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# CRISPR-mediated *BnaIDA* editing prevents silique shattering, floral organ abscission, and spreading of *Sclerotinia sclerotiorum* in *Brassica napus*

Dear Editor,

Rapeseed (*Brassica napus*) oil is a major source of vegetable oil around the world. Stem rot disease caused by the necrotrophic fungus *Sclerotinia sclerotiorum* and silique shattering during harvest are two major contributors to yield loss in *B. napus* (Zhang et al., 2021). Infection by *S. sclerotiorum* occurs when ascospores land on detached petals where they germinate and form mycelia that ultimately infects the leaves (Ding et al., 2021). Consistent with this mode of infection, reduced damage from stem rot has been achieved by generating apetalous rapeseed that are resistant to petal-mediated infection (Yu et al., 2016). We propose that preventing petal shedding should also reduce leaf infections. Moreover, attached floral organs will promote rapeseed flower tourism and provide an additional economic benefit.

The peptide inflorescence deficient in abscission (IDA) and its homologs is conserved in flowering plants and play important roles in regulating floral organ abscission and other cell-separation processes (Sto et al., 2015; Shi et al., 2019). To investigate the function of IDA in rapeseed plants, we identified five IDA homologs in *B. napus* using the Genoscope database (https://www.genoscope.cns.fr/brassicanapus/). We refer to these homologs as *BnaIDA-A07* (BnaA07g27400D), *BnaIDA-C06* (BnaC06g29530D), *BnaIDA-C02* (BnaC02g18450D), *BnaIDA-C04* (BnaC04g26010D), and *BnaIDA-A02* (BnaA02g13980D). Protein sequence alignments revealed that BnaIDA-A07, BnaIDA-C06, BnaIDA-C02, BnaIDA-C04, and BnaIDA-A02 are 88.3%, 89.2%, 85.3%, 86.8%, and 87.0% identical to Arabidopsis AtIDA, respectively. The last 14 amino acids at the C-terminus of these homologs are particularly well-conserved (Supplemental Figure 1). Phylogenetic tree analysis further demonstrated that *BnaIDA-A07* and *BnaIDA-C06* share the highest similarity with *AtIDA* (Figure 1A). According to the *B. napus* transcriptome database (https://brassica.biodb.org/), transcripts of *BnaIDA-A07* and *BnaIDA-C06* are specifically expressed in flowers and mature siliques, which has been confirmed using qRT–PCR (Figure 1B). This suggests that *BnaIDA-A07* and *BnaIDA-C06* may be involved in floral organ abscission in *B. napus*.

To further investigate the function of *BnaIDA-A07 and BnaIDA-C06* in *B. napus*, we knocked out both genes simultaneously using CRISPR–Cas9. Guide RNAs targeting two sites in the coding region of each gene were selected to ensure effective editing using the CRISPR–Cas9 multiplex editing system (Figure 1C). Four T0 transgenic lines were verified by PCR and self-pollinated to produce T1 progeny lines. Sanger sequencing of *BnaIDA-A07* and *BnaIDA-C06* in both T0 and T1 lines confirmed successful editing and identified both single- and multi-base insertions and deletions. We refer to these gene edited lines as *ida-double* (*ida-d*) mutants. *In silico analysis of potential off-target editing sites identified 38 regions where off-target editing could occur. However, only two of these regions (BnaC01g29010D and BnaA10g08600D) were in the coding sequence of a gene. Subsequent sequence analysis confirmed that none of the predicted off-target genome editing sites were present in the ida mutant lines* (Supplemental Figure 2). Because all *ida-d* mutant lines exhibited floral organ persistence, we chose to use the *ida-d17* line for further characterization (Figure 1D). We also demonstrated that expression of *BnaIDA-A07* and *BnaIDA-C06* was unaffected in the *ida-d2/17* mutants (Supplemental Figure 3). These results demonstrate that simultaneous knock out of multiple genes in *B. napus* can be achieved without exogenous T-DNA using CRISPR–Cas9, which can be useful for molecular breeding in rapeseed.

In wild-type (WT) *B. napus*, floral organs such as petals, sepals, and stamens begin shedding around position 8 after pollination with no floral organs attached at position 10 (Figure 1E and Supplemental Figure 4). By contrast, the floral organs of *ida-d17* remained attached throughout flowering and silique maturation (Figure 1F). Floral organs remained attached even after the siliques had completely dried and the petals turned white (Figure 1G). The number of flowers in WT plants reached a maximum around 30 days after anthesis and then decreased due to flower shedding with a typical flowering period of 60 days. By contrast, the *ida-d17* mutants developed more than 150 flowers per plant that remained fully attached even after 70 days post-anthesis (Figure 1H). We also generated plants overexpressing *BnaIDA-A07* (*OE-BnaIDA-A07*) and found that the rate of floral organ detachment was greater than in the WT line (Supplemental Figures 5 and 6).

Dehiscence is a developmental process of cell separation that is required to open silique valves and enable seeds to spread from the mother plant. We previously demonstrated that premature silique dehiscence can result in yield losses of up to 50% during the mechanical harvesting of *B. napus* (Li et al., 2021). *AtIDA* expression has been detected at the silique dehiscence zone, but the role of IDA in dehiscence has yet to be explored (Butenko et al., 2006). Given that *BnaIDA-A07* and *BnaIDA-C06* are also highly expressed in mature siliques (Figure 1B), we were curious to know if BnaIDA is involved in silique dehiscence. To test this hypothesis, we harvested siliques from WT and *ida-d17* mutant plants as siliques began turning a light yellow.

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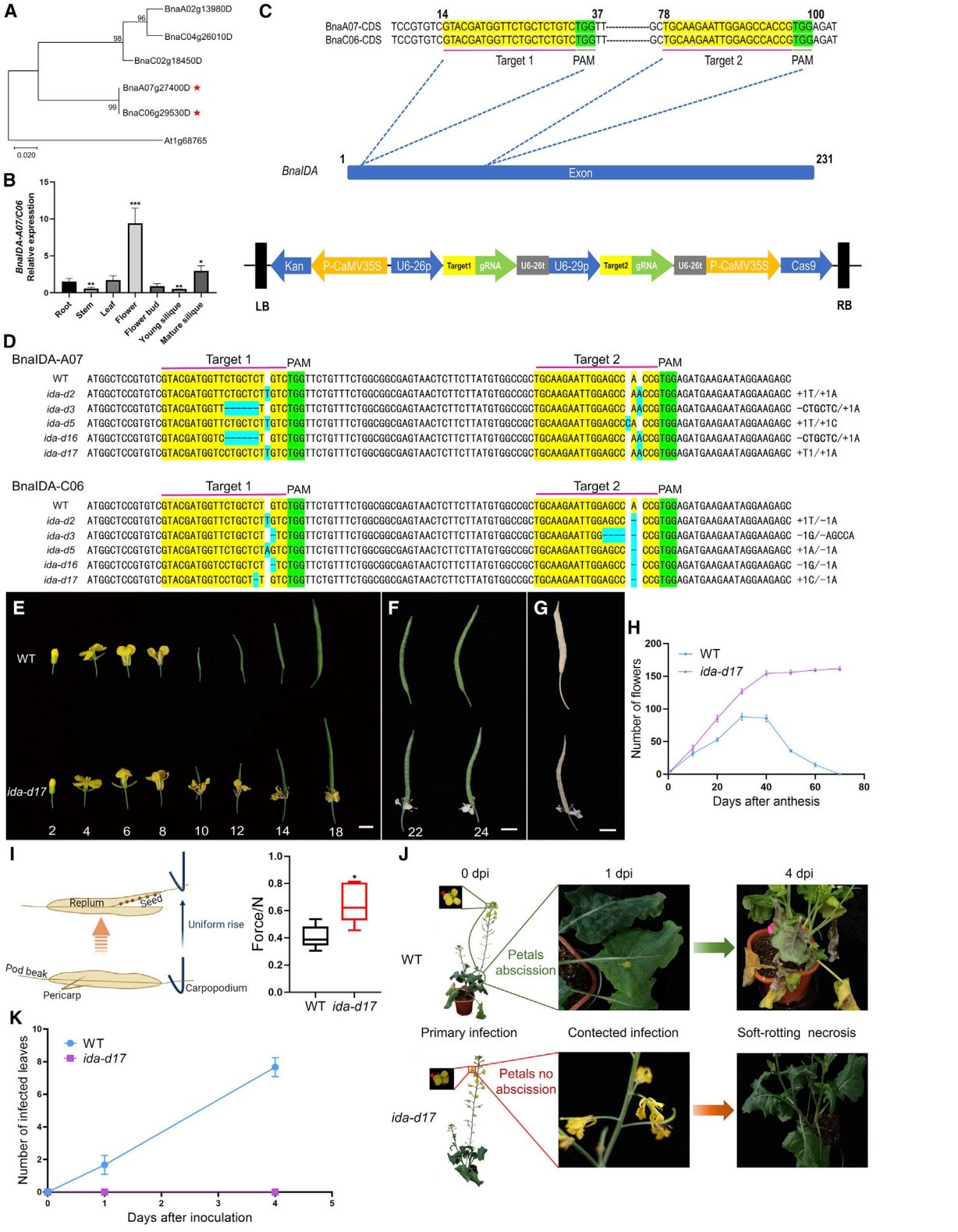


**Figure 1. Functional analysis of two IDA homologs in *B. napus* by gene editing.**

**(A)** Phylogenetic tree of BnaIDA and AtIDA constructed using the neighbor-joining algorithm.

**(B)** qRT–PCR analysis of the expression of *BnaIDA-A07/C06* genes in various tissues of WT plants. The same primers were used to amplify *BnaIDA-A07* and *BnaIDA-C06* due to their high sequence similarity. Significant differences in gene expression were determined using Student's *t*-test with n=3: ***p > 0.001; **p > 0.01; *p > 0.05. Bars indicate standard deviation.

*(legend continued on next page)*

We then measured the force required for silique dehiscence using a texture analyzer (Li et al., 2021). The maximum tensile strength of the WT siliques was about 0.3–0.5 N, while siliques from *ida-d17* plants had a maximum tensile strength of 0.6–0.8 N (Figure 1I). This result suggests that BnaIDA-A07 and BnaIDA-C06 play a crucial role in silique dehiscence. We believe this mutant line has great potential to improve the yield of rapeseed by limiting grain loss due to premature silique dehiscence during mechanical harvesting.

Stem rot disease caused by *S. sclerotiorum* is a major and devastating disease in rapeseed plants (Ding et al., 2021). We sought to determine if reduced floral organ abscission in *ida-d17* could limit the spread of *S. sclerotiorum* by limiting the number of infected petals that fell on leaves. To test this, we manually inoculated the petals of *ida-d17* and WT plants with *S. sclerotiorum*. The inoculated petals of WT plants started to detach and fall on the leaves below at one day post-inoculation. All ten WT plants exhibited soft-rotting necrosis on almost all leaves at four days post-inoculation. However, no disease symptoms were observed on the leaves of *ida-d17* plants (Figure 1J and 1K) and only one of the ten *ida-d17* plants developed mild disease on petals. The attached petals in *ida-d17* plants appear to prevent *S. sclerotiorum* from spreading between infected petals and healthy leaves, thereby reducing the damage caused by stem rot disease.

To further investigate whether editing of *BnaIDA-A07/C06* affected plant growth and development, we compared the overall plant morphology, seed weight, seeds per silique, branch number, plant height, and seed germination rate between *ida-d17* and WT plants. Aside from floral organ attachment, *ida-d17* plants were indistinguishable from WT plants (Supplemental Figure 7). Therefore, important agronomic traits were unaffected by the simultaneous knock out of *BnaIDA-A07* and *BnaIDA-C06* genes in *B. napus*.

In conclusion, we simultaneously edited two IDA homologs (*BnaIDA-A07* and *BnaIDA-C06*) in *B. napus* using the CRISPR–Cas9 system. We revealed that loss-of-function of these IDA homologs resulted in reduced floral organ abscission, silique dehiscence, and disease severity caused by *S. sclerotiorum*. These traits could improve yield in *B. napus by reducing seed loss due to* premature silique dehiscence during mechanical harvesting and losses due to stem rot. There is also the potential economic benefit from botanical tourism by travelers who wish to see the beautiful yellow flowers of *B. napus. The ida-d17 mutant retains its flowers for a longer period of time*, which extends the rapeseed flower-viewing season. Future studies will further investigate these important traits.

## SUPPLEMENTAL INFORMATION

Supplemental information is available at *Plant Communications Online*.


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## AUTHOR CONTRIBUTIONS

R.G. performed experiments and wrote the manuscript. X.L.-T. and R.B.A. conceived the project and revised the manuscript. Other authors analyzed the data and revised the manuscript. All authors who contributed to the study have read and approved the final version of the manuscript.


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**(C)** Diagram of the regions of *BnaIDA-A07* and *BnaIDA-C06* targeted by CRISPR–Cas9. Targeted sites in the conserved coding sequence region of *BnaIDA-A07/C06* are indicated.
**(D)** Mutation analysis of *ida-d2/3/5/16/17* plants. Target sequences, mutations, and PAMs are indicated in yellow, blue, and green, respectively.
**(E–G)** Floral organ abscission after anthesis in WT and *ida-d17* mutant plants at different flower positions, mature siliques, and dried siliques. Scale bar equals 1 cm.
**(H)** Quantitative measurements of WT and *ida-d17* mutant flowers taken over 70 days (n = 10). Bars indicate standard deviation.
**(I)** Schematic diagram of the texture analyzer and quantification of the force required to open siliques. Significant differences were determined using Student's *t-test with n=5:* *p > 0.05. Bars indicate standard deviation.
**(J)** Progression of stem rot caused by *S. sclerotiorum* infection at 0, 1, and 4 days post-inoculation. Red arrows indicate sites of inoculation.
**(K)** Quantification of disease progression (n = 10). Bars indicate standard deviation.

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