Comparison of the Fertility Restorer (*Rfo*) in *Brassica napus*

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Introduction

- Ogu-INRA Cytoplasmic Male Sterility (CMS) is a pollination control system used for hybrid development in *Brassica napus.*
- The CMS system requires 3 lines: A-lines (male-sterile), B-lines (fertile maintainer lines) and R-lines which contain the *Rfo* gene and restore fertility.
- The *Rfo* gene was introgressed into *B. napus* from radish (*Raphanus sativus*).
- Along with the fertility restorer gene, a large piece of radish chromosome was introgressed and has been linked to poor agronomic performance and high glucosinolate levels.
- The *Rfo* introgression contains 17 pentatricopeptide (PPR) motif repeats which confer fertility restoration.

Objective

- Identify and compare changes in the restorer fragment over time following multiple crosses and selection for improved agronomic performance.

Methods

- Ten restorer lines were selected for this analysis.
- Six are early restorer lines with poor agronomic performance.
- Four restorer lines have been recently improved for agronomic performance.
- All plants were grown under the same greenhouse conditions and were self pollinated.
- DNA extraction was performed using the CTAB method on fresh young tissue.
- PCR products were obtained using 2 sets of primers designed based on the PPR-B region.
- Purified PCR products were sequenced by Macrogen USA.
- Sequences were compared using BLAST® NCBI.
- Consensus sequences for each line were then compared using MultAlin.

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Figure 1. Selfed *Brassica* restorer lines **A.** Early restorer line **B.** Improved restorer line.

Figure 2. Rating scale for pods; 1 represents a straight full pod, and 9 represents an aborted pod with no seed.

Results and Discussion

Phenotypic Differences

- Early restorer germination rate was around 60%. Considerably lower compared to the 100% germination rate of improved restorers.
- Days to flowering was 10-14 days later for the early restorers compared to the improved restorers.
- Plants were rated using the 1-9 rating scale (Fig 2.).

Table 1. Rating of 10 restorer lines grown in the greenhouse in Winnipeg, MB, in 2014

Line	UM01	UM02	UM03	UM04	UM05	UM06	UM07	UM08	UM9	UM10
Pod rating	7	6	3	3	6	3	2	6	7	6

- Previous reports have stated the *Rfo* locus and the PPR genes are likely to evolve as a result of intergenic and intragenic recombination.
- All restorer lines exhibited a highly conserved PPR-B region (Figure 3).
- Several mismatches were found for both primer sets, particularly when comparing the original restorer to improved restorers (Figure 3).

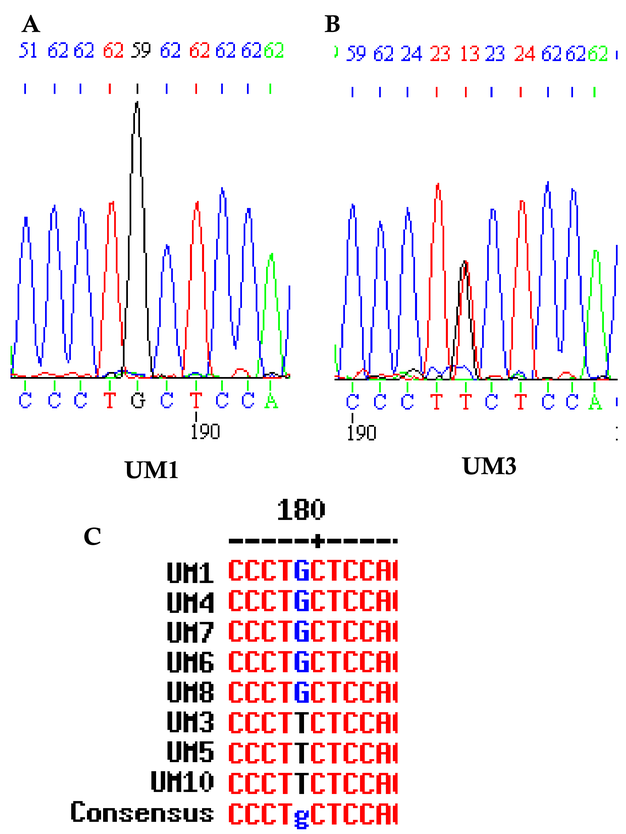

Figure 3. A. Chromatogram from UM1 (original restorer) **B.** Chromatogram from UM5. **C.** Aligned sequences highlight a mismatch between lines.

- Chromatogram in Fig. 3 B, shows two peaks for position 194, that could indicate heterozygosity.
- Chromatograms compared on Fig. 3 A and B suggest that there may be divergence from the original restorer.

Future work

- Future research will focus on studying the flanking regions to this conserved PPR region.
- We will correlate these differences with agronomic performance and seed quality traits.
- This information will aid in the understanding of the evolution of the restorer fragment and its impact on the agronomic performance.

Acknowledgements

The technical assistance of Ralph Kowatsch, Judith Nugent-Rigby and everyone in the Brassica breeding program is greatly appreciated. Special thanks to all of our sponsors listed below for their support.