Development of KASP Markers for Restorer Homozygosity in the Ogu-INRA CMS System in *Brassica napus* L.

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Introduction

- Rapeseed (*Brassica napus* L.), is the second most grown oilseed crop worldwide.
- In Canada, current total rapeseed production is around 1,872 kg/ha and the industry's goal by 2025 is to achieve 2,871 kg/ha.
- To keep up with the increasing demand and production goals from the industry, breeders have targeted high hybrid heterosis.

- Ogu-INRA Cytoplasmic Male Sterility (CMS) is a pollination control system used for hybrid development in *B. 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*) (Delourme et al., 1998).
- The present study addresses the need for an *Rfo* allele-specific marker.

Materials and Methods

- Two sets of isogenic restorer and non restorer lines (5 replications of each) were genotyped with the *B. napus* Illumina Infinium® SNP array.
- The two sets differed in their pedigree and restorer donor background (*Rfo* donor 1 and *Rfo* donor 2).
- Allele calling for each locus was performed using GenomeStudio genotyping software v2011 focusing on the polymorphic SNPs (figure 1) present on linkage group C09, where the *Rfo* gene was introgressed in *B. napus*.

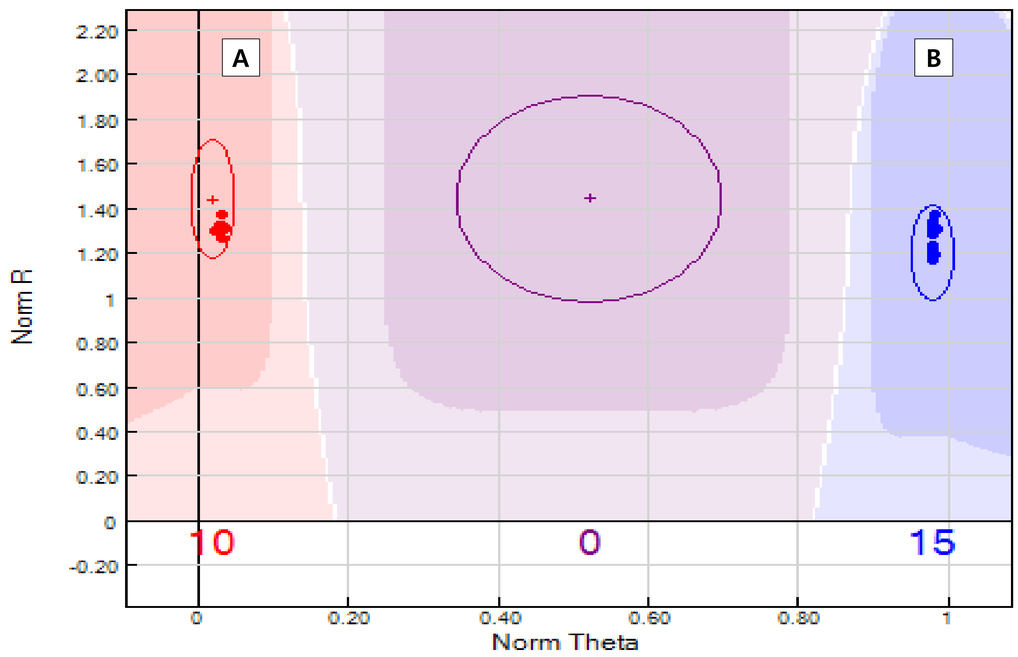

Figure 1. Allele calling for a polymorphic SNP in GenomeStudio.
Samples were separated into two groups: all of the restorer lines **(A)** (5 from each restorer donor) and the 15 non-restorer lines **(B)**.

- Only polymorphic SNPs between restorer and non-restorer lines that were consistent across the 5 replicates from each group and had no missing data, were used for further analysis.
- The sequence containing the SNP with at least 50bp flanking on either side were blasted against the reference genome using the *B. napus* Genome Browser BLAT from Genoscope©.
- For the KASP™ (Kompetitive Allele Specific PCR) assay development, 24 initial SNPs were submitted to LGC© (Herts, UK) for their primer design service.
- These assays were initially tested on 22 genotypes consisting of homozygous restorers, non-restorers and hemizygous restorers.
- Primers that were successful in differentiating homozygous and hemizygous restorers were then validated using 94 different samples.

Results and Discussion

- Out of the 52,157 SNPs on the 60k SNP chip, 1,780 corresponded to C09.
- Due to the high level of similarity between the A and C genomes, a few of the flanking SNP sequences were found to align exclusively to the C09 chromosome.
- When comparing polymorphic SNPs on both isogenic sets, differences were found among the restorer lines depending upon their restorer source.
- Thus, a polymorphic SNP for one set of lines would not be polymorphic for the other and vice versa.
- The initial test of 22 KASP assays resulted in 8 useful markers. These markers had no false positives among the homozygous restorers.
- Differences between restorer sources led to difficulties finding a marker that was able to separate hemizygous and homozygous restorers (Figure 2).

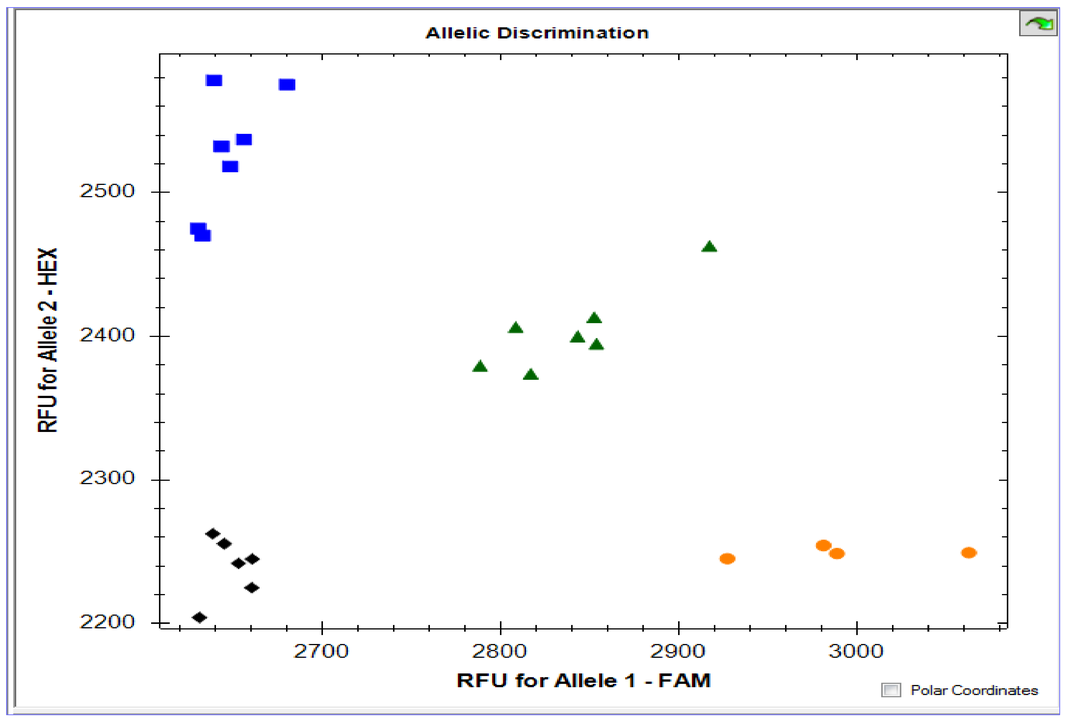

Figure 2. KASP allelic discrimination output based on HEX and FAM fluorescence. Blue squares correspond to homozygous (*Rfo* donor 1) restorer lines, green triangles correspond to hemizygous (*Rfo* donor 1) restorers, orange circles are homozygous non-restorers and black diamonds are two NTC no template control samples and four homozygous restorer (Restorer donor 2) genotypes.

- Further validation showed 2 of the 8 markers identified all homozygous restorers from the *Rfo* donor 1 background.

Conclusions and Future Work

- This codominant marker will aid Brassica breeding programs using the Ogu-INRA CMS system.
- Sequencing data indicates that multiple markers might be required for *Rfo*/*Rfo* identification in each population depending on their background.
- Future research will continue to validate markers on a wider variety of restorer genotypes.

Acknowledgements

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