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Uncovering novel genetic diversity in canola seed traits through haploid mutagenesis and proteo-genomic sequencing

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Background:

Canola (*Brassica napus L.*) is one of the most important oilseed crops worldwide with global production estimated at 75 million tonnes in 2021. Most of canola's economic value comes from its oil, which makes up about 45% of the seed. After crushing, the oil-free meal is a valuable source of protein used mainly in livestock feed. Compared to soybean meal, the nutritional quality of canola meal (CM) is limited by lower levels of crude protein, lower energy density, high insoluble fibre, and the presence of various antinutritional compounds such as glucosinolates, phytates, and saponins. Addressing these deficiencies would allow more extensive use of CM in swine, poultry and fish feeds, and in making protein concentrate and protein isolate for high value pet and human food use.

A key challenge for the genetic improvement of canola is the limited natural variation available for key traits. The creation and manipulation of new variation is further impacted by the paucity of functional knowledge about genes controlling commercially important traits. The ploidy and complex history of genomic duplications in canola further confounds the rapid translation of functional knowledge from the model plant Arabidopsis.

Results:

Here we report a novel functional genomics platform that could serve as a resource for rapid gene/trait discovery, characterization, and optimization. First, using haploid mutagenesis, a powerful genetic tool that can create novel genetic variation in plants, we established a double haploid (DH) mutant population of 1248 lines derived from chemically mutagenized (ethyl methane sulfonate, EMS) microspores of the spring canola line DH4079. Second, we established a platinum-quality reference genome and gene annotations for DH4079, along with a comprehensive messenger RNA, small non-coding RNA and protein expression atlas obtained from developing seeds. The entire mutant population was genotyped using an exome capture array, and subsequently phenotyped in field conditions across multiple locations. Analysis of near-infrared spectroscopy (NIRS) data obtained from DH mutant lines grown in the field revealed lines with desirable seed composition modifications such as increased protein content, reduced fibre, and reduced antinutritional compounds. Using exome sequencing data, potential causal variants for differentially expressed compositional traits in mutant lines were identified. In addition, using a genetic mapping population created using a selected mutant line and the parental line, novel quantitate trait loci (QTLs) associated with different seed composition traits were identified, providing insight into the genetic basis of seed traits. Finally, using exome sequencing data of the entire mutagenized population, we established a "TILLED" variant web database.

Conclusions:

Overall, this study provides valuable information for canola breeding and genetic improvement efforts aimed at enhancing seed composition traits in Canola. We will discuss the salient features of this platform and provide a few examples of gene discovery as well as practical breeding applications.