Variation in Normalized Difference Vegetative Index (NDVI) in canola germplasm

J. Sergio Moroni[1,2], Neil Wratten[1] and David J. Luckett[1]
[1] EH Graham Centre (an alliance between NSW Department of Primary Industries and Charles Sturt University), NSWDPI, Agricultural Institute, Pine Gully Road, Wagga Wagga NSW 2650, Australia, david.luckett@dpi.nsw.gov.au
[2] EH Graham Centre (an alliance between NSW Department of Primary Industries and Charles Sturt University), CSU, School of Agricultural and Wine Sciences, Borooma Street, Wagga Wagga NSW 2650, Australia, smoroni@csu.edu.au

ABSTRACT

We aim to select canola (*Brassica napus*) traits that are related to crop performance under water limited conditions. Rapid fractional ground cover (FGC) is a trait that may minimize evaporation loss early in the season which could lead to more water being available for later use by the plant. In addition, the association of FGC to vigour may result in plants with rapidly-growing and deeper roots with greater access to moisture.

A GreenSeeker was used to estimate normalised difference vegetation index (NDVI) as a surrogate for FGC. Twenty-nine *B. napus* genotypes and one *B. juncea* genotype were sown in a three-replicate plot trial at the Wagga Wagga Agricultural Institute. The 8-row plots were 10 m long and 1.5 m wide, arranged in a three-range x 31-row grid. Before flowering, four estimates of FGC were done on each plot.

Differences for FGC were identified among the genotypes under study and these differences were correlated with yield. All tested genotypes showed a FGC that followed a sigmoidal shape but did not reach total ground coverage at early flowering. The differences between genotypes were characterised by non-parallel curves. Normalised difference vegetation index (NDVI), as measured by the GreenSeeker, is a rapid and robust method of estimating FGC which can be used under different field lighting conditions.

The genotypes tested here are the subject of further studies particularly with regard to root architecture.

Key words: fractional ground cover – NDVI – vigour – drought – GreenSeeker.

INTRODUCTION

Long-term climate change and short-term droughts in south-eastern Australia have greatly increased the need for improved canola (*Brassica napus*) performance under low-rainfall conditions. A pre-breeding project is underway at Wagga Wagga to investigate canola traits that are related to crop performance under water limited conditions, with the aim of identifying genes and associated DNA markers that can be exploited in commercial Australian canola breeding programs to produce improved cultivars.

We are using an indirect (or analytical) approach (as opposed to the classical breeding approach of empirical selection for yield *per se*), based on an understanding of *Brassica napus'*s physiology, to target key traits that may be limiting yield under drought stress (Araus et al., 2002). Secondary or physiological traits should fulfil specific requirements before they are included in a breeding program (Araus et al., 2002): (1) the selective trait must exhibit enough genetic variability, (2) a high genetic correlation with yield and (3) a higher heritability than yield itself in genetic populations representative of those being evaluated (Jackson et al., 1996). The traits under consideration are: phenology, fractional ground cover, canopy temperature depression, rooting depth, transpiration efficiency and plant partitioning.

Rapid fractional ground cover (FGC) relies on good seedling establishment and thus in early plant 'vigour'. A rapid shading of the soil would minimize evaporation (van Herwaarden and Passioura, 2001). Also, a vigorous crop is able to rapidly capture available moisture and progress sooner to harvestable grain, while minimising the impacts of terminal drought in late-spring and early-summer. In addition, a vigorous crop may have more-rapidly-growing and deeper roots with greater access to moisture and, therefore, be able to endure in-season dry periods with minimal damage and yield loss.

When using the indirect selection approach for breeding, rapid and non-destructive techniques to quantify traits of interest are essential. A non-destructive method to estimate FGC relies on the use of digital image analysis (Purcell, 2000; Adamsen, 1999) but is tedious and time consuming, and is dependent on field lighting conditions. Hand held remote optical sensor technology offers the opportunity to make rapid, accurate and non-destructive measurements of the crop canopy under any lighting conditions in the field.

In this work, we used a GreenSeeker to determine normalised difference vegetation index (NDVI) as a surrogate for FGC (Carlson and Ripley, 1997). We screened a diverse range of canola germplasm to determine whether genetic differences could be quantified, whether differential genotypes could be identified, and as a precursor to more detailed investigation of canola response to water limited conditions.

MATERIALS AND METHODS

Twenty-nine *B. napus* genotypes and one *B. juncea* genotype were chosen for inclusion in the experiment (Table 1). They represented a broad range of diversity and covered the main genotypes available to Australian canola growers. Some breeding lines that had appeared to do well under dry conditions were also included. *Brassica juncea* (Indian mustard) has been reputed to be a better performing species than *B. napus* under dry conditions so the B. *juncea* cultivar OASIS-CL was included. Most genotypes were open-pollinated cultivars, however, three important commercial F_1 hybrids were included. The hybrids have the advantage of possessing some degree of unquantified hybrid vigour and this is exhibited, for example, in generally increased seed size. However, in the context of this pre-breeding research, hybrids have the disadvantage that their beneficial non-additive genetic component cannot be fixed for use in other open-pollinated genotypes.

The genotypes were sown in a three-replicate plot trial at Wagga Wagga (35°03'07" S; 147°21'06" E) on a Chromic Luvisol. The trial design was blocked for maturity to aid machine harvest, and designed using DiGGer software to optimise the spatial layout. Cultivar RIVETTE was replicated twice. The 8-row plots were 10 m long and 1.5 m wide, arranged in a three-range x 31-row grid. Normal agronomic practice was followed for crop nutrition, weed control, and pest control. The trial was sown on 11-June-2008. In season rainfall (184.2 mm from April to October inclusive) was well below the historic long-term average for Wagga Wagga Agricultural Institute (335 mm for years 1898-2003 inclusive). Consequently, crop growth was water limited. However, up to flowering, the effects were not too great and good NDVI measurements were possible.

The first estimate (30 July) of fractional ground cover (FGC) was done using digital image analysis prior to the acquisition of a GreenSeeker. Four digital colour images were taken of each plot in good sunlight around mid-day. The photos were centred on a 1 m^2 quadrant so that the quadrant filled the field of view. In order to quickly convert the digital images to FGC estimates, they were then uploaded to the website www.totaloilseedcare.com where an image analysis tool is available to calculate GAI (green area index). These data were then converted to FCG values using a calibration curve.

The subsequent estimates of FGC were done with a Greenseeker Hand Held ™ Optical Sensor Unit (NTech Industries, Ukiah, CA) using normalised difference vegetation index (NDVI) as a surrogate for FGC. The unit generates light at two specific wavelengths bands in the visible (red, 660 nm) and infra-red (NIR, 770 nm) regions and measures the light reflected off the target. This internal illumination allows the user to use the unit in any light condition, day or night. A microprocessor within the sensor analyses the reflected light and calculates NDVI = $[(\rho NIR-\rho VIS)/(\rho NIR+\rho VIS)]$.

Greenseeker readings were taken on three occasions (22 August, 4 September, 16 September) around noon and on clear, sunny days at about 1 meter above the canopy. The earliest genotypes began flowering 89 days after sowing (8 Sept). The mean time to first flower per plot was 96 days (15 Sept). Between 47 and 106 (mean =79) individual reflectance readings were taken per plot. These were converted to NDVI and averaged. The NDVI index was used to estimate soil fraction coverage using a previously established calibration curve. The count of readings (including n=4 for photo-based measurements) was used as a weight in the statistical analysis. Data analysis was carried out using Genstat Version 11 software REML commands to estimate spatial and genotype effects over time.

Table 1. List of 30 genotypes used in this study for assessing fractional ground cover.

Genotype name	Species	Type	Origin	Flowering time	Notes
46C76	*B. napus*	OP[1]	Cultivar	Mid	CL
44C73	*B. napus*	OP	Cultivar	Early	CL
AG-OUTBACK	*B. napus*	OP	Cultivar	Early	-
AG-SPECTRUM	*B. napus*	OP	Cultivar	Mid	-
ATR-HYDEN	*B. napus*	OP	Cultivar	Mid	TT[2]
ATR-COBBLER	*B. napus*	OP	Cultivar	Early	TT
AV-OPAL	*B. napus*	OP	Cultivar	Early	-
BLN2852-03W02	*B. napus*	OP	Breeding line	Mid	-
BLN2737-CO0203-1	*B. napus*	OP	Breeding line	Early	-
BLN3303-CR0302	*B. napus*	OP	Breeding line	Early	-
BLN3343-CO0401	*B. napus*	OP	Breeding line	Early	-
BLN3343-CO0402	*B. napus*	OP	Breeding line	Early	-
BLN3614	*B. napus*	OP	Breeding line	Early	-
CB-PILBARA	*B. napus*	OP	Cultivar	Early	TT
CB-TANAMI	*B. napus*	OP	Cultivar	Early	TT
CB-TELFER	*B. napus*	OP	Cultivar	Early	TT
CB-TRIGOLD	*B. napus*	OP	Cultivar	Early	TT
CHARLTON	*B. napus*	OP	Cultivar	Mid	-
HYOLA50	*B. napus*	F1 hybrid	Cultivar	Early	-
HYOLA75	*B. napus*	F1 hybrid	Cultivar	Mid	-
HYOLA76	*B. napus*	F1 hybrid	Cultivar	Mid	-
KAROO	*B. napus*	OP	Cultivar	Early	TT
MONTY	*B. napus*	OP	Cultivar	Early	-
OASIS-CL	*B. juncea*	OP	Cultivar	Early	CL[3]
RIVETTE	*B. napus*	OP	Cultivar	Early	-
SARDI604	*B. napus*	OP	Cultivar	Early	-
SARDI607	*B. napus*	OP	Cultivar	Early	-
SKIPTON	*B. napus*	OP	Cultivar	Mid	-
SURPASS400	*B. napus*	OP	Cultivar	Early	-
TARCOOLA	*B. napus*	OP	Cultivar	Early	-

[1] OP = open-pollinated; [2] TT = triazine herbicide tolerant; [3] CL = ClearField herbicide tolerant

RESULTS

Due to poor seed quality the genotypes Karoo and Charlton germinated and established very poorly, and consequently, they were excluded from the analysis.

Fractional ground cover as estimated by digital image analysis was significantly correlated (r = 0.99) with NDVI measurements (data not presented). Importantly, the NDVI measurements were also significantly correlated (r = 0.71) with final yield (data not presented).

Figure 1 shows the progression of FGC over time for a subset of contrasting genotypes. The genotypes that are not shown in Figure 1 are those that clustered in the centre at each observation time and did not show any outstanding features. Fractional ground cover, as estimated by the combined digital image measurements and NDVI, showed that genotype effects were highly significant (P<0.001) at all four observation times when analysed separately, and when analysed together with recording date included in the model. The predicted genotype means for FGC ranged from 40.1% for Oasis-CL to 69.8% for Hyola-50 (overall mean = 56.8, $LSD_{[5\%]}$ = 6.00%).

DISCUSSION AND CONCLUSION

The use of the GreenSeeker to estimate normalised difference vegetation index (NDVI) is a rapid and robust method of estimating FGC; a method which offers the flexibility of being able to be used under different lighting conditions in the field. Furthermore, the measurements gathered by the sensor can be used to estimate the variability of each individual plot. Calibration curves of NDVI with biomass or leaf area index can further expand the utility of this technology.

In this first year of screening for FGC, genetic differences were identified. Furthermore, these differences appear to be correlated with yield. All tested genotypes showed a FGC that followed the expected sigmoidal shape (Fig. 1) but did not reach total ground coverage at flowering. The differences between genotypes are characterised by non-parallel curves. Some genotypes increased FGC relatively fast (e.g. HYOLA50 and TARCOOLA) while others increased FGC significantly more slowly (e.g. OASIS-CL and ATR-HYDEN). However, at the end of the recorded period, genotypes also reacted differently to the increasing drought stress. Some that had increased FGC slowly in the early stages continued to increased (e.g. ATR-HYDEN) while others appeared to decline in FGC (e.g. BLN3343-CO0401, SARDI607 and TARCOOLA – dotted lines in Fig. 1).

How FGC relates to "plant vigour" is unclear at the moment due to the fact that "plant vigour" is not a well defined concept. Genotypes of similar biomass may differ in their prostrate versus erect habit which would influence NDVI readings. Other genotypes may differ in their leaf appearance rates, or in individual leaf expansion rates. This is the subject of further experimentation.

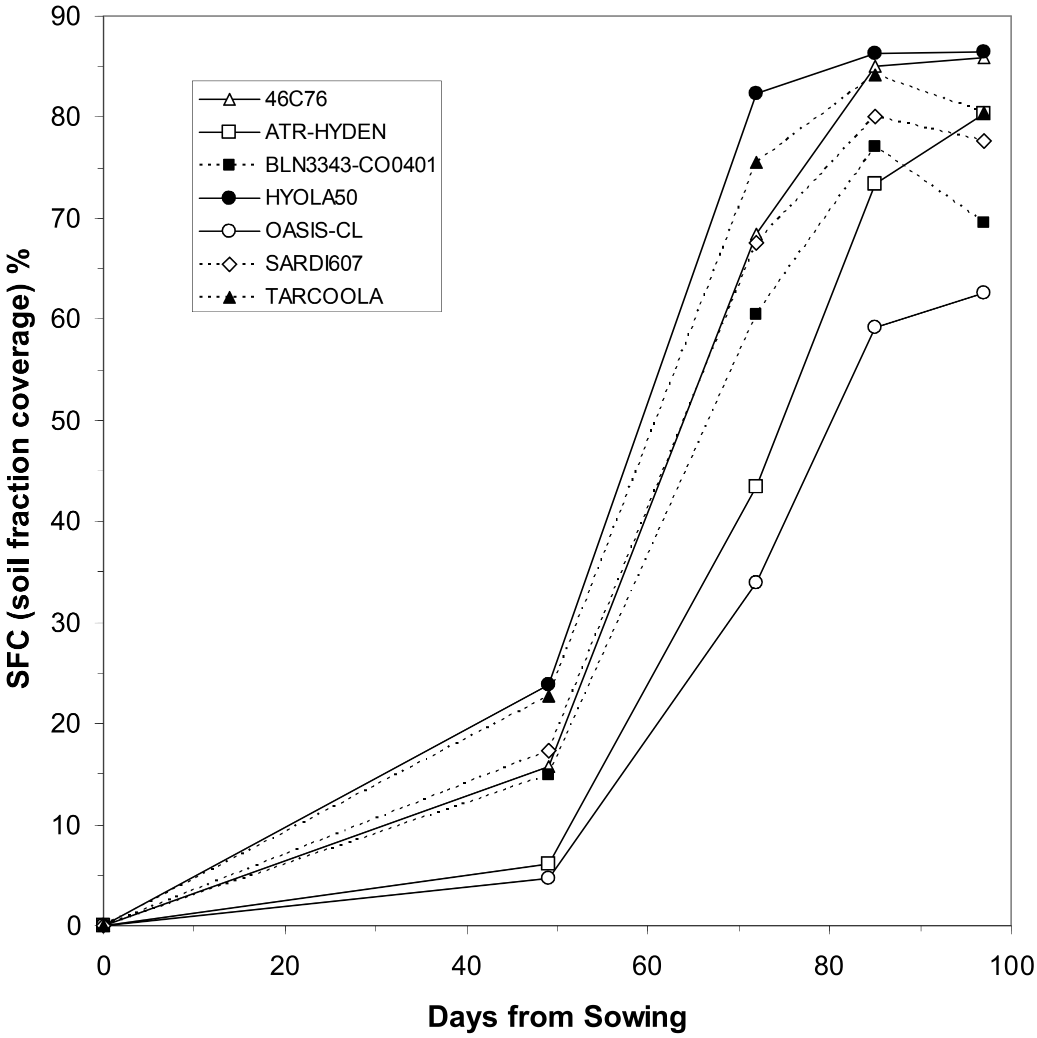

Fig. 1. Predicted genotype mean FGC over time. The LSD (5%) for comparison between means = 8.8%. Only a subset of genotypes is presented to illustrate contrasting and significantly different growth patterns. All genotypes are canola (*B. napus*) except Oasis-CL which is *B. juncea*.

Rapid early growth and over-use of available soil moisture is not a desirable trait if yield is subsequently limited (Ludlow and Muchow, 1990). In contrast, rapid early growth may produce a deeper root system which is better able to withstand longer periods of drought stress, or stress that begins earlier in the season, by accessing more water from the subsoil (Angus et al, 2001). The genotypes tested here are the subject of further studies particularly with regard to root structure. Once clear differential genotypes are identified, the genetic control of these characters will be investigated.

ACKNOWLEDGEMENTS

This research is part-funded by growers through the GRDC (Grains Research & Development Corporation of Australia) – project DAN00108. Dr Iain Hume is thanked for his advice on remote sensing technology. Peter Heffernan and Peter Deane are thanked for their technical assistance.

REFERENCES

Adamsen, F.J., Pinter, P.J., Barnes, E.M., Lamorte, R.L., Wall, G.W., Leavitt, S.W., B.A. Kimball, 1999: Measuring wheat senescence with a digital camera. Crop Sci. 39, 719-724.

Angus, J.F., Gault, R.R., Peoples, M.B., Stapper, M., A.F. van Herwaarden, 2001: Soil water extraction by dryland crops, annual pastures, and lucerne in south-eastern Australia. Aust. J. Agric. Res. 52, 183–192.

Araus, J.L., Slafer, G.A., Reynolds, M.P., C. Royo, 2002: Plant breeding and drought in C3 cereals: what should we breed for? Ann. Bot. 89, 925-940.

Carlson, T.N. and D.A. Ripley, 1997: On the relation between NDVI, fractional vegetation cover and leaf area index. Remote Sens. Environ. 62, 241-252.

Jackson, P., Robertson, M., Cooper, M., G. Hammer, 1996: The role of physiological understanding in plant breeding from a breeding perspective. Field Crops Res. 49, 11-39.

Ludlow, M.M., and R.C. Muchow, 1990: A critical evaluation of traits for improving crop yields in water-limited environments. Adv. Agron. 43, 107-153.

Purcell, L.C., 2000: Soybean canopy coverage and light interception measurements using digital imagery. Crop Sci. 40, 834-837.

van Herwaarden, A.F., and J.B. Passioura, 2001: Improving estimates of water-use efficiency in wheat. Australian Grain. 11, 3-5