Carbon isotope discrimination in canola: the effect of reduced water availability in a rain-out shelter experiment

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ABSTRACT

Genetic differences exist between canola genotypes for carbon isotope discrimination (CID). CID is one measure of the efficiency of the photosynthetic mechanism and, consequently, is expected to influence water-use efficiency, crop productivity and yield. The time at which crop plant tissue samples are taken for the CID assay affects the results. In addition, CID rates may be influenced by the stress levels experienced by the plant, and the interaction with growth stage. The major stress experienced by canola crops growing in Australia is limited water availability, either through transient mid-season dry spells, or the terminal drought at the end of the season (late spring and early summer). We measured CID rates in nine canola (*Brassica napus*) genotypes grown in a rain-out shelter at Wagga Wagga in 2010. Three water regimes were imposed (wet, dry, and very dry) and CID was measured on three occasions during the growth of the plants. There was a strong effect of sampling time on CID but no interaction with genotype. The water treatments were not severe enough to cause very large differences in grain yield, and no relationship was seen between CID and grain yield. Further experiments are required to clarify whether or not CID will be useful as a surrogate character for drought tolerance.

Key words: *Brassica napus* – CID – drought tolerance – water-use efficiency

INTRODUCTION

Carbon isotope discrimination (CID, also known in the literature as delta, Δ) has been linked to improved efficiency in the photosynthetic system through improved leaf gas-exchange efficiency (Farquhar *et al.* 1984), potentially leading to increased carbon fixation per unit of water transpired, and hence improved water-use efficiency (WUE), biomass and grain yield (Condon *et al.* 1997). It has proven difficult to realise improved CID in increased grain yield by indirect selection (Condon *et al.* 2004; Matus *et al.* 1995; Matus *et al.* 1997).

In wheat there are several reports of the positive correlation between CID and grain yield in favourable environments (Rebetzke *et al.* 2008; Zhu *et al.* 2010) although the confounding effects of phenology and plant size must be taken into account. In less favourable (lower yielding) conditions this relationship can be reversed. The expectation is that CID will be a character that is consistent throughout the growing cycle of the plant, and will have moderate to high heritability making breeding, using CID as a surrogate trait for yield, a possibility. Research suggests that the genetic control of CID is polygenic and complex in wheat (Rebetzke *et al.* 2008).

In canola (*Brassica napus* L.) the range in CID seen was greater in one year (1992) than another (1991) but the range was similar to that found in other C3 crop species (~3×10^{-3} for Δ) (Condon 1993).

At Wagga Wagga we have shown (Luckett *et al.* 2011) that CID varies between TT (triazine-tolerant) and conventional cultivars (Fig. 1) and that there is an indication of a correlation with yield (in different directions) for the two groups (Figure 1). What is not clear is whether CID is affected by drought stress and whether some genotypes respond differently.

MATERIALS AND METHODS

In 2010, nine canola (*Brassica napus*) genotypes were grown in small plots in a rain-out shelter as part of an on-going project to investigate the effect of drought on canola performance. The nine genotypes were six open-pollinated commercial cultivars (46C76, AG-OUTBACK, CB-TRIGOLD, RIVETTE, SKIPTON, and TARCOOLA), one commercial F_1 hybrid (HYOLA50), and two breeding lines (BLN3343-CO0401 and SARDI607).

The rain-out shelter consisted of 63 plots in a 7 x 9 (columns x rows) rectangular grid. Each plot consisted of 4 rows of hand-sown plants, each row spaced 20 cm apart, and each plant spaced 10 cm apart. Sowing occurred on 22 May 2010. Multiple seeds were sown at each sowing location, and then thinned to one seedling after emergence. Each set of 9 plots in a column was allocated an irrigation treatment consisting of either “wet”, “dry” or “very-dry”. There was unequal replication of these treatments: three dry, two wet, and two very-dry.

Each column of 9 plots contained the 9 genotypes, arranged in a spatially optimised fashion using the DiGGer package in the software suite R. The blocks were watered using calibrated drip irrigation. The plots in positions 1, 3, 5, 7 and 9 of each column contained a neutron probe access tube used to measure soil water content.

Plots were harvested by hand on 21 December. The outer two rows of each plot and the end plants on each row were discarded. The remaining plants were split into two groups. The first group of 10 random plants were measured and threshed individually. The remaining plants were counted and threshed together as a bulk. For the purposes of this paper, the total plot grain yields are reported (converted to tonnes per hectare).

Leaf samples for CID samples were collected on three occasions (74, 104, and 166 days after sowing). The first sampling time (4 August) was in the middle of the vegetative growth phase, the second (3 September) was at early-to-mid flowering, and the last (4 November) was during pod fill. The two early composite samples were taken by cutting 12-40 random fully-expanded young leaves near the top of the crop and from different plants. In the third sampling green, immature pods and the supporting raceme material was collected from multiple plants. Flowers and unopened buds were avoided. The samples were collected with an extra two within-plot duplicates per column chosen at random. The overall set of 231 (63 + 14 x 3) samples were subjected to restricted randomisation for analysis over three days so that the effect of field and laboratory variation could be separated in the analysis.

Samples were collected in paper bags and dried at 80°C for 4 days in a forced-air dehydrator. They were then ground to a fine powder (< 20 μm) and sent for isotope-ratio mass spectrometry. In the third set of samples, the immature seeds had to be removed before grinding to prevent the high oil content blocking the machine.

Data was returned as carbon isotope composition values ($\delta^{13}C$) which were converted to $\Delta^{13}C$ using the atmospheric partial pressure of air as -0.008%. Analysis of $\Delta^{13}C \times 10^3$ was carried out in R 2.13.0 software using ASREML.

The aim was that withholding water in the dry and very-dry treatments would be targeted to stress the plants and achieve grain yields of 50% and 25%, respectively, of the wet treatment. Unfortunately too much water was available and differences of the intended magnitude were not achieved. The rain-out shelter roof was closed at sowing and remained so until harvest, however, too much pre-season rainfall had occurred to get soil moisture levels low enough to cause the desired yield depression.

RESULTS and DISCUSSION

The fixed main effects of Genotype, Water-treatment (Water) and Sampling-time (Stime) were all highly significant for CID ($\Delta^{13}C \times 10^3$) ($P<0.001$). However, only the “Genotype*Stime” and “Water*Stime” interaction terms were significant ($P<0.001$ and $0.001<P<0.01$, respectively). The random effect of Laboratory-day was not significant but there was a significant autocorrelation within each Laboratory-day (Laboratory-sequence).

Fig. 2 shows the CID values for each Id.Stime combination (across all water treatments). The CID values are much lower at the third sampling time (166 days after sowing), reflecting the onset of terminal drought that the plants were experiencing at that time. CB-TRIGOLD has a consistently higher value for CID due to it being triazine-tolerant. The degree of interaction while statistically significant does not appear to be striking – there was not a genotype which was obviously performing quite differently from the others.

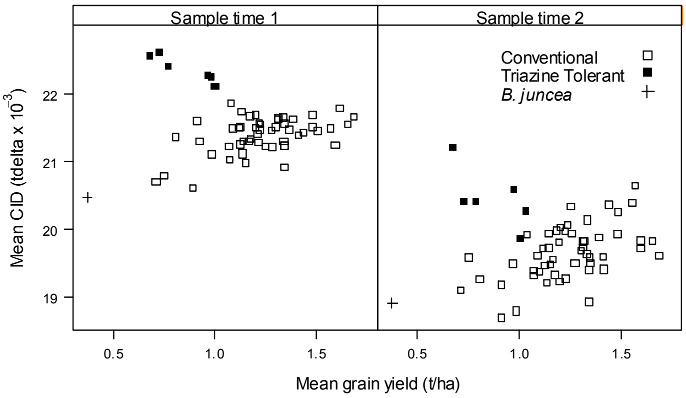

Fig. 1. Relationship between CID and grain yield (both simple means) in a field-grown plot trial at Wagga Wagga in 2009 with two sampling times for CID (Luckett *et al.* 2011). Three groups of *Brassica* germplasm were used: conventional canola (□), triazine tolerant canola (■), and one *B. juncea* (+). Correlation coefficients (r) were: conventional canola genotypes at sample time1 = 0.48 ($p<0.001$); conventional genotypes at time2 = 0.53 ($P<0.001$); TT genotypes at time1 = -0.94 ($P=0.0057$); and TT genotypes at time2 = -0.86 ($P=0.134$).

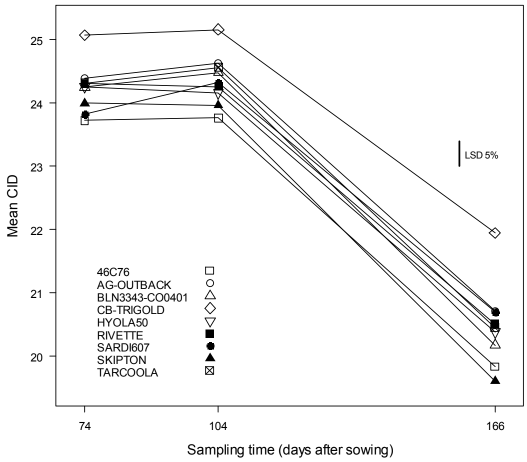

Fig 2. Relationship between Sampling-time (days after sowing) and mean CID ($\Delta^{13}C \times 10^{-3}$) for nine genotypes across all three water treatments in 2010. Responses are mainly parallel, with little interaction. CB-TRIGOLD (a triazine-tolerant genotype) has consistently higher CID than the conventional types (both open pollinated and hybrid).

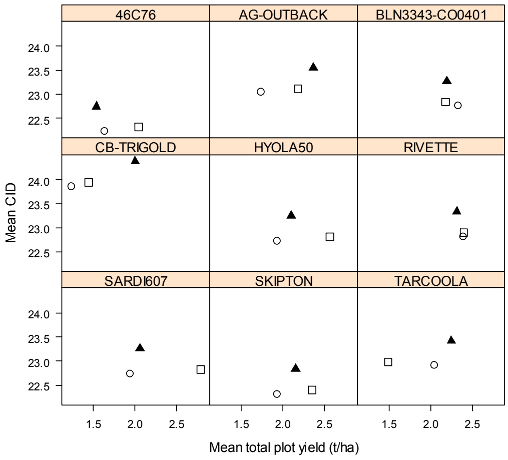

Fig. 3. Relationship between plot grain yield (t/ha) against mean CID ($\Delta^{13}C \times 10^{-3}$) for each genotype in 2010. Water treatments were: wet (▲), dry (□) and very-dry (o). LSD (5%) for CID = 0.243, and for yield = 0.697.

Total grain yield per plot (expressed in tonnes/ha) was recorded at harvest and plotted against CID (Fig.3). There was no obvious relationship. This may have been due to the fact that the drought stress imposed was insufficiently severe to cause an effect, as indicated by the quite high yields, even in the “very-dry” treatment (Fig. 3).

Grain yields were also surprising – the highest yield was not always obtained with the most water. For example, for the genotype 46C76, the wet treatment yield was the lowest of the three. The expected trend was seen for genotypes AG-OUTBACK and CB-TRIGOLD. It is possible that some other unknown factor was influencing yield.

Further research is required to examine the effect of drought stress on CID in different cultivars, and the water treatments applied need to be much more severe.

ACKNOWLEDGEMENTS

We thank Peter Heffernan, David Roberts, Peter Deane and Scott Clark for technical assistance. Dr Hilary Stuart-Williams conducted the carbon isotope analyses at RSBS, ANU, Canberra. This research was partly funded by growers through the GRDC (project DAN00108, “National Brassica Germplasm Improvement Program”, aka NBGIP).

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