

The long and the short of it: How longer hypocotyls could improve canola establishment

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Take home message

- The hypocotyl is the elongating stem between the root and cotyledons of canola seedlings
- Long hypocotyls are associated with better emergence of canola from deep sowing (50 mm)
- All Australian varieties have short-to-medium length hypocotyls, while several overseas varieties have been identified with long hypocotyls
- Long hypocotyl genes from overseas varieties are being incorporated into Australian canola varieties to improve establishment in deeper sown crops.

Aims

1. To incorporate long hypocotyl genes from overseas varieties into locally adapted germplasm
2. To confirm these genes improve establishment of Australian canola
3. To provide improved germplasm and molecular markers to canola breeders.

Introduction

Canola (*Brassica napus* L.) production provided on average \$4.1 billion per annum to the Australian economy over the last five years, making it Australia's second most valuable crop (ABARES, 2023). Canola underpins the production of other important crops through its function as an important break crop in cereal-based rotations by breaking disease cycles and enabling the control of weeds.

One of the major challenges with canola is unreliable establishment (50% of germinable seeds establish on average; McMaster *et al.*, 2019), which increases seed cost, and can reduce yield potential, increase weed-management requirements, and in extreme cases requires costly resowing. The direct and indirect costs of poor establishment to Australian growers can be conservatively estimated at between \$100M–\$200M annually. This problem is expected to worsen under a changing climate and with sowing into more marginal (hotter, drier) conditions.

Between 2019 and 2023, CSIRO and GRDC co-invested in a four-year research project (CSP1907-001RTX) to improve the genetics of canola establishment. After consultation with industry and reviewing the international scientific literature (Nelson *et al.*, 2022a,b), long hypocotyls and early vigour (that is, fast and strong growth from germination through to around the four-leaf stage) were identified as critical targets for genetic improvement of canola establishment (Figure 1). Multiple lab- and field-based experiments were used to search for genetic diversity for these traits and to test their impact on canola establishment (Nelson *et al.*, 2023). Among a diverse set of 255 open-pollinated canola varieties (comprising 101 historic Australian varieties and 154 overseas varieties) and 28 current Australian (mainly hybrid) varieties, it was discovered that current and historic

Australian varieties all have short- to medium-length hypocotyls when screened using efficient and repeatable lab-based assays. The longest hypocotyl varieties all came from the major canola growing regions overseas (Nelson *et al.*, 2023).

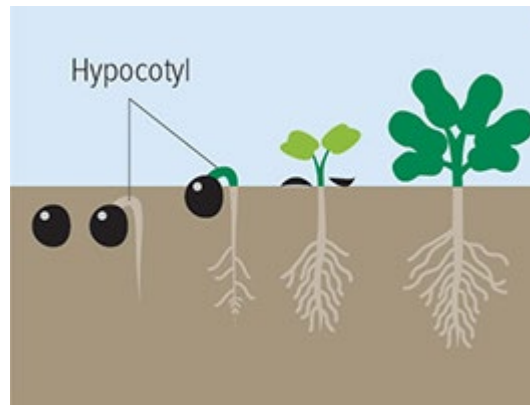


Figure 1. Schematic of early growth stages of canola seed with hypocotyl indicated. Image adapted from GRDC GroundCover (<https://groundcover.grdc.com.au/crops/oilseeds/canola-establishment-under-the-scope>)

A subset of 20 contrasting varieties plus five current Australian varieties were then evaluated for field emergence and establishment from conventional (20 mm) and deep (50 mm) sowing in eight field experiments in NSW and WA in 2021 and 2022. Results showed the long hypocotyls identified in the lab were associated with improved emergence when canola was sown deep (Figure 2). It was also determined that hypocotyl length is controlled by several genes in contrast to the long coleoptile trait in wheat, which is controlled chiefly by one gene (Rebetzke *et al.*, 2007).

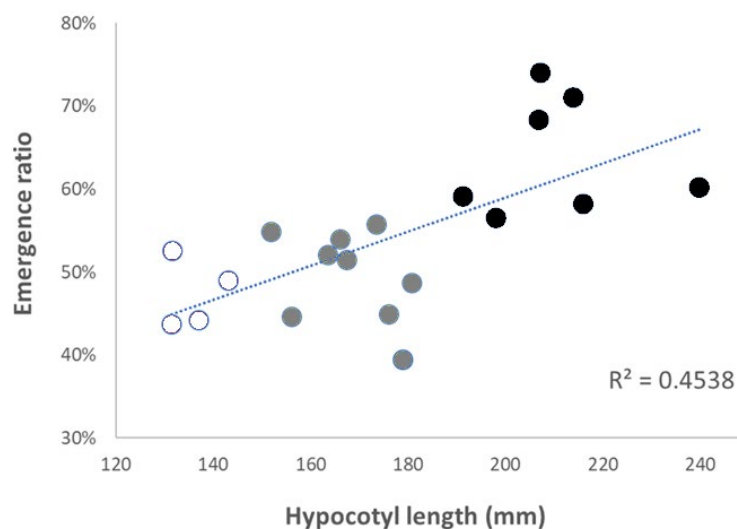


Figure 2. The close relationship between hypocotyl length measured in controlled lab conditions and effective field emergence from deep (50 mm) relative to conventional (20 mm) sowing depths for 20 international varieties averaged across seven field experiments in NSW and WA in 2021 and 2022. White circles denote historic Australian varieties with short hypocotyls, grey circles denote overseas varieties with intermediate hypocotyl lengths, and black circles denote long hypocotyl overseas varieties.

Based on these positive results, in 2023 CSIRO and GRDC co-invested in a second phase of research through a five-year project (CSP2307-002RTX) that aims to transfer the long hypocotyl trait from overseas varieties into Australian varieties using rapid generation breeding, and to confirm the value of long hypocotyls for improving canola establishment. The project will provide long hypocotyl

germplasm along with efficient selection tools to commercial breeding programs to develop the next generation of canola varieties with improved establishment. Below is an overview of the strategy to introduce the long hypocotyl trait into Australian canola.

Experimental approach

Selecting parents for crossing

From the previous lab-based screening of 255 open-pollinated varieties the longest hypocotyl varieties (between 206.4 – 240.0 mm in length) were from Canada, Czechia, France, Germany, Portugal, Japan, and the UK. Genetic analysis revealed that these 12 varieties represented four distinct genetic groupings so one variety from each of the four genetic groupings was selected to maximise the diversity of, and increase the likelihood of success in selecting, long hypocotyl genes (Figure 3). For the Australian canola crossing parents, two varieties were selected with short hypocotyls (to provide a contrast to the long hypocotyl donors) with high levels of early vigour. It had previously been discovered that the long hypocotyl trait was most effective in enabling emergence from deep sowing when combined with early vigour (Nelson *et al.*, 2023).

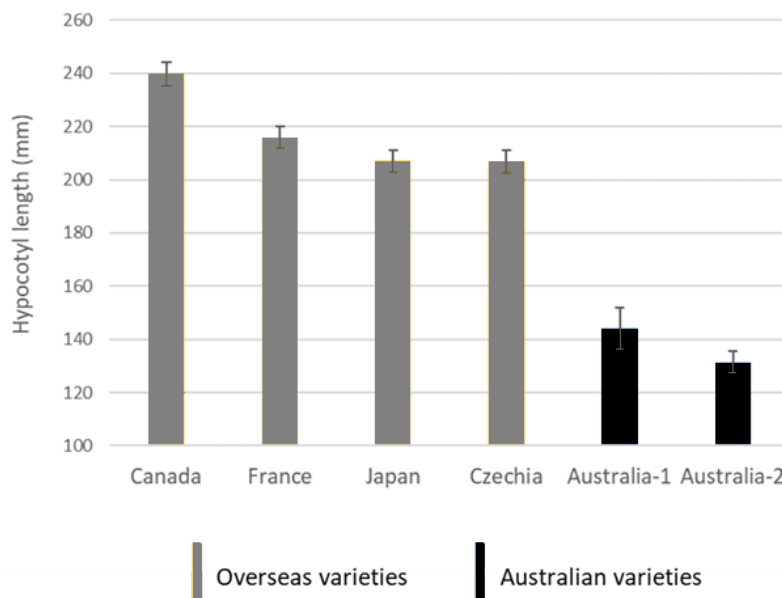


Figure 3. Hypocotyl length of four overseas canola varieties (grey bars) selected as long hypocotyl trait donors to two vigorous Australian varieties (black bars). Error bars denote standard error of each variety mean.

Crossing strategy

Due to the poor adaptation of overseas varieties to Australian growing environments, two phases of population development were designed with the aim of producing breeding lines containing the long hypocotyl trait together with local Australian adaptation and quality:

1. Introgression of long hypocotyl genes into Australian varieties through a backcrossing approach (2023 – 2026). This will involve crossing each of the four long hypocotyl donors to an Australian variety three times (while selecting for the long hypocotyl trait) followed by single seed descent for four further generations to produce 200 BC2S4 individuals per population. Rapid generation cycling methods will enable these populations to be produced within three years. The populations will be used for genetic mapping and molecular marker development for the long hypocotyl trait.

2. Using the best individuals and the markers developed from Phase-1 population development, long hypocotyl alleles will be pyramided from the four donor sources into Australian varieties to create long hypocotyl 'super-donors'. These will be provided to breeding companies to incorporate into their breeding programs. This process will take a further two years (2026 – 2028).

Field validation of long hypocotyl lines

The crossing activities described above will produce Australian-adapted lines incorporating long hypocotyl genes from overseas varieties. We will then confirm the effectiveness of the long hypocotyl trait for improving emergence, establishment, and final grain yield. This will involve two distinct experimental approaches during the 2026/27 and 2027/28 growing seasons:

1. Single row-based evaluation of contrasting population 'tails' (that is, genetically related material with contrasting long versus short hypocotyls). These carefully managed, hand-sown experiments will be undertaken at six sites across NSW, SA, and WA with the primary aim of confirming the effectiveness of the long hypocotyl trait for improved emergence and establishment from conventional (20 mm) and deep (50 mm) sowing depths. Figure 4 gives an impression of the precise, manually-intensive nature of this approach using images taken during similar experiments in the previous project.
2. Plot-based evaluation of the same contrasting population tails in cooperation with canola breeding companies targeting a minimum of eight sites per year. Some experiments will be located at NVT sites to facilitate their dual use as grower demonstration plots. The plots will be assessed for emergence, establishment, and vigour, and will be the first opportunity to measure the effect of the long hypocotyl trait on grain yield.



Figure 4. Illustrative images from single-row establishment experiments carried out at the Boorowa Agricultural Research Station in 2021 and 2022. Each seed was placed carefully in a specified position at 20mm or 50 mm depth, and then monitored closely until the fourth leaf stage. Photo: John Kirkegaard

Summing-up

In the previous project, overseas canola varieties with long hypocotyls were identified and were able to emerge better from deep sowing than any Australian variety tested. In this project, these long hypocotyl genes will be transferred into Australian varieties using rapid generation breeding, their value for improving canola establishment assessed, and then determine if the long hypocotyl trait influences final grain yield. The project will provide long hypocotyl germplasm along with efficient selection tools to commercial breeding programs thereby enabling the development of the next generation of canola varieties with improved establishment potential.

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