

## Improving water-use efficiency and drought tolerance in canola – potential contribution from improved carbon isotope discrimination (CID)

David J. Lockett, Raymond Cowley, Sergio Moroni & Harsh Raman

EH Graham Centre for Agricultural Innovation,  
(an alliance between Industry & Investment NSW and Charles Sturt University)  
Wagga Wagga NSW 2650 Australia  
Corresponding author: david.lockett@industry.nsw.gov.au

### Introduction

Carbon isotope discrimination (CID, also known in the literature as delta,  $\Delta$ ) 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). However, some authors argue that improving WUE will lead to less soil water overall being captured by plants leading to lower “effective use of water” (EUW) (Blum 2009). It has often 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 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. The cost of CID testing is quite high (currently about USD15 per sample in Australia) and so marker-assisted selection is the ultimate aim. Research suggests that the genetic control of CID is polygenic and complex in wheat (Rebetzke *et al.* 2008).

In canola (*Brassica napus* L.) only a little is known about the genetic variability for CID, and its potential to improve WUE, and even less about its ability to improve grain productivity and drought tolerance (Condon 1993). The data range seen in canola was greater in one year (1992) than another (1991) but the range was similar to that found in other C3 crop species ( $\sim 3 \times 10^{-3}$  for  $\Delta$ ).

The overall aims of this work are to search for genetic variation in CID in canola germplasm, to determine its relationship to grain yield, to map the genes responsible, to see how CID varies under drought stress, and to provide Australian canola breeders with markers for those genes to facilitate marker-assisted selection leading to improved cultivars. This paper reports on some phenotyping for CID and grain yield in a range of canola germplasm.

### Materials and Methods

Several sets of *Brassica* germplasm were grown in small-plot field trials at Wagga Wagga, NSW, Australia in 2008 and 2009. Six experiments were grown: four in 2008, two in 2009. Two were drip-irrigated under a rain-out shelter (ROS), the rest were rain-fed (Table 1). There was significant concurrence of genotypes between experiments. Field plots (9 m x 1.4 m, 8 tynes rows) were sown with a cone seeder, and harvested with a plot header. The ROS experiments were sown and harvested by hand. They consisted of 4 rows x 2 m long, and plants thinned to 10 cm spacing. The experiments were managed according to normal commercial practice (fertilizer and pest control). Experiments were grown in a rectangular plot grid and designed using the DiGger package in R (Coombes 2009).

**Table 1.** Number of genotypes included in each experiment conducted at Wagga Wagga, NSW, Australia, used to assess the effect of Carbon Isotope Discrimination (CID) on yield of *Brassica napus* and *B. juncea* cultivars and breeding lines.

| Experiment   | Genotypes    |                   |                 | Total |
|--------------|--------------|-------------------|-----------------|-------|
|              | Conventional | Triazine-tolerant | <i>B.juncea</i> |       |
| 2008 field-1 | 22           | 7                 | 1               | 30    |
| 2008 field-2 | 30           | 0                 | 0               | 30    |
| 2008 field-3 | 182          | 1                 | 1               | 184   |
| 2008 ROS     | 23           | 7                 | 1               | 31    |
| 2009 field   | 52           | 7                 | 1               | 60    |
| 2009 ROS     | 7            | 2                 | 0               | 9     |

Experiments in 2008 were sampled once for CID analysis, those in 2009 were sampled twice. In addition, the 2009 experiments consisted of a multi-phase design to allow separation of the field and the laboratory errors (Smith *et al.* 2006). Leaf samples were taken at the vegetative stage of growth when the plants had received autumn-winter rainfall (or irrigation) and were not water-limited. Where a second leaf sample was taken, this was done at early-flowering when some drought stress was beginning to show in some of the rain-fed experiments. Samples consisted of young, fully-expanded leaves taken at random from multiple plants within the plot. Samples were rapidly dried at 70 °C for at least 48 hrs and then very finely ground ready for CID analysis.

## Results

The CID and yield data presented here are from a single experiment (2009 field) used as an example. Analysis of the entire dataset will be presented elsewhere.

The CID values ( $\Delta \times 10^{-3}$ ) observed were comparable with other C3 species and in the range 18.5 to 23.5 (depending on sampling time). Within a sampling time the range in this example was reduced (e.g. 18.5 to 21.0). Figure 1 shows the relationship between the simple means for CID and grain yield for the two sampling times from the 2009 field experiment.

Figure 1 show how the relationship between CID and yield depends upon the type of germplasm being considered. The correlations between CID and yield were mostly significant but varied in direction: normal genotypes (*B. napus*) showed a positive correlation between CID and grain yield (Figure 1), whereas triazine-tolerant (TT) genotypes showed the opposite (Figure 1). The single mustard (*B. juncea*) genotype present had the lowest grain yield and a low CID value at both sampling times.

Extreme genotypes for the CID trait were identified from these experiments, and those genotypes are being used as parents for mapping populations. One already existing fully-genotyped population (Skipton x Ag-Spectrum) was identified as segregating for CID differences. The search for QTLs affecting CID is now underway (Raman *et al.* 2011).

## Discussion

Triazine tolerance (TT) in canola causes reduced photosynthetic performance and this is seen in a yield penalty for TT cultivars (in the absence of weeds) (Robertson *et al.* 2002). Our data confirms these findings and shows that TT cultivars have a generally higher CID level, which is also significantly negatively correlated with grain yield. It appears that Australian canola breeders wanting to use CID as an indirect selection method will need to apply opposite selection pressure in TT genotypes compared to conventional genotypes.

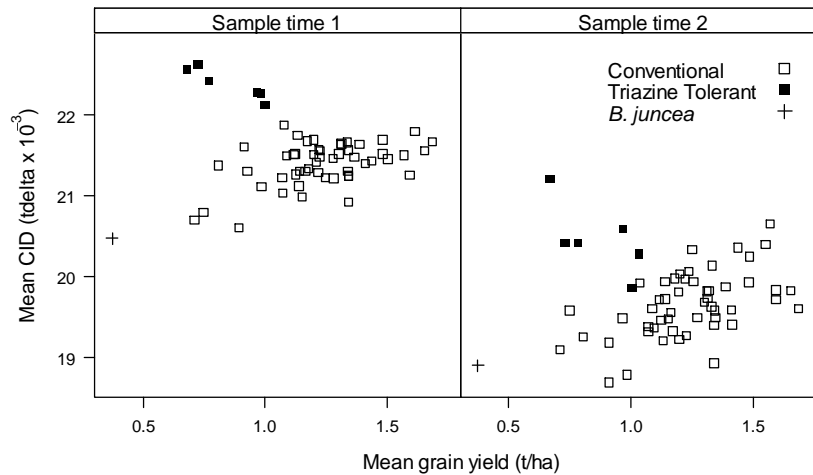
In a single cultivar, 'Westar' (Knight *et al.* 1994) showed that CID declined with increasing water stress and this was reflected in lower WUE. However, those authors did not measure grain yield.

Our studies are continuing, to examine the changes in CID as the season progresses and the terminal drought takes effect. Irrigation treatments are being used to enhance the differences. Mapping populations are being phenotyped and genotyped with the aim of rapidly identifying the genes involved, and markers closely linked to those genes.

Condon (1993) gives the example of a decrease in  $\Delta$  of  $\sim 1 \times 10^{-3}$  that would theoretically increase transpiration efficiency by  $\sim 25\%$ . Not all this increased efficiency would be captured as improved WUE, but the question remained: how much, if any, would be captured as improved grain

yield? These early results suggest that at least some progress for grain yield could be made via the CID character.

We are interested in whether CID values for certain cultivars are less responsive to drought stress indicating that their photosynthetic and gas-exchange characteristics are more resilient in the stressed environment. The relationship may even flip from positive to negative depending on water availability (Ludlow *et al.* 1990) potentially making selection inefficient and progress slow.



**Figure 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. Three groups of Brassica germplasm were used: conventional canolas ( $\square$ ), triazine tolerant canolas ( $\blacksquare$ ), 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 ( $P = 0.134$ ).

### Acknowledgements

We thank Peter Heffernan, David Roberts and Peter Deane for technical assistance, and Dr Hilary Stewart-Williams for conducting CID analysis at ANU, Canberra.

### Bibliography

Blum A (2009) Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. *Field Crops Research* **112**, 119-123.

Condon AG (1993) Variation in carbon isotope discrimination in Brassicas. In 'Ninth Australian Research Assembly on Brassicas (ARAB)'. Wagga Wagga, NSW, Australia. (Eds N Wratten and RJ Mailer) pp. 146-150

Condon AG, Hall AE (1997) Adaptation to diverse environments: variation in water-use efficiency within crop species. In 'Ecology in Agriculture'. (Ed. LE Jackson) pp. 79-116. (Academic Press)

Condon AG, Richards RA, Rebetzke GJ, Farquhar GD (2004) Breeding for high water-use efficiency. *Journal of Experimental Botany* **55**, 2447-2460.

Coombes N (2009) DiGGER: DiGGER design generator under correlation and blocking. R package version 0.2-1. <http://www.austatgen.org/files/software/downloads>

Farquhar GD, Richards RA (1984) Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Australian Journal of Plant Physiology* **11**, 539-552.

Knight JD, Livingston NJ, Kessel Cv (1994) Carbon isotope discrimination and water-use efficiency of six crops grown under wet and dryland conditions. *Plant, Cell and Environment* **17**, 173-179.

- Ludlow MM, Muchow RC (1990) A critical evaluation of traits for improving crop yields in water-limited environments. *Advances in Agronomy* **43**, 107-153.
- Matus A, Slinkard AE, Van Kessel C (1995) Carbon isotope discrimination and indirect selection for seed yield in lentil. *Crop Science* **35**, 679-684.
- Matus A, Slinkard AE, Van Kessel C (1997) Genotype x environment interaction for carbon isotope discrimination in spring wheat. *Crop Science* **37**, 97-102
- Raman H, Raman R, Prangnell R, Eckermann P, Edwards D, Batley J, Coombes N, Taylor B, Wratten N, Lockett D, Dennis L (2011) Genetic dissection of natural variation for flowering time in rapeseed. In '13th International Rapeseed Congress'. Prague, Czech Republic p. (this volume)
- Rebetzke GJ, Condon AG, Farquhar GD, Appels R, Richards RA (2008) Quantitative trait loci for carbon isotope discrimination are repeatable across environments and wheat mapping populations. *Theoretical and Applied Genetics* **118**, 123-137.
- Robertson MJ, Holland JF, Cawley S, Potter TD, Burton W, Walton GH, Thomas G (2002) Growth and yield differences between triazine-tolerant and non-triazine-tolerant cultivars of canola. *Australian Journal of Agricultural Research* **53**, 643-651.
- Smith AB, Lim P, Cullis BR (2006) The design and analysis of multi-phase plant breeding experiments. *Journal of Agricultural Science* **144**, 393-409.
- Zhu L, Li S, Liang Z, Zhang Z, Xu X (2010) Relationship between yield, carbon isotope discrimination and stem carbohydrate concentration in spring wheat grown in Ningxia Irrigation Region (North-west China). *Crop & Pasture Science* **61**, 731-742.