Developing Canola Restorer Lines without Negative Agronomic Traits Associated with the *Rfo* Introgression from Radish

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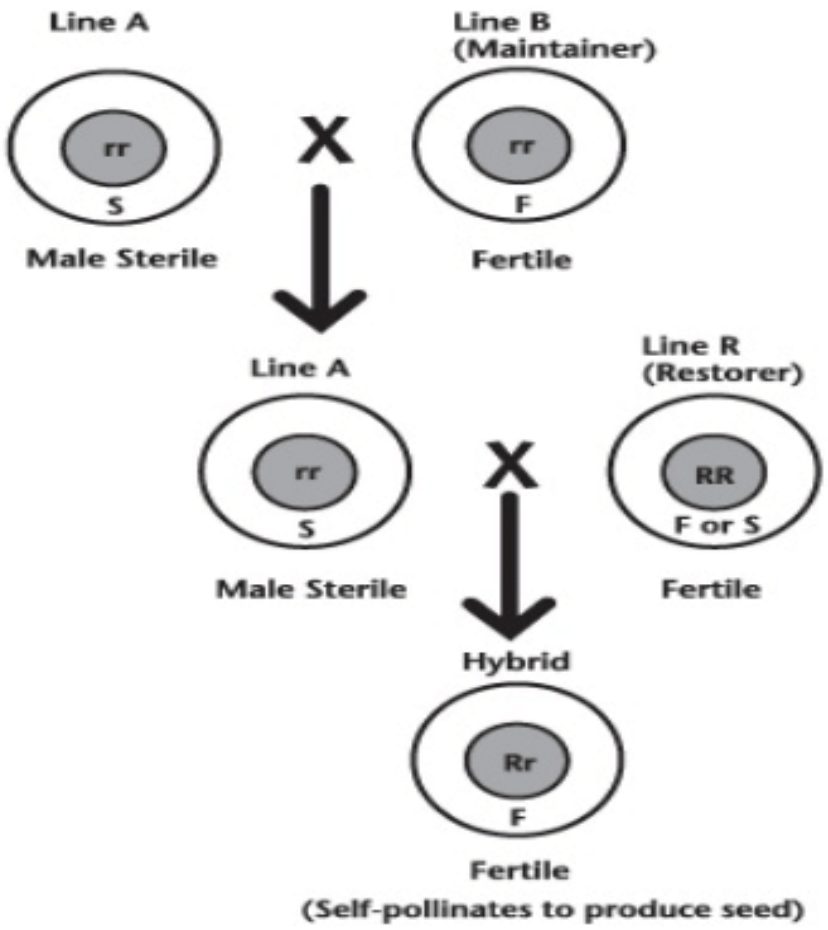
Introduction



- Male sterility is the plant failure to produce dehiscent anthers or functional pollen
- Ogura Cytoplasmic Male Sterility (CMS) is the most widely applied in cruciferous crops stable sterility and complete abortion
- It was first reported in radish (Ogura 1968).
- Ogura (CMS) is caused by the mitochondrial gene *Orf138*, which disrupts pollen production **without affecting female fertility**.

Introduction

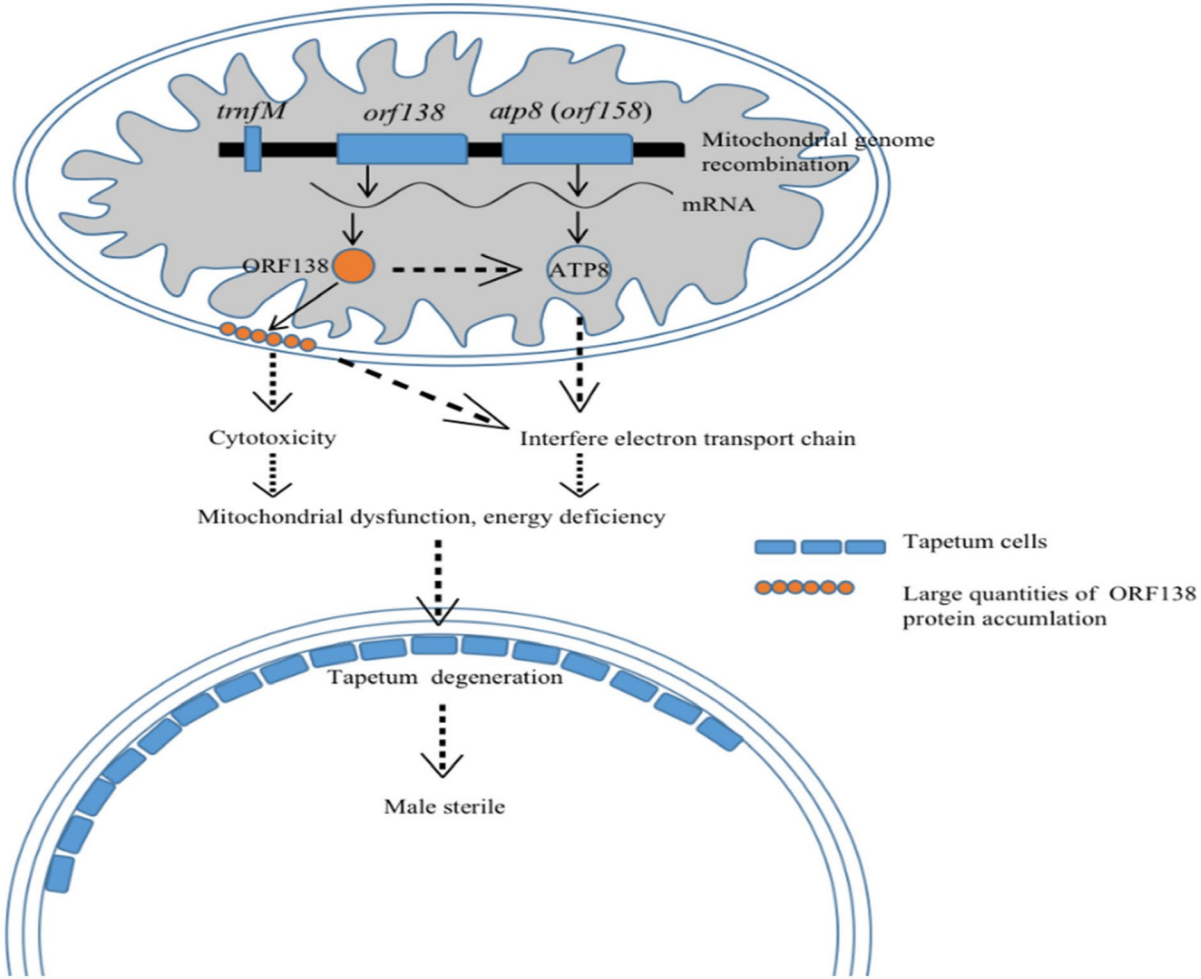
- Ogura-CMS and *Rfo* gene have been introduced from radish to Brassica species by **interspecific crosses** (Bannerot et al. 1974) and **protoplast fusion** (Pelletier et al. 1983).

Is *Orf138 expressed only in the anthers?*

- ORF138 protein is expressed in the **reproductive organs** and in the **vegetative tissues** (Krishnasamy and Makaroff, 1994; Bellaoui et al., 1999).

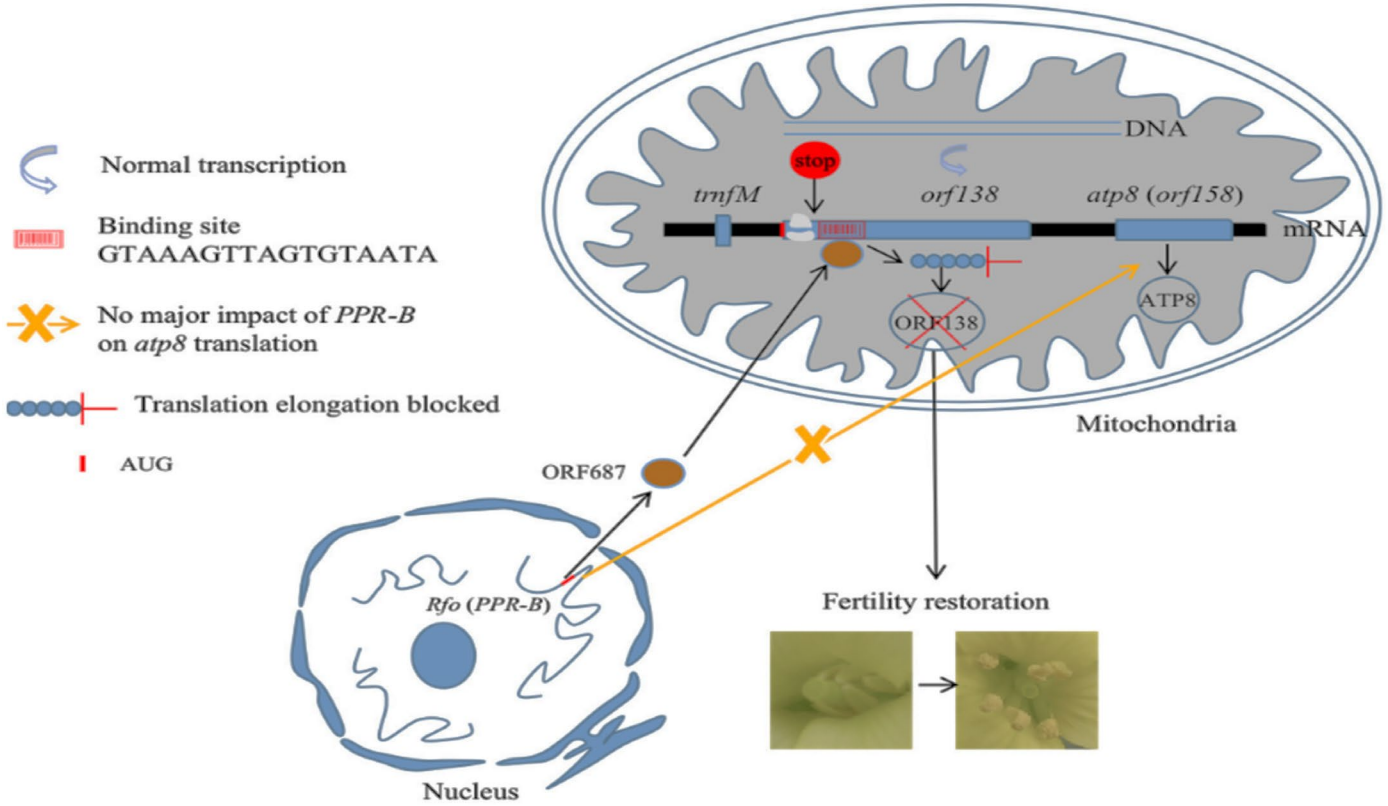
- It is still unknown how the mitochondrial gene participates in the CMS process without affecting plant development.

How orf138 induces Ogura CMS?



Ren, W., Si, J., Chen, L., Fang, Z., Zhuang, M., Lv, H., ... & Zhang, Y. (2022). Mechanism and utilization of ogura cytoplasmic male sterility in cruciferae crops. *International Journal of Molecular Sciences*, *23*(16), 9099.

- ***Rfo (PPR-B)*** *is* a nuclear gene that restores male fertility by altering the expression of *Orf138* at the post-transcriptional level.



Wang, C., Lezhneva, L., Arnal, N., Quadrado, M., & Mireau, H. (2021). The radish Ogura fertility restorer impedes translation elongation along its cognate CMS-causing mRNA. *Proceedings of the National Academy of Sciences*, *118*(35), e2105274118.

Objectives

1- To introduce and **overexpress *PPR-B* gene**, into *Brassica napus*, excluding non-essential regions of the radish *Rfo* fragment.

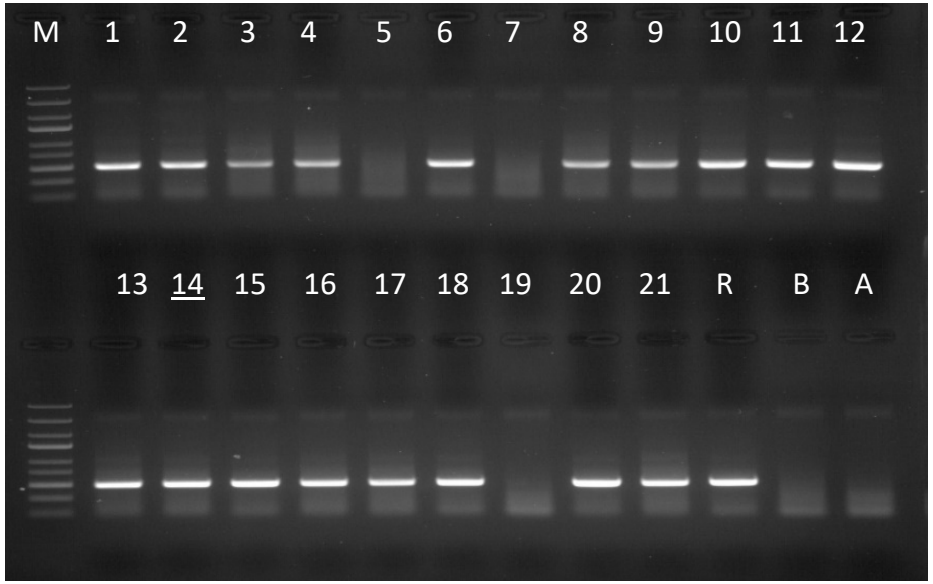
2-To assess the morphological parameters, pod characteristics and **agronomic performance** of the transformed *B. napus*.

Methodology

- RNA extraction and cDNA synthesis
- *PPR-B* (2064 bp) isolation by PCR
- *E. coli* transformation using the **Gateway cloning** protocol
- Sequencing: Sanger sequencing and Nanopore sequencing
- *Agrobacterium* transformation by **electroporation**
- Plant transformation using spray method
- Phenotyping

Antibiotic and PCR screening revealed high transformation rate

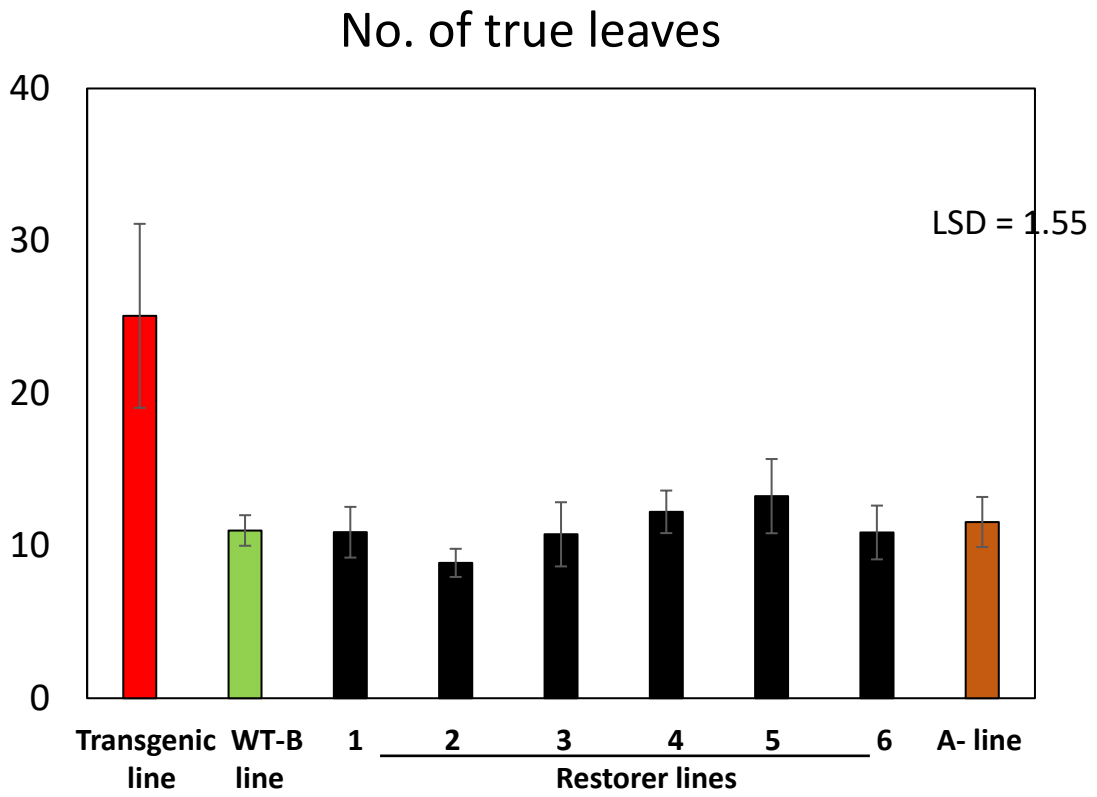
Genotyping by PCR



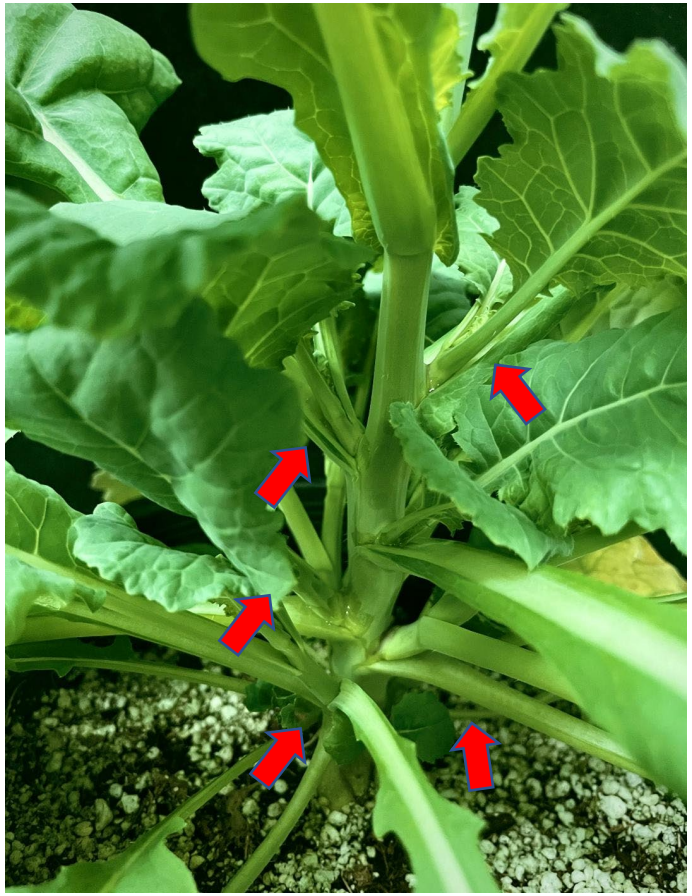
Transformed plants exhibit greater biomass

- **Morphology of the plants 30 days after seeding**

Transgenic line WT – B line Restorer line



More lateral branches in transformed plants



Transgenic line (PPRB)

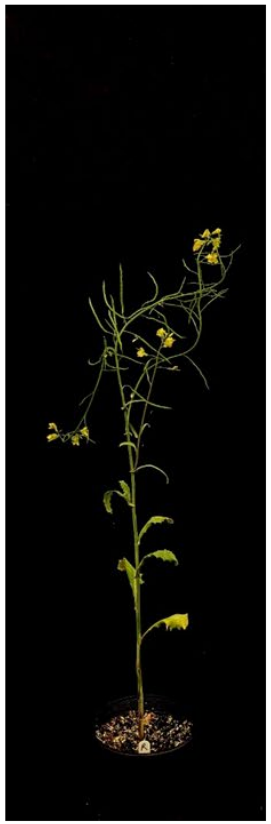
WT – B line

Transgenic plants show more flowers and siliques


Transgenic-PPRB


WT – B line

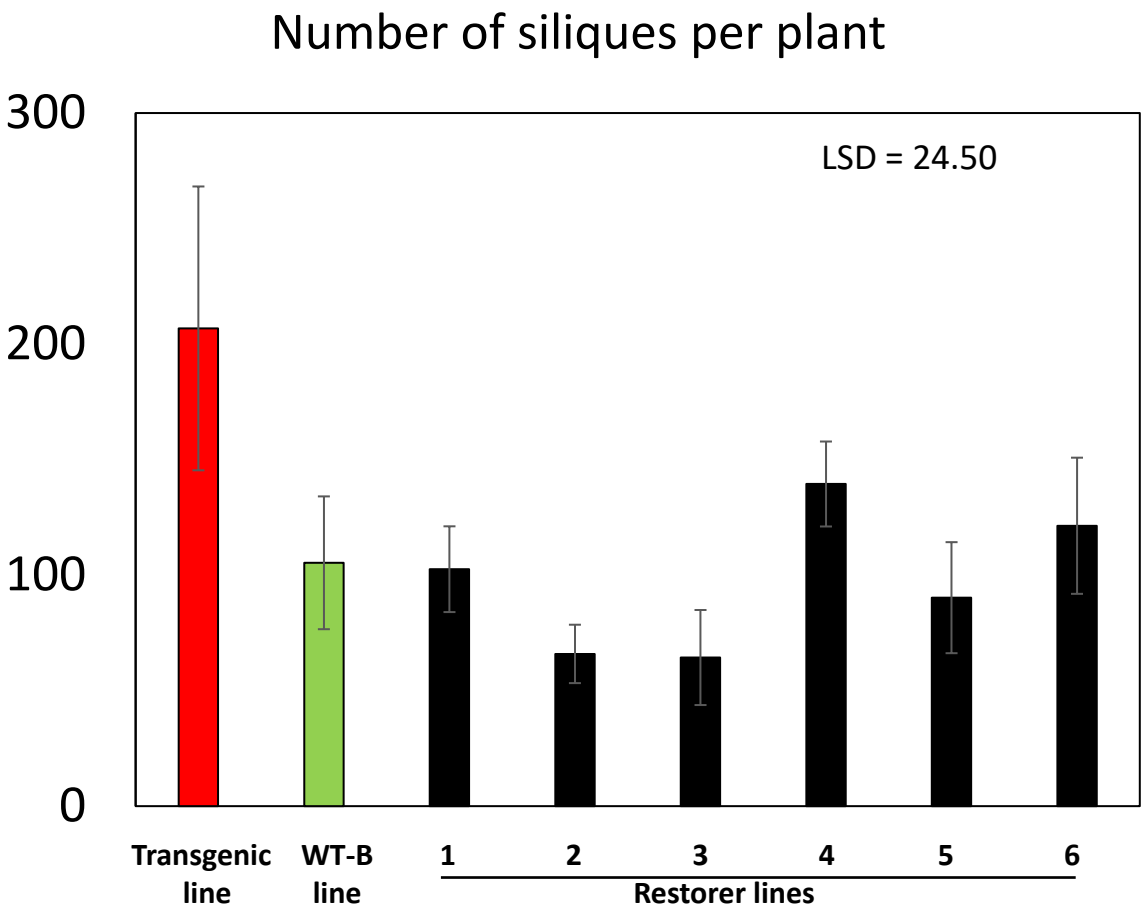

Restorer line 1

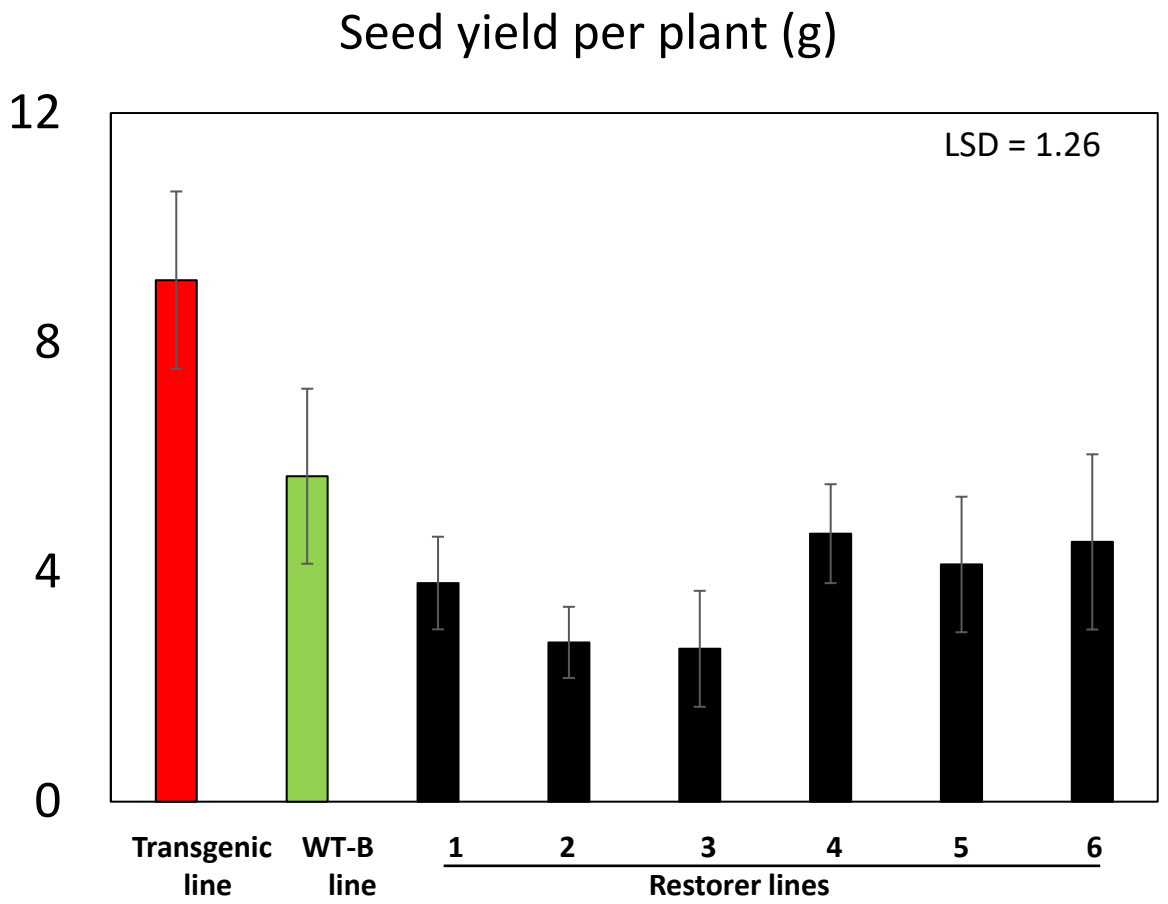

Restorer line 2


Restorer line 3

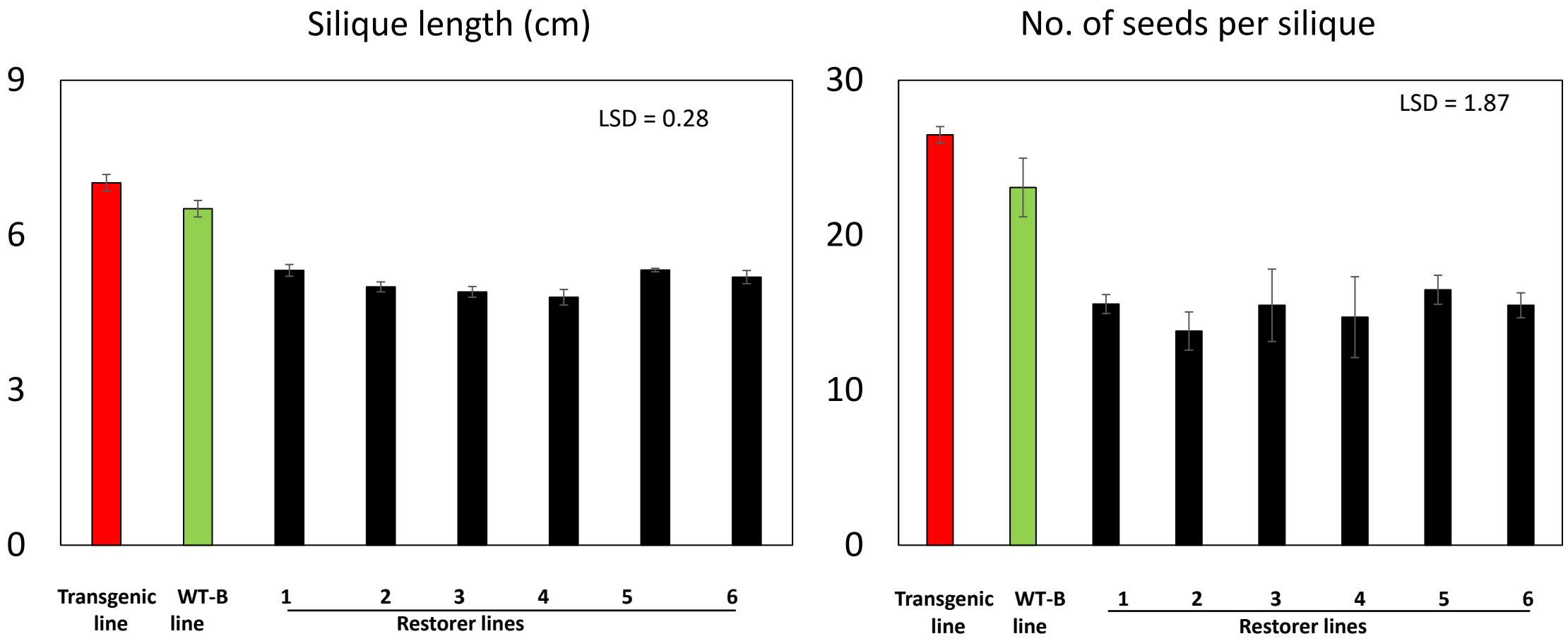

A-line (Male sterile)

Transgenic plants exhibit better agronomic performance





Transgenic plants exhibit better agronomic performance



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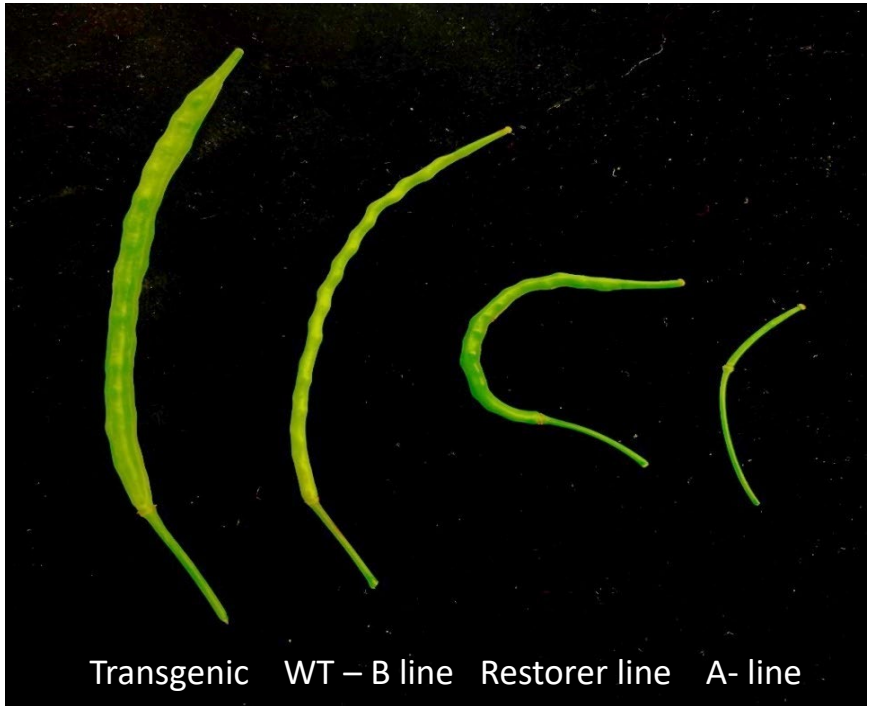
Silique morphology



Transforming A line with *PPR-B* resorted the fertility

PPR-B (2064 bp)

Conclusion

- The transformed genotypes demonstrated enhanced biomass production and improved yield characteristics.

- By isolating the restorer gene (PPR-B), it becomes possible to develop high-yielding restorer plants without inheriting the negative agronomic traits often associated with the complete Rfo introgression from radish.

- The transgenic materials generated in this study have the potential to serve as valuable restorer lines in canola breeding programs.

Acknowledgments

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