

# Genes implicados en la dehiscencia vegetal

Genética, Genómica y Mejora Vegetal

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DE GRANADA

**Dehiscence** is the splitting of a mature plant structure along a built-in line of weakness to release its contents. This is common among **fruits**, **anthers** and **sporangia**. Sometimes this involves the complete detachment of a part; structures that open in this way are said to be **dehiscent**. Structures that do not open in this way are called **indehiscent**, and rely on other mechanisms such as decay or predation to release the contents.

FLORACIÓN + FECUNDACIÓN



MADURACIÓN DEL FRUTO



DISPERSIÓN DE LA SEMILLA



ÉXITO  
REPRODUCTIVO



DOMESTICACIÓN  
HUMANA

# MICROESTRUCTURA DE UNA SILICUA:

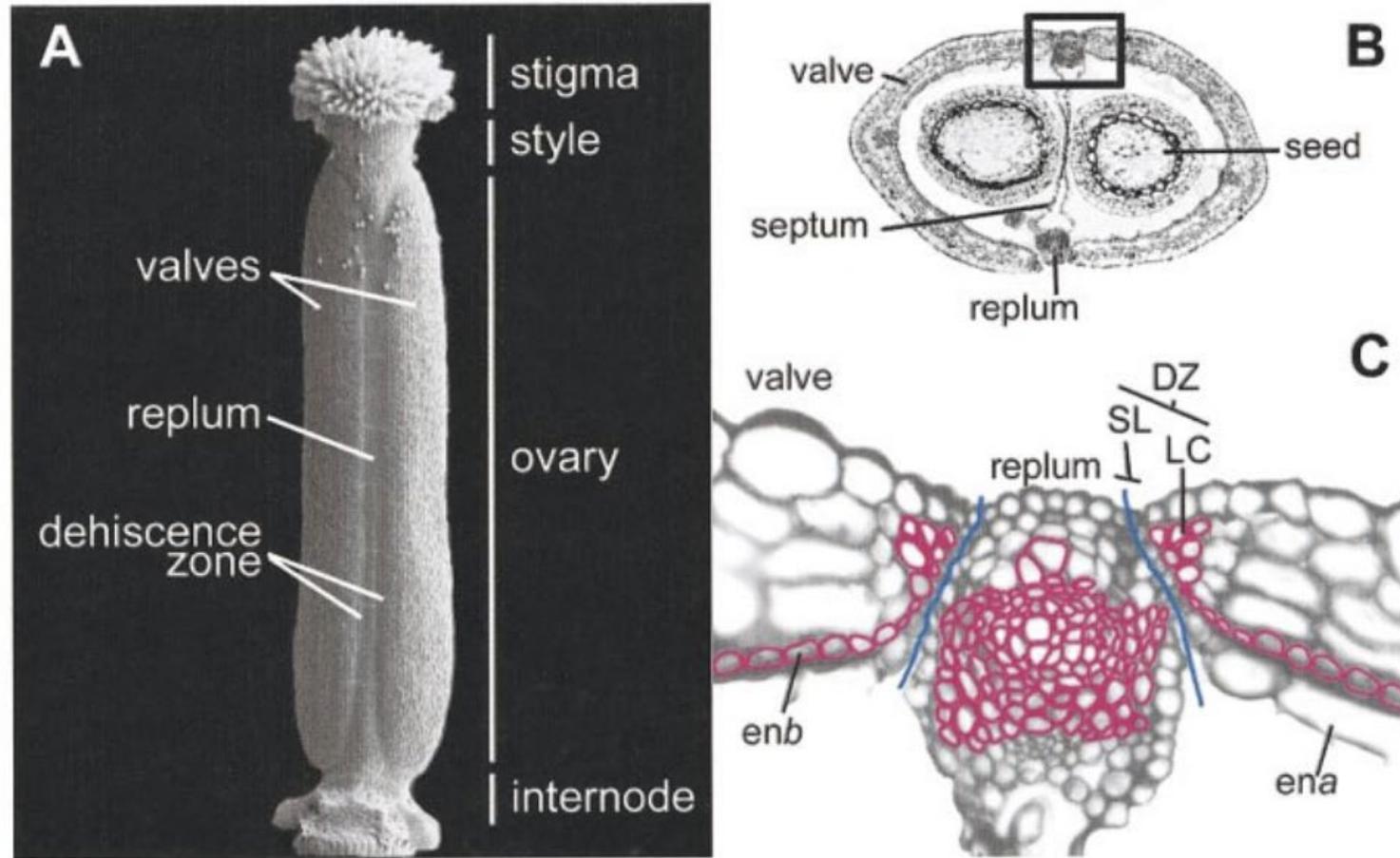
**Valves | Valvas:** cada una de las dos mitades de un fruto. Son el resultado de la fusión de dos carpelos que encierran a las semillas.

**Replum (external septum) | Replum (septo externo):** tabique que permanece después de la dehiscencia y al que quedan adheridas las semillas.

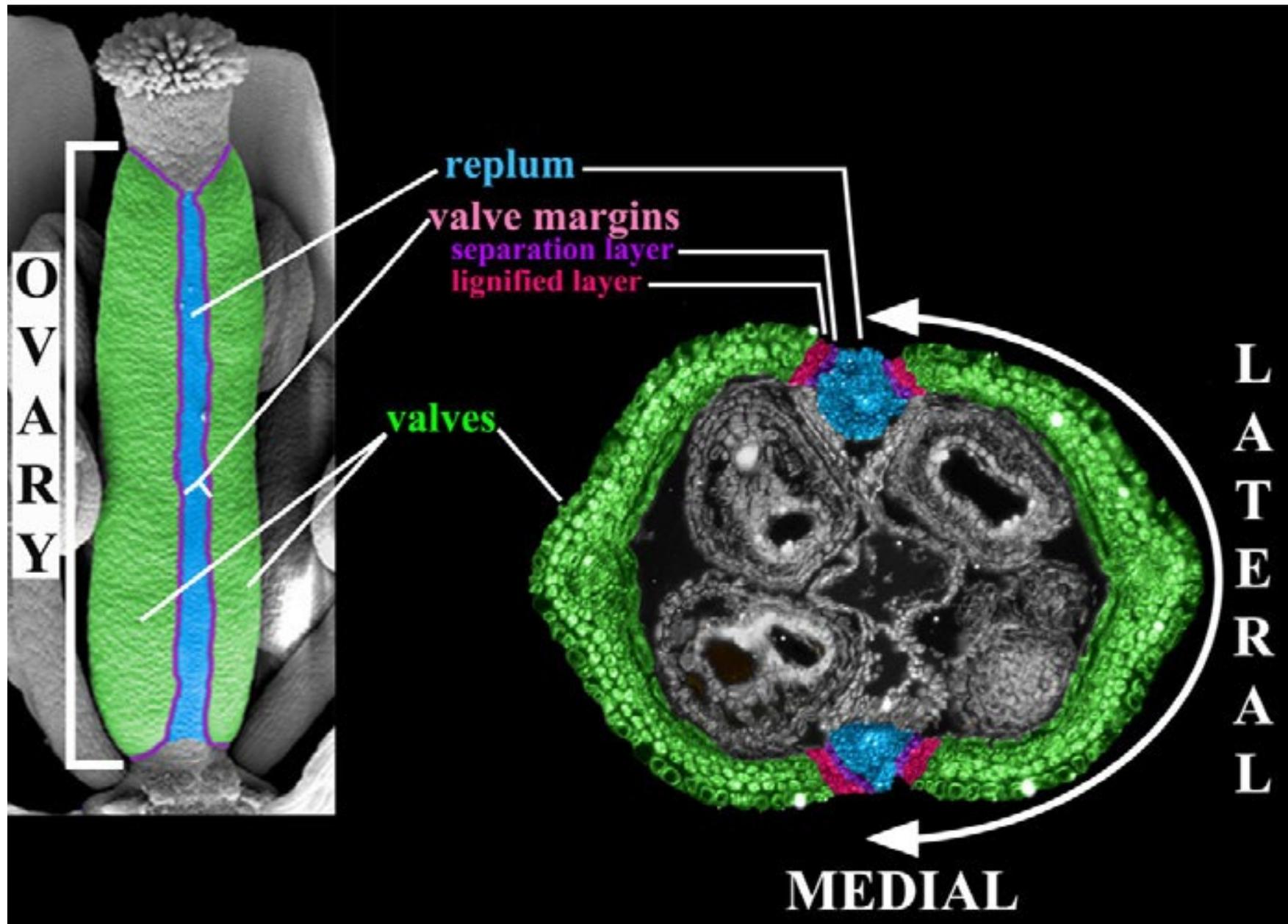
**Dehiscence zone (DZ) | Zona de dehiscencia:** banda de unas pocas células de grosor que se extiende a todo lo largo del fruto entre las valvas y el replum. Comprende a las células lignificadas y a la capa de separación:

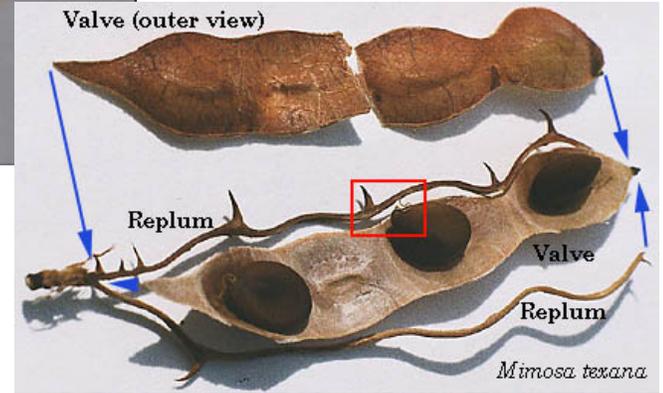
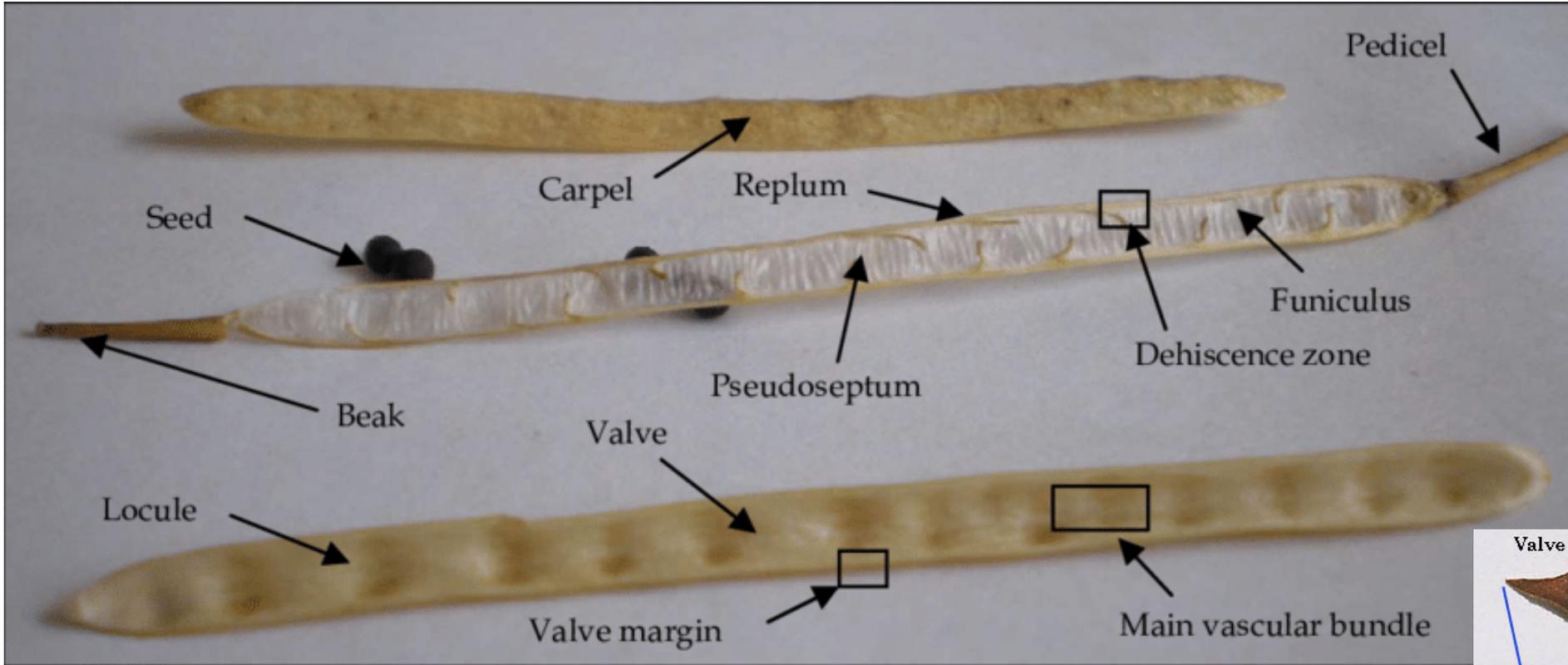
***Lignified cells (LC) | Células lignificadas:*** grupo de células endurecidas por la acumulación de ligninas.

***Separation layer (SL) | Capa de separación:*** grupo de células existente entre dos grupos de células lignificadas por donde se produce la separación de las valvas. Se diferencian bien de las células adyacentes porque las células de esta capa se diferencian pronto durante el desarrollo de la silicua y permanecen en una especie de estado de latencia. Cuando se degradan por autólisis, se produce la dehiscencia.



**Fig. 1.** The *Arabidopsis* fruit. Scanning micrograph of a silique shortly after pollination (stage 14, according to Smyth *et al.*, 1990). The different parts of the fruit are indicated. Transverse section of the ovary of a mature silique (stage 17B). A close-up of the boxed area is shown in (C). Section of the dehiscence zone of a stage 17B silique. Lignified cell walls have been traced in pink for clarity, and the fracture surface in the separation layer is noted by a blue line. DZ, dehiscence zone; SL, separation layer; LC, lignified cells at the valve margin mesocarp; ena, endocarp, already disintegrating in the left valve; enb, endocarp.





FLORACIÓN + FECUNDACIÓN



MADURACIÓN DEL FRUTO



DISPERSIÓN DE LA SEMILLA



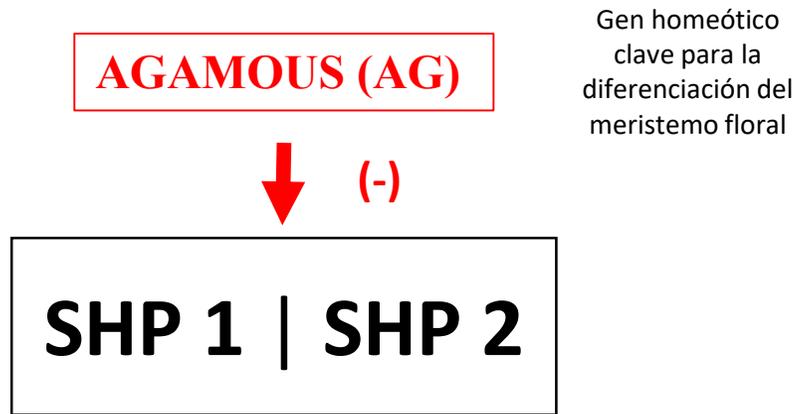
ÉXITO  
REPRODUCTIVO



DOMESTICACIÓN  
HUMANA

Regulación temprana: diferenciación celular.

Actividad enzimática: para que ocurran, de forma progresiva, los cambios que dan lugar físicamente a la dehiscencia (composición de la pared celular, lignificación y desintegración de la capa de separación).

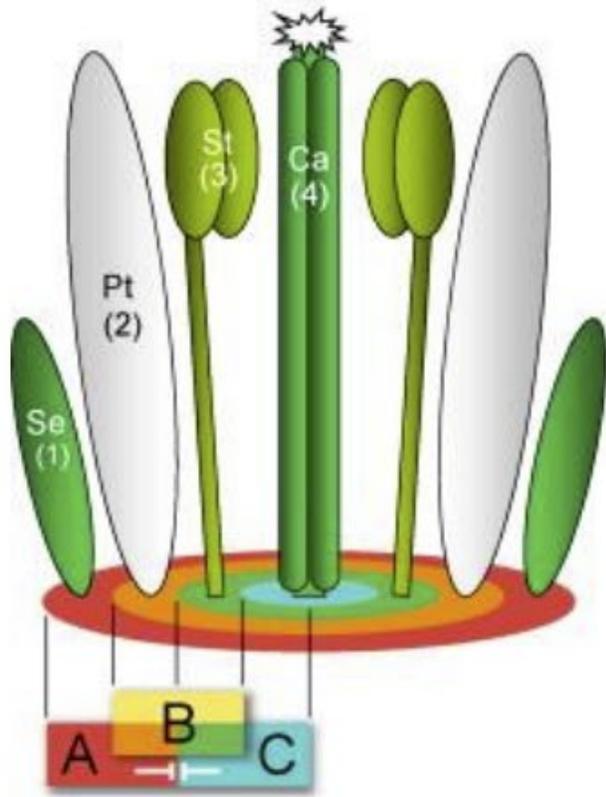


### DIFERENCIACIÓN DE LA ZONA DE DEHISCENCIA

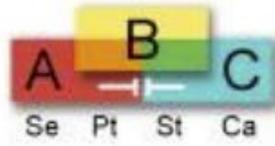
Al principio de la cadena intervienen dos **factores de transcripción**, los genes MADS-box *SHATTERPROOF1* (SHP1) y *SHATTERPROOF2* (SHP2).

SHP1 y SHP2 tienen funciones redundantes, por lo que serían genes duplicados. Son necesarios para la correcta lignificación y **diferenciación celular** de los márgenes de las valvas y la zona de dehiscencia. Para el correcto desarrollo del fruto, es crucial que estos genes se expresen en la DZ.

Están regulados por *AGAMOUS* y presentan un patrón de expresión idéntico.



Wild-type

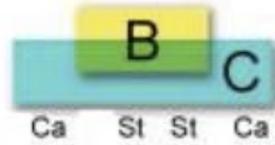


Verticilo 1 (V1)  
Verticilo 2 (V2)  
Verticilo 3 (V3)  
Verticilo 4 (V4)

Sépalos (Se)  
Pétalos (Pe)  
Estambres (Es)  
Carpelos (Ca)

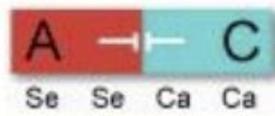


A-function mutant



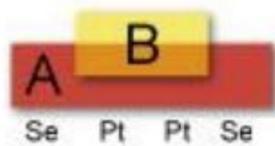
*apetala2*

B-function mutant



*pistillata*

C-function mutant



*agamous*

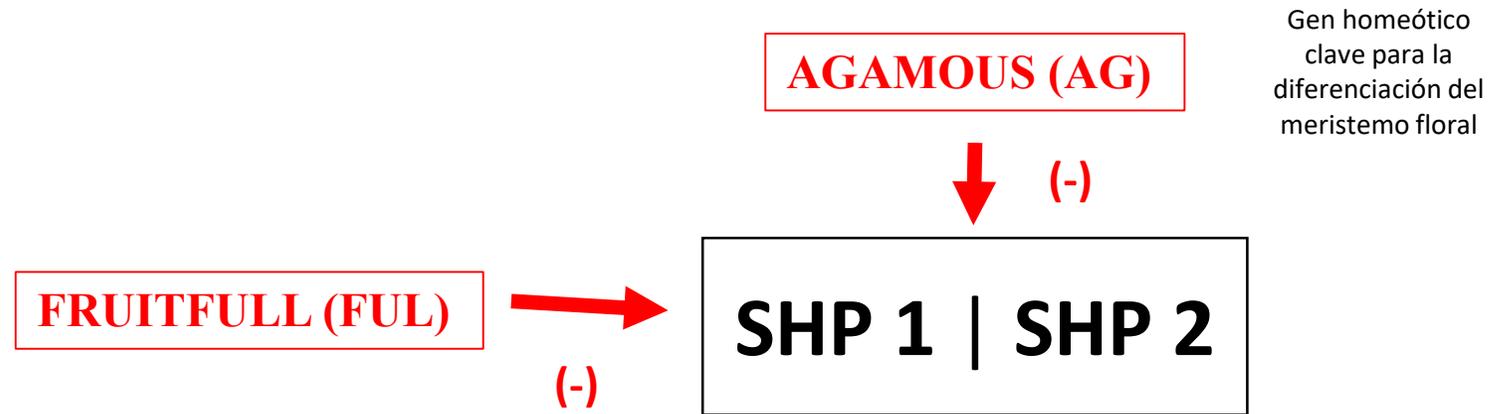
**A** (APETALA2, AP2),  
**B** (APETALA3 y PISTILLATA, AP3/PI)  
**C** (AGAMOUS, AG).

***A. thaliana***

**Función A** *APETALA2 (AP2) APETALA1 (AP1)*

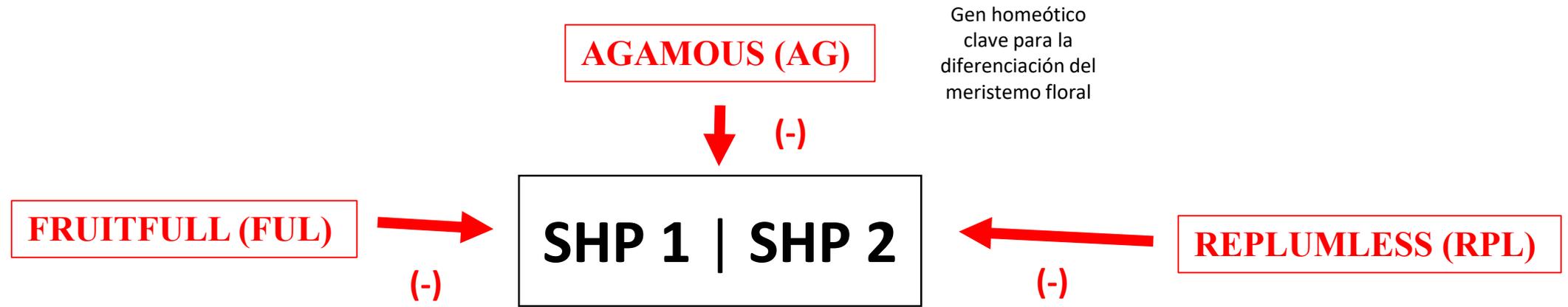
**Función B** *APETALA3 (AP3) PISTILLATA (PI)*

**Función C** *AGAMOUS (AG)*



### DIFERENCIACIÓN DE LAS VALVAS

FRUITFULL (FUL) pertenece a la familia de **genes MADS-box**. Está relacionado con la regulación del desarrollo de la flor y del fruto. Se expresa en los carpelos y se acumula en el meristemo floral promoviendo el inicio del desarrollo floral. Posteriormente, expresándose en las valvas favorece la correcta diferenciación de éstas y la extensión del fruto.



El gen **REPLUMLESS (RPL)**, homeobox del tipo (BEL1-like), se expresa en el replum y es regulado negativamente por el gen floral homeótico **APETALA2 (AP2)**.

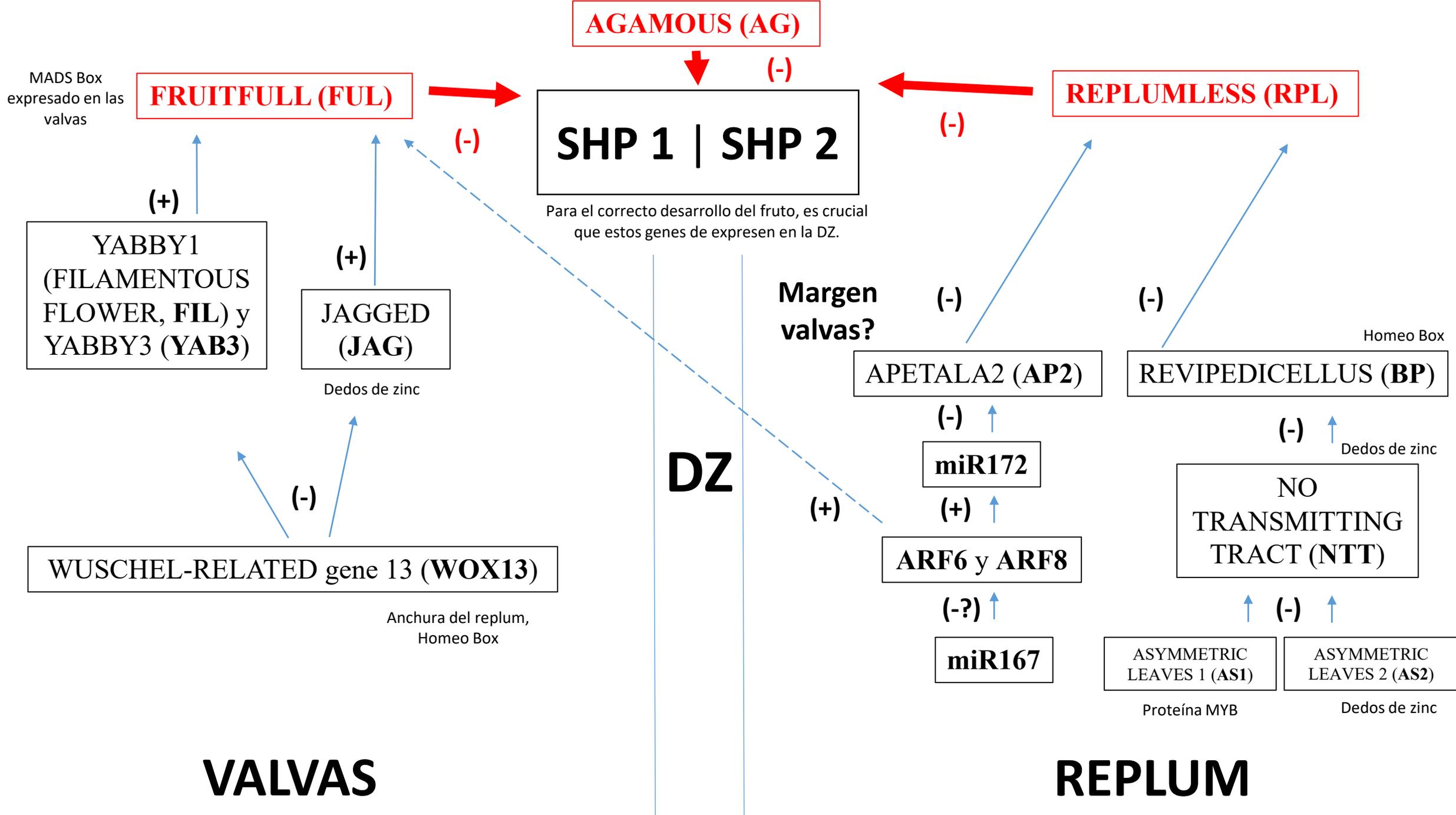
\* Otras denominaciones: *PENNYWISE, BELLRINGER, VAAMANA, BLH9.*

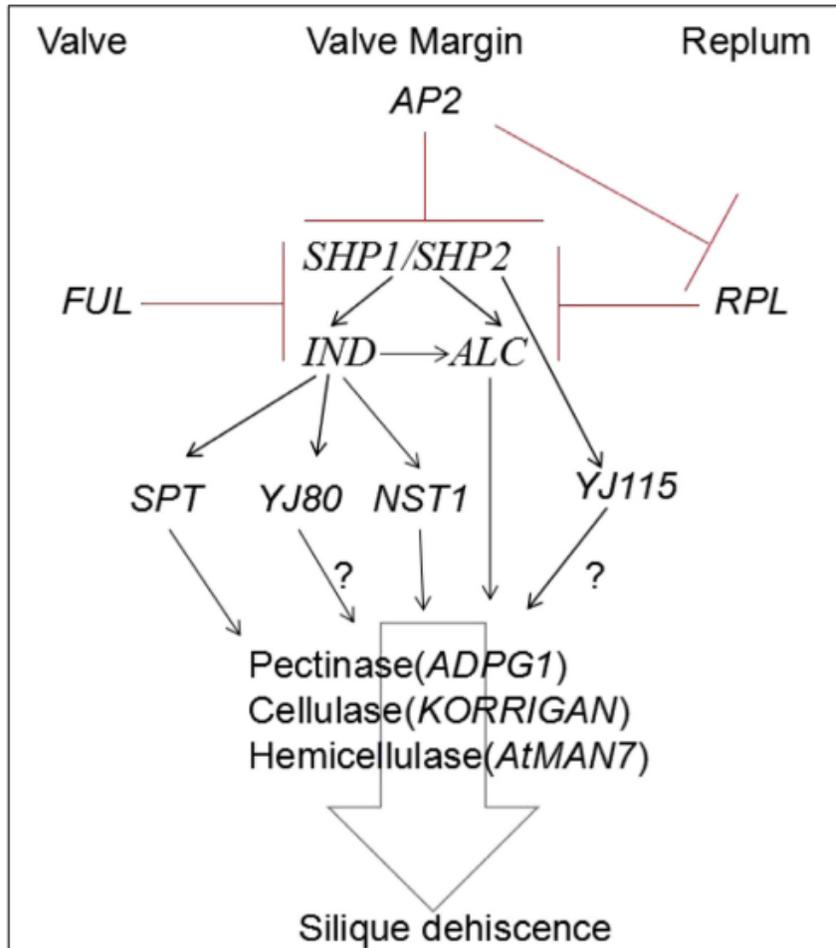
***A. thaliana***

**Función A** *APETALA2 (AP2) APETALA1 (AP1)*

**Función B** *APETALA3 (AP3) PISTILLATA (PI)*

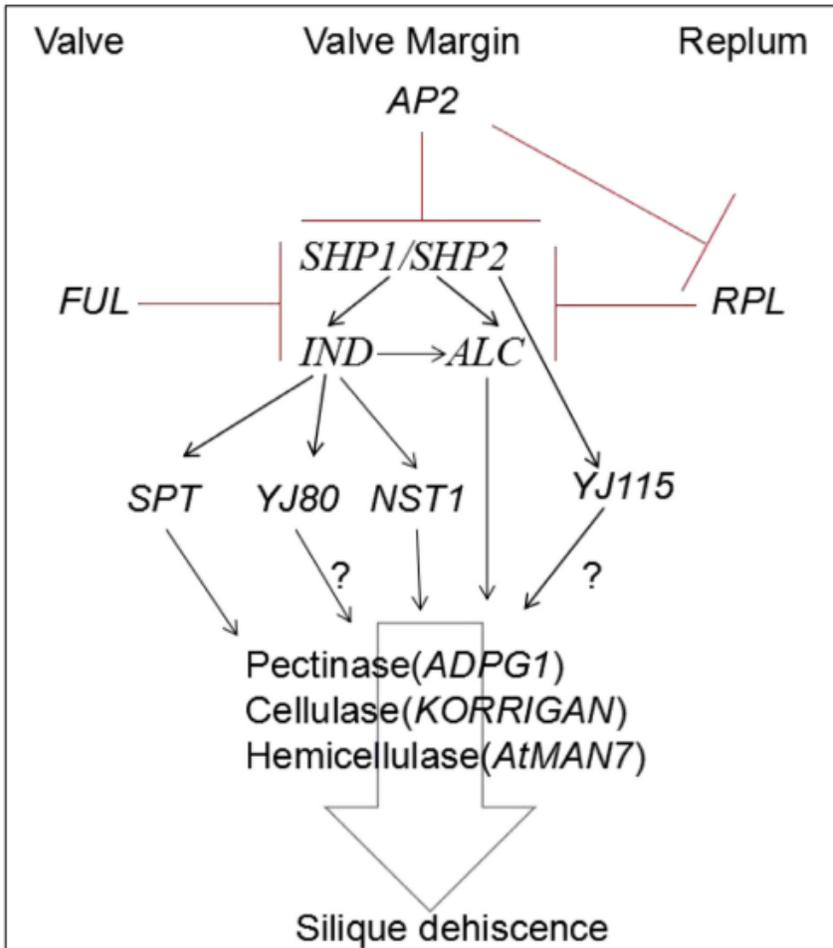
**Función C** *AGAMOUS (AG)*





**FIGURE 3** | Model for regulation of silique dehiscence in *Arabidopsis thaliana*. The major transcription factors and their regulatory interactions, as well as degradation enzyme of cell wall are included. Hormone coordinate signal transduction in silique dehiscence which not shown in figure. Negative regulation represented by red lines, Positive regulation represented by black arrow. Question marker means hypothetical relationships not well supported by experimental data.

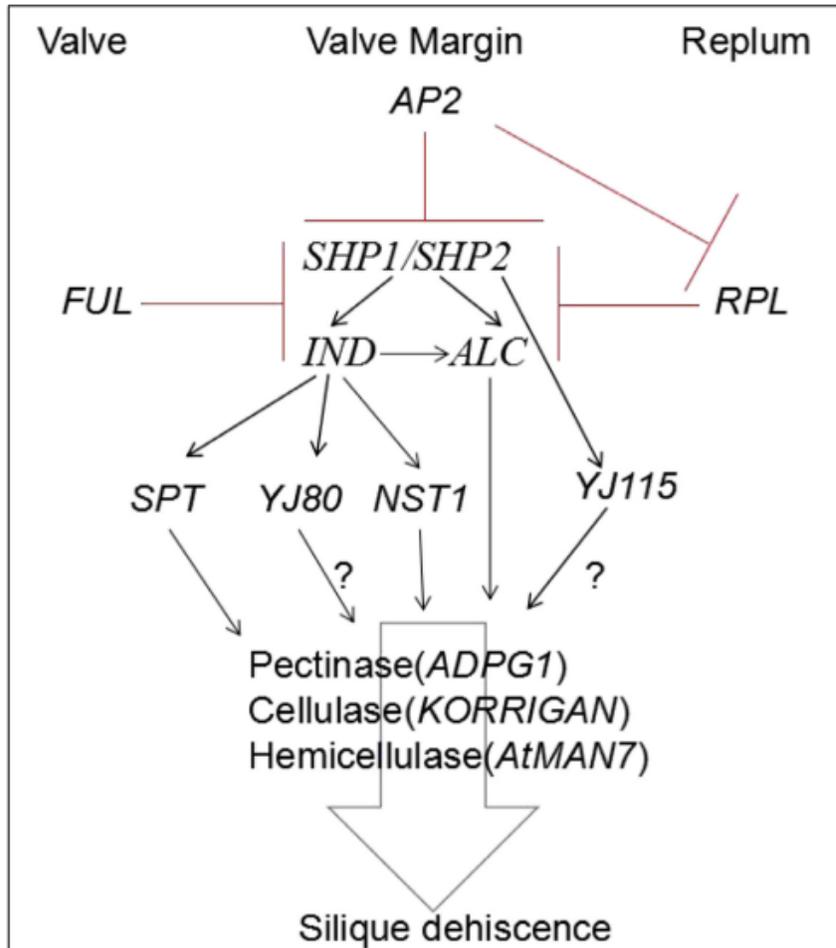
SHP1 y SHP2 activan a los genes INDEHISCENT (IND), ALCATRAZ (ALC) y SPATULA (SPT), que son factores de transcripción del tipo *helix-loop-helix*. Contribuyen también al desarrollo de la DZ.



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El gen INDEHISCENT1 (IND1) es imprescindible para la diferenciación de la DZ. Los mutantes para este gen presentan falta de lignificación en los márgenes de la valva y el fruto no se abre.

\* Regulado positivamente por SHP y, por tanto, negativamente por FUL.



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ALCATRAZ (ALC) es un factor de transcripción bHLH, expresado en los márgenes de las valvas fundamentalmente. En los estadios finales del desarrollo, su expresión está restringida al puente de células lignificadas.

Interviene en el desarrollo de la SL, ya que los mutantes presentan una DZ con morfología normal pero con una línea de fractura imprecisa.

**COMO NORMA GENERAL, LOS  
GENES QUE SE EXPRESAN EN EL  
REPLUM REGULAN  
NEGATIVAMENTE A LOS QUE SE  
EXPRESAN EN LAS VALVAS Y  
VICEVERSA**

**FRUITFULL (FUL)**



**SHP 1 | SHP 2**

**AGAMOUS (AG)**



**(-)**



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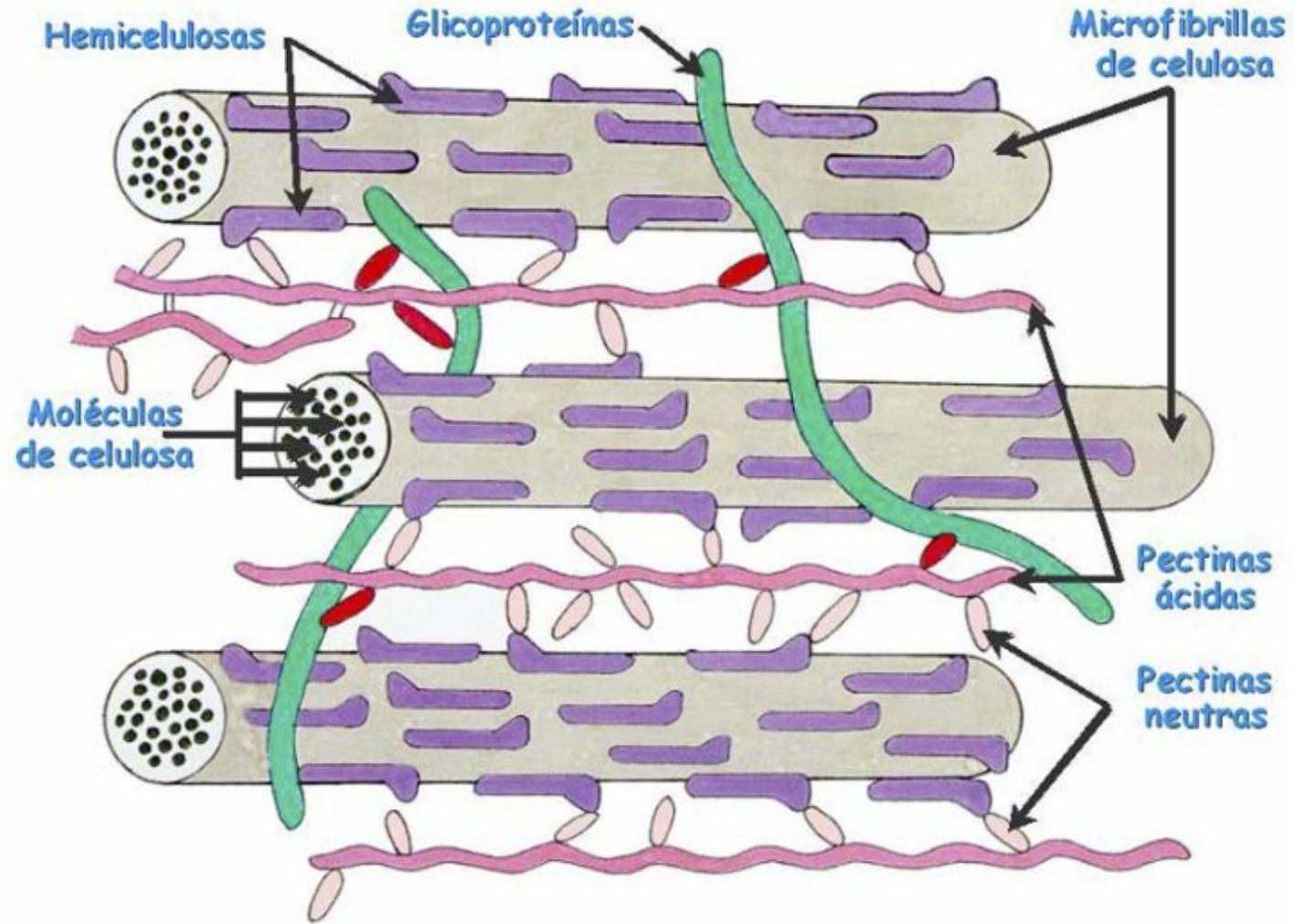
**REPLUMLESS (RPL)**

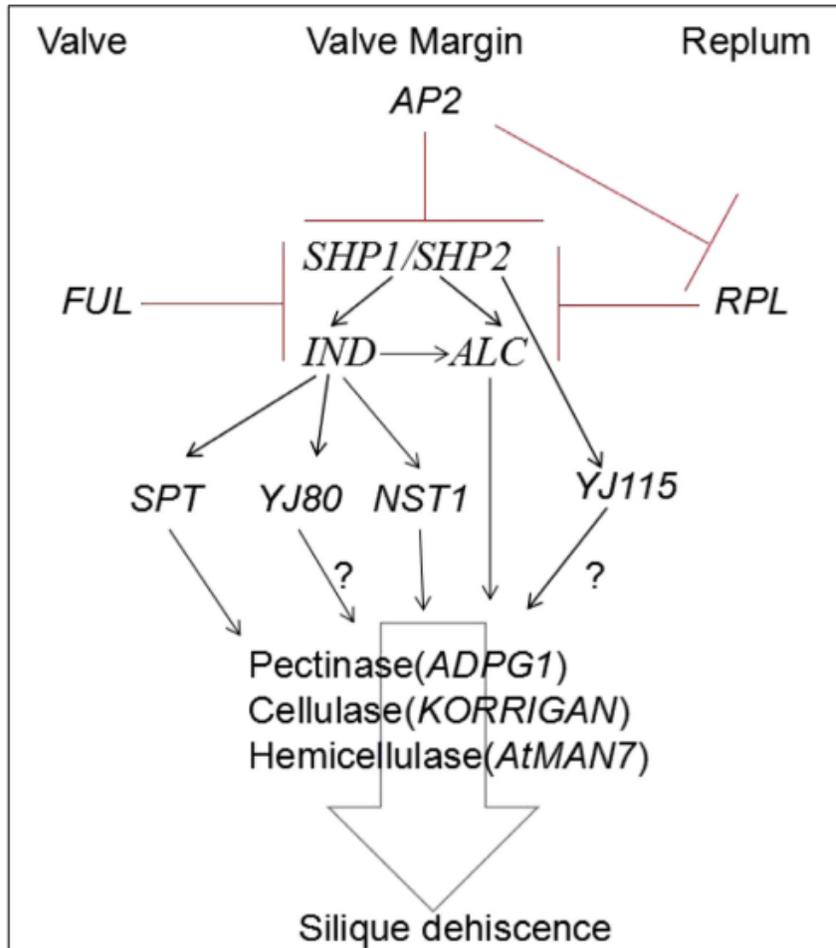
**DZ**

**VALVAS**

**REPLUM**

Degradación de la pectina, la celulosa y la hemicelulosa, componentes principales de la pared celular.





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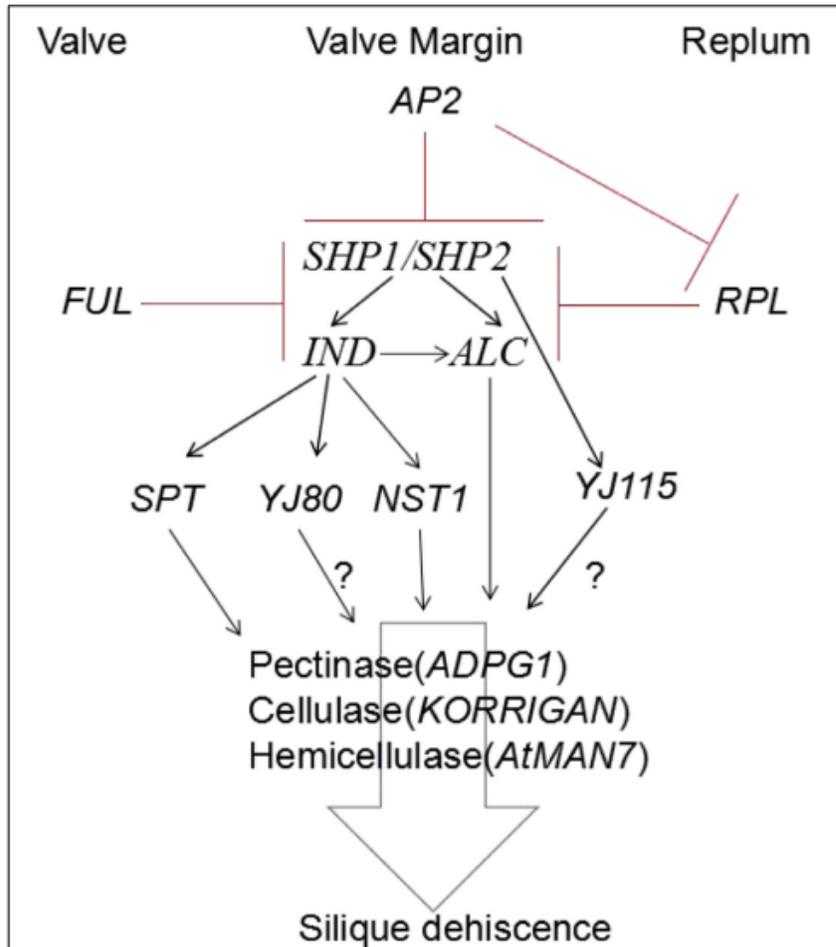
# Efectores aguas abajo

Gen de la poligalacturonasa (**ADPG1**) es una hidrolasa implicada en la degradación de la pectina y de las paredes celulares. Se expresa en células de la DZ en las que se produce la separación.

\* Sinónimos: SAC66, RDPG1, PG.

ADPG1 es regulado por IND (en la DZ) y por HECATE3 (en la zona de abscisión)

Presenta un dominio N-terminal que bloquea la secreción de ADPG1 a la pared celular. Cuando la planta recibe las señales, se elimina el dominio y la proteína madura es transportada para cumplir su función.



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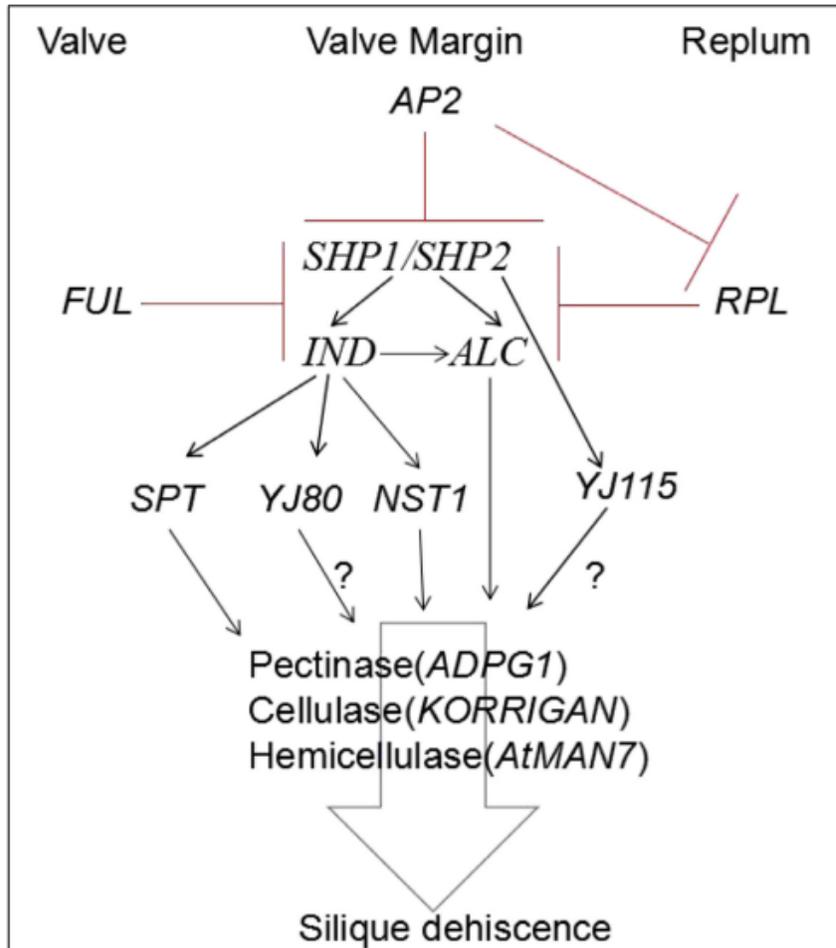
# Efectores aguas abajo

Se ha relacionado el incremento de la actividad de la celulasa b-1,4-glucanasa y de la hemicelulasa con la dehiscencia/abscisión.

En Arabidopsis se han descrito 25 celulasas diferentes. Si bien, se ha demostrado la implicación directa en la dehiscencia de la celulasa CELLULASE6 (CEL6) y de la hemicelulasa MANNANASE7 (MAN7).

En la silicua, su expresión está regulada por los factores de transcripción IND y ALC.

Controlan el tiempo de diferenciación de las valvas y promueven la degradación celular de las células de la capa de separación.

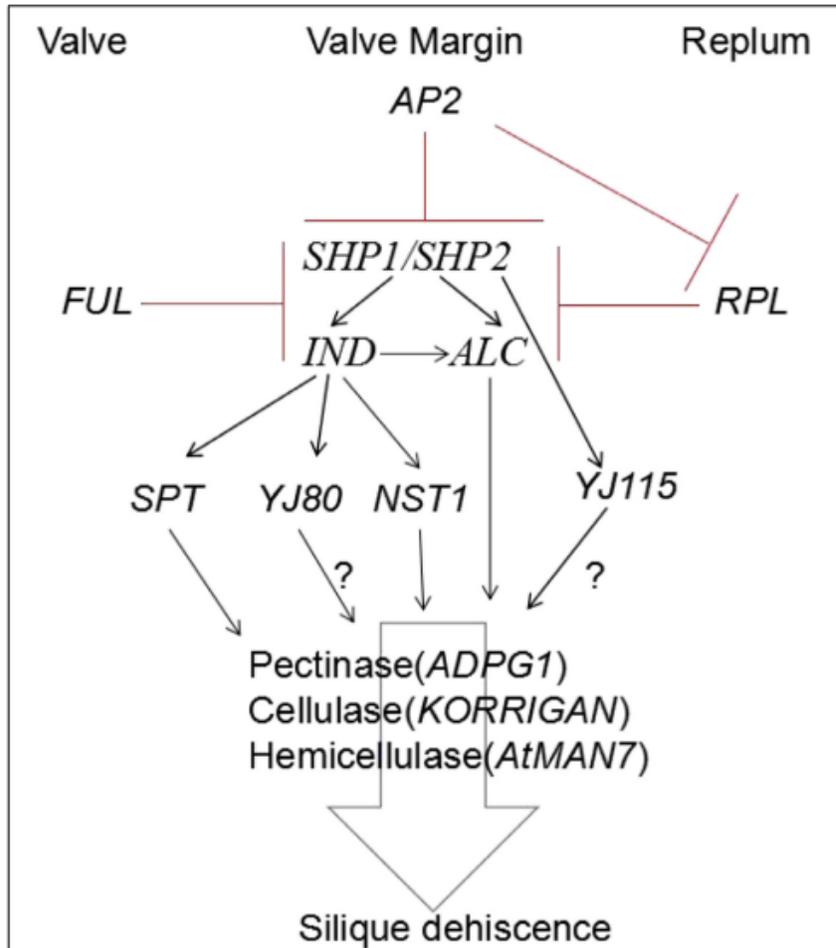


**FIGURE 3** | Model for regulation of silique dehiscence in *Arabidopsis thaliana*. The major transcription factors and their regulatory interactions, as well as degradation enzyme of cell wall are included. Hormone coordinate signal transduction in silique dehiscence which not shown in figure. Negative regulation represented by red lines, Positive regulation represented by black arrow. Question marker means hypothetical relationships not well supported by experimental data.

# Efectores aguas abajo

Se ha relacionado el incremento de la actividad de la celulasa b-1,4-glucanasa con la dehiscencia/abscisión.

La celulasa KORRIGAN se ha asociado con este proceso y con la elongación celular rápida.



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YJ80 se expresa en el margen de las valvas y en la zona de abscisión. Podría regular al gen XET, una glicosilasa que en *B. napus* se expresa en las etapas finales del desarrollo del fruto.

El gen YJ115 se expresa en el replum y en el margen de las valvas y está regulado por los genes SHP1 y SHP2. Se encuentra aguas arriba de un gen desconocido que contiene un dominio transmembrana putativo.

## Evidence that an evolutionary transition from dehiscent to indehiscent fruits in *Lepidium* (Brassicaceae) was caused by a change in the control of valve margin identity genes

Andreas Mühlhausen<sup>1,†</sup>, Teresa Lenser<sup>2,†</sup>, Klaus Mummenhoff<sup>1,†</sup> and Günter Theißen<sup>2,\*;‡</sup>

<sup>†</sup>Department of Biology, Botany, University of Osnabrück, Barbarastraße 11, D-49076 Osnabrück, Germany, and

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Possible candidates are BRANCHED1 (BRC1), an ortholog of which may determine whether dehiscent or indehiscent fruits develop on the dimorphic plant *Aethionema arabicum*, and TCP FAMILY TRANSCRIPTION FACTOR 4 (TCP4) which may regulate YABBY3. These two genes were found to be DDEGs. Our study elucidates differences in gene expression patterns between dehiscent and indehiscent fruits and reveals BRC1 and TCP4 as possible causes for the evolutionary transition from dehiscent to indehiscent fruits in *Lepidium*.

## BARRIDO SELECTIVO EN PISTACHO DEL GEN YAB

The nuts of cultivated *P. vera* have higher nut splitting ratios than their wild relatives; therefore, this seed shattering habit might have been one of the most notable obstacles to overcome during domestication. A strong selective sweep was observed for the *YAB* gene (Figure 6a), which encodes the axial regulator, *YABBY* 1-like isoform X2. A homolog of *YAB*, in the grass family, *Shattering1* (*Sh1*), encodes a *YABBY* TF that controls seed shattering in sorghum, rice, and maize, and has also led to extreme fruit size during tomato domestication (Cong et al., 2008; Lin et al., 2012; Tang et al., 2013). Interestingly, the homologous gene *Sh1* was under parallel selection during the domestication of both monocot (sorghum, rice, maize) and eudicot crops (pistachio), although selection occurred in opposite directions for the shattering versus non-shattering phenotypic effects of *YABBY* homologs. Besides, as noted above, *AGL11(SEEDSTICK)*, was also found in controlling the structure and mechanical properties of the seed coat and fruit size by regulating cytokinin levels and *FRUITFULL* (Pinyopich et al., 2003; Mizzotti et al., 2012). Finally, *PSY1* catalyzes the reaction from prephytoene diphosphate to phytoene during the late stages of fruit ripening in *Arabidopsis* (Zhou et al., 2015).

**Abscission** (from Latin *ab-* 'away', and *scindere* 'to cut') is the **shedding** of various parts of an **organism**, such as a **plant** dropping a **leaf, fruit, flower, or seed**.

*ADPG1* promoter was expressed in regions where cell separation occurred such as at the anther dehiscence site and the DZ.

A tomato pectinase, *positional sterility-2* (*PS-2*), is a homologous gene of *ADPG1*, which is required for anther dehiscence

Interestingly, *SAC70* (*B. napus*) expression was also detected in regions where two other processes of cell separation occur: in the junction between the seed and the funiculus prior to **seed abscission**, and in cells adjacent to the **anther dehiscence** site.

Similar to pectinase genes, the expression of cellulase genes in the pedicel abscission zone of tomato are also regulated by the MADS-box transcription factors, J, MC, and SIMBP21.

Yu Y-K, Li Y-L, Ding L-N, Sarwar R, Zhao F-Y and Tan X-L (2020) **Mechanism and Regulation of Silique Dehiscence, Which Affects Oil Seed Production.** *Front. Plant Sci.* 11:580. doi: 10.3389/fpls.2020.00580

Ripoll JJ, Roeder A, Ditta G & Yanofsky M (2011) **A novel role for the floral homeotic gene APETALA2 during *Arabidopsis* fruit development.** *Development*, 138. 5167-76. 10.1242/dev.073031.

Hossain S, Kadkol G, Raman R, Salisbury P, Raman H (2012) **Breeding *Brassica napus* for Shatter Resistance.** *Plant Breeding*, 10.5772/29051.

# ESTRATEGIAS PARA LA CARACTERIZACIÓN DE GENES IMPLICADOS EN LA DEHISCENCIA:

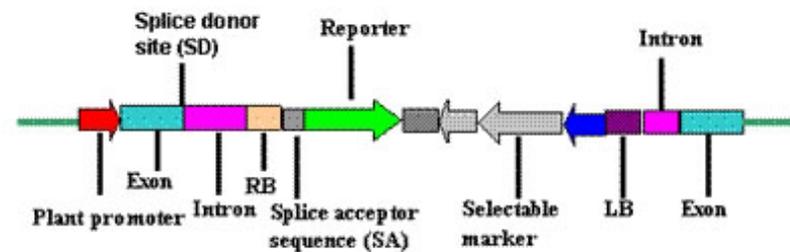
ANÁLISIS DE MUTANTES | GENÉTICA INVERSA: generación de mutaciones dirigidas y restreo de las mutaciones.

ESTUDIOS DE EXPRESIÓN DE GENES: estudio de transcriptomas. Utilizando ARNm aislado del tejido implicado en la dehiscencia (margen de las valvas, fundamentalmente).

# Gene trapping

Esta técnica genera mutaciones (inserciones) de pérdida de función al azar y permite reportar la expresión del gen implicado. Permite seleccionar *in vivo* los fenotipos de interés.

El vector contiene un **gen reporter** y un **marcador seleccionable**. Cola poli-A



**Figure 3. Gene trap vector.** The promoterless reporter gene contains splice acceptor (SA) sequence. The promoterless reporter gene can be expressed when insertion of a promoter trap vector occurs in an intron and results in a transcriptional fusion due to splicing from the chromosomal splice donor (SD) site to the SA sequence.

These experiments are based on the random insertion of reporter genes in the Arabidopsis genome, so that when inserted in the proximity of an enhancer or within a gene, the expression of the reporter is driven by the neighbouring regulatory sequences. Several of these lines showing reporter expression in the valve margins have allowed the cloning of new DZ-related genes and the identification of different cell identities within the valve margin.