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How is the Silique Fruit Dismantled over its Maturation?

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ABSTRACT

In dehiscent fruits, such as the silique of *Arabidopsis*, housed ripe seeds are dispersed into the surrounding environment through a process known as pod shattering. This seed-expelling process is a consequence of the partial and gradual dismantling of the silique architecture. The shattering occurs at a precise site in the silique (i.e. valve margins, made up of a separation layer and adjacent lignified layer) and involves a network of tightly regulated genes. Thus, (i) *INDEHISCENT* (*IND*) primarily directs the differentiation of the valve-margin cells into the separation and lignified layers; (ii) *SHATTERPROOF* (*SHP1*, *SHP2*), *ALCATRAZ* (*ALC*) and *IND* directs the valve-margin identity and pod shattering; (iii) *SHP*, *ALC*, *IND* and *FRUITFULL* (*FUL*) are required for lignification of the most internal valve-cell layer (en*b*); (iv) *REPLUMLESS* (*RPL*) and *FUL* have been found to set the boundaries of the genes that confer valve-margin identity; (v) *FUL* acts primarily in the valve to restrict the expression of *IND*, *SHP*, and *ALC* to the valve margin, rather than by playing a major role itself in specifying valve identity; (vi) *RPL* maintains the replum boundary by restricting the expression of *SHP* to the valve margin; (vii) *JAGGED* (*JAG*), that promotes lateral organ growth, and *YABBY3* (*YAB3*) and *FILAMENTOUS FLOWER* (*FIL*), which are both related to establishing abaxial polarity in lateral organs, are necessary for expression of *FUL* and *SHP* in the valve and valve margin, respectively; and (viii) *RPL* regulates *SHP* indirectly by restricting *JAG* and *FIL* expression from the replum. In this review, knowledge concerning the opening of *Arabidopsis* fruit is compared with other still less-known crucifer and non-crucifer species.

Keywords: Arabidopsis, Brassica, *Lotus corniculatus*, canola, dehiscence, pod-opening-zone, pod-shatter, silique, soybean, valve-margin
Abbreviations: AG, agamous; **ALC**, alcatraz; **bHLH**, basic helix-loop-helix; **CW**, cell wall; **en*b***, endocarp-*b* layer; **ET**, ethylene; **FIL**, filamentous flower; **FUL**, fruitful; **IND**, indehiscent; **JAG**, jagged; **RPL**, replumless; **SHP**, shatterproof; **STK**, seedstick; **SPOZ**, soybean pod opening zones; **WT**, wild type; **YAB**, YABBY

CONTENTS

INTRODUCTION

Brassicaceae is a large plant family (338 genera and 3,700 species) of major scientific and economic importance (Bailey *et al.* 2006). *Brassica* is the most economically important genus of Brassicaceae. Several species of this genus have been cultivated from ancient times. Thus, canola (*B. napus*, *B. rapa* and *B. juncea*) has emerged as an important agricultural plant and is now the second largest oilseed crop with an annual worldwide production of 38 million tons of oil (Economic Research Service 2001). In addition to its widespread use in food preparation, it is also used as a biofuel for transportation. A recent directive from the European Union aims to promote a step-wise substitution of conventional fuel, such as diesel and gasoline, by crop-derived biofuels (European Parliament 2003), thereby dramatically increasing the demand for an efficient breeding programme. *B. juncea* (Indian mustard) is becoming the oilseed crop of choice in both India and Australia as a result of its increased heat and drought tolerance in comparison with *B. napus* (Burton *et al.* 2003; Gupta *et al.* 2004). *B. juncea* can be also used in phytoremediation projects to clean up contaminated soils, as it is able to accumulate heavy metals more efficiently than other *Brassica* species (Epstein *et al.* 1999; Clemente *et al.* 2005).

Many plants have developed mechanisms to self-disperse seeds by highly modifying their fruit structure. Our ancestors began domesticating crop plants by selecting grains and legumes that had reduced seed-shattering characteristics. Thus, in the course of rice domestication, the artificial selection of non-shattering habit makes easy harvest and decreases harvest losses (Li *et al.* 2006). *Brassica* and *Arabidopsis* (both members of the Brassicaceae) are estimated to have diverged approximately 20 million years ago (Yang *et al.* 1999). This close relationship is also reflected in their similar overall fruit morphologies. *Arabidopsis* and *Brassica* plants disperse their seeds by a pod-shattering mechanism known as fruit dehiscence. Fruit dehiscence or pod shattering results in the opening of a seed pod and in the dispersal of its seeds. The pod shatters in the premature shedding of seeds from siliques prior to and during harvest. This process effectively discloses the mature seeds, which

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can then be released and scattered by rain or wind. Therefore dehiscence is an effective way for plants to optimise the chances of survival for the following generations. However, unsynchronised pod shattering constitutes huge losses for canola farmers. It has been reported that 11-25% of harvests are lost as a result of unsynchronised maturation (Price *et al.* 1996), and losses of up to 50% have been estimated in seasons when adverse weather conditions have delayed harvesting. Moreover, the prematurely released seeds fall to the ground where they germinate to become weeds (volunteers), hindering the crop rotation practice used by many farmers. In oilseed rape, pod shattering can cause a loss of up to 50% of the potential seed yield if harvesting is delayed by adverse conditions. Moreover, seeds that are shed and persist in the soil give rise to weed oilseed plants contaminating crops that are subsequently grown. These studies suggest that genetic strategies for controlling pod shattering could have global importance for canola farmers.

Pod shattering is not an isolated problem for *Arabidopsis* and *Brassica* plants, but has been recognized in several other dry-fruited crop plants including such legumes as birdsfoot trefoil (*Lotus corniculatus*) (Grant 1996; García-Díez and Steiner 2000), soybean (Philbrook and Oplinger 1989), and sesame (Day 2000). The mechanism of silique opening has been studied in detail in *Arabidopsis* and *Brassica* by means of microscopy and molecular biology techniques (Spence *et al.* 1996; Ferrándiz *et al.* 1999; Patterson *et al.* 2001; Ferrándiz *et al.* 2002; Roberts *et al.* 2002; Dinneny and Yanofsky 2005; Lewis *et al.* 2006). Data from dehiscence in *Arabidopsis* are helping to understand the pod-shattering mechanism (Christiansen *et al.* 2002). However, it is still not clear whether the genes identified in legumes share equivalent functions with their counterparts in *Arabidopsis*.

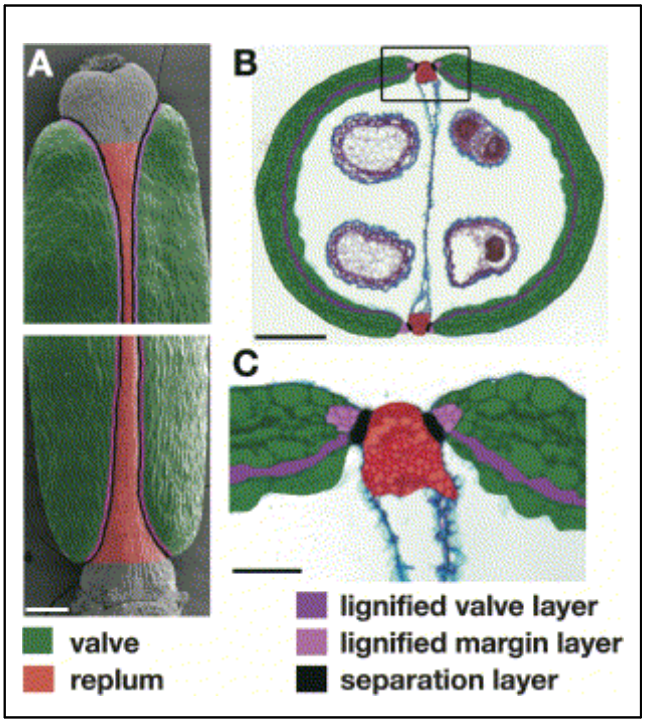

Fig. 1 General and transversal views of mature *Arabidopsis* fruit. WT silique (**A**) scanning electron micrograph of the apex and base of a mature WT of *Arabidopsis*, with the regions of the silique colorized as indicated. (**B**) Transverse section on a WT silique with the cell types colorized as in (**A**). (**C**) Close up of the valve margin region of the transverse section boxed in (**B**). Scale bars in (**A**) and (**B**) represent 200 μm, and in (**C**) represent 50 μm. Adapted from Liljegren *et al.* (2004).

SILIQUE DEVELOPMENTAL PATTERN IN OILSEED RAPE AND ARABIDOPSIS: STRUCTURAL ALTERATIONS

A study of oilseed rape (*B. napus*) pods shows that the fruit is a bivalve silique (i.e. carpel houses two seed-containing valves separated by a pseudoseptum and a replar region) (Picart and Morgan, 1984). The silique architecture of this crucifer and its importance for pod shattering has also been described in detail (Morgan *et al.* 1998). Development of this pod is divided into three stages: (1) the first one (0-20 days after anthesis, DAA), in which the silique reaches its maximum length and the two dehiscence zones (i.e. region 1-3 cells wide that separates the vascular tissue from the valve edges) are distinguished at the carpel margins adjacent to the septum and runs the whole length of the silique (20 DAA) (Meakin and Roberts, 1990); (2) during the second stage (20-50 DAA), secondary cell wall (CW) material is deposited in the walls of valve-edge cells, and the replum (pod framework which remains after the valves drop off) becomes progressively lignified (maximum of lignification at 35 DAA) (Meakin and Roberts 1990); after 35 DAA, the dehiscence zones are enclosed by thickened tissues, and the cells exhibit a progressive reduction in both volume and organellar content, and from 40 DAA onwards CW degradation occurs in dehiscence zones, resulting in a loss in cellular cohesion (Petersen *et al.* 1996); and (3) the third developmental stage, from 50-70 DAA (third stage of development) the lignified cells undergo senescence (i.e. pods becomes desiccated, tensions in the silique wall caused primarily by the lignification of the endocarp cells surrounding the dehiscence zones are created, and the weakened dehiscence zone CW eventually gives way, resulting in the shattering of the pod and release of the seeds) (Spence *et al.* 1996). In *B. juncea* lines that have a reduced tendency to shatter the endocarp layer (end*b*) is not completely lignified (Spence *et al.* 1996). Although the *Arabidopsis* fruit structure is typical of several thousand species of *Brassicaceae*, including oil-seed crops such as canola, the replum morphology varies considerably (Brücker 2000). Thus, at the replum of *Allairia petiolata* of Cavara et Grande fruit is very large and protrudes from the fruit in a manner that is reminiscent of the *ful* mutant replum. In contrast, *B. napus* fruit forms a suture with no external replum where the valve margins come together in a V shape (Meakin and Roberts 1990), which is reminiscent of the *rpl-3* mutant fruit.

By contrast to oilseed rape, the female floral organ of *A. thaliana* (**Fig. 1**) was extensively studied. Thus, gynoecium is divided into four different parts: (a) the apex, a stigmatic tissue on which the pollen grain adheres and germinates; (b) the transmitting tract, a connecting apex-ovary tissue that exude sugars, proteins and signals that feed and guide the pollen tube to the ovules (Johnson and Preuss 2004); (c) the ovary, the longest part of gynoecium that houses the ovules; and (d) the gynophore, that attaches the ovary to the flower. While all of the tissue layers present in the mature silique are already formed in the gynoecium before fertilization (Spence *et al.* 1996; Vivian-Smith *et al.* 2001), tissues of the valve and valve margin region require as yet totally unknown signals produced by post-fertilization processes to acquire their final differentiated state. That is, correct spatial regulation of where the dehiscence zone is drawn and when dehiscence occurs is crucial for successful seed dispersal. The signalling carried out by the housed seeds may be key in the pod-shattering process (Chaudhury *et al.* 1997; Vivian-Smith *et al.* 2001).

The general pattern of pod development in *Arabidopsis* is similar to that of oilseed rape, although the whole process occurs at a much faster rate (Ferrándiz *et al.* 1999). No differences in silique dehiscence have been recorded within different ecotypes of *Arabidopsis*. In the *Arabidopsis* maturating silique (**Fig. 2**), the valve consists of several cell layers (Spence *et al.* 1996; Vivian-Smith *et al.* 2001): (1) the outermost one (epidermic layer) has undifferentiated stomata before fertilization; after fertilization, these stomatic apparatus end their differentiation, and the gas exchange is initiated; (2) under epidermic layer there are three cell layers that include photosynthetic cells which are transformed

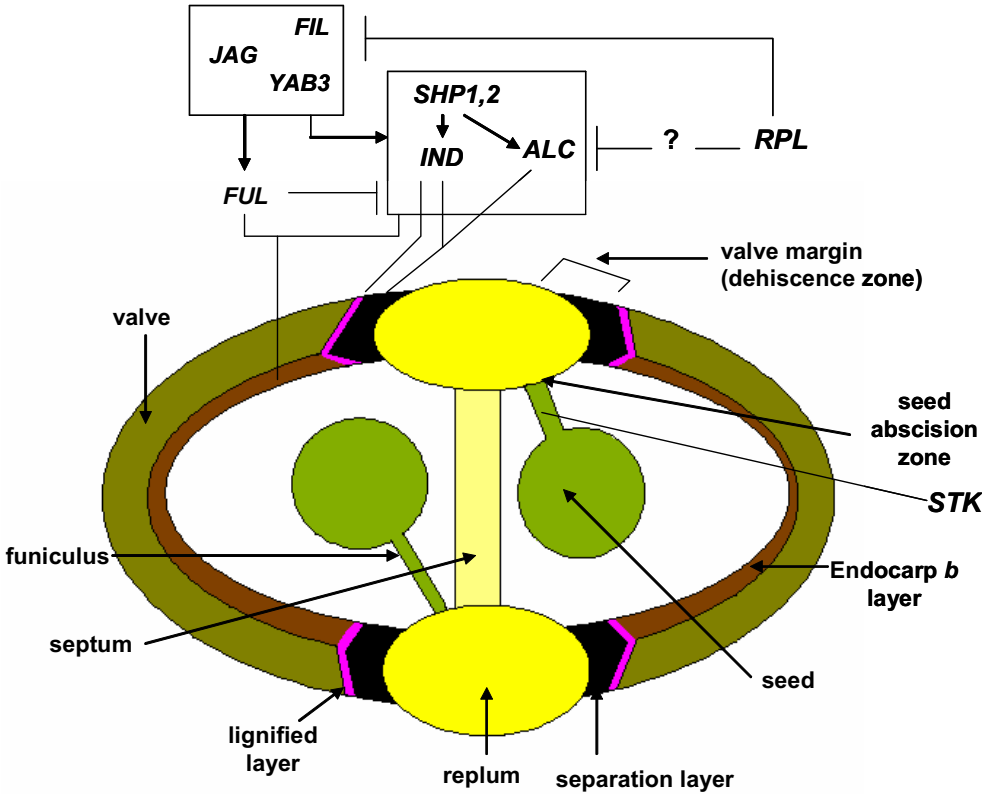

Fig. 2 Diagramatic view of the transverse cross section of mature silique of *Arabidopsis* and genetic pathway controlling its development. The role of two set of genes and individual genes is described within the manuscript.

after fertilization in auxiliary vascular strands; (3) the most-internal cell layers are termed endocarp layer-*b* (en*b*) and endocarp layer-*a* (en*a*), respectively; whereas en*a* degenerates during silique maturation, en*b* is enriched in lignin. On the other hand, at the valve margins, two tissue layers differentiated into the dehiscence zone where the valves will separate from the replum. These cell layers are termed lignified and separation layer, respectively; the first one being continuous with the en*b* and will form the spring-like tension mechanism that drives the separation of the valves from replum during pod shattering (Spence *et al.* 1996). A separation process is considered an event that dissolves the adhesive substance (middle lamella) that holds plant cells together and/or that degrades their CW. Thus, dehiscence describes events that involve the release of an organ's internal contents, such as when a fruit opens to scatter its seeds. During the dehiscence event, separation usually occurs in specialized, narrow bands of cells termed dehiscence zones. In *Arabidopsis*, the separation layer degenerates the middle lamella between adjacent CW and separate from each other during dehiscence, CW dismantling enzymes (i.e. polygalacturonases) being involved (Petersen *et al.* 1996; Jenkins *et al.* 1999). A similar process where tensions in the pod and a zone of weakness contribute to pod dehiscence has also been described for other species, including sesame (Day 2000) and soybean (Tiwari and Bhatia 1995).

ANATOMIC DIFFERENCES OF THE POD OPENING ZONE BETWEEN SOYBEAN AND CRUCIFERS

Soybean, an ancient crop, has gained increasing importance as an inexpensive source of protein and edible oil in the past few decades. This fruit also undergoes shattering, this trait being highly dependent on the cultivar (Tiwari and Bhatnagar 1991). Anatomy of two-valvar soybean pod has been studied in detail (Esau 1977) and certain structures are important for resistance to shattering. Thus, length and thickness of the bundle cap as well as the thickness of the pod wall have been found to correlate negatively with shatter susceptibility (Tiwari and Bhatia 1995). As referred before, the problem of pod shattering has undoubtedly attracted the greatest attention in oilseed rape and in the model plant *Arabidopsis*. Soybean pods consist of a single carpel that encloses the central cavity where the seeds are housed. Each of the two sides of the pod, has a suture, the dorsal and ventral, where the pods open at maturity (**Fig. 3**). Soybean pod opening zones (SPOZ) present a dehiscence zone beneath each suture. SPOZ are functionally equivalent to those found in crucifers but not exact copies, as the ventral dehiscence zone does not span the entire pod wall. This fact corroborates the contention that pods have evolved from a single leaf where the leaf margins have merged at the dorsal suture, thus squeezing the seeds. Therefore the soybean ventral suture should be a remnant of the leaf midrib. In contrast, cruciferous siliques have evolved from two merging leaves. Likewise, whereas the parenchyma cells in the SPOZ are clearly distinguishable from the surrounding valve-edge cells by their morphology and CW, the dehiscence zone in crucifers at roughly the same stage are distinguishable only by their size (Meakin and Roberts 1990). The middle lamella has largely disappeared in the late stage of the dehiscence zone of the mature yellow pod, thereby weakening adhesion between the opposite edges of the valve. The valve-edge cells have all synthesised a large secondary CW, as opposed to the dehiscence-zone cells that have not. A remarkable difference with regard oilseed rape is that at the time of silique opening, cells in the dehiscence zone are floating freely in the extracellular matrix with their primary wall severely thinned (Petersen *et al.* 1996). Pod shattering usually commences on the dorsal side of the pod (Tiwari and Bhatia 1995). This is consistent with the feature that the ventral dehiscence zone does not span the mesocarp and therefore greater force is required to break open the pod on the ventral side than on the dorsal side where only the fibre cap cells connect the valve edges at maturity. In oilseed rape the dehiscence-zone cells, although stripped of most of their CW, remain viable and retain their size and shape even at the point of dehiscence. Pod opening in soybean is a consequence of the weakening of the dorsal and ventral dehiscence zone combined with tension building up in the senescing pod. For a extensive and comprehensive study related to the dehiscence zone in pod soybean, see Christiansen *et al*. (2002).

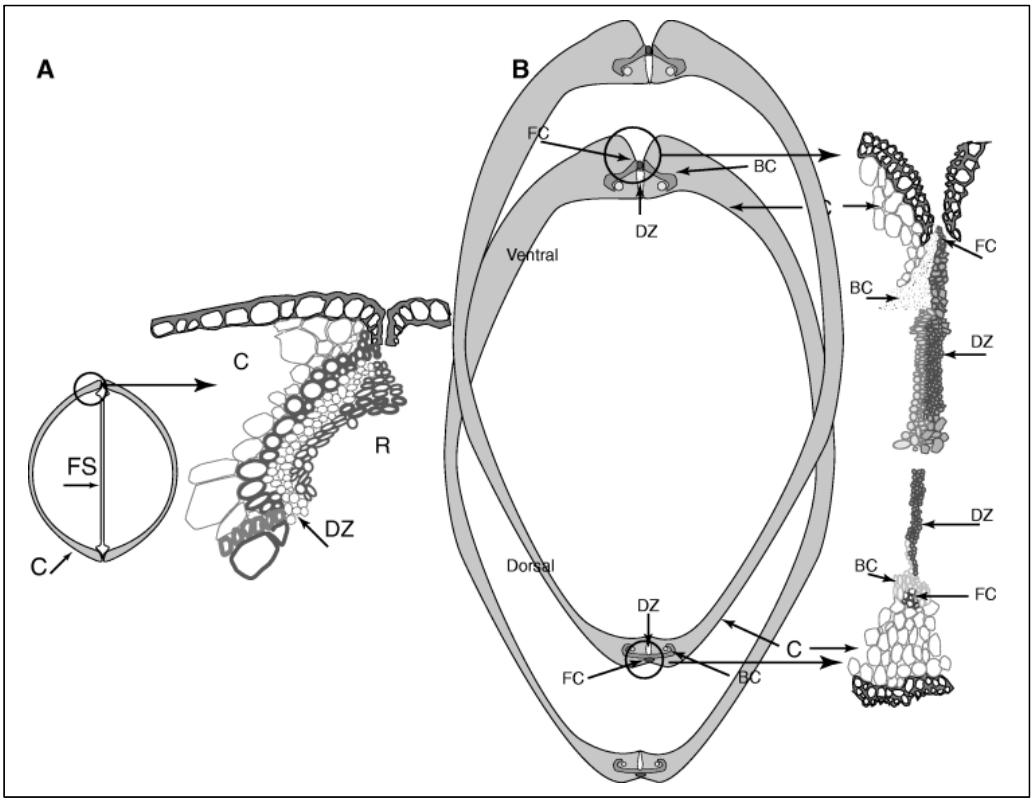

Fig. 3 Representation of cruciferous silique and soybean pod. (**A**) Cross-section of a silique. The encircled area is enlarged to visualize the area containing one of the dehiscence zone. (**B**) Cross-sections of soybean pods. The areas around the dorsal and ventral sutures are depicted and the dehiscence zones are enlarged for comparison with the silique. BC, bundle cap; C, carpel; DZ, dehiscence zone; FS, false septum; R, replum; FC, fibre cap cells. Adapted from Christiansen *et al.* (2002).

MUTATIONS THAT AFFECT SILIQUE OPENING

In the recent years, a great number of genes involved in both development and disruption of silique have been isolated and studied in depth. Some of genes involved in dismantling of dehiscence zone of *A. thaliana* are included in **Table 1**. During the development of the gynoecium, the transcription factors SHATTERPROOF1 and 2 (*SHP1* and *SHP2*; two MADS-box genes previously known as *AGL1* and *AGL5* that share 87% identity at the amino-acid sequence level and show almost identical expression patterns in developing fruit) are expressed at the valve margin. The results of this expression appear to indicate that both genes may function both to specify the valve margin and to direct dehiscence-zone development in the mature fruit. The SHP mutants also deserve attention in that they provide an example of the importance of differentiation of unique cells within the region of cell separation. Although both single mutants have putative loss-of-function alleles, *shp1-1* and *shp2-1*, the fruit show no detectable differences from WT fruit (*SHP1* and *SHP2* are functionally redundant). However, *shp1-1 shp2-1* double mutants have a striking phenotype, as the mature fruit is unable to shatter. Studies of *shp1 shp2* fruits, and of plants constitutively expressing *SHP1* and *SHP2*, show that these two genes control dehiscence-zone differentiation and promote the lignification of adjacent cells (Ferrándiz *et al.* 2000; Liljegren *et al.* 2000). In mature *shp1 shp2* fruits, scanning electron reveals the absence of dehiscence zones whereas stain with phloroglucinol a notable reduction in valve-margin-cell lignification was observed (Liljegren *et al.* 2000). On the other hand, the enhancer-trap marker line, YJ80, which is expressed in the valve margin, is still expressed at the apex of *shp1 shp2* fruits (Liljegren *et al.* 2004). All of these results suggest that *SHP1* and *SHP2* probably represent the top of the hierarchy regulating dehiscence zone formation.

FRUITFULL (*FUL*), which corresponds to the *AGL8* MADS-box transcription factor gene, is required for specifying a valve development and valve-cell fate in the mature gynoecium (Gu *et al.* 1998; Liljegren *et al.* 1998, 2004). Probably, *FUL* acts to prevent style elongation in WT fruits. Thus, the *ful* mutant siliques cannot elongate after fertilization and cell division, and the mesocarp cells lignify ectopically (Liljegren *et al.* 2000). That is, cells in the mesophyll tissue layers become lignified late in fruit development and are much smaller than in WT (Ferrándiz *et al.* 2000). Due to scant valve elongation, the small viable seeds are strongly compacted into a reduced space. The inhibition of elongation affects only valves since replum and septum cells continue to elongate. Mature *ful* siliques fail to dehisce nor-

Table 1 Summary of genes controlling gynoecium and fruit development in *A. thaliana*

Gene name	Role	References
AGAMOUS (*AG*)	Regulates the identitiy of the carpels	Dinneny *et al*. 2005
ALCATRAZ (*ALC*)	Contributes in the formation of a strip of labile nonlignified cells link with partly lignified valve and replum, with provide the tension for pod dehiscence	Rajani and Sundaresan 2001
FRUITFULL (*FUL*)	Represses expression of the valve-margin identity genes in the valves; promotes the lignification of the *enb* layer	Liljegren *et al*. 1998, 2004
INDEHISCENT (*IND*)	Controls the development of the valve-margin separation layer and lignified layer; promotes the lignification of the *end* layer	Liljegren *et al*. 2004; Dinneny and Yanofsky 2005
FILAMENTOUS FLOWER (*FIL*)	Regulate the polarity of tissues in lateral organs	Eshed *et al*. 2004
JAGGED (*JAG*)	Promotes the growth of tissues in lateral organs	Ohno *et al*. 2004
REPLUMLESS (*RPL*)	Represses expression of valve-margin identity genes in the replum	Roeder *et al*. 2003
SEEDSTICK (*STK*)	Controls cell expansion and division in the funiculus;essential for seed abscission	Pinyopich *et al*. 2003
SHATTERPROOF 1,2 (*SHP 1,2*)	Act together to promotes valve-margin development through activation of *IND* and *ALC* expression; are essential for the lignification of the *enb* layer	Liljegren *et* al. 2000, 2004
YABBY3 (*YAB3*)	Establish abaxial polarity in lateral organs	Eshed *et al*. 2004

mally, most likely owing to the abnormal valve-replum boundary, so that the growing seeds press against both valves, rupturing them (Gu *et al.* 1998). *Ful* negatively regulates the *SHP* genes in the valves, as the *SHP* genes become ectopically expressed in the valves of *ful* loss-of-function mutants. Thus, fruits from plants constitutively expressing *FUL* are indehiscent due to a complete lack of dehiscent differentiation with conversion of all cells into valve-cell identity (Ferrándiz *et al.* 2000). Recent results have led to the hypothesis that loss-of-function mutations in valve-margin-identify genes should rescue *ful* valve development (Dinneny and Yanofsky 2005).

INDEHISCENT (*IND*; formerly GT140), a basic helix-loop-helix (bHLH) gene that is required for fruit dehiscence, is now known to be the primary factor that directs the differentiation of the valve margin into separation and lignified layers, since the *ind* mutation was able to rescue many aspects of the *ful*-mutant phenotype and could suppress the ectopic lignification of *ful* valves (Liljegren *et al.* 2004; Dinneny and Yanofsky 2005). Thus, of all the mutations that affect dehiscence, loss-of-*IND* function has the strongest effect on valve-margin development (i.e. mutant siliques lack the lignified patches at the valve margins and are unable to shatter). In strong alleles of *ind*, both the lignified layer and separation layer are eliminated throughout the fruit. On the other hand, *ALCATRAZ* (*ALC*), which encodes a myc/bHLH transcription-factor gene, also controls fruit dehiscence in *Arabidopsis* (Liljegren *et al.* 2000; Rajani and Sundaresan 2001; Liljegren *et al.* 2004). The *alc* mutation, which affects only a select set of valve-margin tissues, has a well-developed lignified layer but lacks separation-layer tissues and lignified cells form a bridge between the en*b* layer and the vascular bundle of the replum, blocking valve detachment after middle lamella disintegration (Rajani and Sundaresan 2001). *ALC* is also ectopically expressed in the valves of *ful* mutants (Liljegren *et al.* 2004). Removal of *ALC* activity in these mutants, however, does not abolish the ectopic valve lignification, even when combined with *shp1,2*, although fruit size is moderately rescued in *ful alc shp1,2* mutants. The *al ful* double mutants show a partial reduction of *ful* phenotypes in the valves, suggesting that *ALC* might be repressed by *FUL* in this tissue, in a similar way to that observed for the *SHP* genes or *IND* (Rajani and Sundaresan 2001).

Thus far, genes controlling replum development have yet to be identified. Recently, the *REPLUMLESS* (*RPL*) gene was characterized in *Arabidopsis* (Roeder *et al.* 2003). RPL belongs to the BELL1 family of homeodomain transcription factors (Becker *et al.* 2002). *RPL* encodes a homeodomain protein that prevents replum cells from adopting a valve-margin-cell fate by negatively regulating expression of the *SHP1* and *SHP2* genes. Both *RPL* and *FUL* are required to limit *SHP1* and *SHP2* expression to a narrow strip of cells so that the valve margin differentiates precisely at the valve/replum boundary. The double mutant termed *rpl ful* affects the plant architecture and appears to lack the replum, whereas the overall fruit morphology of the *rpl-1* single mutant is similar to WT, except that the mutant fruits are about half as long as WT (Roeder *et al.* 2003). As an extreme case (i.e. *rpl-3* fruit), the valves appear to have encroached on the replum region. The SHP activity was removed by constructing the *rpl-1 shp1 shp2* triple mutant or *ful rpl-1 shp1 shp2* quadruple mutant which possesses replum; replum restoration indicated that the ectopic expression of the *SHP* genes is largely responsible for the loss of replum development in *rpl* mutants. Lastly, the dehiscence studies in *rpl* mutants suggest that one role for the outer replum is to prevent the valve-margin lignified layers from fusing together and inhibiting dehiscence (Roeder *et al.* 2003).

A crucial aspect of fruit development that is not well understood is how the pattern of gene activities that control valve-margin formation is initially established. Recently, it was demonstrated that the *Filamentous flower* (*FIL*) and *YABBY3* (*YAB3*) genes (two YABBY-family transcription factors), which regulate the polarity of tissues in lateral organs (Eshed *et al.* 2004), are required to promote the expression of *FUL* and *SHP* in the valves and valve margin, respectively (Dinneny *et al.* 2005). The unrelated gene, *JAGGED* (*JAG*; a C_2H_2 zinc-finger transcription factor), which promotes the growth of tissues in lateral organs (Dinneny *et al.* 2004; Ohno *et al.* 2004), acts redundantly with *FIL* and *YAB3* to promote the expression of *FUL* and *SHP*, with *jag fil yab3* triple mutants lacking *FUL* and *SHP* expression in the valves or valve margins. In *fil yab3* fruit, which is indehiscent, the expression of *FUL* is absent throughout the valves (Dinneny *et al.* 2005). On the other hand, it has been found that the expression of the floral homeotic gene, *AGAMOUS* (*AG*; member of monophyletic clade of MADS-box genes that includes *SHP1*, *SHP2 and STK*), which regulates the identity of the carpels, is unaffected in *fil yab3* mutants. This feature indicates that *FIL/YAB* and *AG* represent independent pathways regulating *FUL* and *SHP* expression (Dinneny *et al.* 2005). Likewise, there are data to suggest that the activation of *FUL* and *SHP* expression may require different levels of *FIL*, *YAB3* and *JAG* activity (i.e. *FUL* expression is strongly affected in *fil yab3* mutants, whereas *SHP* expression is lost in only part of the fruit). That is, *FIL*, *YAB3* and *JAG* redundantly contribute to proper valve and valve-margin development by promoting the expression of *FUL* and *SHP* in a region-specific manner (Lewis *et al.* 2006). Finally, replum formation is restored in *jag-5D* fruit by removing SHP activity, further demonstrating that RPL might indirectly regulate *SHP* by restricting *JAG* from the replum (Dinneny *et al.* 2005).

Once the silique opens, the attached seeds are released from funiculus. Recently, SEEDSTICK (STK), a MADS-domain transcription factor closely related to SHP1 and SHP2, has been functionally characterized in *Arabidopsis*. The results point out that STK controls cell expansion and division in the funiculus (i.e. an umbilical-cord-like structure that connects the developing seed to the fruit), and is essential for seed abscission (Pinyopich *et al.* 2003). However, other gene signals appear to be involved in the abscission zone of higher plants (von Stackelberg *et al.* 2003). One candidate might be the gene product that is affected in a pea mutant, *development funiculus* (*def*): funiculi in *def* pods also lack seed abscission zones, preventing seed dispersal. The phenotype of *stk* loss-of-function mutants was studied in depth, and a failure of the seeds to be released from the mature fruits was observed as the most striking characteristic. The separation of abscission zone cells (located immediately adjacent to the seed body in WT) fails to occur in *stk* fruit (Pinyopich *et al.* 2003). In rice plants, shattering habit has been shown to be controlled by the formation of abscission layer, which occurs at the juncture between the sterile lemma and pedicel (Watanabe *et al.* 2003). By classical genetic analysis and molecular analysis, several genes have been identified to control seed shattering in rice (Thomson *et al.* 2003). It has been speculated that two dominants genes located on chromosome 1 and 4, respectively, may be important for seed shattering in rice (Cai and Morishima 2000). During abscission of seed in rice plant, the gene of chromosome 1 (*qsh1*) controls the formation of abscission layer at the base of sterile lemma; a mutant, g to t occurring in the regulatory region located 12kb away from *qsh1*, leads to the absence of abscission layer in *japonica* rice (Konishi *et al.* 2006).

UPDATE ON FRUIT DEHISCENCE PROCESS IN BRASSICACEAE

The fruit of *Arabidopsis* and *Brassica* (i.e. silique) are composed of three major tissues: the replum, with its attached seeds; the valves, a protective ovary walls; and the valve margin (dehiscence zone), located between the replum and valves and constituted by a separation layer and an adjacent layer of lignified cells (**Figs. 1, 2**). The lignified and rigid cells of the margin valves together the en*b*, produce a ten-

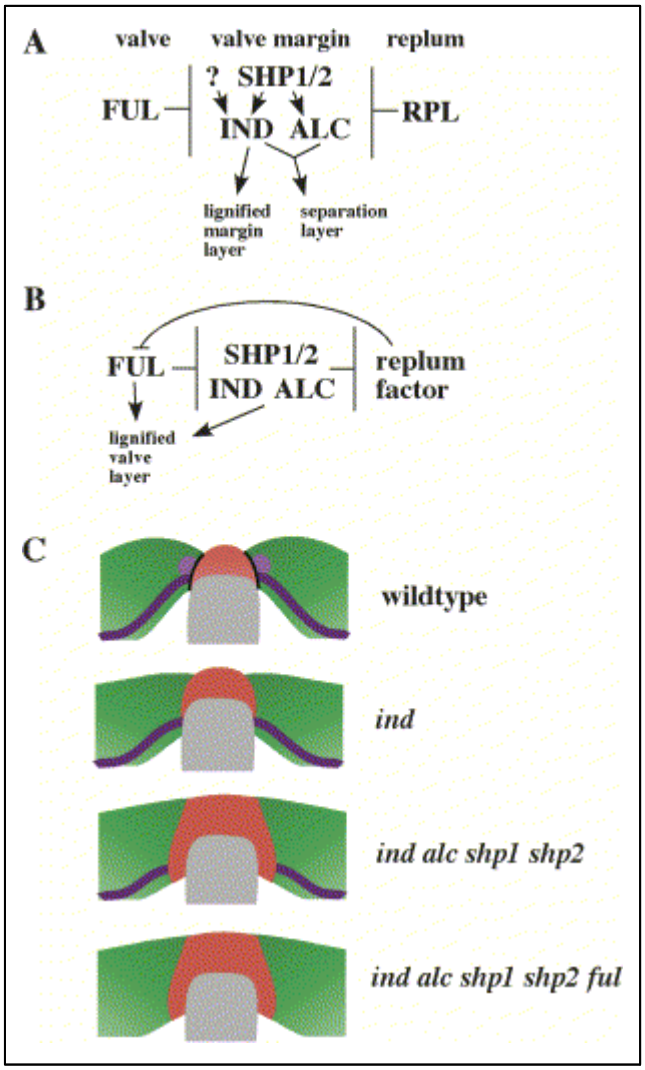

Fig. 4 Setting the silique valve margin in *Arabidopsis*. A model of the regulatory network specifying valve margin development (**A**) and a model for the regulation of the valve lignified layer (**B**). Representation of WT, *ind*, *ind alc shp1 shp2*, and *ind alc shp1 shp2 ful* fruit cross-sections depicting the replum (red), valves (green), lignified margin layer and lignified valve layer (magenta), and separation layer of the margin (black). Adapted from Liljegren *et al.* (2004).

sion within the drying silique that contributes to an active fruit-opening process termed dehiscence (i.e. the valves detach from the replum, allowing the seed to be released). In recent years, substantial molecular and genetic evidence has been accumulated to identify the major genes controlling fruit dehiscence in Brassicaceae (**Figs. 2, 4**). At present, it is known that: (i) *SHATTERPROOF1* and *2* (*SHP1* and *SHP2*; two redundant MADS-box genes) act together to constitutively regulate valve-margin differentiation, control dehiscence zone differentiation and promote the lignification of adjacent cells, both genes being essential for normal pod dehiscence (Liljegren *et al.* 2000, 2004). (ii) *INDEHISCENT* (*IND*), a basic helix-loop-helix bHLH gene, primarily directs the differentiation of the valve-margin cells into the separation and lignified layers (Liljegren *et al*. 2004) (**Fig. 4**). (iii) *IND*, *SHP1*, *SHP2* and *ALCATRAZ* (*ALC*; contributes to the formation of a strip of labile nonlignified cells linked with partly lignified valve and replum, which provide the tension for pod dehiscence) control valve-margin identity and pod shattering (Liljegren *et al.* 2000; Rajani *et al*. 2001; Liljegren *et al.* 2004), and IND, SHP, ALC and FRUITFULL (FUL) are required for en*b* lignification (Liljegren *et al.* 2004) (**Fig. 4**). (iv) REPLUMLESS (RPL), a transcription factor expressed in the replum (Roeder *et al.* 2003), and FUL have been found to set the boundaries of the genes that confer valve-margin identity. (v) FUL acts primarily in the valve to restrict the expression of *IND*, *SHP*, and *ALC* to the valve margin, rather than by playing a major role itself in specifying valve identity (Gu *et al.* 1998; Ferrándiz *et al.* 2000; Liljegren *et al.* 2004); that is, FUL negatively regulates the *SHP* genes in the valves, as the *SHP* genes become ectopically expressed in the valves of full loss-of-function mutants (Dinneny and Yanofsky 2005). Conversely, fruits from plants constitutively expressing *FUL* are dehiscent due to a complete lack of dehiscent-zone differentiation with conversion of all cells into a valve-cell identity (Ferrándiz *et al*. 2000). This phenotype in somewhat more severe than the *shp1* and *shp2* phenotype, suggesting that *FUL* probably not only acts through *SHP r*epression, but is also able to regulate other factors involved in dehiscence-zone cell-fate specifications; *FUL*'s main role in valve development is to suppress the expansion of valve-margin-identity gene expression within the valves (Dinneny and Yanofsky 2005). (vi) *RPL* maintains the replum boundary by restricting the expression of *SHP* to the valve margin (Roeder *et al.* 2003). (vii) JAGGED (JAG), which promotes lateral organ growth, and YABBY3 (YAB3) and FILAMENTOUS FLOWER (FIL), both related to establishing abaxial polarity in lateral organs, are necessary for expression of *FUL* and *SHP* in the valve and valve margin, respectively (Dinneny *et al.* 2005). (viii) RPL is known to regulate *SHP* indirectly by restricting *JAG* and *FIL* expression from the replum (Dinneny *et al.* 2005), and *RPL* gene is required to prevent the ectopic expression of valve-margin markers in the replum (Roeder *et al.* 2003); that is, RPL is not directly required for replum formation, but is instead required to prevent the expression of *SHP* in replum cells (Roeder *et al.* 2003). (ix) While *FIL*, *YAB3* and *JAG* are expressed in both the valves and presumptive valve margin, *FUL* and *SHP* are expressed in mutually exclusive domains in these tissues (Dinneny *et al.* 2005).

For growers of many oilseeds crops, such as canola (*Brassica napus*, *B. rapa*, and *B. juncea*), pod shattering still causes significant harvest losses (Price *et al.* 1996). Recently, it has been demonstrated how studies of fruit patterning and dehiscence in *Arabidopsis* can be applied to improve the seed yields of important crops such as canola. Ectopic expression of *FUL* MADS-box gene is found to prevent pod shattering in *B. juncea*, demonstrating that genetic interactions that control valve-margin development are conserved between closely related plants (Østegard *et al.* 2006). When the *FUL* gene is constitutively expressed from CaMV 35S promotor, *SHP* expression is absent from the valve margin. Consequently, *35S::FUL* fruit fail to differentiate valve margins (Østegard *et al.* 2006) and in these shatter-resistant fruit the pod fails to open and the seeds are trapped inside. The overall similarity between *Arabidopsis* and *Brassica* suggests that similar genetic pathways may be responsible for fruit development in these species. On the other hand, CaMV 35S promotor was used to drive the expression of the *Arabidopsis FUL* gene in transgenic *B. juncea* to test whether constitutive *FUL* expression could prevent fruit opening as in *Arabidopsis*. The expression of *FUL* resulted in shatter-resistant fruit with seed trapped inside the pods (Østegard *et al.* 2006). In *35S::FUL Arabidopsis* plants, early flowering, terminal flowers, and increased seed weight were reported, in addition to the indehiscent fruit (Ferrándiz *et al.* 2000). Early flowering was also shown in *35S::MADSB* transgenic oilseed rape plants (Chandler *et al.* 2005). Likewise, several loci that regulate seed shattering in crop plants (e.g. *Sht1* and *Sh1* in buckwheat and sorghum, respectively) have been gradually known (Matsui *et al.* 2004). Finally, the *B. juncea* orthologue of the *Arabidopsis SHP1* (*BjSHP1*) gene was expressed in narrow strips at the margin, and in developing ovules. As expected, valve margin expression of *BjSHP1* could not be detected in the *35S::FUL 1* and *35S::FUL 2* transgenic lines (Østegard *et al.* 2006). However, the expression of *BjSHP1* was still detected in the ovules of these

transgenic lines, as previously reported for *Arabidopsis* (Ferrándiz *et al.* 2000), indicating that *FUL* is not sufficient to repress *SHP1* expression in ovules. That is, ectopic expression of *FUL* in *B. juncea* is sufficient to negatively regulate *BjSHP1* expression in the valve margin and inhibit valve-margin differentiation. These data suggest that a similar mechanism of gene regulation is used to control pod shattering in *Arabidopsis*, *B. juncea*, and, most likely, in other *Brassica* species.

IS ETHYLENE INVOLVED IN SILIQUE DISMANTLING?

During the pod-shattering process all of the cells of the dehiscence zone undergo CW breakdown at approximately the same time. Likewise, pod shattering occurs at the same time as seed abscission. For this to occur, there must be some trigger that starts the process of separation, and the same signal or a second one may coordinate the events that result in pod shattering. However, little data are available to confirm this supposition. Unlike abscission (González-Carranza *et al.* 1998), there is little evidence to suggest that ethylene (ET) acts as a regulator of pod-shattering. For example, in *A. thaliana*, with a non-functional ET receptor (i.e. *etr1*), there was a normal time course of valve separation (Roberts *et al.* 2002). As an attractive hypothesis, the ET produced from housed seeds should trigger pod-dehiscence. Thus, mustard and canola seeds produce significant amounts of ET during embryogenesis, specifically in the early pre-desiccation stages (Child *et al*. 1998). However, the role of valves in controlling the ET production from seeds and the capacity of their own valves to produce it, is at present unknown. In parthenocarpic siliques of *B. napus* the peak in ET occurs 20 days later than that observed in seeded pods (Child *et al*. 1998), whereas dehiscence is delayed only in the former compared to the latter by a few days. On the other hand, the inhibition of ET synthesis by the addition of aminoethoxyvinylglycine (inhibitor of ACC-synthase activity) to pods of *B. napus* has a weak effect on the timing of pod dehiscence (Child *et al*. 1998). Recently, Matilla's group demonstrated that when the silique wall and housed seeds of *Brassica rapa* cv. 'Rapa' tend to turn yellow, the last step of ET biosynthesis is strongly inhibited, so much so that ACC-oxidase activity is undetectable during the two last phases of desiccation (Rodríguez-Gacio and Matilla 2001). Likewise, the expression of *BrACO1* gene was undetectable in both silique-wall and housed seeds during desiccation (Rodríguez-Gacio *et al.* 2004). On the other side, due to the similarities between valve separation and abscission process (Roberts *et al.* 2002), it was also proposed that ABA could be involved in shattering in crucifers (Ferrándiz 2002). At the desiccation phase of silique valves of *B. rapa* cv. 'Rapa' a major peak of free-ABA was detected (Puga-Hermida *et al.* 2003).

FUTURE PERPECTIVES OF SHATTERING

The gynoecium, constituted by many different cell types (**Fig. 1**) and controlled by complex genetic interactions (**Figs. 2, 4**), is perhaps the most complex organ structure in higher plants (Scutt *et al.* 2006). The process of pod-shatter requires a patterning mechanism to draw a dehiscence line in the ovary. The dehiscence process in mature fruits of the Brassicaceae family is perhaps one of the latest important steps of gynoecium development, since the seed dispersal is involved. In-depth knowledge of this process will provide tools to engineer shatter-resistant seed pods to prevent crop loss in plants of agronomic importance. *Brassica* species are closely related to *Arabidopsis* and represent ideal candidates for model-to-crop approaches as they include important crop plants, such as canola. Although the pod-shattering mechanism is an advantage in nature, unsynchronised pod-shatter constitutes one of the biggest problems for canola farmers. Several strategies for breeding crops with a reduced capacity to shatter are currently underway. *B. napus* displays little variation in resistance to shattering between current cultivars; however, resistant lines have been found within the crop's diploid parents (*B. oleraceae* and *B. rapa*). Synthetic *B. napus* lines have been generated from different lines of *B. oleraceae* and *B. rapa*, and these show increased variation in pod-shattering susceptibility (Morgan *et al.* 1998). These synthetic lines, however, contain many agronomically deleterious traits that make them unsuitable as cultivars (Morgan *et al.* 1998). Intergeneric crosses between *B. napus* and *Sinapis alba* (yellow mustard) are also being generated, in an attempt to transfer a number of beneficial agronomic traits to oilseed rape, including resistance to drought stress as well as pod shattering (Brown *et al.* 1997). As with *B. napus*, there is little variation in pod-shattering resistance among individuals of birdsfoot trefoil (*Lotus corniculatus*), and breeding to reduce shatter through recurrent selection has been unsuccessful (Grant 1996). At present, attempts are being made to transfer the indehiscent seed-pod trait from distantly related species (e.g. interspecific somatic hybridisation) (Grant 1996). In soybean, studies are currently underway to identify QTL that confers resistance to pod dehiscence (Bayley *et al.* 1997). Five potentially independent RFLP markers were associated with pod dehiscence, one of which accounted for 44% of the variation in shatter. In sesame the only varieties with high seed retention are those that are homozygous for the recessive indehiscent gene (*id*) (Day 2000).

As described above in detail, notable advances on fruit dehiscence were carried out over the past two years. With this new understanding of the genes that control dehiscence, it may be possible to modify crops to inhibit pod shattering and prevent such heavy seed loss, particularly under adverse weather conditions. For example, the *FUL* key role during *Arabidopsis* silique opening was indefectibly demonstrated (Dinneny *et al.* 2005; Lewis *et al.* 2006). The first successful attempt to transfer *FUL* MADS-box gene from *Arabidopsis* into *B. juncea* was carried out by Yanofsky's group (Østegard *et al.* 2006). Ectopic expression of the *FUL* gene in *B. juncea* leads to a lack of valve-margin specification in the fruit and, consequently, to a failure in fruit opening. Likewise, it was also confirmed that the genetic pathway for dehiscence-zone development is regulated similarly in *Arabidopsis* and *Brassica* (e.g. canola). If this strategy is confirmed, it should also be applicable for controlling pod-shatter in other important *Brassica* crop species, and sets the direction for future work in this field. In addition, valve-margin-identity genes could also be used to identify and characterize naturally occurring genetic variation in the form of QTL affecting dehiscence. Since completely indehiscent fruit present their own problems, making seed harvesting more difficult, QTLs of moderate effect may represent more useful tools for fine tuning the dehiscence process.

Once a set of genes belonging to valve margins were identified in *Arabidopsis*, the future work should be the functional analysis of those transcripts (Somerville *et al.* 2004; Yong *et al.* 2005). The knowledge emerging from these studies should be applied to plants with high agronomic interest (e.g. canola and rice). Quantitative genetics and genome analysis are also being used to characterize loci that regular seed shattering in crop plants, such as *Sh1* in sorghum and *Sht1* in buckwheat (Paterson *et al.* 1995; Matsui *et al.* 2004). Likewise, it was recently demonstrated that seed shattering in rice is controlled by a single dominant gene, *Shattering1* (*SHA1*), and that *g237t* mutation in *SHA1* accounts for the elimination of seed shattering (Lin *et al.* 2006). Lin *et al*. (2006) demonstrated that *SH4*, an allelic gene of *SHA1* (98% amino acid identity), is involved in abscission-layer development. Consequently, understanding the molecular basis of the non-shattering grains grown today will be particularly satisfying, as they underlie some of the first traits incorporated by our ancestors into their crops.

Although knowledge on ET signalling has advanced considerably in the third millennium (de la Torre *et al.* 2006), almost nothing has been discovered concerning the implications of the action mechanism of ET during the pod-

shattering process. If it is inequivocally demonstrated that ET is a hormonal signal related to the dismantling of the silique, then it will be possible to make a precise and thorough study of the protein factors directly involved. Polygalacturonases and other enzymes that hydrolyse the structure of the CW should be carefully analysed, both molecularly and biochemically, in relation to the mutants described in this review. Several previous studies made in the last decade (Jenkins *et al.* 1996; Petersen *et al.* 1996; Sander *et al.* 1996; Jenkins *et al.* 1999; Whitelaw *et al.* 1999; Sander *et al.* 2001; Christiansen *et al.* 2002; Roberts *et al.* 2002; Rodríguez-Gacio *et al.* 2004) will no doubt serve as the basis for beginning to decipher the complex puzzle that constitutes the dismantling of such a complex structure as the dehiscent fruit.

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REFERENCES

Adams Phillips L, Barry C, Giovannoni J (2004) Signal transduction systems regulating fruit ripening. *Trends in Plant Science* **9**, 331-338

Bailey MA, Mian MAR, Carter TE, Ashley DA, Boerma HR (1997) Pod dehiscence of soybean: identification of quantitative trait loci. *Journal of Heredity* **88**, 152-154

Bailey CD, Koch MA, Mayer M, Mummenhoff K, O'Kane SL, Warwick SI, Windham MD, Al-Shehbaz IA (2006) Toward a global phylogeny of the Brassicaceae. *Molecular Biology and Evolution* **23**, 2142-2160

Becker A, Bey M, Bürglin TR, Saedler H, Theissen G (2002) Ancestry and diversity of BEL1-like homeobox genes revealed by gymnosperm (*Gnetum gnemon*) homologs. *Development Genes and Evolution* **212**, 452-457

Blázquez MA, León J (2006) Reproductive development. In: Hedden P, Thomas SG (Eds) *Plant Hormone Signaling, Annual Plant Reviews* (Vol 4), Blackwell Publishing Ltd., Oxford, UK, pp 293-310

Brown J, Brown AP, Davis JB, Erickson D (1997) Intergeneric hybridization between *Sinapis alba* and *Brassica napus*. *Euphytica* **93**, 163-168

Brüker C (2000) Interpreting Botanical Progress - Clarification of the carpel number in *Papaverales*, *Capparales*, and *Berberidaceae*. *Botanical Review* **66**, 155-307

Burton WA, Salisbury P, Potts D (2003) The potential of canola quality *Brassica juncea* as an oilseed crop for Australia. In: Proceedings of the 13th Biennial Australian Research Assembly on Brassicas, Tamworth, NSW, Australia, 2003, pp 62-64

Cai HW, Morishima H (2000) Genomic regions affecting seed shatering and seed dormancy in rice. *Theoretical and Applied Genetics* **100**, 840-846

Chaudhury AM, Ming L, Miller C, Craig S, Dennis ES, Peacock WJ (1997) Fertilization-independent seed development in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences USA* **94**, 4223-4228

Chandler J, Corbesier L, Spielmann P, Detterdorfer J, Stahl D, Apel K, Melzer S (2005) Modulating flowering time and prevention of pod shatter in oilseed rape. *Molecular Breeding* **15**, 87-94

Child RD, 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

Christiansen LC, Degan F, Ulvskov P, van Onckelen HA (2002) Examination of the dehiscence zone in soybean pods and isolation of a dehiscence-related endopolygalacturonase gene. *Plant Cell and Environment* **25**, 479-480

Clemente R, Walker DJ, Bernal MP (2005) Uptake of heavy metals and As by *Brassica juncea* grown on contaminated soil in Aznalcollar (Spain): the effects of soil amendments. *Environmental Pollution* **138**, 46-58

Day JS (2000) Anatomy of capsule dehiscence in sesame varieties. *Journal of Agriculture Science* **134**, 45-53

de la Torre F, Rodríguez-Gacio MC, Matilla AJ (2006) How ethylene works in the reproductive organs in higher plants. A signaling update from the third millennium. *Plant Signaling and Behavior* **1**, 231-242

Dinneny JR, Yadegari R, Ficher RL, Yanofsky MF, Weigel D (2004) The role of *JAGGED* in shaping lateral organs. *Development* **131**, 1101-1110

Dinneny JR, Weigel D, Yanofsky MF (2005) A genetic framework for fruit patterning in *Arabidopsis thaliana*. *Development* **132**, 4687-4696

Dinneny JR, Yanofsky MF (2005) Drawing lines and bordes: how the dehiscence fruit of *Arabidopsis* is patterned. *BioEssays* **27**, 42-49

Economic Research Service (2001) Oil crops situation and outlook. USDA, OCS-2000, 66 pp

Epstein AL, Gussman CD, Blaylock MJ, Yermiyahu U, Huang JW, Orser CS (1999) EDTA and Pb-EDTA accumulation in *Brassica juncea* grown in Pb-amended soil. *Plant and Soil* **208**, 87-94

Eshed Y, Izhaki A, Baum SF, Floyd SK, Bowman JL (2004) Asymmetric leaf development and blade expansion in *Arabisopsis* are mediated by *KANAD1* and *YABBY* activities. *Development* **131**, 2997-3006

Etheridge N, Chen YF, Schaller GE (2005) Dissecting the ethylene pathway of *Arabidopsis*. *Briefs in Functional Genomics and Proteomics* **3**, 372-381

European Parliament (2003) Directive 2003/30/EC of the European Parliament and the Council of 8 May 2003 on the Promotion of the Use of Biofuels or Other Renewable Fuels for Transport (OJ L 123, 17.5.2003), European Union, 42 pp

Ferrándiz C, Pelaz S, Yanofsky MF (1999) Control of carpel and fruit development in Arabidopsis. *Annual Review of Biochemistry* **68**, 321-354

Ferrándiz C, Liljegren SJ, Yanofsky MF (2000) Negative regulation of the SHATTERPROOF genes by FRUITFULL during *Arabidopsis* fruit development. *Science* **289**, 436-438

Ferrándiz C (2002) Regulation of fruit dehiscence in Arabidopsis. *Journal of Experimental Botany* **53**, 2031-2038

Geldner N, Friml J, Stierhof YD, Jurgens G, Palme K (2001) Auxin transport inhibitors block PIN1 cycling and vesicle trafficking. *Nature* **413**, 425-428

Grant WF (1996) Seed pod shattering in the genus *Lotus* (Fabaceae): A synthesis of diverse evidence. *Canadian Journal of Plant Science* **76**, 447-456

Gupta V, Mukhopadhyay A, Arumugam N, Sodhi YS, Pental D, Pradhan AK (2004) Molecular tagging of erucic acid trait in oilseed mustard (*Brassica juncea*) by QTL mapping and single nucleotide polymorphisms in *FAE1* gene. *Theoretical and Applied Genetics* **108**, 743-749

Heisler MG, Atkinson A, Bylstra YH, Walsh R, Smyth DR (2001) *spatula*, a gen that controls development of carpel margin tissues in Arabidopsis, encodes a bHLH protein. *Development* **128**, 1089-1098

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 oil-seed rape (*Brassica napus* L.). *Journal of Experimental Botany* **47**, 111-115

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

Johnson MA, Preuss D (2004) Plotting a course: multiple signals guide pollen tubes to their targets. *Developmental Cell* **2**, 273-281

Konishi S, Izawa T, Lin SY, Ebana K, Fukuta Y, Sasaki T, Yano M (2006) An SNP caused loss of seed shattering during rice domestication. *Science* **312**, 1392-1396

Lewis MW, Leslie M, Liljegren SJ (2006) Plant separation: 50 ways to leave your mother. *Current Opinion Plant Biology* **9**, 59-65

Li CB, Zhou AL, Sang T (2006) Rice domestication by reducing shattering. *Science* **311**, 1936-1939

Liljegren SJ, Ditta GS, Eshed Y, Savidge B, Bowman JL, Yanofsky MF (2000) *SHATTERPROOF* MADS-box genes control seed dispersal in *Arabidopsis*. *Nature* **404**, 766-770

Liljegren SJ, Roeder AHK, Kempin SA, Gremski K, Østergaard L, Guimil S, Reyes DK, Yanofsky MF (2004) Control of fruit patterning in *Arabidopsis* by INDEHISCENT. *Cell* **116**, 843-853

Lin Z, Griffith ME, Li X, Zhu Z, Tan L, Fu Y, Zhang W, Wang X, Xie D, Sun Ch (2007) Origin of seed shattering in rice (*Oriza sativa* L.). *Planta* **226**, 11-20

Liu Z, Meyerowitz EM (1995) LEUNING regulates AGAMOUS expression in Arabidopsis flowers. *Development* **121**, 975-991

Matsui K, Kiryu Y, Komatsuda T, Kurauchi N, Ohtani T, Tetsuka T (2004) Identification of AFLP markers linked to non-seed shattering locus (*Sht1*) in buckwheat and conversion of STS markers for marker-assisted selection. *Genome* **47**, 469-474

Meaking PJ, Roberts JA (1990) Dehiscence of fruit in oil-seed rape (*Brassica napus* L.). *Journal of Experimental Botany* **41**, 995-1002

Morgan CL, Bruce DM, Child R, Ladbrooke ZL, Arthur AE (1998) Genetic variation for pod shatter resistance among lines of oilseed rape development from synthetic *B. napus*. *Field Crops Research* **58**, 153-165

Nemhauser JL, Feldman LJ, Zambryski PC (2000) Auxin and ETTIN in Arabidopsis gynoecium morphogenesis. *Development* **127**, 3877-3888

Ohno CK, Reddy GV, Heisler MG, Meyerowitz EM (2004) The *Arabidopsis JAGGED* gene encodes a zinc finger protein that promotes leaf tissue development. *Development* **131**, 1111-1122

Østegaard L, Kempin SA, Bies D, Klee HJ, Yanofsky MF (2006) Pod shatter-resistant *Brassica* fruit produced by ectopic expression of the *FRUITFULL* gene. *Plant Biotechnology Journal* **4**, 45-51

Parcy F, Nilsson O, Busch MA, Lee I, Weigel D (1998) A genetic framework for floral patterning. *Nature* **395**, 561-566

Paterson AH, Lin Y-R, Li Z, Schertz KF, Doebley JF, Pinson SRM, Liu S-C, Stansel JW, Irvine JE (1995) Convergent domestication of cereal crops by independent mutations at corresponding genetic loci. *Science* **269**, 1714-1718

Patterson SE (2001) Cutting loose. Abscission and dehiscence in *Arabidopsis*. *Plant Physiology* **126**, 494-500

Petersen 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

Philbrook B, Oplinger ES (1989) Soybean field losses as influenced by harvest delays. *Agronomy Journal* **81**, 251-258

Picart JA, Morgan DG (1984) Pod development in relation to pod shattering. *Aspects of Applied Biology* **6**, 101-110

Price JS, Neale MA, Hobson RN, Bruce DM (1996) Seed losses in commercial harvesting of oilseed rape. *Journal of Agricultural Engineering Research* **80**, 343-350

Pinyopich A, Ditta GS, Savidge B, Liljegren SJ, Baumann E, Wisman E, Yanofsky MF (2003) Assessing the redundancy of MADS-box genes during carpel and ovule development. *Nature* **424**, 85-88

Puga-Hermida MI, Gallardo M, Matilla AJ (2003) The zygotic embryogenesis and ripening of *Brassica rapa* seeds provokes important alterations in the levels of free and conjugated ABA and polyamines. *Physiologia Plantarum* **111**, 279-288

Rajani S, Sundaresan V (2001) The *Arabidopsis* myc/bHLH gene *ALCATRAZ* enables cell separation in fruit dehiscence. *Current Biology* **11**, 1914-1922

Roberts JA, González-Carranza ZA (2002) Abscission, dehiscence and other cell separation processes. *Annual Review of Plant Biology* **53**, 131-158

Roberts JA, Elliot KA, González-Carranza ZA (2002) Abscission, dehiscence and other cell separation processes. *Annual Review of Plant Biology* **53**, 131-158

Rodríguez-Gacio MC, Matilla AJ (2001) The last step of the ethylene biosynthesis pathway in turnip tops (*Brassica rapa*) seed: alterations related to development and germination and its inhibition during desiccation. *Physiologia Plantarum* **111**, 273-279

Rodríguez-Gacio MC, Nicolás C, Matilla AJ (2003) The final step of the ethylene biosynthesis pathway in turnip tops (*Brassica rapa* L. cv. Rapa): Molecular characterization of the ACC oxidase *BrACO1* throughout zygotic embryogenesis and germination of heterogeneous seeds. *Physiologia Plantarum* **121**, 132-140

Rodríguez-Gacio MC, Nicolás C, Matilla AJ (2004) Cloning and analysis of a cDNA encoding and endo-polygalacaturonase expressed during the desiccation period of the silique-valves of turnip-tops (*Brassica rapa* L. cv. Rapa). *Journal of Plant Physiology* **161**, 219-227

Roeder AHK, Ferrándiz C, Yanofsky MF (2003) The role of the REPLUMLESS homeodomain protein in patterning the *Arabidopsis* fruit. *Current Biology* **13**, 1630-1635

Sander L, Bottermann J, Ulvskov P, Borkhardt B (1996) Nucleotide sequence of a gene encoding a pod dehiscence zone specific endo-polygalacturonase from *B. napus*. *Plant Physiology* **111**, 1354-1359

Sander L, Child R, Ulvskov P, Albrechtsen M, Borkhardt B (2001) Analysis of a dehiscence zone endo-polygalacturonase in oilseed rape (*B. napus*) and *A. thaliana*: evidence for roles in cell separation in dehiscence and abscission zones, and in stylar tissues during pollen tube growth. *Plant Molecular Biology* **46**, 469-479

Scutt ChP, Vinauger-Douard M, Fourquin Ch, Finet C, Dumas Ch (2006) An evolutionary perpective on the regulation of carpel development. *Journal of Experimental Botany* **57**, 2143-2152

Somerville C, Bauer S, Brininstool G, Facette M, Hamann T, Milne J, Osborne E, Paredez A, Persson S, Raab T, Vorwerk S, Youngs H (2004) Toward a systems approach to understanding plant cell walls. *Science* **306**, 2206-2211

Spence J, Vercher Y, Gates P, Harris N (1996) 'Pod shatter' in *Arabidopsis thaliana*, *Brassica napus* and *B. Juncea*. *Journal of Microscopy* **68**, 195-203

Thomson MJ, Tai TH, McClum AM, Lai XH, Hinga ME, Lobos KB, Xu Y, Matínez CP, McCouch SR (2003) Mapping quantitative trait loci for yield, yield components and morphological traits in an advanced backcross population between *Oryza rufipogon* and *Oryza sativa* cultivar Jefferson. *Theoretical and Applied Genetics* **107**, 479-493

Tiwari SP, Bhatia VS (1995) Characters of pod anatomy associated with resistance to pod-shattering in soybean. *Annals of Botany* **76**, 483-485

Vivian-Smith A, Koltunow AM (1999) Genetic analysis of growth-regulator-induced parthenocarpy in *Arabidopsis*. *Plant Physiology* **121**, 437-451

Vivian-Smith A, Luo M, Chaudhury A, Koltunow A (2001) Fruit development is actively restricted in the absence of fertilization in Arabidopsis. *Development* **128**, 2321-2331

von Stackelberg M, Lindermann S, Menke M, Riesselmann S, Jacobsen HJ (2003) Identification of AFLP and STS markers closely linked to the *def* locus in pea. *Theoretical and Applied Genetics* **106**, 1293-1299

Watanabe K, Oba S, Horiuchi T (2003) Allelic test of rice shattering genes *sh1* and *sh2* in an F_2 population derived from the cross between Momigaredatsu and Dee-Geo-Woo-Gen (*Oryza sativa* L.) SABRAO. *Journal of Breeding and Genetics* **35**, 57-64

White PJ (2002) Recent advances in fruit development and ripening: an overview. *Journal of Experimental Botany* **53**, 1995-2000

Yang YW, Lai KN, Tai PY, Li WH (1999) Rates of nucleotide substitution in angiosperm mitochondrial DNA sequences and dates of divergence between *Brassica* and other angiosperm lineages. *Journal of Molecular Evolution* **48**, 597-604

Yong W, Link B, O'Malley R, Terwari J, Hunter CT, Lu C-A, Li X, Bleecker AB, Koch KE, McCann MC, McCarty DR, Patterson SE, Reiter WD, Staiger Ch, Thomas SR, Vermerris W, Carpita WC (2005) Genomics of plant cell wall biogenesis. *Planta* **221**, 747-751