Improved Ammonium Utilization and Greater Root Area Growth are Favored in Hybrid Canola Under Intensive Mono-cropping

Raju Soolanayakanahally, Yi Hu, Shankar Pahari, Robert Guy

Agriculture and Agri-Food Canada
Saskatoon, Saskatchewan, Canada
raju.soolanayakanahally@agr.gc.ca

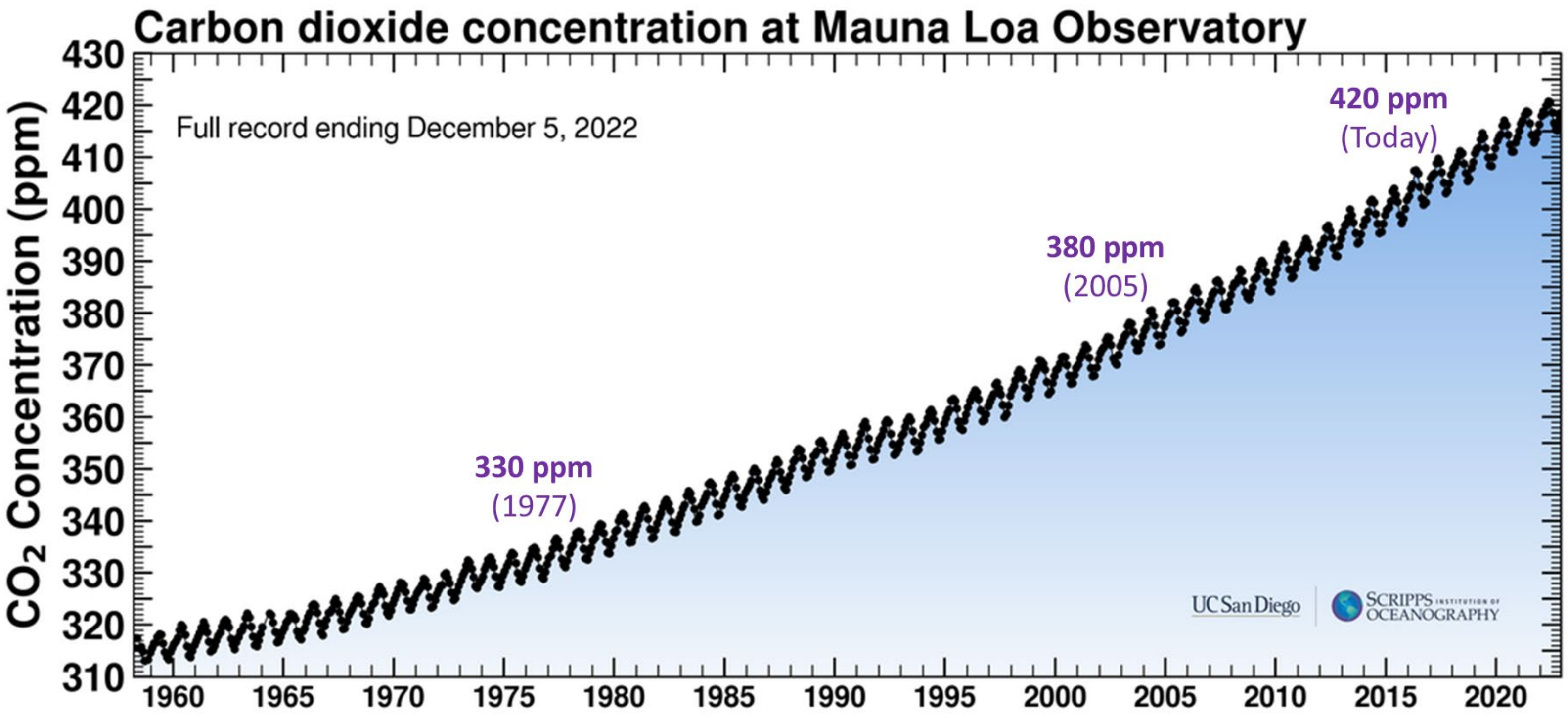
@raju_aafc

16th IRC, Sydney-Australia
25 September 2023

Land Acknowledgement

I would like to acknowledge that the SRDC-AAFC is located on Treaty Six Territory and the Homeland of the Métis. We pay our respect to the First Nation ancestors of this place and reaffirm our relationship with one another. We would also like to recognize that some may be attending this congress from other traditional Indigenous lands. In doing so, we are actively participating in reconciliation as we navigate our time in learning and supporting each other at 16th IRC 2023.

The Keeling Curve hits 420 ppm



- High CO_2 levels inhibit the conversion of biochemical step: **NO_3^- → NO_2^- → NH_4^+** (NADH, NR [nitrate reductase] & NiR [nitrite reductase])

- Reliance on **NH_4^+ fertilizers** might be a solution to avoid bottlenecks with nitrate fertilizer, but we need to understand canola plants' ability to deal with NH_4^+ toxicity.

Research Hypothesis

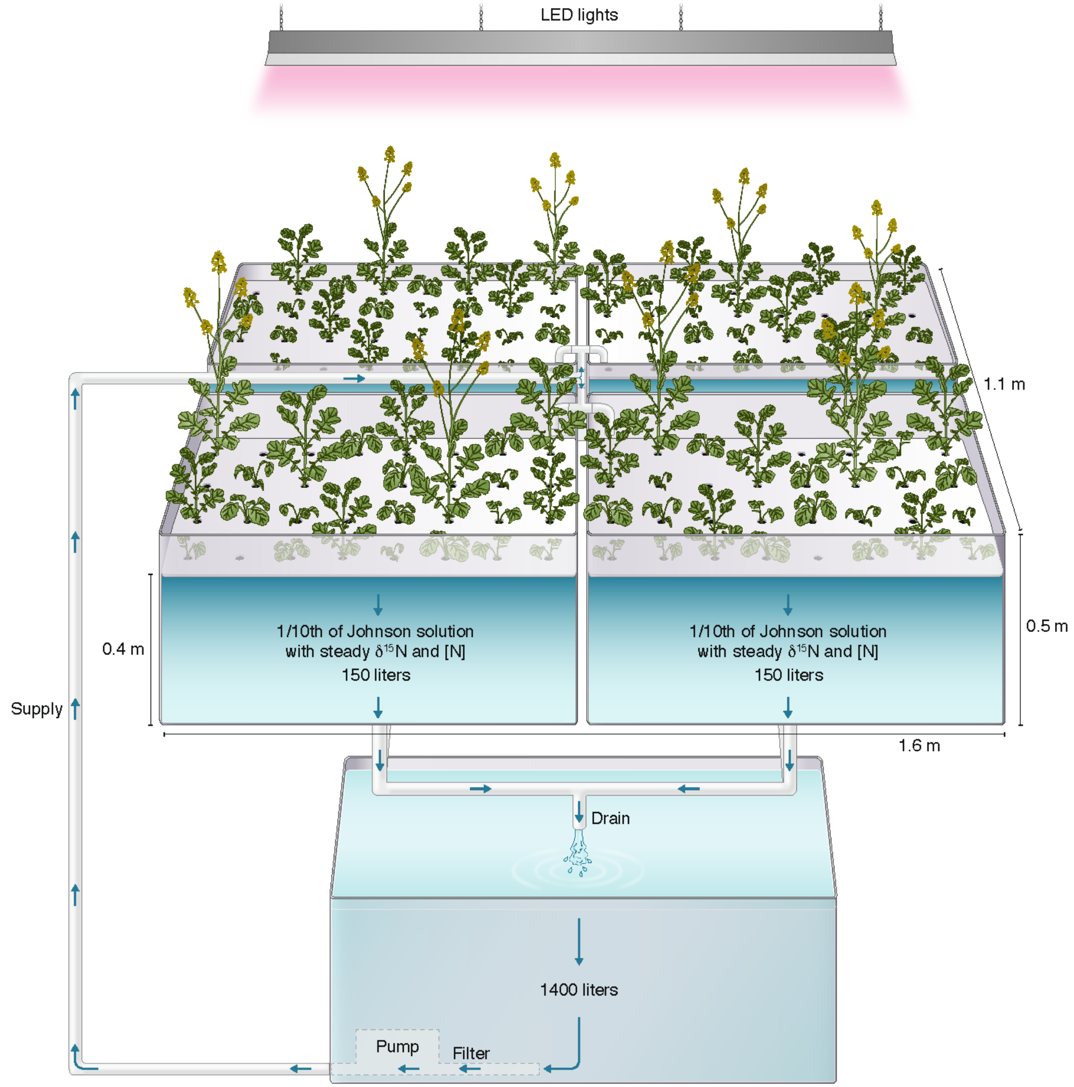
Canola has been under strong selection in breeding programs for higher yield and better oil quality. "***We hypothesized that direct selection for yield in canola may have resulted in the indirect selection of N-uptake efficiency, NUE and WUE.***"

In this study, we selected 23 Canadian canola lines developed from 1942 to 2017, including open-pollinated (OP) lines developed prior to 2005 as well as more recent commercial hybrids (CH).

In four separate experiments, 23 canola lines were grown hydroponically in a greenhouse:

- Low ammonium (0.5 mM)
- High ammonium (5.0 mM)
- Low nitrate (0.5 mM)
- High nitrate (5.0 mM)

Steady-State Hydroponic System



- Up to 1400 liters of hydroponic solution
- 32 plants with four replicates (128 plants in total)
- Ambient light with LED lighting
- 18/6 day/night cycling
- Nutrient solution replaced regularly to avoid substantial changes in source $\delta^{15}N$

Hu et al. (2022) https://doi.org/10.3389/fpls.2022.1024080

Harvesting and $\delta^{15}N$ measurement

Hydroponically grown canola

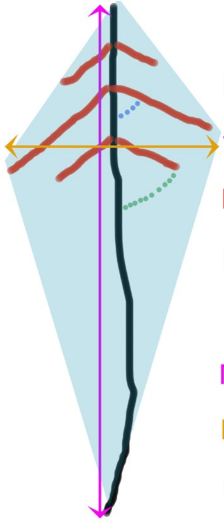
Harvesting into leaves, stem and roots

Isotope ratio mass spectrometer (EA-IRMS) & elemental analysis

2D root imaging

Canola lines → Experimental setup → Image acquisition → Root feature extraction → Trait analysis

- **23 canola lines, 12 replicates**
  - 14 Open-pollinated (OP)
  - 9 Commercial hybrids (CH)
- **Modified Hoagland nutrient (150L)**
  - High nitrate (HN, 10mM)
  - Low nitrate (LN, 0.5mM)
- **Steady-state hydroponics**
  - Regular aeration
  - Nutrient solution replaced weekly
- **Controlled environment**
  - pH, EC, light. temperature



Pahari & Soolanayakanahally (unpublished)

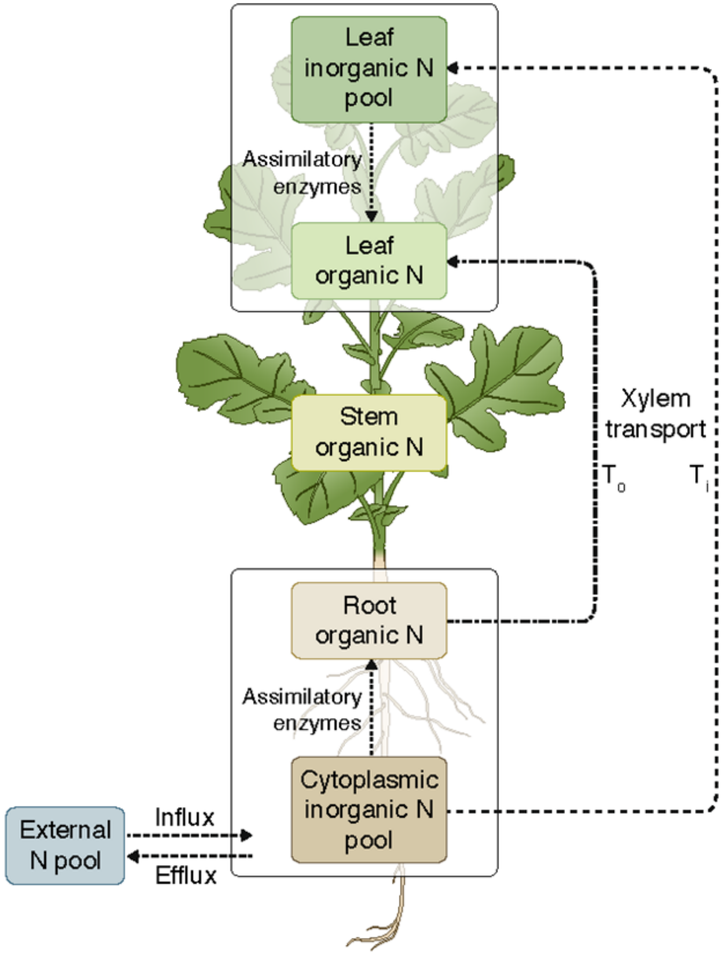
Isotope Mass Balance (IMB) Model

“all models are wrong, but some are useful.”

George E.P. Box (1919-2013)

Assumptions:

- Homogenous external N conditions (steady [N] and $\Delta^{15}N$).
- N assimilation only in roots and leaves.
- Negligible fractionation during root-to-shoot transport (xylem transport), shoot-to-root transport (phloem transport), and senescence.

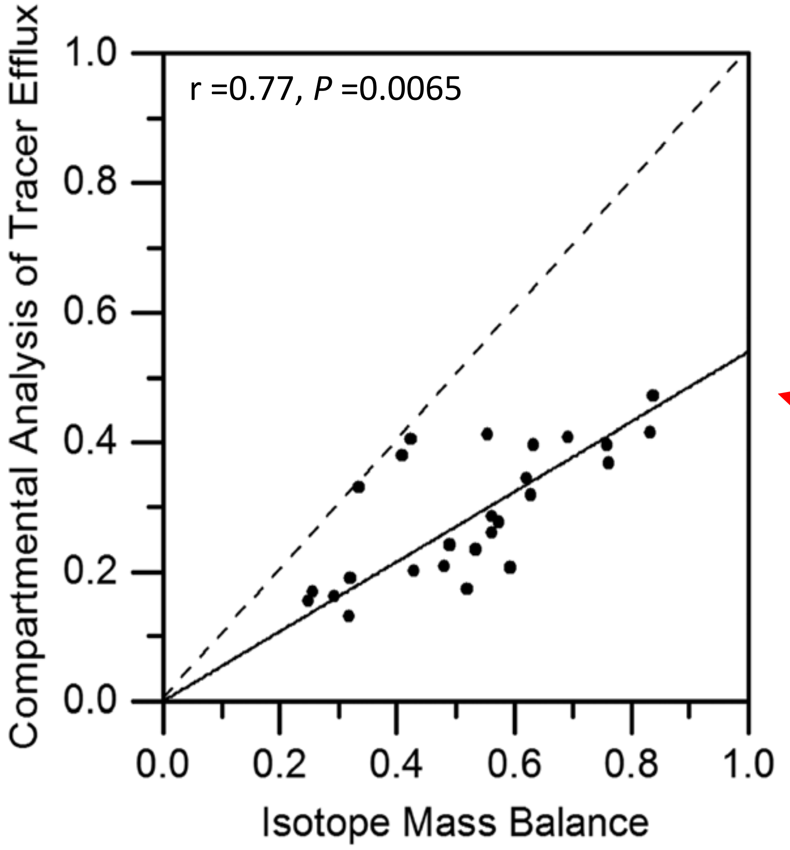


$$\Delta^{15}N_{plant} = \Delta_{enzyme} * P_{root} * \frac{E}{I}$$

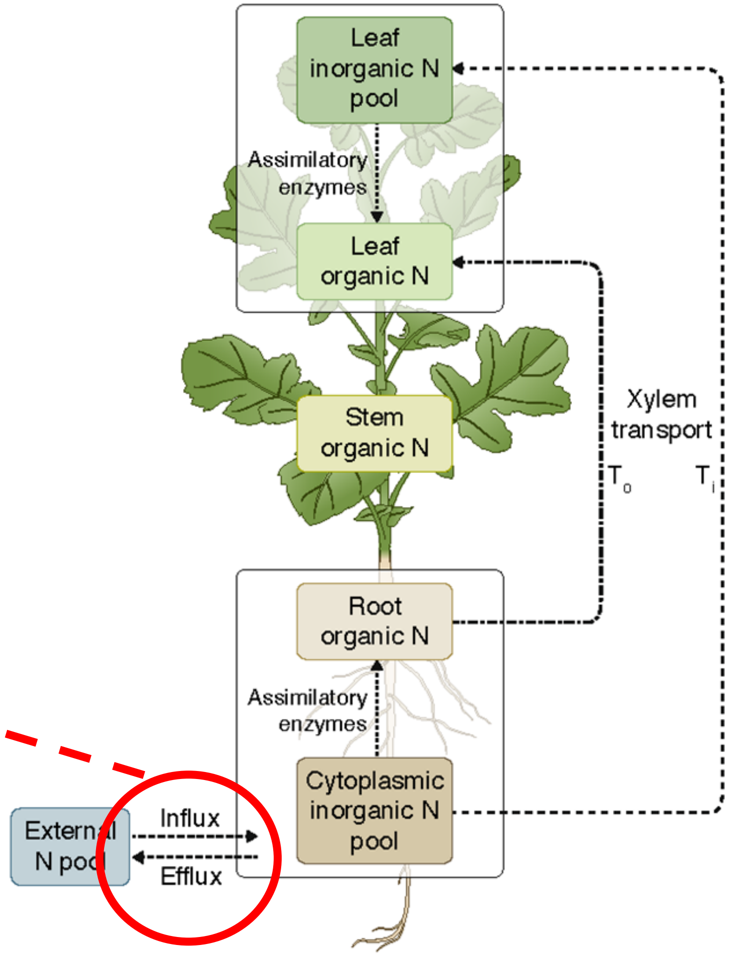
Hu et al. (2022) https://doi.org/10.3389/fpls.2022.1024080

Isotope Mass Balance (IMB) Model

The IMB model predicted efflux/influx (E/I) vs. other method



Kalcsits & Guy (2016) *Plant, Cell and Environment*



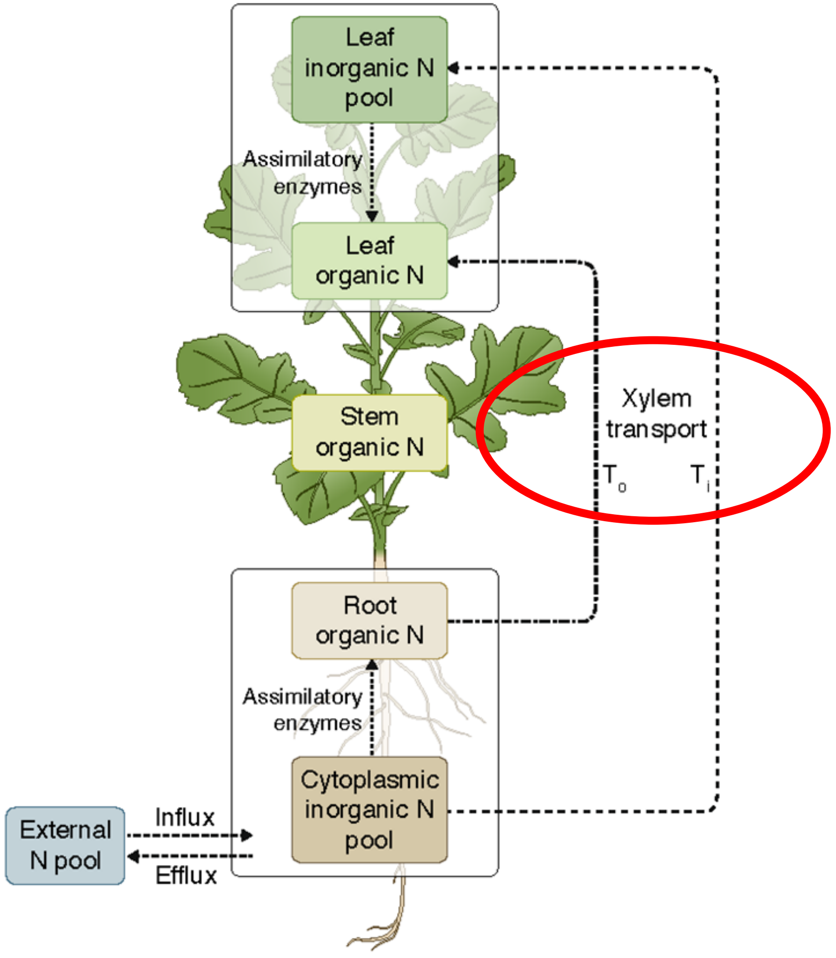
$$\Delta^{15}N_{plant} = \Delta_{enzyme} * P_{root} * \frac{E}{I}$$

Isotope Mass Balance (IMB) Model

Remaining IMB model predictions need to be verified

- Percentage ratio of inorganic nitrogen (*Ti*) relative to total nitrogen (*Tt*) translocated to the leaves (*Ti*/*Tt*)
- Proportion of inorganic N assimilated in roots (P_{root})
- Difference in $\delta^{15}N$ between organic and inorganic N should be similar to the $\Delta^{15}N_{assimilatory\ enzyme}$

$$\Delta^{15}N_{plant} = \Delta^{15}N_{enzyme} * P_{root} * \frac{E}{I}$$



$$\Delta^{15}N_{plant} = \Delta_{enzyme} * P_{root} * \frac{E}{I}$$

Considerable discrimination against ^{15}N in low ammonium

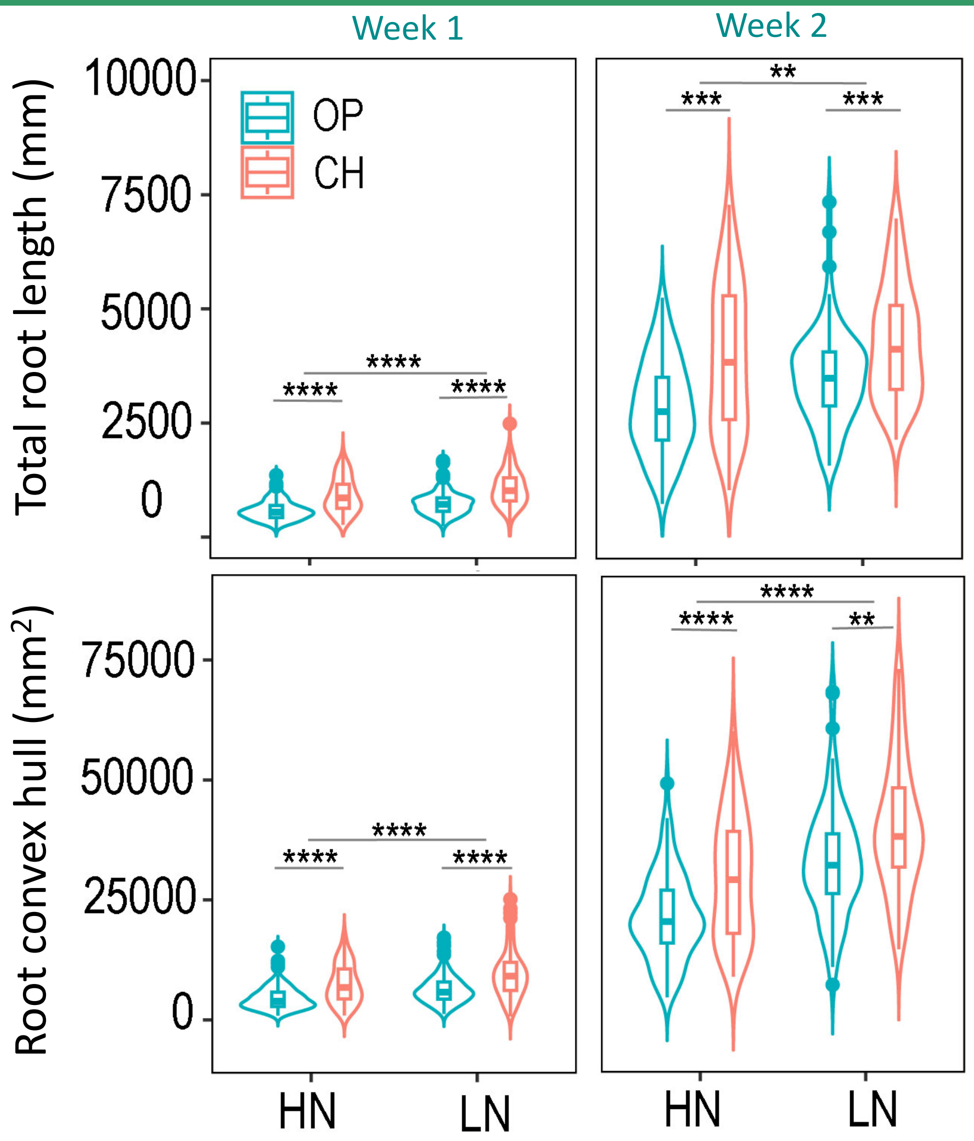
Trait	Open-pollinated (Mean value ± SD)	Commercial hybrids (Mean value ± SD)	Group Diff
WP biomass (g)	3.25 ± 1.24	5.70 ± 2.15	**P<0.001**
R:S ratio	0.24 ± 0.07	0.25 ± 0.08	ns
Leaf $\Delta^{15}N$ (‰)	6.0 ± 0.8	6.1 ± 0.9	ns
Root $\Delta^{15}N$ (‰)	6.9 ± 1.0	7.5 ± 0.8	**P<0.001**
Stem $\Delta^{15}N$ (‰)	8.1 ± 1.0	8.2 ± 0.9	ns
WP $\Delta^{15}N$ (‰)	6.5 ± 0.8	6.8 ± 0.8	**P<0.05**
Ti/Tt	0.05 ± 0.04	0.08 ± 0.04	**P<0.001**
P_{root}	0.98 ± 0.02	0.96 ± 0.03	**P<0.001**
E/I	0.39 ± 0.05	0.41 ± 0.05	**P<0.01**
WP $\delta^{13}C$ (‰)	-31.6 ± 1.0	-31.1 ± 1.0	**P<0.05**
WP C/N	9.95 ± 2.03	10.4 ± 2.44	ns

Unlike the ammonium experiment, there were no statistically significant differences between the OP and the CH groups in either the low nitrate experiment or the high nitrate experiment.

OP and CH differences

- CH lines exhibited superior ammonium utilization as the sole nitrogen source.
- CH lines displayed greater biomass and a lower proportion of inorganic nitrogen assimilation in roots (P_{root}), as well as higher translocation of inorganic nitrogen to shoots (*Ti*/*Tt*), suggesting that the CH lines had a relatively higher flow of ammonium to the shoot.
- Gross N influx and efflux averaged 244±18 and 97±14 mg N per plant for the OP lines, and 430±38 and 182±33 mg N per plant for the CH lines.
- This slightly higher efflux implies an even faster rate of ammonium uptake by the CH lines.

2D root imaging



CH lines display greater primary root length, total root length, and convex hull root to forage nitrogen

Conclusions

- Urea is the predominant source of nitrogen used to fertilize canola. Given that urea is converted to ammonium by urease enzymes and hydration in the soil, intensive mono-cropping may have favored an increased ability to tolerate and/or utilize ammonium.
- So, increasing canola plant tolerance and/or utilization of ammonium is imperative for increasing NUE.

CAP Funding Acknowledgments:

SaskCanola Saskatchewan Canola Development Commission

Manitoba Canola Growers

ALBERTA CANOLA PRODUCERS COMMISSION

canola council OF CANADA

Agriculture and Agri-Food Canada Agriculture et Agroalimentaire Canada