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-d17 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.

Published by the Plant Communications Shanghai Editorial Office in association with Cell Press, an imprint of Elsevier Inc., on behalf of CSPB and CEMPS, CAS.

This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
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.

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.

**REFERENCES**

Butenko, M.A., Stenvik, G.E., Alm, V., Saether, B., Patterson, S.E., and Aalen, R.B. (2006). Ethylene-dependent and -independent pathways controlling floral abscission are revealed to converge using promoter::reporter gene constructs in the ida abscission mutant. J. Exp. Bot. 57:3627–3637. https://doi.org/10.1093/jxb/erl130.

Ding, L.N., Li, T., Guo, X.J., Li, M., Liu, X.Y., Cao, J., and Tan, X.L. (2021). Sclerotinia stem rot resistance in rapeseed: recent progress and future prospects. J. Agric. Food Chem. 69:2965–2978. https://doi.org/10.1021/acs.jafc.0c07351.

Li, Y.L., Yu, Y.K., Zhu, K.M., Ding, L.N., Wang, Z., Yang, Y.H., Cao, J., Xu, L.Z., Li, Y.M., and Tan, X.L. (2021). Down-regulation of MANNANASE7 gene in Brassica napus L enhances silique dehiscence-resistance. Plant Cell Rep. 40:361–374. https://doi.org/10.1007/s00299-020-02638-5.
Correspondence

Yu, K., Wang, X., Chen, F., Chen, S., Peng, Q., Li, H., Zhang, W., Hu, M., Chu, P., Zhang, J., et al. (2016). Genome-wide transcriptomic analysis uncovers the molecular basis underlying early flowering and apetalous characteristic in Brassica napus L. Sci. Rep. 6:30576. https://doi.org/10.1038/srep30576.

Zhang, X., Cheng, J., Lin, Y., Fu, Y., Xie, J., Li, B., Bian, X., Feng, Y., Liang, W., Tang, Q., et al. (2021). Editing homologous copies of an essential gene affords crop resistance against two cosmopolitan necrotrophic pathogens. Plant Biotechnol. J. 19:2349–2361. https://doi.org/10.1111/pbi.13667.