THE FAST FLUORESCENCE KINETICS: A SENSITIVE TOOL FOR EARLY DETECTION OF WATER STRESS IN SUGARCANE

By

DJ OLSEN[1], R SHAFIEI[1], FC BOTHA[1,2]

[1]*Sugar Research Australia, Indooroopilly*
[2]*The Queensland Alliance for Agriculture and Food Innovation* (*QAAFI*)
University of Queensland, St Lucia
dolsen@sugarresearch.com.au

KEYWORDS: Chlorophyll, Fluorescence, Performance Index, Maximum Quantum efficiency, OJIP-test, Water Stress.

Abstract

WATER STRESS IS a major constraint for sugarcane production in many regions of the world, including Australia. Sensitive and non-destructive early measurement of the crop response to water stress would be of great value for producers, advisors, and researchers. Chlorophyll-a (Chla) fluorescence is well established as a tool for measurement of photosynthetic efficiency. Changes in the kinetics of Chla fluorescence can provide valuable insight into the structure and function of the photosynthetic apparatus and chloroplast membrane integrity. The parameter Fv/Fm is often used to describe the effect of stress on the quantum yield of photosystem two (PSII). In this study the polyphasic OJIP fluorescence transient was used to evaluate the response of the sugarcane photosynthetic electron transport system. Chlorophyll fluorescence was measured on three leaves in the canopy of KQ228$^{(\!)}$ over a five-day water stress period, and the response analysed using the OJIP-test. The results show that several of the parameters that can be derived from the OJIP test are more sensitive and a better reflection of water stress than the Fv/Fm ratio. Evidently PSII is much more sensitive to water stress than photosystem one (PSI). In late stages of stress there are signs of a loss in membrane integrity and a disruption of water splitting in PSII.

Introduction

Sugarcane is one of the most commercially important crops in Australia. It belongs to a NADP-ME (tropical/subtropical) C4 grass family (Poaceae) which has high rates of photosynthesis and high productivity (Moore *et al.,* 1997; Botha and Moore, 2013). Sugarcane production is often limited by water stress (Inman-Bamber *et al.,* 2012 and references therein). The physiological responses of sugarcane to water stresses have been studied but are generally still poorly understood (Lakshmanan and Robinson, 2013 and references therein).

Constant and severe drought stress leads to stomatal closure, reduction of CO_2 availability and transpiration rates as well as loss of chlorophyll, which causes leaf chlorosis (Oukarroum *et al.,* 2007). Early measurement of the water stress before severe impacts, such as chlorosis, set in is important from a crop management and production perspective.

Variable fluorescence has been broadly used for measuring water stress in plants (for review Brestic and Zivcak, 2013). Chlorophyll fluorescence is known to be the most sensitive tool to measure the efficiency of photosynthesis and, consequently, can be used as an indicator of plant general vitality (Maxwell and Johnson, 2000).

Fluorescence can give insights into the ability of a plant to tolerate environmental stresses and also the extent to which those stresses have damaged the photosynthetic apparatus (Maxwell and Johnson, 2000).

Measurement of maximum photochemical efficiency (Fv/Fm), or quantum yield of PSII, is reported to be the most employed and reliable parameter utilising the chlorophyll fluorescence extremes of minimal (F_0) and maximal (Fm).

The kinetics of chlorophyll fluorescence after dark adaptation can provide valuable additional information on the organisation, efficiency, and linkage of electron transport (Strasser *et al.,* 2004).

The polyphasic rise in chlorophyll-a (Chla) fluorescence (OJIP) can be used to investigate the behaviour of PSII with functional parameters calculated by the JIP-test (Strasser and Strasser, 1995). Analysis of the fast fluorescence rise according to the JIP-test allows establishment of structural and functional parameters, providing a quantification of the system's behaviour (Strasser and Strasser, 1995; Kruger *et al.,* 1997). Additionally, the OJIP fluorescence curve analysis can be used to monitor the effect of various biotic and abiotic stresses affecting the structure and function of the photosynthetic apparatus (Strasser *et al.,* 2004).

Under intense actinic light, a sequence of Chla fluorescence energy states is evident as a series of steps (OJIP). The O-J steps represent the electron acceptor of PSII from the ground state or minimal ($O=F_0$) to the J peak (J=Fj) which occurs at 2 ms after the light exposure. The O-J step has been described as corresponding to the photochemical phase (Schreiber and Neubauer, 1987; Eullaffroy *et al.,* 2009) Whereas, J-I phase corresponds to plastoquinone (PQ) quenching which (FI) takes place between 20–30 ms post illumination. The I-P (Fp=Fm) peak is the result of maximum concentration of fluorescence yield resulting from oxidation of plastocyanin (PC) as well as photo-oxidation of P700+ in and about PSI (Strasser *et al.,* 2000; Schansker *et al.,* 2003; Oukarroum *et al.,* 2009; Tóth *et al.,* 2011)

In addition to F_0 and Fm, which represent the size of plastoquinone pool during reduction, variable fluorescence (Fv) can also be determined to describe the maximum quantum yield (Fv/Fm) of PSII photochemistry (Schansker *et al.,* 2003). The OJIP test calculates other biophysical parameters, such as energy flux of absorption (ABS), trapping (TR), and electron transport (ET), as well as an overall Performance Index (PIabs) for the assessment of plant physiological status (Strasser *et al.,* 2004).

Unlike the widely used Fv/Fm parameter, which is calculated solely from the Chla fluorescence endpoints F_0 and Fm, PIabs is comprised of the primary photochemical process of electron absorption (ABS), trapping (TR) of excitation energy, electron transport (ET) supplementary to primary plastoquinone (QA), and dissipation of excess excitation energy. Because it captures the kinetics of electron flow through the photosynthetic apparatus, from PSII to PSI, not only at its endpoints, it is one of the most sensitive and informative phenomenological parameters (van Heerden *et al.,* 2007; Brestic and Zivcak, 2013). In particular, PIabs is much more sensitive to changes in relative water content (RWC) than Fv/Fm, making it a valuable tool for the detection of water stress (Brestic and Zivcak, 2013). Fv/Fm is highly insensitive to stomatal effects or to any other effects occurring in moderate drought stress, although it does draw attention to the effect of co-occurring stresses, such as heat stress and photo-inhibition (Brestic and Zivcak, 2013).

The PIabs, which measures the overall fitness of electron transport, has been used in agricultural systems as a screening tool for a range of plant stressors, such as water stress (Oukarroum *et al.,* 2007; van Heerden *et al.,* 2007; Oukarroum *et al.,* 2009), cold stress (van Heerden, 2014), and pathogen stress (Gonçalves *et al.,* 2005).

The OJIP test parameters, such as PIabs, have been used in sugarcane previously to determine the effect of biotic and abiotic stress on the photosynthetic electron transport system. Recently the effect of frost stress (van Heerden, 2014) and aluminium-induced water stress (Ecco *et al.,* 2013) was studied in sugarcane using the OJIP test, including the PIabs parameter. Although well described as a useful tool for the assessment of a range of biotic and abiotic stresses, the usefulness of the OJIP test, including the PIabs parameter, as a tool for the early detection of water stress in sugarcane has not been studied (Ecco *et al.,* 2013).

In this study we have evaluated the OJIP test as a sensitive, early detection tool for water stress in sugarcane. In addition, we report that major changes in electron transport occur early on during the onset of water stress. Furthermore, we show that individual component of combined parameters, such as PIabs, can be further dissected.

Methods and materials

Material

The pot trial was planted on 10 June 2014 on the grounds of the Sugar Research Australia (SRA) Research Station at Brandon, QLD (19° 34.177′S, 147° 193.565′E). Variety was KQ228$^{\phi}$ planted as one-eye setts, one per pot. Pots were 280 mm diameter × 270 mm height and each was filled with 14 kg of sandy loam soil, sourced from a fallow block on the SRA Burdekin Station (0–20 cm depth). Following soil nutrient testing, the soil was fertilised according to Six Easy Steps nutrient recommendations and fertilised bi-weekly with a complete soluble fertiliser until trial end. Irrigation was by a pressurised system using dripper emitters and managed by a timer. Each pot contained one PR2/4 soil moisture probe (Delta-T Devices Ltd, Cambridge UK). During the experiment soil moisture (%vol) was measured daily at 9 am and results presented as an average of the six replicates.

Water stress treatment

There were two water treatments arranged in a completely randomised design. Treatments were 1) fully irrigated (maintained at 80% field capacity), and 2) water stressed. All plants were maintained stress free and fully irrigated prior to commencement of the experiment. The experiment was conducted over five days from 4 May to 8 May 2015 corresponding to 11 months after planting. During this time the water stress treatment was created by removing all irrigation and allowing the pots to dry down over the course of the five-day trial period.

Fluorescence measurements

Chlorophyll-a fluorescence was measured daily between 9.00 am and 11.00 am during the trial period. Measurements were taken on leaves +1, +3 and +5, where leaf +1 was the first fully expanded leaf corresponding to the top visible dewlap. Measurements were taken using a PEA fluorescence meter (Hansatech Instruments Ltd., Kings Lynn, Norfolk, PE 30 4NE, UK). The transients were induced by a red light (peak at 650 nm) of 3200 μmol/m^2/s provided by the PEA instrument through an array of six light-emitting diodes (van Heerden, 2014).

Leaf clips were placed near the middle of the leaf blade and allowed to dark adapt for a minimum of 15 minutes before measurement. The polyphasic rise in Chla fluorescence was used to investigate the behaviour of PSII with functional parameters calculated by the OJIP-test (Strasser and Strasser, 1995).

A range of agronomic measurements, including stalk height, total leaf number, and leaf number of the last green leaf, were taken each day prior to the fluorescence measurements.

Phenotypic description of stress

Each day, prior to measuring Chla fluorescence, leaves +1, +3 and +5 were rated for their phenotypic response to the treatments. Leaf yellowing was scored according to the severity rating key shown in Table 1. The rating system consisted of a severity scale from 0–4 (Table 1).

Table 1—Water stress severity rating key.

Severity rating	Description (degree of yellowing on leaves)
0	Green leaf
1	Yellowing is present in 1–25% of the leaf. Yellowing extending from the tip towards the leaf base. Drying evident from the leaf tip
2	Yellowing is present in 26–50% of the leaf. Yellowing extending from the dead tip towards the leaf base.
3	Yellowing is present in 51–75% of the leaf. Advanced yellowing across the entire leaf blade, extending from the dead tip towards the leaf base
4	Dead/senesced leaves (>75% of leaf yellow/dead)

Statistical analyses

Analysis of agronomic indicators (Figure 1.) was undertaken using Statistix10 at a 95% confidence level. Data was first checked for normality by Shapiro-Wilk test and transformations made where required to ensure normality. A split-plot design was used with main factor (irrigation) and sub-factor (day). Difference between groups was determined by a Tukey's HSD all-pairwise comparisons test (displayed as letter separations a, ab, b etc.).

Results

Soil moisture deficit

As expected, soil moisture % decreased over the course of the trial period in the stressed treatment while remaining fairly constant in the control pots (Figure 1A). Soil moisture was significantly lower in stressed pots throughout the treatment period ($p<0.05$). Means were 14% and 21% lower on days one and two respectively, and were 23% lower on days three, four, and five.

Phenotypic response to stress

Plants began the treatment period with between seven and 10 leaves per stalk, and there was no difference in total leaf numbers during days one to four ($p<0.05$) ($p<0.05$, Figure 1C). By day five, however, many of the lower leaves on the stressed plants were completely brown and dead. Total leaves per stalk dropped significantly on day five with stressed plants having an average of four total leaves compared with eight for the control plants.

On day one, yellowing was observed on leaf +6 and lower for both treatments, typical of natural senescence. However, as the trial period progressed, yellowing in stressed plants occurred higher in the canopy, while remaining at a relatively constant level in the control plants.

Yellowing progressed rapidly up the stalk from leaf +6 to leaf +1 in the stressed plants over the five-day period, and was significantly different each day from day two onwards ($p<0.05$) (Figure 1B). On day two, the average leaf number of the last green leaf was +7.1 in control plants and +3.2 in stressed plants. Given that no phenotypic response to the stress was seen on day two in leaf +3, it was decided to focus on this leaf for this study.

The severity of yellowing observed on leaf +3 of stressed plants became progressively more severe over the course of the trial period while corresponding leaves on control plants remained green. Leaves were significantly yellower than control on days four and five, displaying strong yellowing on 50–75% of their lamina extending from a dead leaf tip ($p<0.05$) (Figure 1D).

It is apparent that by day four, the stressed plants have reached their phenotypical response endpoint. Values for soil moisture%, leaf number of last green leaf, and severity of yellowing remain unchanged between days four and five. The only real phenotypic difference between these two days is the significantly lower number of leaves per stalk ($p<0.05$). Senescing leaves on day four were fully brown and dead on day five.

For reasons described above, it was decided to focus on days one, two and four and leaf +3 in this study. Day one is included as the first day with significant differences in soil moisture, baseline day, while day two represents the earliest point where Chla fluorescence differs between treatments (see leaf fluorescence section to follow), while phenotypic response in leaf +3 remains the same. Day four represents the logical endpoint of the study based on phenotype and fluorescence data.

Leaf fluorescence

At the commencement of the experiment control and stress plants show very similar fluorescence transients (Figure 2A). However, by day two the OJIP curves showed separation between the control and treated plants (Figure 2 B). In stressed plants F_0 was elevated, F_m was reduced and there was separation at around 2 ms corresponding to the J-step. By Day four, even more separation can be seen (Figure 2 C). Maximal fluorescence (F_m) was much reduced in the stressed plants resulting in a greater area above the curve, and the OJIP curve deviated markedly from the control curve from 300 μs onwards.

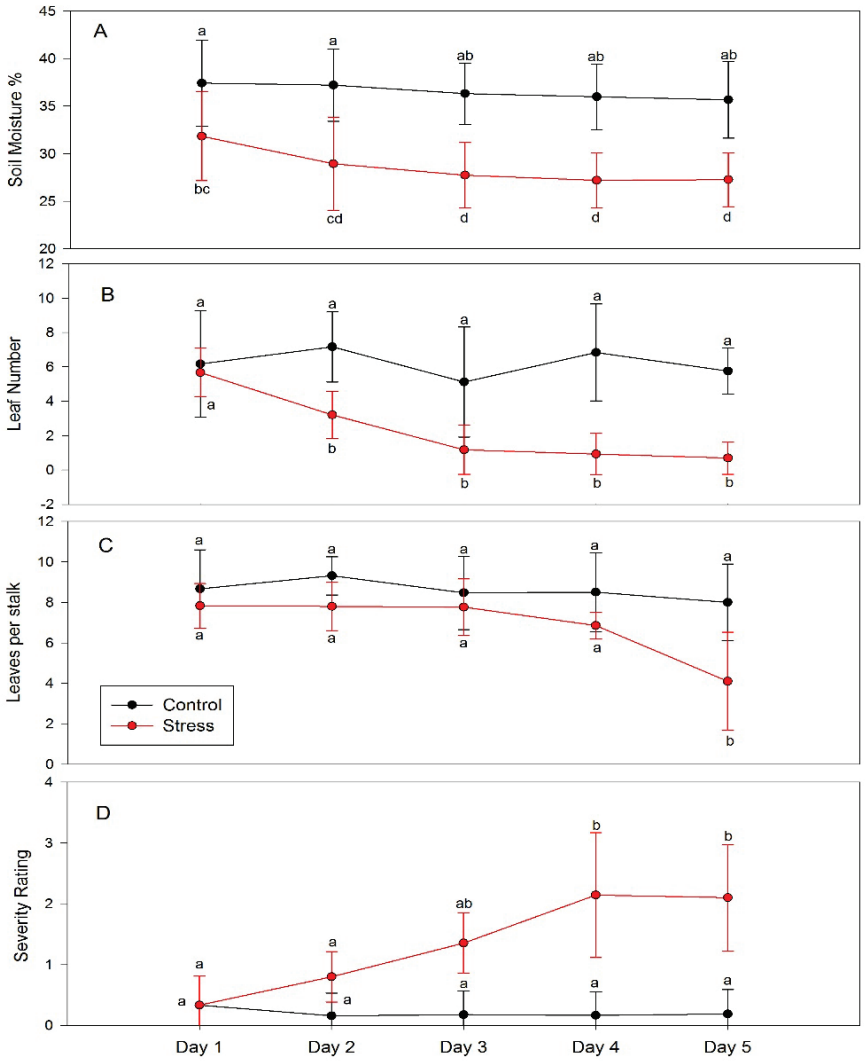

Fig. 1—Agronomic indicators during the five-day trial period. Soil moisture % (A), leaf number of the last green leaf per stalk (B), total leaves per stalk (C) and severity of yellow symptoms (D). Statistical significance is shown by letter separations (a, ab, b etc.) at $p<0.05$.

The Chla transients (Figure 2A, B, C) were normalised at the minimal (F_0) and maximal (Fm) endpoints to highlight differences between the treatments (Figure 2D, E, F).

The normalised curves were similar on day one but showed marked divergence on days two and four between the control and the stressed plants. There is especially clear separation in the curves around 2 ms corresponding to the J step.

The specific effects of water stress on the OJIP fluorescence rise kinetics were visualised by compiling delta variable fluorescence (ΔFv) curves (van Heerden *et al.,* 2007). For the construction of ΔFv curves, the normalised values (Figure 2D, E, F) derived from transients recorded for the control treatment were subtracted from that for the stress treatment (Figure 2G, H, I).

Clearly there is little difference at day one, however at day two a broad peak is evident around the J step at 2 ms.

Besides being even more pronounced, by day four the peak has also skewed left so that it peaks at an earlier time point, around 300 µs. On this day the curve is no longer smooth with data points becoming more erratic.

The presence of a peak at this point is indicative of a disruption in the water splitting activity of PSII (Strasser *et al.,* 2004; Tsimilli-Michael and Strasser, 2008, 2013).

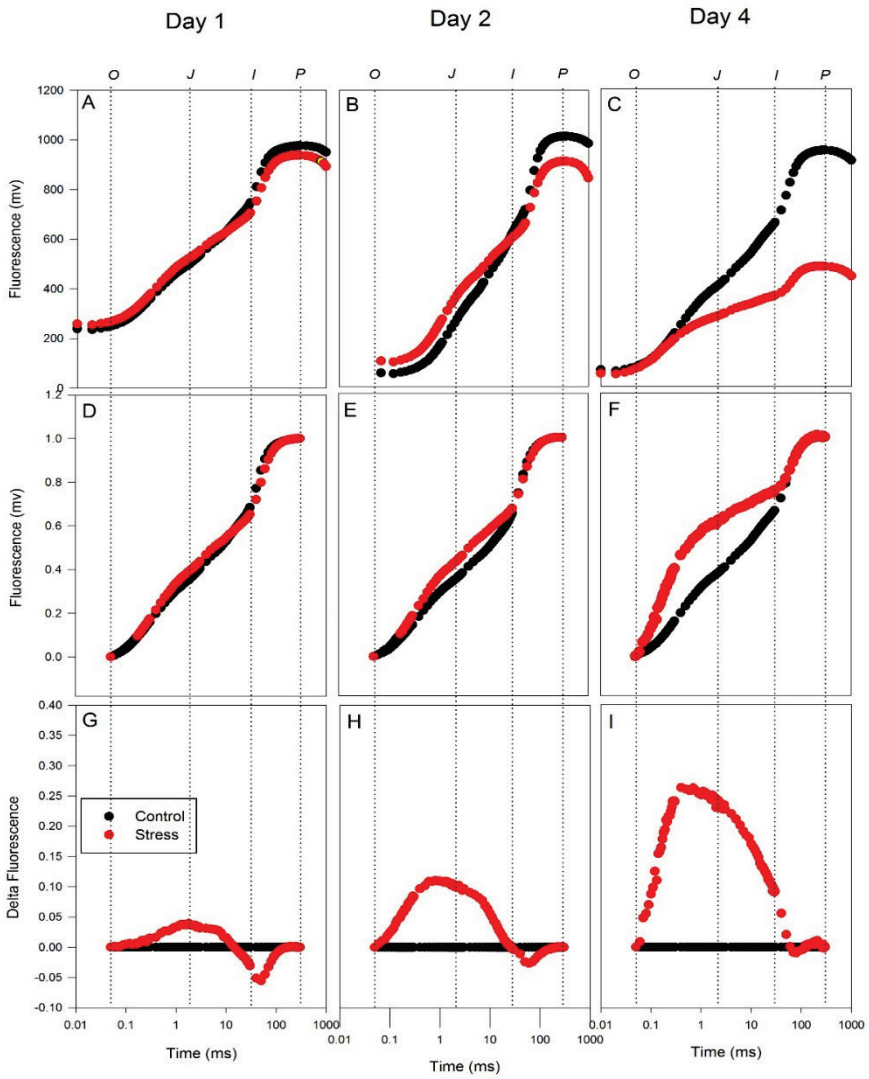

Fig. 2—Leaf +3 fluorescence transients measured on day 1, day 2 and day 4. Non-normalised fluorescence transients (A), (B) and (C). Transients normalised between F0 and Fm in (D), (E) and (F). Delta fluorescence shown in (G), (H) and (I). Time points corresponding to OJIP are shown by vertical dashed lines.

Individual fluorescence parameters

The most used OJIP parameters as previously defined (Strasser *et al.,* 2000; Strasser *et al.,* 2004; Tsimilli-Michael and Strasser, 2013) are captured in Table 2. For this study we have chosen to express the data on the reaction centre basis. The expression of the data in this way gives an accurate picture of the efficiency of individual reaction centres. However, all of these parameters can be expressed on the cross section basis using either (range zero-maximum). When expressed on a cross section basis the data will provide additional information on the density of the reaction centres (for review Tsimilli-Michael and Strasser, 2013)

Most of the parameters show significant variation over the treatment period (Table 2). Only the most informative parameters are presented in Figure 3.

The radar plots (Figure 3) show the effect of the stress event on six key parameters simultaneously. On day one there was no statistical difference between treatments for any of the parameters measured (Figure 3A). However, on day two the stressed curve clearly shrinks inward from the control. On this day only PIabs was significantly reduced in stressed plants. Compared to control, PIabs was reduced by 60%, while Fv/Fm showed a non-significant 9% decrease. (Figure

3B). On day four, five parameters were significantly reduced in stressed plants; Fv/Fm, Fm, RC/SCo, PIabs and Area. PIabs and Fv/Fm were 81% and 22% reduced respectively, compared with the control (Figure 3C). The parameter RC/SCo, an indicator of the density of reaction centres per excited leaf cross section, was significantly reduced on day four indicating the closing down of reaction centres.

Table 2—OJIP-test parameters on days 1, 2 and 4. Values are means ± SD, n=6.

	t for Fm (ms)	Area	Fm	Fv/Fm	PI abs	ABS/RC	TRo/RC	ETo/RC	DIo/RC	RC/Cso
Day 1										
Control	288.33 ± 11.7	16438.17 ± 5780.0	976.67 ± 289.7	0.77 ± 0.02	2.62 ± 1.00	2.15 ± 0.23	1.64 ± 0.15	1.01 ± 0.10	0.50 ± 0.09	118.85 ± 35.4
Stress	281.67 ± 7.5	16743.50 ± 2847.1	938.67 ± 122.4	0.74 ± 0.04	2.10 ± 1.31	2.19 ± 0.34	1.61 ± 0.19	0.92 ± 0.06	0.58 ± 0.16	125.42 ± 20.1
Day 2										
Control	255.00 ± 41.23	17665.25 ± 1851.6	1045.75 ± 86.35	0.77 ± 0.02	3.77 ± 1.05	1.75 ± 0.1	1.35 ± 0.06	0.88 ± 0.02	0.4 ± 0.06	147.03 ± 13.6
Stress	260.00 ± 33.67	14799.50 ± 2563.6	961.00 ± 151.9	0.71 ± 0.06	1.54 ± 1.13	2.44 ± 0.5	1.72 ± 0.22	0.92 ± 0.07	0.73 ± 0.29	126.07 ± 21.7
Day 4										
Control	281.67 ± 61.8	19224.17 ± 6300.8	995.00 ± 72.3	0.76 ± 0.02	2.50 ± 0.84	2.11 ± 0.34	1.59 ± 0.23	0.97 ± 0.17	0.51 ± 0.11	128.09 ± 18.1
Stress	246.67 ± 76.6	8174.33 ± 7412.0	604.33 ± 323.7	0.55 ± 0.16	0.48 ± 0.69	4.92 ± 3.08	2.29 ± 0.43	0.79 ± 0.22	2.63 ± 2.75	73.01 ± 47.3

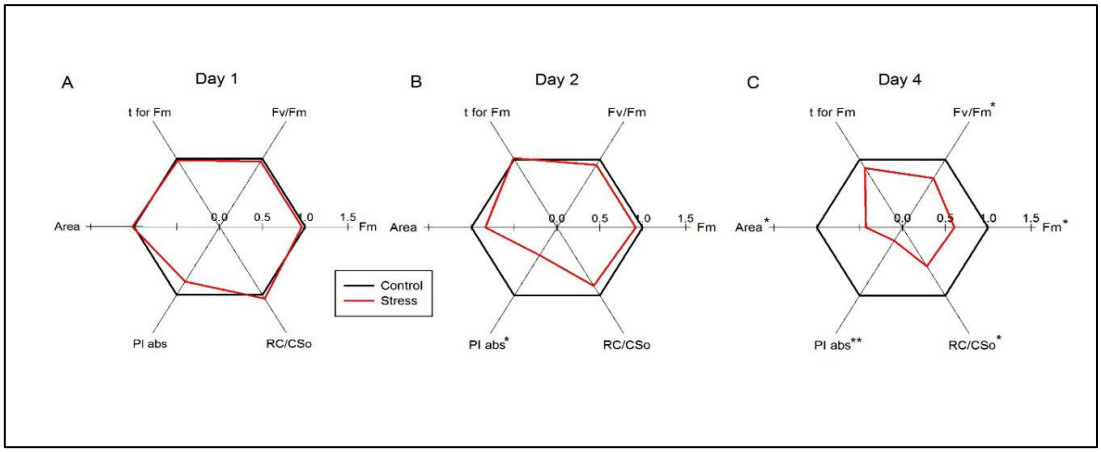

Fig. 3—Radar plots comparing leaf +3 fluorescence parameters on three days. Day 1 (A), Day 2 (B), and Day 4 (C). For each plot, values for the stress treatment have been normalised to their corresponding control so that the control has values of 1 for all parameters and the stress treatment is expressed as a proportion of the control. Statistical significance is shown *p<0.05, **p<0.01 and ***p<0.001.

Discussion

This study illustrated the usefulness of variable Chla fluorescence as a measure of the effects of water stress on sugarcane and identifies the parameter PIabs as a useful tool for the early detection of this stress. Additionally, the data indicated that several key components of the electron transport system are affected before visual phenotypic symptoms of stress are evident.

Environmental stress has highly impacted the growth and productivity of agricultural yields due to the extreme weather events such as prolonged drought and flooding. Constant and severe drought stress leads to stomatal closure, reduction of CO_2 availability and transpiration rates.

It is well documented and understood that when water stress is severe enough it will lead to stomatal closure and decreases in mesophyll conductance.

Prolonged periods of limitations on photosynthesis will also lead to a loss of chlorophyll resulting in leaf chlorosis. However, measuring stomatal conductance and CO_2 assimilation under field conditions require sensitive expensive equipment.

It is known that the production and growth of plants is highly dependent on an intact fully functional photosynthetic electron transport system to regulate electron capture and flux. In a healthy leaf, the solar energy is absorbed by a chlorophyll molecule in fully active reaction centres (RCs). The absorbed energy can be use in either the photochemical pathways, which lead to the production of NADPH and ATP, or dissipated in non-photochemical processes as fluorescence and heat (Strasser *et al.,* 2004). Over-excitation of photosynthetic apparatus not only leads to increased fluorescence but also to an increase in the production of reactive oxygen species (ROS) by transferring absorbed energy to oxygen. Increased levels of ROS will result in cell death (Hortensteiner and Krautler, 2011; Saga and Tamiaki, 2012; Saga *et al.,* 2013).

The measurement of Chla fluorescence is one of the most widely used methods to probe photosynthesis (Kalaji *et al.,* 2014 and references therein). The Chla fluorescence signal is rich in its content, is very sensitive to changes in photosynthesis, and can be recorded with great precision. Commercially available and affordable instruments make it easy to measure Chla fluorescence, however the interpretation of such measurements is still very contentious (Kalaji *et al.,* 2014). The most widely used is the fluorescence rise from F_0 to Fm, i.e. the variable fluorescence (Fv). The dominant interpretation assumes that the variable fluorescence is determined by the redox state of QA, the first quinone acceptor of PSII. This measurement is generally expressed as the ratio between Fv and Fm.

Recently, PIabs has been used to study the effect of stress on many crop species, however little work has been done in sugarcane. In this study we observed a greater sensitivity of PIabs to water deficit than for the commonly used Fv/Fm parameter (Table 2 and Figure 3). PIabs was a sensitive early indicator of stress, showing significant reduction on day two in the absence of phenotypic response. Fv/Fm was less sensitive and only became a significant indicator on day three after the onset of yellow symptoms. The sensitivity of PIabs over Fv/Fm is well described in the literature and has its origin in the parameters used for its calculation (Brestic and Zivcak, 2013). Whereas Fv/Fm is calculated from the two endpoints of the Chla fluorescence transient, PIabs is a composite of the kinetics parameters of electron absorption (ABS/RC), trapping (TR/RC) and electron transport from PSII to PSI (ET/RC). As a result, PIabs has advantages in comparison with Fv/Fm as it can indicate the physiological status of the membrane integrity. A reduction PIabs signals a disruption in electron transport of photosynthetic apparatuses and downregulation of PSII function (Strasser *et al.,* 2004; van Heerden *et al.,* 2007).

While PIabs describes overall fitness of the photosynthetic apparatus, its component elements of absorption (ABS/RC), trapping (TRo/RC) and electron transport (ETo/RC) can be individually studied to give further insight into the location of the disruption within photosynthetic apparatuses.

In this study, the significant increase in ABS/RC on day four for stressed plants (Table 2), occurring between O–J phase, was indicative of the depletion of the PSII core in response to the shutting down of reaction centres. Over the course of the trial, the trapping flux energy TRo/RC increased gradually in stressed plants while electron transport ETo/RC remained relatively unchanged. This indicates that the stress event caused major disruption at the front end of the electron transport chain, that is, at the electron absorption and trapping level of PSII. As the stress progressed, reaction centres were shut down resulting in a reduced turnover of QA reduction and subsequent electron transport to PSI.

The closure of reaction centres caused an increase in electron absorption (ABS/RC) as the remaining RC's shared the increased load. The fact that ETo/RC remained relatively unchained suggests that the disruption was not caused at the interface of electron transfer to PSI, but rather was centralised in the photochemical processed of PSII (Strasser *et al.,* 2000; Tsimilli-Michael and Strasser, 2013).

The parameter RC/CS (active reaction centres per excited cross-section) measured the concentration of the reaction centres that been impacted by the drought stress, and together with the

PIabs parameters can provide further confirmation of the size and density of the RC pool. Our data show this parameter significantly decreased by day four, which agrees with our conclusion that the stress event caused major disruption around PSII.

As the leaf's photosynthetic capacity was progressively more affected by the inhibition of the electron flux through the photochemical pathway from PSII to PSI, there was a significant increase in the re-emission of fluorescence.

The radar plots (Figure 3) also describe of the same structural and functional patterns of change among the PIabs elements and other essential parameters and confirm the sensitivity of PIabs as an early detection tool for water stress in sugarcane.

Evidently, the reactions around PSII are more sensitive to water stress than electron flow from PSI to the carbon fixation processes are least sensitive to water stress to drive carbon fixation. Early in water stress there is a strong ΔJ peak appearing (disruption of PSII) and then an apparent even earlier disruption with the development of a ΔK peak (disruption of water splitting on PSII). The appearance of a ΔK peak is indicative of temperature stress (van Heerden *et al.,* 2007). It is also interesting that that the data in the Δ curves become much more erratic late in the stress period. This is taken as an indication of membrane changes and a special separation of the components of the electron transport system (Reto Stasser, pers. comm.).

Conclusions

The OJIP test is a very sensitive method to determine early water stress in sugarcane. When sugarcane plants are exposed to water stress initial changes are a disruption of electron absorption and trapping in PSII. It is probably this super charging of PSII that leads to ROS production causing membrane damages and photo oxidation of chlorophyll.

By the time first yellow is observed membrane disruption and separation of the components of the electron transport system has developed to a point where the water splitting activity of PSII is also compromised.

Acknowledgements

The authors would like to thank SRA technicians Angela Zeilstra and Jane Brownlee.

REFERENCES

Botha FC, Moore PH (2013) Biomass and bioenergy. In 'Sugarcane: Physiology, biochemistry, and functional biology.' pp. 521–540. (John Wiley & Sons Ltd: Chichester, UK).

Brestic M, Zivcak M (2013) PSII fluorescence techniques for measurement of drought and high temperature stress signal in crop plants: Protocols and applications. In 'Molecular stress physiology of plants.' (Eds GR Rout, AB Das) pp. 87–131. (Springer: New York, USA).

Ecco M, Santiago EF, Lima PR (2013) Chlorophyll a fluorescence in two varieties of sugar cane subjected to aluminum and water stress. *African Journal of Agricultural Research* **8**, 4941–4948.

Eullaffroy P, Frankart C, Aziz A, Couderchet M, Blaise C (2009) Energy fluxes and driving forces for photosynthesis in Lemna minor exposed to herbicides. *Aquatic Botany* **90**, 172–178.

Gonçalves MC, Vega J, Oliveira JG, Gomes MMA (2005) Sugarcane yellow leaf virus infection leads to alterations in photosynthetic efficiency and carbohydrate accumulation in sugarcane leaves. *Fitopatologia Brasileira* **30**, 10–16.

Hortensteiner S, Krautler B (2011) Chlorophyll breakdown in higher plants. *Biochimica et Biophysica Acta* **1807**, 977–88.

Inman-Bamber NG, Lakshmanan P, Park S (2012) Sugarcane for water-limited environments: Theoretical assessment of suitable traits. *Field Crops Research* **134**, 95–104.

Kalaji H, Schansker G, Ladle R, Goltsev V, Bosa K, Allakhverdiev S, Brestic M, Bussotti F, Calatayud A, Dąbrowski P, Elsheery N, Ferroni L, Guidi L, Hogewoning S, Jajoo A, Misra A, Nebauer S, Pancaldi S, Penella C, Poli D, Pollastrini M, Romanowska-Duda Z, Rutkowska B, Serôdio J, Suresh K, Szulc W, Tambussi E, Yanniccari M, Zivcak M (2014) Frequently asked questions about in vivo chlorophyll fluorescence: practical issues. *Photosynthesis Research* **122**, 121–158.

Kruger GHJ, Tsimilli-Michael M, Strasser RJ (1997) Light stress provokes plastic and elastic modifications in structure and function of photosystem II in camellia leaves. *Physiologia Plantarum* **101**, 265–277.

Lakshmanan P, Robinson N (2013) Stress physiology: Abiotic stresses. In 'Sugarcane: Physiology, biochemistry, and functional biology.' pp. 411–434. (John Wiley & Sons Ltd: Chichester, UK).

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence—a practical guide. *Journal of Experimental Botany* **51**, 659–668.

Moore PH, Botha FC, Furbank RT, Grof CPL, Keating BA, Wilson JR (1997) Potential for overcoming physio-chemical limits to sucrose accumulation. In 'Intensive sugarcane production: meeting the challenges beyond 2000'. Proceedings of the Sugar 2000 Symposium, Brisbane, Australia, 20–23 August 1996. pp. 141–155.

Oukarrou A, Madidi SE, Schansker G, Strasser RJ (2007) Probing the responses of barley cultivars (Hordeum vulgare L.) by chlorophyll a fluorescence OLKJIP under drought stress and re-watering. *Environmental and Experimental Botany* **60**, 438–446.

Oukarroum A, Schansker G, Strasser RJ (2009) Drought stress effects on photosystem I content and photosystem II thermotolerance analysed using Chl a fluorescence kinetics in barley varieties differing in their drought tolerance. *Physiologia Plantarum* **137**, 188–199.

Saga Y, Hirai Y, Sadaoka K, Isaji M, Tamiaki H (2013) Structure-dependent demetalation kinetics of chlorophyll a analogs under acidic conditions. *Photochemistry and Photobiology* **89**, 68–73.

Saga Y, Tamiaki H (2012) Demetalation of chlorophyll pigments. *Chemistry & Biodiversity* **9**, 1659–1683.

Schansker G, Srivastava A, Govindjee Strasser RJ (2003) Characterisation of the 820-nm transmission signal paralleling the chlorophyll a fluorescence rise (OJIP) in pea leaves. *Functional Plant Biology* **30**, 785–796.

Schreiber U, Neubauer C (1987) The polyphasic rise of chlorophyll fluorescence upon onset of strong continuous illumination. II. Partial control by photosystem II donor side and possible ways of interpretation. *Zeitschrift für Naturforschung* **42c**, 1255–1264.

Strasser BJ, Strasser RJ (1995) Measuring fast fluorescence transients to address environmental questions: the JIP-test. . In 'Photosynthesis: from light to biosphere.' (Ed. P Mathis) Vol. 5 pp. 977–980. (Kluwer Academic Publishers: The Netherlands)

Strasse RJ, Srivastava A, Tsimilli-Michael M (2000) The fluorescent transient as a tool to characterise and screen photosynthetic samples. In 'Probing photosynthesis: Mechanisms, regulation and adaptation.' (Eds M Yunus, U Pathre, P Mohanty) pp. 445–483. (Taylor and Francis: London).

Strasser RJ, Tsimilli-Michael M, Srivastava A (2004) Analysis of the chlorophyll a fluorescence transient. In 'Chlorophyll a fluorescence: A signature of photosynthesis' (Eds G Papageorgiou, Govindjee) Advances in Photosynthesis and Respiration Vol. 19 pp. 321–362. (Springer: Dordrecht, Netherlands).

Tóth SZ, Nagy V, Puthur JT, Kovács L, Garab G (2011) The physiological role of ascorbate as photosystem ii electron donor: Protection against photoinactivation in heat-stressed leaves. *Plant Physiology* **156**, 382–392.

Tsimilli-Michael M, Strasser R (2008) Experimental resolution and theoretical complexity determine the amount of information extractable from the chlorophyll fluorescence transient OJIP. In 'Photosynthesis. Energy from the sun.' 14th International Congress on Photosynthesis Volume 1. (Eds J Allen, E Gantt, J Golbeck, B Osmond) pp. 697–701. (Springer: Dordrecht, Netherlands).

Tsimilli-Michael M, Strasser R (2013) The energy flux theory 35 years later: formulations and applications. *Photosynthesis Research* **117**, 289–320.

van Heerden PDR (2014) Differential acclimation capacity to frost in sugarcane varieties grown under field conditions. *Plant Growth Regulation* **72**, 181–187.

van Heerden PDR, Swanepoel JW, Kruger GHJ (2007) Modulation of photosynthesis by drought in two desert scrub species exhibiting C3-mode CO_2 assimilation. *Environment and Experimental Botany* **61**, 124–136.