Characterization of Cruciferin Protein in a *Brassica napus* Nested Association Mapping Population

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Introduction

- Canola (*Brassica napus* L.) is grown largely for its edible oil, while the protein-rich meal is often used as livestock feed.[1]
- Commercial *B. napus* meal contains up to 39% protein on a 12% moisture basis.[2]
- Cruciferin's relative abundance and functional properties make it a potential source of protein for human consumption.[1]

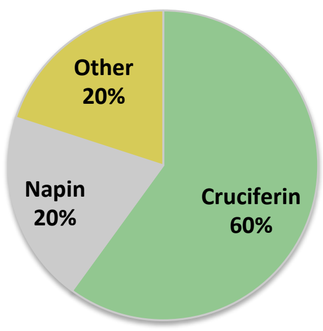

Figure 1: Seed storage protein composition of mature *Brassica napus* seeds.[1]

- Development of a rapid method to quantify cruciferin protein and an improved understanding of existing phenotypic variation will aid breeding efforts and contribute to the demand for plant-based protein sources.

Objective

- Evaluate phenotypic variation in cruciferin content and the effect of genotype and environment on cruciferin content in the parental lines of a Nested Association Mapping (NAM) population.

Materials and Methods

Population Development and Field Evaluation

- The NAM population was developed at Agriculture and Agri-Food Canada in Saskatoon, SK. The 51 parental lines were selected to represent the diversity across spring *B. napus*. Field trials took place over 2 years in Winnipeg, MB.

Protein Extraction and Quantification of Cruciferin

- *B. napus* meal was defatted and total soluble protein (TSP) was extracted (Figure 2).

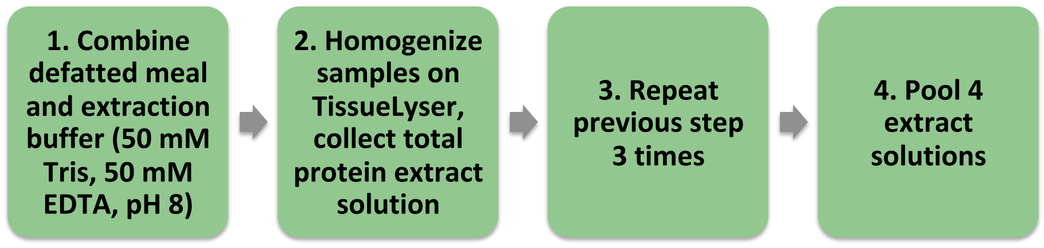

Figure 2: Protocol for extraction of total soluble protein from *B. napus* seed.

- Rabbit polyclonal antibodies were raised against the cruciferin alpha chain in *B. napus*, and validated by Western blot to confirm no cross-reactivity.
- Total soluble cruciferin content relative to a Westar standard was determined by indirect quantitative-ELISA.

Analysis of Seed Oil and Protein Content

- Total seed protein content and seed oil content were determined by near-infrared spectroscopy.

Results and Discussion

Table 1: Mean and range observed in total seed protein content, oil content, and cruciferin content in 51 NAM parental lines in 2016 and 2017.

	2016			2017		
	Oil (%)	Total Protein (%)	Cruciferin (% of Westar)	Oil (%)	Total Protein (%)	Cruciferin (% of Westar)
Mean ± SD	37.35 ± 3.53	29.83 ± 2.03	77.94 ± 19.88	45.32 ± 4.01	23.51 ± 2.78	74.05 ± 19.86
Range	28.26-44.76	23.45-35.89	37.99-144.35	33.48-52.59	17.50-32.24	37.20-154.91

- Variation in cruciferin content was observed (Figure 3), ranging from 37.20-154.91% of the Westar control across 2016 and 2017 (Table 1).
- While total growing season and post-flowering precipitation was lower in 2017 than 2016, mean oil content was higher and mean total seed protein was lower in 2017 when compared with 2016.

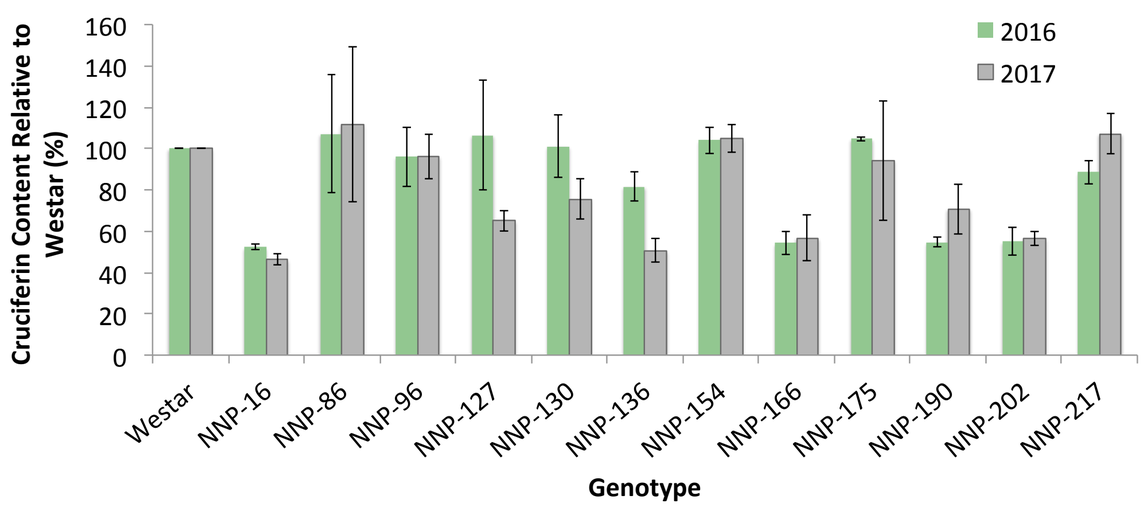

Figure 3: Mean cruciferin content relative to a Westar control in a selection of NAM parental lines, as determined by quantitative-ELISA. Error bars represent the standard deviation of three field replicates in 2016 and 2017.

- Analysis of variance revealed that both genotype and interactions between genotype and environment impacted cruciferin content significantly ($p<0.05$), while year had no significant ($p>0.05$) effect on cruciferin content.

Conclusion

- Phenotypic variation was observed in NAM parental lines, indicating that natural diversity exists in spring *B. napus* for cruciferin content, however genotype-by-environment interactions significantly impacted cruciferin content.
- Future research will evaluate additional site-years for cruciferin content and incorporate genotypic data for the identification of potential regions controlling cruciferin content.

Acknowledgements

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References

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2. Canola Council of Canada. 2015. Canola meal feed industry guide, 5th Edition.