

**SHORT NON-REFEREED PAPER****CAN DRONE PHENOTYPING AID PLANT BREEDING? PRELIMINARY RESULTS**HOFFMAN, N<sup>1</sup>, SINGELS A<sup>1,2</sup>, JOSHI, S<sup>1,3</sup> AND MOODLEY, D<sup>1</sup><sup>1</sup>South African Sugarcane Research Institute, P/Bag X02, Mount Edgecombe, 4300, South Africa<sup>2</sup>School of Agriculture, Earth and Environmental Sciences, University of Kwazulu-Natal, Private Bag X01, Scottsville 3209, South Africa<sup>3</sup>School of Life Sciences, College of Agriculture, Engineering and Science, University of KwaZulu-Natal

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**Abstract**

Drone phenotyping of key plant traits, i.e. canopy cover and photosynthetic efficiency, could enhance sugarcane breeding. The objectives of this study were to assess: (1) the impact of these traits on yield; (2) the feasibility of estimating these traits with drone-captured crop reflectance parameters; and (3) the feasibility of predicting yield from these parameters. A trial comprising 53 genotypes, with two water treatments and three replications, was planted near Komatipoort. The well-watered (WW) treatment was fully irrigated, while stress was imposed in the water deficit (WD) treatment 160 days after planting (DAP) at canopy closure. Ground measurements were taken on ten reference genotypes: fortnightly for fractional interception of photosynthetically active radiation (FIPAR), a proxy for green canopy cover, and stomatal conductance ( $g_s$ ), a proxy for photosynthetic efficiency, on seven occasions, coinciding with drone flights. Normalized Difference Vegetation Index (NDVI) and canopy temperature ( $T_c$ ) were derived from drone imagery for all plots on seven occasions. Stalk dry matter (SDM) yield was measured at approximately 340 DAP. SDM correlated significantly with FIPAR in the WW treatment at 98 ( $r=0.73^*$ ) and 109 DAP ( $r=0.66^*$ ), along with seasonal average  $g_s$  ( $r=0.88^*$ ). WD SDM was negatively correlated with  $g_s$  in the pre-stress period ( $r=-0.81^*$ ), but was positively correlated in the stress period ( $r=0.77^*$ ). This suggests that high  $g_s$  is desirable only under non-water-stressed conditions. The results confirm that FIPAR and  $g_s$  are influential traits for determining yield. FIPAR correlated significantly with NDVI ( $r=0.75^*$ ), and  $g_s$  with  $T_c$  ( $r=-0.50^*$ ) for water stress-free crops with partial canopy cover. SDM showed mostly significant correlations with NDVI for both treatments, and declined with crop age. SDM also correlated significantly with  $T_c$  only for water stress-free crops in the WW ( $r=-0.61^*$ ) and WD ( $r=-0.48^*$ ) treatments. These results suggest that NDVI and  $T_c$  of water stress-free crops before canopy closure could be used to identify high-yielding genotypes. Initial results are promising in that they show drone phenotyping could assist plant breeding. Refinements in the trial and data capture methodologies are needed to better understand the impacts of stress and to improve the reliability of drone phenotyping for breeding application.

**Keywords:** High throughput phenotyping, plant breeding, canopy cover, canopy temperature, NDVI, stomatal conductance.

**Introduction**

Sugarcane breeding is resource intensive and time consuming, due to the phenotyping of large populations for several traits throughout the crop growth cycle at multiple locations and with replicated trials. Indirect selection criteria, with limited traits, are used in the early stages on large populations of genotypes. Physiological knowledge about water use efficiency and

drought tolerance could be used to increase the selection efficiency in the early stages of the breeding programme by eliminating unsuitable genotypes and ultimately, accelerating the genetic gains in crop performance.

Stomatal conductance ( $g_s$ ) is a key trait for which genetic variation exists and it can be exploited in breeding (Inman-Bamber and Smith 2005; Smit and Singels 2006; Eksteen *et al.* 2014; Basnayake *et al.* 2015).  $g_s$  controls water loss (and carbon fixation) from the crop canopy (Jarvis and McNaughton, 1986), with the rate of water loss strongly influencing canopy temperature ( $T_c$ ).  $g_s$ , together with crop canopy cover, determine yield as they represent the productivity and size of the photosynthetic system, respectively.  $g_s$  can be estimated through remotely-sensed  $T_c$ , and green canopy cover can be estimated from remotely-sensed Normalized Difference Vegetation Index (NDVI).

We report on the findings from an early-stage investigation into the use of drone-sensed crop reflectance information for monitoring genotype performance. The objectives of this study were to assess: (1) the impact of green canopy cover and  $g_s$ , on yield; (2) the feasibility of estimating these traits with drone-captured NDVI and  $T_c$ ; and (3) the feasibility of predicting yield from NDVI and  $T_c$ .

### Materials and Methods

A field trial comprising 53 genotypes subjected to two water regimes, was established near Komatipoort (October 2018 – September 2019). The trial area included three blocks (~1 ha each) spaced apart, with each block divided into the two water treatments. The well-watered (WW) treatment received adequate water throughout the crop cycle. The water deficit (WD) treatment received sufficient irrigation during the early stages of crop growth up to 160 days after planting (DAP). Thereafter, water deficit was imposed by withholding irrigation during nine stress periods that lasted between 9 and 29 days each, depending on rainfall.

Ground measurements included: (1) soil water content, which was measured continuously in selected plots with Aquacheck capacitance probes; (2) fractional interception of photosynthetically active radiation (FIPAR) by the green canopy, which was measured fortnightly for ten reference genotypes with a ceptometer; and (3)  $g_s$ , which was measured with the CIRAS-3 photosynthesis system for reference genotypes on seven occasions.

Crop reflectance in the visible (RGB, 380 – 750nm), near infrared (NIR, 800 - 875nm) and infrared (thermal, 7.5 – 13.5 $\mu$ m) bands were captured on seven occasions, which coincided with  $g_s$  measurements (Table 1). In three separate flights per measurement date, a DJI Phantom 4 drone was flown with: (a) a standard DJI FC330 RGB camera; (b) Sentera Precision NIR single sensor; and (c) a FLIR Vue Pro R camera. Image processing (radiometric calibration, georectification and image stitching) was carried out in the Pix4D, ArcGIS and QGIS software packages. Plot average values for NDVI and  $T_c$  were estimated from a net plot area that excluded the edge effects. Estimates of  $T_c$  were obtained after soil and senesced leaf pixels were masked through supervised (maximum likelihood algorithm) image classification procedures. Stalk dry matter (SDM) yield was measured at harvest (~340 DAP).

The crop canopy and crop water status were quantified in terms of FIPAR and a crop water satisfaction index (CWSI), calculated following the soil water deficit factor used in the Ceres models (Jones and Kiniry, 1986):

$$CWSI = 2ASW/ASWC \quad \text{Equation 1}$$

where ASW is the profile average plant available soil water content as measured with probes, and ASWC is the profile average plant available water content of the soil at field capacity, estimated from soil texture.

## Results and Discussion

### *Trait impacts on yield*

The correlations between SDM and FIPAR for the WW treatment were strongest and significant ( $p = 0.05$ ) at 98 ( $r = 0.73^*$ ) and 109 DAP ( $r = 0.66^*$ ) when FIPAR differed significantly between genotypes (data not shown). The relationship was not significant for the WD treatment, presumably because water stress was imposed when the canopy was fully developed. These results suggest that measurements of FIPAR can be used as an indicator of early vigour to identify high- and low-yielding genotypes for water stress-free crops.

SDM also correlated well with  $g_s$  for each measurement, except at 161 DAP, and with seasonal average  $g_s$  ( $r = 0.88^*$ ) for the WW treatment (Table 1). The relationship varied over time for the WD treatment, where SDM and  $g_s$  showed a significant negative correlation ( $r = -0.81^*$ ) in the pre-stress period at 118 DAP, and a significant positive correlation ( $r = 0.77^*$ ) shortly after the imposition of mild water stress at 189 DAP (Table 1). This suggests that high  $g_s$  is desirable only in stress-free environments, and that relatively low  $g_s$  may be advantageous in environments where water deficit is likely.

### *Trait phenotyping*

Overall, FIPAR correlated well ( $r = 0.75^*$ ) with NDVI for both water treatments when crops had medium canopy cover and high water satisfaction (Flights 1-3) (results not shown). However, the range in NDVI values was limited, and measurements are needed earlier in the growth cycle, during partial canopy, to reveal more about early vigour. Crop water stress significantly reduced NDVI in the WD treatment for high canopy cover (flights 4-7), which is in agreement with previous research (Begue *et al.* 2010).

Furthermore,  $g_s$  showed a significant correlation with  $T_c$  when averaged over the season (Table 1), and when the CWSI was high ( $r = -0.50^*$ ).

### *Yield phenotyping*

SDM correlated significantly with NDVI throughout the season for both treatments, except at 265 DAP for the WD treatment (Table 1). The correlation for the WW treatment declined with crop age and was stronger than that of the WD treatment.

$T_c$  differed significantly between genotypes under WW and WD conditions for the first two flights, and for the WW treatment of the third flight only (data not shown). The  $T_c$  values measured in the subsequent flights showed significant spatial variation in an east-west direction, possibly due to the soil effects, which obscured the genotypic differences in  $T_c$ . Statistical correction with REML analysis for spatial trends did not improve the correlations. Overall, SDM correlated significantly with  $T_c$  for stress-free crops (flights 1-2) when the spatial variation did not affect  $T_c$  estimates in the WW ( $r = -0.61^*$ ) and WD ( $r = -0.48^*$ ) treatments. These correlations increased further ( $r = -0.65^*$  and  $-0.54^*$ , respectively) when considering erect cane only, as lodging was found to affect  $T_c$  estimates. These results highlight the importance of phenotyping erect crops that are grown on uniform soils.

Interestingly, the study found a significant correlation between SDM of the WD treatment when expressed relative to the corresponding WW values, and the differences in  $T_c$  between the WD and WW treatments that were measured during flights 1 to 3 ( $r = -0.55^*$ ) (data not shown). This relationship could be further explored as the basis for phenotyping of drought tolerance.

**Table 1. Correlation between stalk yield (SDM) and stomatal conductance ( $g_s$ ), Normalized Difference Vegetation Index (NDVI) and canopy temperature ( $T_c$ ); between canopy cover (FIPAR) and NDVI; and between  $g_s$  and  $T_c$ , for the well-watered (WW) and water deficit (WD) treatments measured on different dates and for the season as a whole. Values in brackets represent correlations for erect (unlodged) cane. The average canopy cover (FIPAR) and crop water satisfaction index (CWSI) values for each flight and treatment were categorized as low (L), medium (M) or high (H). Values in bold (with an asterisk) indicate statistical significance at  $p=0.05$ .**

Flight	Date (DAP)	FIPAR		CWSI		SDM vs $g_s$		FIPAR vs NDVI		$g_s$ vs $T_c$		SDM vs NDVI		SDM vs $T_c$	
		WW	WD	WW	WD	WW	WD	WW	WD	WW	WD	WW	WD	WW	WD
1	20 Feb 19 (118)	0.71 (M)	0.70 (M)	1.74 (H)	1.30 (H)	<b>0.91*</b>	<b>-0.81*</b>	<b>0.49*</b>	<b>0.63*</b>	-0.55	-0.40	<b>0.64*</b>	<b>0.36*</b>	<b>-0.59*</b> (-0.66*)	<b>-0.48*</b> (0.44*)
2	12 Mar 19 (138)	0.75 (M)	0.73 (M)	1.65 (H)	1.17 (H)	<b>0.75*</b>	0.27	<b>0.69*</b>	<b>0.51*</b>	-0.57	-0.11	<b>0.56*</b>	<b>0.32*</b>	<b>-0.56*</b> (-0.50*)	<b>-0.40*</b> (-0.47*)
3	4 Apr 19 (161)	0.87 (H)	0.77 (M)	1.80 (H)	1.30 (H)	0.06	0.22	<b>0.61*</b>	<b>0.18*</b>	-0.36	0.12	<b>0.53*</b>	<b>0.28*</b>	<b>-0.24*</b> (-0.21*)	-0.00 (-0.06)
4	2 May 19 (189)	0.81 (H)	0.78 (M)	1.64 (H)	0.68 (M)	<b>0.84*</b>	<b>0.77*</b>	0.02	-0.23	0.08	0.18	<b>0.54*</b>	<b>0.53*</b>	-0.00 (-0.03)	-0.20 (-0.15)
5	30 May 19 (217)	0.86 (H)	0.85 (H)	1.74 (H)	0.54 (M)	<b>0.78*</b>	0.05	0.03	-0.15	-0.22	0.63	<b>0.40*</b>	<b>0.29*</b>	-0.17 (-0.13)	-0.19 (-0.20)
6	17 July 19 (265)	0.87 (H)	0.86 (H)	1.76 (H)	0.40 (L)	-	<b>0.57*</b>	0.05	-0.19	-	0.40	<b>0.45*</b>	0.22	0.00 (0.04)	0.00 (0.00)
7	26 Sep 19 (336)	0.89 (H)	0.89 (H)	1.69 (H)	0.34 (L)	<b>0.57*</b>	-	0.04	0.08	-0.32	-	<b>0.32*</b>	<b>0.37*</b>	-0.20 (-0.02)	-0.20 (-0.22)
	Seasonal average	0.82	0.80	1.72	0.82	<b>0.88*</b>	0.01	<b>0.38*</b>	<b>-0.49*</b>	<b>-0.44*</b>	0.21	<b>0.61*</b>	<b>0.35*</b>	<b>-0.54*</b> (-0.55*)	<b>-0.46*</b> (-0.48*)

## Conclusions

The study confirmed that FIPAR and  $g_s$  are influential traits for determining yield. Initial results show promise for the use of drone spectral imagery to assist plant breeding by identifying high-yielding genotypes in stress-free crops. This suggests that drone-captured NDVI and Tc of stress-free crops before canopy closure could be used to identify high-yielding genotypes. Refinements in trial and data capture methodologies are needed to better understand the impacts of stress and to improve the reliability of drone phenotyping for breeding application.

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