

## Improved ammonium utilization and greater root area growth are favoured in hybrid canola under intensive mono-cropping

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### Background:

Nitrogen (N) serves as the primary limiting nutrient for most plants in both natural and agricultural ecosystems. Among the inorganic nitrogen sources, nitrate ( $\text{NO}_3^-$ ) and ammonium ( $\text{NH}_4^+$ ) are the most critical for plant growth. However, modern agricultural practices rely heavily on nitrogen fertilizers to boost crop yields. Unfortunately, more than half of the nitrogen applied to fields is lost to the environment through leaching, causing greenhouse gas emissions, and groundwater contamination.

### Objective:

A better understanding of how diverse canola (*Brassica napus* L.) lines utilize and partition nitrogen can improve nitrogen use efficiency (NUE) and promote sustainable agriculture. We hypothesized that the selection for yield in canola may have caused a parallel selection for traits such as N-uptake efficiency, NUE, and water use efficiency. Our study employed an isotope mass balance model to explore whether the selection for canola yield has also led to the inadvertent selection of traits linked to nitrogen utilization.

### Methods:

We conducted three separate hydroponic experiments in a greenhouse involving 23 Canadian canola lines, which were developed between 1942 and 2017, including both open-pollinated (OP) lines developed before 2005 and recent commercial hybrids (CH), under either 0.5 mM ammonium, 0.5 mM nitrate or 5 mM nitrate nutrient conditions. Later, in a separate greenhouse study, we used a 2D root phenotyping platform that utilizes blue blotter germination paper as a base for root growth to determine the genetic variation in nitrogen acquisition traits among OP and CH canola lines.

### Results:

While there were minimal consistent disparities observed between OP and CH canola lines under nitrate conditions, the CH lines exhibited superior ammonium utilization as the sole nitrogen source. Overall, the CH lines displayed greater biomass and a lower proportion of inorganic nitrogen assimilation in roots ( $P_{\text{root}}$ ), as well as higher translocation of inorganic nitrogen to shoots ( $T_i/T_t$ ), suggesting that the CH lines had a relatively higher flow of ammonium to the shoot. In addition, the CH lines showed slightly higher average root and whole-plant  $\Delta^{15}\text{N}$ , indicating a minor increase in efflux/influx ( $E/I$ ). The slightly higher efflux implies an even faster rate of ammonium uptake by the CH lines. By harvest, we calculate that gross N influx and efflux averaged  $244 \pm 18$  and  $97 \pm 14$  mg N per plant for the OP lines, and  $430 \pm 38$  and  $182 \pm 33$  mg N per plant for the CH lines, respectively. Under low nitrogen conditions, the primary root length, total root length, and convex hull root area showed a significant increase, and among the canola lines, the CH lines exhibited greater values for these traits as compared to the OP lines.

### Conclusions:

The improved ability of modern canola hybrids to withstand and/or utilize ammonium may be attributed to the intensive mono-cropping practices. Furthermore, our findings suggest that CH lines possess superior root traits, which enable them to better meet the nitrogen demands of their sinks. These results could potentially inform breeding strategies aimed at enhancing the nitrogen use efficiency of canola crop.