

# Screening chickpea for adaptation to water stress: Associations between yield and crop growth rate

Lachlan Lake and Victor O. Sadras

South Australian Research and Development Institute, Hartley Grove, Urrbrae 5064, lachlan.lake@sa.gov.au

## Abstract

Robust associations between yield and crop growth rate in a species-specific critical developmental window have been demonstrated in many crops. In this study we focus on genotype-driven variation in crop growth rate and its association with chickpea yield under drought. We measured crop growth rate using Normalised Difference Vegetative Index (NDVI) in 20 diverse chickpea lines, after calibration of NDVI against biomass accounting for morphological differences between Kabuli and Desi types. Crops were grown in eight environments resulting from the combination of seasons, sowing dates and water supply, returning a yield range from 152 to 366 g m<sup>-2</sup>. For both sources of variation, environment and genotype, yield correlated with crop growth rate in the window 300 °Cd before flowering to 200 °Cd after flowering. In the range of crop growth rate from 0.07 to 0.91 g m<sup>-2</sup> °Cd<sup>-1</sup>, the relationship was linear with zero intercept, as with other indeterminate grain legumes. Genotype-driven associations between yield and crop growth rate were stronger under water stress than under favourable conditions. Despite this general trend, lines were identified with high crop growth rate in both favourable and stress conditions. We demonstrate that calibrated NDVI is a rapid, inexpensive screening tool to capture a physiologically meaningful link between yield and crop growth rate in chickpea.

## Keywords

Abiotic Stress, Seed Number, Spectral Reflectance.

## Introduction

Chickpea is one of the most important pulse crops grown in over 50 countries, however, yield remains unstable and unreliable mainly as a result of poor adaptation to stress; water and heat stress are the dominant abiotic stress for Australian agriculture (Rubiales and Mikic 2015; Soltani and Sinclair 2012; Lake et al. 2016). Yield is associated with crop growth rate in a species-specific critical window in maize, wheat, canola, sunflower, pea and soybean (Tollenaar et al. 1992; Andrade et al. 2002; Guilioni et al. 2003; Zhang and Flottmann 2016). Crop growth rate integrates environmental and genotypic sources of variation, and is thus a trait often used in modelling, with potential applications in breeding (Wiegand and Richardson 1990). Both the timing of the critical period for yield and the models describing the relationship between yield and crop growth rate differ among species. The most critical period is before flowering in small grain cereals, and after flowering in pulses (Sadras and Dreccer 2015). Models vary from linear relationships with zero intercept (soybean) to non-linear (hyperbolic) with a non-zero intercept indicating a minimum crop growth rate for reproduction (maize and sunflower) (Egli and Yu 1991; Vega et al. 2001). The shape of the model is important because a linear relationship indicates a tight coupling between vegetative and reproductive growth, whereas non-linearity indicates decoupling. The decoupling can be morphological as in maize and sunflower where strong apical dominance constrains seed set under high availability of resources, or physiological as speculated for field pea (Andrade et al. 2005; Sadras et al. 2013).

Crop growth rate can be derived from destructive measurements of biomass (Tollenaar et al. 1992; Guilioni et al. 2003; Zhang and Flottmann 2016) or with morphometric measurements based on allometric relationships (Vega et al. 2001). Both methods are time consuming. A non-destructive option is spectral reflectance, which can provide high throughput alternatives (Sadras et al. 2013).

There is scarce information in chickpea about the association of yield and crop growth rate within physiologically meaningful critical periods (Lake and Sadras 2014), the nature of the association (linear/non-linear) or the consistency of the relationship for different varieties and environments. This research aims to test the association between crop growth rate within the critical period and yield in a collection of chickpea lines grown in an environmental range from nearly yield potential to agronomically meaningful water stress.

## Methods

### *Plant material and experimental design*

Fifteen Desi and five Kabuli chickpea lines (Lake and Sadras 2016) that represent a broad range in agronomic adaptation, yield, morphology and phenology were evaluated. Crops were grown at Roseworthy in South Australia; eight environments resulted from a combination of two seasons (2013 and 2014), two sowing dates (recommended or late) and two water regimes (irrigated and dry).

The two water regimes were either sprinkler irrigated or rainout shelter canopy for the first sowing date and sprinkler irrigated and rainfed for the late sowing. Water regimes were intended to provide conditions suitable for high yield, and water deficit around the critical period for yield determination. Water stress was quantified using actual weather data (including irrigation) and APSIM modelling software. Treatments were laid out in a split-split-plot design of three replicates with sowing date as main plot, water regime as secondary plot, and varieties randomised within each plot. Plot size was 7.25 m<sup>2</sup>, comprised of six rows (spaced 24cm) of five meters length.

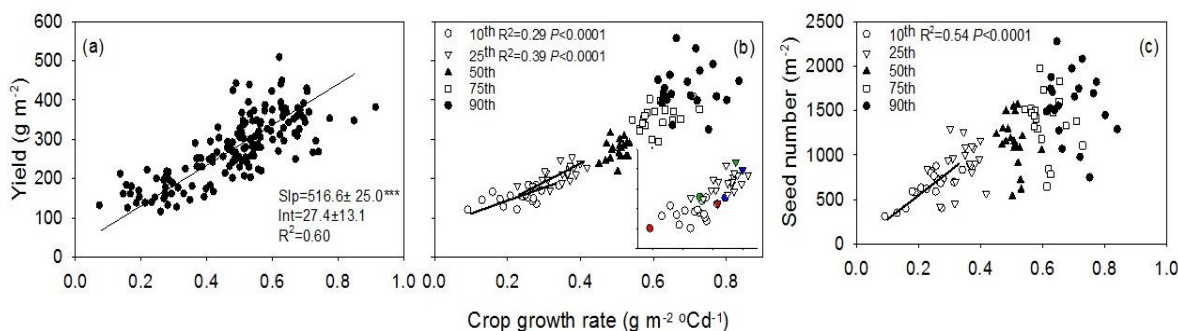
### *Phenology, crop growth rate and yield*

We scored phenology weekly to establish time to: flowering, pod emergence, end of flowering and maturity. We used a thermal time scale to express phenology, calculated from daily mean temperature and base temperature of 0°C. Initially we calibrated the Normalised Difference Vegetative Index (NDVI) readings for biomass using field plots with four Desi and two Kabuli types; the calibrations produced separate equations for the morphologically distinct Desi ( $R^2 = 0.75$ ) and Kabuli ( $R^2 = 0.87$ ) types. We then estimated crop growth rate using weekly measurements of NDVI until maturity. We fitted polynomials to plot the progression of NDVI with time and used the calibration equations to convert NDVI to biomass and calculate crop growth rate (Sadras et al. 2013). We used three criteria to define the time-window for the calculation of crop growth rate; it had to be consistent with the physiology of the species (Lake and Sadras 2014), relate to yield, and fit within the confines of the NDVI ability to detect biomass changes before saturation. These criteria returned a window from 300 °Cd before to 200 °Cd after flowering. Hereafter, crop growth rate refers to this window. Yield and components were measured from 2x1 lineal m cuts taken from inner rows of plots.

## Results and Discussion

The analysis of variance revealed a significant effect of environment, line and their interaction on crop growth rate (all  $P < 0.0001$ ). There was a positive relationship between crop growth rate and phenology for all phenological stages ( $P < 0.0001$ ); however the primary driver of this relationship was environment and the relationship became non-significant when the effect of environment was removed. Water stress was the dominant driver of environmental variation with a significant positive relationship with crop growth rate ( $R^2 = 0.68$ ,  $P < 0.011$ ).

Figure 1 shows the relationship between yield, seed number and crop growth rate. Across lines and environments, yield was closely related to crop growth rate (Figure 1a). The association between yield and crop growth rate for lines averaged across environments was significant but more scattered ( $R^2 = 0.22$ ,  $P = 0.0374$ ). We further analysed this association calculating yield and growth rate for the 10th, 25th, 50th, 75th and 90th percentiles for each line, thus accounting for the environmental variation from more stressful (10th percentile) to more favorable (90th) (Figure 1b). The association between yield and crop growth rate was stronger under stress (10th and 25th percentiles), and this is reinforced by the association between seed number and crop growth rate in the 10th percentile (Figure 1c). Among the 20 lines investigated, there were lines such as CICA 1016 and PBA Boundary which had relatively high crop growth rate in both the 10th and the 25th percentile environments with no significant difference to other lines in the 90th percentile (Figure 1b inset). In all cases, the relationships between yield and crop growth rate were linear (i.e. quadratic term not significant,  $P = 0.14$ ) and intercept not different from zero ( $P > 0.9$ ).



**Figure 1. (a) Relationship between yield and crop growth rate for the combination of 20 lines and 8 environments. Relationship between crop growth rate and (b) yield and (c) seed number. In both (b) and (c), the 10th, 25th, 50th, 75th and 90th percentiles are presented to represent a range from more stressful to more favorable conditions. Lines are Model II (Reduced Major Axis) regressions accounting for error in both variables (Ludbrook 2012), and are not plotted when not significant ( $P > 0.05$ ). Inset in 1(b) zooms in on the 10th (circles) and 25th (triangles) percentiles for yield and crop growth rate and highlights three contrasting lines; red is Almaz, blue is PBA Boundary and green is CICA 1016. Crop growth rate is for the window from 300 °Cd before flowering to 200 °Cd after flowering. Note the difference in the x-scale among panels, reflecting the difference in range of crop growth rate depending on source of variation. Slope (Slp) and intercept (Int) are shown with standard errors. Significance is denoted as  $P < 0.0001$ \*\*\*,  $P < 0.01$ \*\* and  $P < 0.05$ \***

This research shows that chickpea conforms with soybean and canola, having a linear relationship between yield and crop growth rate. Physiologically this grouping of the determinate species maize and sunflower and the indeterminate species soybean, canola and chickpea reflects the reproductive plasticity of the pulses and brassicas allowing them to adjust their seed number to the environment, while maize and sunflower respond linearly only up to a ceiling determined by the number of inflorescences (Vega et al. 2001; Guilioni et al. 2003). Our linear models have intercepts that were not significantly different from zero indicating that there is no minimum threshold for yield, a result also reported in soybean and pea (Egli and Yu 1991; Guilioni et al. 2003).

## Conclusions

This research demonstrates the use of NDVI to calculate a physiologically relevant trait, namely crop growth rate in a critical window. We have demonstrated that NDVI is suitable for high-throughput phenotyping of chickpea which could be applied within breeding programs provided it satisfies the criteria of a useful secondary trait; it must be associated with improved yield in target environment with greater heritability than yield. We have also demonstrated the link between yield and crop growth rate under water stress and provided information on lines contrasting for ability to grow fast under stress and non-stress conditions. With careful selection it should be possible to produce chickpeas with increased crop growth rate in water stress environments without suffering a yield penalty in favorable environments.

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