. *Programmed cell death in animals and plants*. Oxford: BIOS Scientific Publishers Ltd., 203–211.

Roberts JA, Schindler CB, Tucker GA. 1984. Ethylene-promoted tomato flower abscission and the possible involvement of an inhibitor. *Planta* **160**: 159–163.

Roberts JA, Coupe SA, Taylor JE, Whitelaw CA. 1997. Spatial and temporal expression of abscission-related genes during ethylene-promoted organ shedding. In: Kanellis AK, Chang C, Kende H, Grierson D, eds. *Biology and biotechnology of the plant hormone ethylene*. NATO Series. 185–190.

Rose JKC, Lee HH, Bennett AB. 1997. Expression of a divergent expansin gene is fruit-specific and ripening related. *Proceedings of the National Academy of Sciences, USA* **94**: 5955–5960.

Ruperti B, Whitelaw CA, Roberts JA. 1999. Isolation and expression of an allergen-like mRNA from ethylene-treated *Sambucus nigra* leaflet abscission zones. *Journal of Experimental Botany* **50**: 733–734.

Sack FD. 1987. The development and structure of stomata. In: Zieger E, Farquhar GD, Cowan IR, eds. *Stomatal function*. Stanford: Stanford University Press, 59–89.

Sargent JA, Osborne DJ, Dunford SM. 1984. Cell separation and its hormonal control in the Gramineae. *Journal of Experimental Botany* **35**: 1663–1667.

Schumacher K, Schmitt T, Rossberg M, Schmitz G, Theres K. 1999. The *Lateral suppressor* (*Ls*) gene of tomato encodes a new member of the VHIID protein family. *Proceedings of the National Academy of Science USA* **96**: 290–295.

Sexton R. 1976. Some ultrastructural observations on the nature of foliar abscission in *Impatiens sultani*. *Planta* **128**: 49–58.

Sexton R, Roberts JA. 1982. Cell biology of abscission. *Annual Review of Plant Physiology* **33**: 133–162.

Sexton R, Durbin ML, Lewis LN, Thompson WW. 1980. Use of cellulase antibodies to study leaf abscission. *Nature* **283**: 873–874.

Spence J, Vercher Y, Gates P, Harris N. 1996. Pod shatter in *Arabidopsis thaliana*, *Brassica napus* and *B. juncea*. *Journal of Microscopy* **181**: 195–203.

Stevens RA, Martin ES. 1978. Structural and functional aspects of stomata. 1. Developmental studies in *Polypodium vulgare*. *Planta* **142**: 307–316.

Szymkowiak EJ, Irish EE. 1999. Interactions between *jointless* and wild-type tomato tissues during development of the pedicel abscission zone and the inflorescence meristem. *The Plant Cell* **11**: 159–175.

Taylor JE, Coupe SA, Picton SJ, Roberts JA. 1994. Isolation and expression of a mRNA encoding an abscission-related β 1,4-glucanase from *Sambucus nigra*. *Plant Molecular Biology* **24**: 961–964.

Taylor JE, Webb STJ, Coupe SA, Tucker GA, Roberts JA. 1993. Changes in polygalacturonase activity and solubility of polyuronides during ethylene-stimulated leaf abscission in *Sambucus nigra*. *Journal of Experimental Botany* **258**: 93–98.

Taylor JE, Tucker GA, Lasslett Y, Smith CJS, Arnold CM, Watson CF, Schuch W, Grierson D, Roberts JA. 1990. Polygalacturonase expression during leaf abscission of normal and transgenic tomato plants. *Planta* **183**: 133–138.

Thompson DS, Osborne DJ. 1994. A role for the stele in intertissue signaling in the initiation of abscission in bean leaves (*Phaseolus vulgaris* L.). *Plant Physiology* **105**: 341–347.

Torki M, Mandaron P, Mache R, Falconet D. 2000. Characterization of a ubiquitous expressed gene family encoding polygalacturonase in *Arabidopsis thaliana*. *Gene* **242**: 427–436.

Torki M, Mandaron P, Thomas F, Quigley F, Mache R, Falconet D. 1999. Differential expression of a polygalacturonase gene family in *Arabidopsis thaliana*. *Molecular and General Genetics* **261**: 948–952.

Tucker GA, Schindler CB, Roberts JA. 1984. Flower abscission in mutant tomato plants. *Planta* **160**: 164–167.

Tucker ML, Sexton R, del Campillo E, Lewis LN. 1988. Bean abscission cellulase: Characterization of a cDNA clone and regulation of gene expression by ethylene and auxin. *Plant Physiology* **88**: 1257–1262.

Warren Wilson J, Warren Wilson PM, Walker ES. 1987. Abscission sites in nodal explants of *Impatiens sultani*. *Annals of Botany* **60**: 693–704.

Warren Wilson PM, Warren Wilson J, Addicott FT, McKenzie RH. 1986. Induced abscission sites in internodal explants of *Impatiens sultani*: A new system for studying positional control. *Annals of Botany* **57**: 511–530.

Webb STJ, Taylor JE, Coupe SA, Ferrarese L, Roberts JA. 1993. Purification of β-1,4-glucanase from ethylene-treated abscission zones of *Sambucus nigra*. *Plant Cell and Environment* **16**: 329–333.

Webster BD, Leopold AC. 1972. Stem abscission in *Phaseolus vulgaris* explants. *Botanical Gazette* **133**: 292–298.

Wilson VR, Gould KS, Lovell PH, Aitken-Christie J. 1998. *In vitro* abscission of kauri (*Agathis australis*) branches. *New Zealand Journal of Botany* **36**: 495–501.

Wing RA, Zhang H-B, Tanksley SD. 1994. Map-based cloning in crop plants. Tomato as a model system: I. Genetic and physical mapping of *jointless*. *Molecular and General Genetics* **242**: 681–688.

Wong CH, Osborne DJ. 1978. The ethylene-induced enlargement of target cells in flower buds of *Ecballium elaterium* L. and their identification by the content of endoreduplicated nuclear DNA. *Planta* **139**: 103–111.

Woycicki Z. 1924. Recherches sur la déhiscence des anthères et le rôle du stomium. *Revue Générale de Botanie* **36**: 196–212.

Wright M, Osborne DJ. 1974. Abscission in *Phaseolus vulgaris*. The positional differentiation and ethylene-induced expansion growth of specialised cells. *Planta* **120**: 163–170.

Yasuda E, Ebinuma H, Wabiko H. 1997. A novel glycine-rich/hydrophobic 16 kDa polypeptide gene from tobacco: similarity to prolin-rich protein genes and its wound-inducible and developmentally regulated expression. *Plant Molecular Biology* **33**: 667–678.