Fruit Indehiscence Caused by Enhanced Expression of *NO TRANSMITTING TRACT* in *Arabidopsis thaliana*

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In flowering plants, fruit dehiscence enables seed dispersal. Here we report that *ntt-3D*, an activation tagged allele of *NO TRANSMITTING TRACT* (*NTT*), caused a failure of fruit dehiscence in *Arabidopsis*. We identified *ntt-3D*, in which the 35S enhancer was inserted adjacent to AT3G-57670, from our activation tagged mutant library. *ntt-3D* mutants showed serrated leaves, short siliques, and indehiscence phenotypes. *NTT*-overexpressing plants largely phenocopied the *ntt-3D* plants. As the proximate cause of the indehiscence, *ntt-3D* plants exhibited a near absence of valve margin and lignified endocarp *b* layer in the carpel. In addition, the replum was enlarged in *ntt-3D* mutants. *NTT* expression reached a peak in flowers at stage 11 and gradually decreased thereafter and *pNTT::GUS* expression was mainly observed in the replum, indicating a potential role in patterning. *NTT::GFP* localized in the nucleus and cytoplasm. *FRUITFULL* (*FUL*) expression was down-regulated in *ntt-3D* mutants and *ntt-3D* suppressed up-regulation of *FUL* in *replumless* mutants. These results indicate that *NTT* suppresses *FUL*, indicating a potential role in patterning of the silique. In seed crops, a reduction in pod dehiscence can increase yield by decreasing seed dispersal; therefore, our results may prove useful as a basis to improve crop yield.

INTRODUCTION

The fruit, a complex structure unique to flowering plants, mediates the maturation and dispersal of seeds. Various types of fruits are found in angiosperms, and the fruits of most plants are derived from the ovary wall and the fertilized ovules. The fruits of more than 3,000 species of *Brassicaceae*, including *Arabidopsis thaliana*, are known as siliques and develop from the fertilized gynoecium. The development of the *Arabidopsis* silique provides an excellent model system for studying the mechanisms that determine a plant organ due to the presence of distinctive morphological characteristics (Dinneny and Yanovsky, 2005). The silique is divided into three major regions

(valve, replum, and valve margin) (Ostergaard, 2009). The valves (or seed pod walls) encircle the developing seeds for protection and, after maturation of seeds, detach to promote seed dispersal in a process called pod dehiscence (or pod shatter). The replum, which connects the two valves, forms a central ridge that attaches the fruit to the plant. The valve margins form at the junction between the valves and the replum and facilitate the opening of the fruit through the actions of two different cell types. On the replum side of the valve margin, the separation layer (or dehiscence zone) detaches the valve from the replum through cell-cell separation mediated by the secretion of hydrolytic enzymes (Meakin and Roberts, 1990a; 1990b). On the valve side of the margin, the lignified cell layer forms. The lignified margin layer is continuous with the lignified valve layer (endocarp *b*). Together, these tissues are thought to create spring-like tension, thereby causing fruit dehiscence (Spence et al., 1996).

Several genes play important roles in regulating valve margin development. *SHATTERPROOF1* (*SHP1*) and *SHP2* are required for the proper development of fruit valve margins (Liljegren et al., 2000). Loss of *SHP1* and *SHP2* activities results in the absence of the lignified layer and separation layer, thereby preventing dehiscence. *INDEHISCENT* (*IND*) and *ALCATRAZ* (*ALC*) act downstream of *SHP* (Liljegren et al., 2004; Rajani and Sundaresan, 2001). Mutations in *IND* lead to defects in the small cells of the separation zone and the adjacent lignified cell layers, and mutation in *ALC* causes the absence of a layer of non-lignified cells at the site of separation. Thus, *IND* is required for specification of both the lignified layer and the separation layer of the valve margin, whereas *ALC* primarily regulates separation layer development. Valve margin development is controlled by *FRUITFULL* (*FUL*) and *REPLUMLESS* (*RPL*), which negatively regulate valve margin identity genes. In *ful* mutants, valve margin identity genes such as *SHP* become ectopically expressed in the valve, resulting in the partial conversion of valve tissues into valve margin-like tissues including lignified and separation layer-like cell types (Ferrandiz et al., 2000; Gu et al., 1998). In *rpl* mutants, ectopic *SHP* expression in the replum region causes replum cells to form into

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narrow files of cells that resemble cells found in the valve margin, which eventually renders *rpl* fruits partially indehiscent (Roeder et al., 2003). Recently, *BREVIPEDICELLUS* (*BP*), the class I KNOX gene, has been known to be involved in replum development (Alonso-Cantabrana et al., 2007). Antagonistic gene activities of these genes determine fruit patterning (Alonso-Cantabrana et al., 2007; Gonzalez-Reig et al., 2012).

NO TRANSMITTING TRACT (*NTT*) plays a role in the differentiation of transmitting tract (Crawford et al., 2007). Normal production of extracellular matrix (ECM) in the transmitting tract facilitates pollen tube growth. Due to the near absence of ECM in *ntt* mutants, pollen tubes experience difficulty in moving through the transmitting tract. The resulting failure of fertilization substantially reduces seed development, specifically in the basal part of the fruit. Although the effect of *ntt* lesion in transmitting tract development has been reported, little is known about the effect of its enhanced expression by activation tagging on fruit development and its potential downstream target(s).

Here we report the analysis of an activation tagged allele of *NTT* (*ntt-3D*), which exhibited a pod indehiscence phenotype. Examination of *NTT* and *FUL* expression in wild-type plants and *ntt-3D* mutants indicate a potential role for *NTT* in regulation of *FUL*. Also, because an activation tagged gene has an enhanced expression level reflecting its endogenous expression pattern (Weigel et al., 2000), unlike ectopic expression driven by the 35S promoter, our results provide clues to the mechanisms by which *NTT* functions in fruit dehiscence.

MATERIALS AND METHODS

Plant materials and growth conditions

Wild-type *Arabidopsis* plants (ecotype Columbia) and transgenic *Arabidopsis* plants were grown in Sunshine Mix 5 (USA) or Murashige and Skoog (MS) medium at 23°C under long-day (LD) conditions (16 h light/8 h dark photoperiod) at a light intensity of 120 $\mu\text{mol m}^{-2}\text{s}^{-1}$. *ntt-1* (SALK_049220) was obtained from the *Arabidopsis* Biological Resource Center (ABRC) (Alonso et al., 2003).

Generation of transgenic plants

To generate the *pNTT::GUS* construct, a 2.5 kb promoter region of *NTT* (AT3G57670) was amplified and fused with the pBI101 vector. To generate the *35S::NTT* construct, the coding region of *NTT* was amplified using Pfu fusion DNA polymerase (NEB, USA), cloned into pGEM3zf, and then inserted into pCHF3. To generate the *35S::NTT::GFP* construct, *NTT* cDNA without its stop codon was amplified and the amplicon was fused with pCHF3-GFP. Oligonucleotide sequences used for cloning these constructs are shown in Supplementary Table 1. These constructs were introduced into plants using the floral dip method and transformants were selected for kanamycin resistance.

Expression analyses

Total RNA was isolated from *Arabidopsis* seedlings with the Trizol reagent (Invitrogen, USA) and first-strand complementary DNA (cDNA) was synthesized from 1–2 μg of total RNA, in accordance with the manufacturer's instructions (Roche Applied Science, USA). Gene expression levels were analyzed via semi-quantitative reverse transcription (RT)-polymerase chain reaction (PCR) or quantitative RT-qPCR methods, as previously described (Hong et al., 2010; Yoo et al., 2011). RT-qPCR was performed in a 384-well plate with a LightCycler 480

(Roche Applied Science, USA) using SYBR Green Master mixture (Roche Applied Science, USA). Threshold cycle (Ct) and PCR efficiency of the primers used were calculated using LinRegPCR (Ramakers et al., 2003). Relative abundance of the transcripts was calculated by the statistical formula from geNorm (Vandesompele et al., 2002). For reference genes for quantification by RT-qPCR, two stably expressed genes [*PP2A43* (AT1G13320) and *SAND* family protein (AT2G28390)] were used to follow the 'The eleven golden rules for quantitative RT-PCR' (Kim et al., 2011; Udvardi et al., 2008). All RT-qPCR experiments were carried out in two or three biological replicates (independently harvested samples at different days) with three technical triplicates, each with similar results. Oligonucleotide sequences used for RT-PCR or RT-qPCR are listed in Supplementary Table 2. To test subcellular localization of *NTT*: GFP protein, transgenic *Arabidopsis* plants expressing *NTT*: GFP under the control of the 35S promoter were generated.

Scanning electron microscopy (SEM)

Fruits of flowers at stage 18 (Smyth et al., 1990) were fixed using 2.5% glutaraldehyde in 25 mM phosphate buffer (pH 7.0) at 4°C, incubated in 1% osmium tetroxide in 25 mM phosphate buffer (pH 7.0) at 4°C, and then washed with 25 mM phosphate buffer. Fixed samples were dehydrated in a graded ethanol series and were replaced by 100% isoamyl acetate. Critical point drying was carried out. Gold particle-coated samples were examined under a scanning electron microscope (JSM-5300, Japan Electric, Japan).

Histological staining

To observe the valve tissues, the silique was fixed in FAA at room temperature. Siliques were dehydrated through a graded ethanol series, then treated with HistoClear (National Diagnostics, USA) and embedded in paraplast. Each sample was sectioned to 8 μm on a microtome and mounted on slides. Sections were de-waxed in HistoClear and washed in 100% ethanol. Slides were stained with 0.05% Toluidine Blue O. To observe the lignified cell, sectioned samples were stained with 2% phloroglucinol in 95% ethanol and then observed in 50% hydrochloric acid (Liljegren et al., 2000).

RESULTS

Isolation and morphological characterization of *S6-003* mutants

To isolate *Arabidopsis* mutants that affect developmental processes, we screened a mutant library of approximately 80,650 activation tagged lines (Ahn et al., 2007; Weigel et al., 2000). We identified a mutant (*S6-003*) that had serrated leaves (Fig. 1A) and, based on subsequent characterization (see below), we named this allele *ntt-3D*. Self-fertilized progeny of a hemizygous *ntt-3D* plant exhibited a similar phenotype as the parental line in the subsequent generation, indicating that the phenotype was dominant and was caused by a single T-DNA insertion. *ntt-3D* plants had narrow cotyledons and small, severely serrated leaves (Fig. 1B). In addition, *ntt-3D* plants produced short internodes and thus were shorter (height = 16.7 ± 3.0 cm) than wild-type plants (23.7 ± 1.0 cm) when grown for 55 days under LD conditions. The flowers of *ntt-3D* plants opened precociously before the petal was more elongated than the sepal (Fig. 1C). In addition, a noticeable phenotype in the fruit structure was seen in *ntt-3D* plants. The siliques of *ntt-3D* plants were short and twisted (Fig. 1D). The seedpods of *ntt-3D* plants barely opened even after their seeds matured and dried (Fig. 1E),

Fig. 1. Phenotypes and characterization of the *ntt-3D* mutants under long-day (LD) conditions. (A) Overall morphology of 25-day-old wild-type (WT) (left) and *ntt-3D* plants (right). (B) Leaf morphology of wild-type plants (Top) and *ntt-3D* plants (Bottom). (C) Inflorescence morphology of wild-type (left) and *ntt-3D* plants (right). (D) Silique phenotypes of wild-type (left) and *ntt-3D* plants (right). (E) Indehiscent silique phenotype of *ntt-3D* plants (Bottom). (F) A T-DNA insertion site in *ntt-3D* (S6-003) plants. (G) Upregulation of *NTT* (AT3G57670) in *ntt-3D* whole seedlings grown for 10 days under LD conditions. *UBQ10* was used as an internal control. (H-I) Rosettes (H), bolted plants (I), and siliques (J) of wild-type, *ntt-3D*, and *35S::NTT* plants.

suggesting that *ntt-3D* plants also showed a failure of pod dehiscence.

Plasmid rescue (Weigel et al., 2000) revealed that a T-DNA containing the 35S enhancer was inserted between AT3G57660 and AT3G57670 in *ntt-3D* plants (Fig. 1F). RT-PCR revealed that, in *ntt-3D* plants, AT3G57670 expression was substantially increased (Fig. 1G). AT3G57670 consists of two exons and encodes a C2H2/C2HC-type zinc finger protein (Englbrecht et al., 2004; Sagasser et al., 2002). This gene was known as *NO TRANSMITTING TRACT* (*NTT*) and functions in transmitting tract development in the carpel of *Arabidopsis* (Crawford et al., 2007). To confirm that the phenotypes seen in *ntt-3D* plants were conferred by enhanced expression of *NTT*, we analyzed the phenotype of plants (*35S::NTT*), in which the coding region of *NTT* was expressed under the control of the 35S promoter. Phenotypes observed in homozygous *35S::NTT* plants were similar to those seen in *ntt-3D* plants, including serrated leaves, short internodes, and short siliques (Figs. 1H-1J). The silique phenotypes of *35S::NTT* transgenic plants could be categorized into three groups: wild-type-like, intermediate, and strong (data not shown). Although tiny fruits with virtually no seeds were produced in strong lines, their fruits

were almost indehiscent. In addition, intermediate lines also showed a weak indehiscence phenotype. These results indicated that the morphological defects seen in *ntt-3D* plants were due to the enhanced expression of *NTT*.

High expression of *NTT* in the carpel

RT-PCR analysis revealed that expression of *NTT* was mainly detected in reproductive tissues such as flower buds and flowers (Fig. 2A), which was consistent with the public microarray data (Schmid et al., 2003). *NTT* expression levels were low in vegetative tissues such as leaves, stems, and roots. This result suggested that *NTT* principally plays a role in the development of reproductive organs.

Because the spatial expression patterns of *NTT* were studied only in developing carpels (Crawford et al., 2007), we analyzed *NTT* expression in more detail using *pNTT::GUS* transgenic plants. *pNTT::GUS* expression was observed in vegetative tissues such as the leaf vasculature and petiole (Fig. 2B). Furthermore, strong *pNTT::GUS* expression was detected in proliferating tissues such as the shoot apex region and root tip (Fig. 2B inset). During flower development, *pNTT::GUS* expression could be detected in the carpel of flowers as early as stage 8

Fig. 2. Expression of *NTT* in wild-type plants. (A) Spatial expression pattern of *NTT*. *UBQ10* was used as a control. RL, rosette leaves; CL, cauline leaves; S, stems; OF, old flowers at stage 15 (Smyth et al., 1990); YF, young flowers at stages 13 and 14; FB, floral buds; R, roots. Floral stages were previously defined (Smyth et al., 1990). (B) *pNTT::GUS* expression in whole seedlings. Magnified views of shoot apex (Bottom right) and root apex (Bottom left) are shown. (C) Time-course expression of *NTT* during flower developmental stages revealed via RT-qPCR. Floral stages were previously defined (Smyth et al., 1990). (D) *pNTT::GUS* expression in the carpel of flowers at stage 12 (Smyth et al., 1990) (Top) and at stage 14 (Bottom). (E) Subcellular localization of *NTT::GFP* protein in *Arabidopsis* epidermal cells.

(Smyth et al., 1990) and strong expression was detected at stage 11 (Fig. 2C). GUS reporter analysis revealed that *pNTT::GUS* expression was observed in the replum of the silique (Fig. 2D, arrows). *NTT::GFP* protein was localized preferentially in the nucleus and the cytoplasm in *Arabidopsis* epidermal cells (Fig. 2E). Expression of *NTT* in the carpel was consistent with the indehiscence phenotype of the activation tagged *ntt-3D* allele of *NTT* (Fig. 1J).

The morphological defects of valve margin and *enb* layer in *ntt-3D* plants

Because *ntt-3D* plants showed morphological defects in the fruit, we further investigated the fruit development of *ntt-3D* plants via scanning electron microscopy (SEM). In flowers at stage 12 (Smyth et al., 1990), which is prior to fertilization, fruit development in *ntt-3D* mutants seemed largely normal, comparing to the fruit development seen in wild-type plants (Fig. 3A). However, at stage 18, dehiscence did not occur in *ntt-3D* mutants and the replum was wider in *ntt-3D* mutants than in wild-type plants (Fig. 3B, right). By contrast, in wild-type siliques, the dehiscence zone, which is normally formed at the valve margin, was well developed and separation of the valves from the replum began to occur at stage 18 (Fig. 3B, left). Our observation suggested that an increased activity of *NTT* affected differentiation of valve margin into the dehiscence zone and formation of the valve margin.

To further characterize the role of *NTT* in valve margin development, we examined the morphology of the valve margin in *ntt-3D* mutants. In the wild-type mature fruits, the valve margin

differentiated into a layer of lignified cells and a separation layer made of small cells (Fig. 3C). The small cell layers were observed in the replum region. However, in *ntt-3D* fruits, separation and lignified margin layers were almost absent and the replum was enlarged. Furthermore, the endocarp *b* layer was nearly absent in *ntt-3D* mutants. This result indicated that the enhanced expression of *NTT* led to the alterations in the differentiation of valve margin into the dehiscence zone.

Because lignification in the mature fruit plays an important role in dehiscence, we investigated lignification of the *ntt-3D* mutants using phloroglucinol, a lignin-specific histological stain. Wild-type fruit showed lignification of valve margin cells adjacent to the dehiscence zone, the vascular bundles, and the endocarp *b* layer (Fig. 3D, pink). However, lignified cells were almost absent in the valve margin of *ntt-3D* fruits (Fig. 3D, arrowhead). By contrast, in *ntt-1* mutants, a loss-of-function allele of *NTT*, the overall lignification pattern, the size of the replum, and the endocarp *b* layer were not clearly different from those in wild-type plants, raising the possibility that *NTT* acts redundantly in carpel development. These results indicated that the enhanced expression of *NTT* affected the lignification of margin cells in the fruit. Taken together, these results suggested that the change in *NTT* activity is important for proper development of the valve margin and endocarp *b* layer of the valve.

Downregulation of *FUL* in *ntt-3D* mutants

ntt-3D mutants showed enlarged replum, decreased silique size, and fruit indehiscence. To identify the gene(s) responsible for the phenotype, we examined the expression levels of fruit pat-

Fig. 3. Defects in endocarp *b* layer, valve margin, and replum seen in the carpel of *ntt-3D* mutants. (A, B) Scanning electron microscopy (SEM) images of the carpel in a flower at stage 12 (Smyth et al., 1990) (A) and at stage 18 (B) of wild-type (WT) and *ntt-3D* plants. Dotted line: putative dehiscence zone. (C) Toluidine Blue O-stained sections of the silique at stage 17 (Smyth et al., 1990) of wild-type (left) and *ntt-3D* (right) plants. Note that the valve margin and the endocarp *b* layer was almost absent in *ntt-3D* plants (arrowhead). (D) Phloroglucinol-stained sections of the silique of a flower at stage 17 (Smyth et al., 1990) of wild-type, *ntt-3D*, and *ntt-1* plants. Arrow: lignified cells stained with phloroglucinol. Note the near absence of lignified cells in *ntt-3D* fruits (arrowhead). enb, endocarp *b* layer; DZ, dehiscence zone; LL, lignified layer; R, replum; SL, separation layer; V, valve; VM, valve margin.

termining genes in *ntt-3D* mutants via RT-qPCR. We found that *FUL* expression is apparently decreased in *ntt-3D* mutants (Fig. 4A), whereas *RPL* expression was unaltered. *BP* expression was slightly increased in *ntt-3D* mutants. However, in *ntt-1* mutants, *FUL* and *RPL* expression levels did not show a dramatic change. *BP* expression levels were slightly decreased in *ntt-1* mutants. We then tested the effect of *rpl* mutation on the expression of *FUL*. *FUL* expression was slightly increased in *rpl-2* mutants (Fig. 4B), consistent with the repression of a valve factor by a replum factor (Romera-Branchat et al., 2013). However, *FUL* expression was decreased in *rpl-2 ntt-3D* mutants, indicating that *ntt-3D* suppressed the effect of *rpl* mutation.

DISCUSSION

From an activation tagging pool, a pleiotropic mutant, *ntt-3D*, was isolated. Ectopic expression of *NTT* resulted in serrated leaves and reduced height and root length in *ntt-3D* plants (Figs. 1A and 1B). *35S::NTT* plants also produce small siliques with small and irregular seeds and thin valves, like those of *ntt-3D* plants (Figs. 1H, 1I, and 1J). These data indicate that increased expression of *NTT* was responsible for the *ntt-3D* mutant phe-

notypes, suggesting that enhanced expression of *NTT* affects the regulation of a variety of processes in plant development and differentiation.

NTT encodes a C_2H_2/C_2HC -type zinc finger protein (Englbrecht et al., 2004; Sagasser et al., 2002). The classical C_2H_2 zinc fingers have been implicated in DNA binding, RNA binding, and protein-protein interactions and are involved in a variety of functions. The classical C_2H_2 zinc finger domain is conserved throughout the genomes of eukaryotes. In *Arabidopsis*, 176 zinc finger proteins are predicted by *in silico* analyses, about 0.7% of the total proteins encoded in the *Arabidopsis* genome (Englbrecht et al., 2004). Only 33 *A. thaliana* zinc finger proteins are conserved in other eukaryotes. By contrast, the majority of the *Arabidopsis* zinc finger proteins (81%) are plant-specific. *NTT* belongs to a plant-specific zinc finger subfamily of six proteins having a conserved C_2H_2/C_2HC zinc finger motif. Among them, only *TRANSPARENT TESTA 1* (*TT1*) was found to be involved in seed coat endothelium development (Sagasser et al., 2002). Interestingly, both *TT1*-overexpressing plants and *NTT*-overexpressing plants exhibited similar phenotypes, including a wide range of defects in leaf morphology, flower and fruit morphology, flowering time, stem and root length. Thus,

Fig. 4. Downregulation of *FUL* in *ntt-3D* mutants. (A) Expression levels of *FUL*, *RPL*, and *BP* in the carpel of flowers at stage 12 (Smyth et al., 1990) in *ntt-3D* mutants. (B) Expression levels of *FUL* in *rpl-2* and *rpl-2 ntt-3D* double mutants. Each gene expression level in wild type plants was arbitrarily set to 1.0. Statistical analysis was done with an unpaired Student's *t*-test. The results are considered statistically significant ($p < 0.05$).

Fig. 5. A proposed model of *NTT* (a replum factor) in regulating *FUL* (a valve factor) in carpel development. Arrows represent promotion effects, whereas T-bars indicate repression effects. A lignified layer (LL) is shown in grey. *enb*, endocarp *b* layer; SL, separation layer.

genes in this subfamily may play important roles in regulating tissue-specific processes.

Pod dehiscence is a mechanism to disperse seeds. It requires proper differentiation of specific cell types in the valve margin, separation layer and lignified layer, and the lignified layer of the valve (endocarp *b*). During differentiation, transcription factors, including *SHP1*, *SHP2*, *IND*, and *ALC* play a role in margin development (Liljegren et al., 2000; 2004; Rajani and Sundaresan, 2001). They are negatively regulated by *FUL* in the valve and *RPL* in the replum to restrict their expression (Roeder et al., 2003). A lesion in any one of these genes induces a partial or complete indehiscent fruit phenotype.

The *ntt-3D* mutants showed a decrease in fruit length and fruit dehiscence, probably due to the near absence of lignified cells in the valve margin and the valve endocarp *b* layer (Fig. 3D). *FUL* is involved in fruit growth after fertilization as well as lignification of the inner valve layer (Gu et al., 1998). *FUL* also indirectly regulates fruit growth by restricting the activity of valve margin genes (*SHP1*, *SHP2*, *ALC*, and *IND*) to the valve mar-

gin. Introduction of a lesion in *SHP*, *ALC* or *IND* into *ful* mutants induced the absence of the lignified valve layer and thus almost completely restored fruit growth, suggesting that *SHP*, *IND*, *ALC* and *FUL* play a redundant role or that *FUL* acts as the primary determinant of endocarp *b* layer lignification (Liljegren et al., 2004). Because the fruit of *ntt-3D* mutants was short and *FUL* expression was decreased in *ntt-3D* mutants (Figs. 1E and 4A), it is possible that the down-regulation of *FUL* is responsible for the fruit phenotype of *ntt-3D* mutants. Expression analyses suggested that *NTT* acts as a replum factor (Fig. 2D) and there is no cross-regulation between *NTT* and *RPL*, which is also a replum factor (Fig. 4A) (K.S.C and J.H.A., unpublished data). Taken together, our results suggest that *NTT* affects *FUL* expression and is involved in fruit patterning for dehiscence as well as transmitting tract cell differentiation (Fig. 5). However, it is unclear how *NTT* negatively regulates *FUL* in their different expression domains. Further analysis at the cellular level would provide a clue as to whether *NTT* non-cell-autonomously regulates *FUL* during carpel development.

In summary, we found that an activation tagged allele of *NTT* exhibited a pod indehiscence phenotype. *NTT* negatively regulates *FUL* expression in the carpel. Loss of pod dehiscence is a useful trait for harvesting seeds, because it can minimize the chance of losing seeds in natural habitats. The pod indehiscence phenotype caused by *NTT* activation may be useful to increase the yield of crop plants.

Note: Supplementary information is available on the Molecules and Cells website (www.molcells.org).

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