

Sun Induced Fluorescence (SIF) as stress indicator in Wheat crop and its relation with key photosynthetic parameters

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Sun Induced Fluorescence (SIF) serves as useful means for detecting the condition of vegetation as it has a close correlation with photosynthesis, thus it is used to derive Gross Primary Product (GPP). SIF could be a good estimator of net photosynthesis levels in crops affected by water deficit and nutritional stress. The field collected spectral signatures of wheat crop are used of different growth stages under control and stress treatments. SIF retrieval for wheat crop is done from spectral reflectance signatures using Spectral Fitting Method (SFM). Relations between SIF signals and key photosynthetic parameters from combined gas exchange and chlorophyll fluorescence at leaf level based on Farquhar–von Caemmerer–Berry leaf photosynthesis model fitting and others. Analysis is done for observed patterns of relationships and thus contributes to the understanding of usefulness of SIF as crucial for remote sensing application.

Introduction

Sun Induced Fluorescence related with photosynthesis and CO₂ assimilation. Accurate Crop (Biotic and Abiotic) stress detection be potentially detected through Sun Induced Fluorescence (SIF). Passive fluorescence retrieval quantifies the absolute values of SIF emission. The SIF is emanated from the light reactions of photosynthesis, and is more closely related to Electron Transport Rate (*J*) in photosystem II than to Gross Primary Product (GPP). There is need to understanding the mechanisms influence the GPP:SIF relationship across time and space. The aim of the present work is to study remotely sensed SIF and photosynthesis process using ground based instrumentation and modelling of crop stress

Methods and Materials

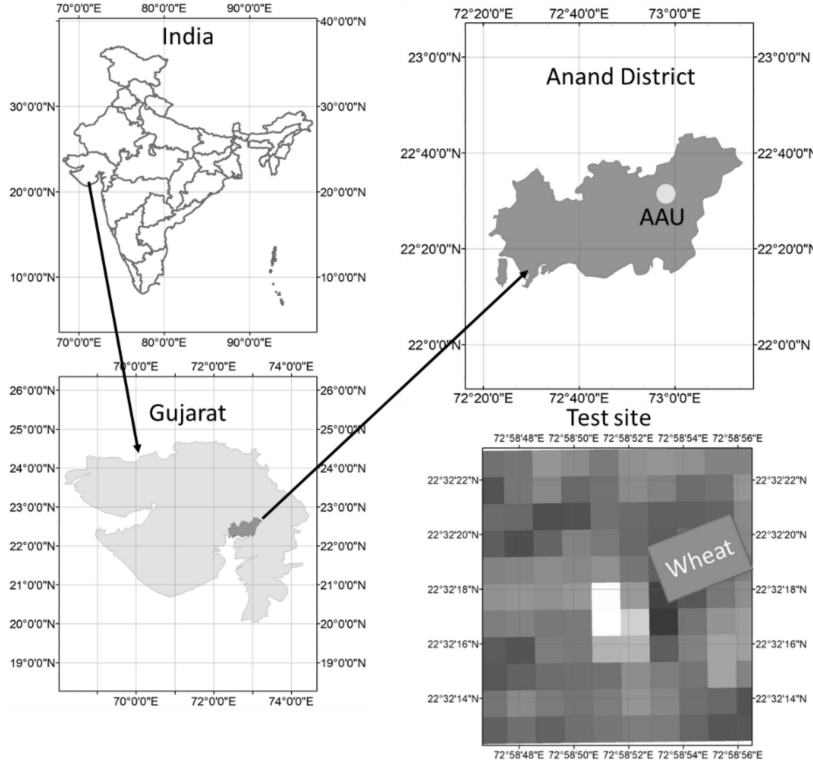
In-situ measurements were taken over wheat crop sown on November 20, 2020 at Anand Agricultural University (AAU) in early 2021 during 3 growth stages - 63, 81 and 93 Days After Sowing (DAS). The site is located in Anand City (22.5645° N, 72.9289° E), Anand District, Gujarat and comes under in Gujarat plain & hill agro-climatic zone. The crop experienced mild water stress on DAS 81. Photosynthesis associated parameters along with those associated with stress were measured on the given dates. The retrieval of actual SIF spectrum between 640 to 850 nm by Spectral Fitting Methods (Cogliati et al 2019) using field spectroscopic measurements (Upwelling Radiance, Downwelling Radiance). Spectral Evolution's field portable and laboratory NIR spectroradiometer which allows UV-VIS-NIR measurements within spectral range 350-2500 nm.

The spectroradiometer has been attached with standard fore-optic with 25° field of view (FOV) through a permanent fibre optic cable.

LI-COR Bioscience's LI-6400XT photosynthesis gas exchange system is used to non-destructively measure amount of CO₂ flux indicated by NIR absorbed in leaf in the Leaf Chamber Fluorometer (LCF) which indirectly indicates of photosynthesis activity of the plant under steady-state conditions. The instrument also measures physiological, climatic and environmental parameters. The change in the CO₂ concentration of the air flowing across the LCF is indicator of photosynthetic carbon assimilation in leaf by wheat crop. An Infrared Gas Analyzer (IRGA) consists of reference and sample CO₂ chambers such that reference chamber gives the actual concentration of CO₂ in air and sample chamber contains the amount of CO₂ present inside the 2 cm² leaf area enclosed in the chamber. The LCF generates rectangular pulses of actinic light on to leaf area consisting RGB-Red (630nm) for measuring and saturation flashes, Blue (470nm), Far red (740nm) Light Source using Pulse-Amplitude Modulation (PAM) of both dark- and light-adapted leaf samples. Measured parameters include minimal fluorescence (F_o), fluorescence under saturating light conditions (F_m), fluorescence under non-saturating light conditions (F), maximal fluorescence during a saturating light flash (F_m'), and minimal fluorescence of a light adapted leaf (F_o'), and calculated parameters include $F_v (=F_m - F_o)$, Maximum quantum yield (F_v/F_m), efficiency of PS II (F_v'/F_m'), effective quantum yield (Φ_{PSII}), Photochemical quenching of fluorescence (qP), Non-photochemical quenching of fluorescence (qN), Non-Photochemical Quenching (NPQ) and Electron transport rate (ETR). Another parameter maximum carboxylation rate (V_{cmax}) may be obtained from measured A/Ci curves fitted the Farquhar-von Caemmerer-Berry model (Farquhar, von Caemmerer, & Berry, 1980a) (Yang et al., 2018).

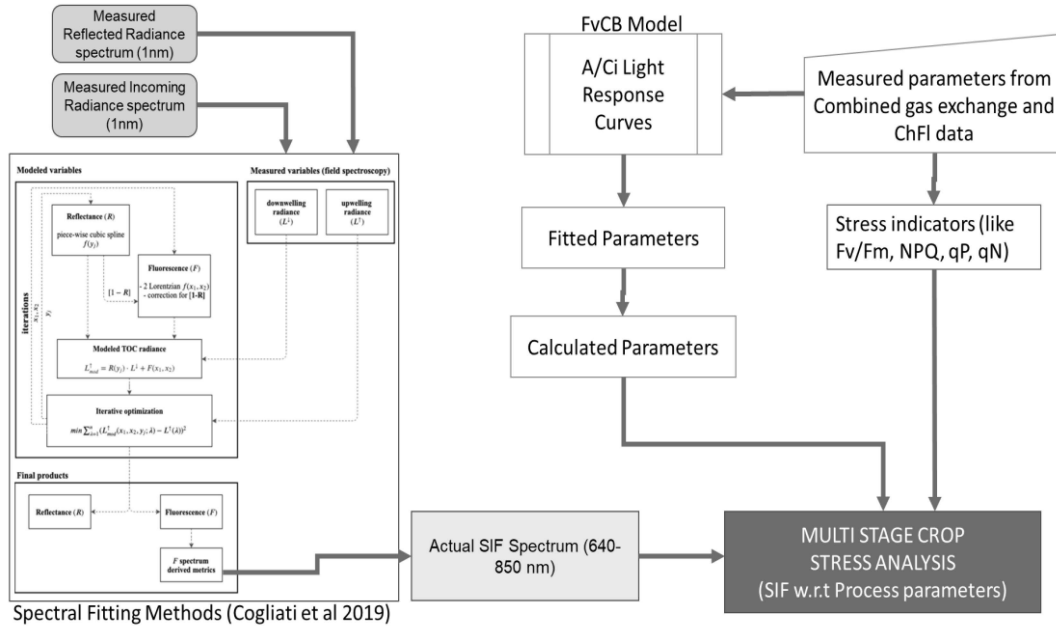
Measurement of Photosynthesis associated parameters require An/Ci Curve fitting based on Farquhar–von Caemmerer–Berry leaf photosynthesis model (FvCB model) (Farquhar et al 1980) from Pulse Amplitude Measurement (PAM) using LICOR-6400XT Portable Leaf Photosynthesis and gas exchange system.

Figure 1. Map showing the study area (Field shown on Top of Landsat 8 satellite data)



$A_{Vc\max}$ is the rate of net CO_2 assimilation determined by the Rubisco limited state ; $A_{j\max}$ is the rate of net CO_2 assimilation determined by the RUBP limited state; $V_{c\max}$ is the maximum velocity of Rubisco for carboxylation; R_d is Day respiration rate; Γ^* is the chloroplastic CO_2 photo compensation point.

Figure 2: Methodology



V_{cmax} , J_{max} , Γ^* and R_d were fitted from the response curves of A_n/C_i measured with Li-6400-XT by using methods of fitting A–C_i curves to estimate photosynthetic parameters of the FvCB model have been used (Ethier and Livingston, 2004).

Using model of (Farquhar et al., 1980), net CO₂ assimilation rate of a leaf is given as : $A_n = \min[A_{Vcmax}, A_j]$

$$A_{Vcmax} = \frac{V_{cmax} \times (C_c - \Gamma^*)}{(C_c + K_{CO})} - R_d$$

In the Rubisco limited phase, the response of net assimilation A to C_c is described using the FvCB (Farquhar et al., 1980) equation defined by $A_{Vcmax} = \frac{V_{cmax} \times (C_c - \Gamma^*)}{(C_c + K_C(1 + O/K_0))} - R_d$

(NOTE: K_C is the Michaelis constant of Rubisco for carbon dioxide; K_0 is the inhibition constant (Michaelis constant) of Rubisco for oxygen; K_{co} is the composite parameter for the Michaelis–Meten constants of RUBP carboxylation and oxygenation, which was assumed to be constant at 807.73 $\mu\text{mol mol}^{-1}$ at 25 °C)

$$\Gamma^* = \frac{0.5 O}{S_{c/o}} \quad \text{and} \quad S_{c/o} = \frac{V_{c,max} K_O}{V_{o,max} K_C}$$

Where S_{co} Rubisco's relative specificity for CO₂ as opposed to O₂ ; and O is O₂ concentration in the media.

The chloroplast CO₂ concentration (C_c) $C_c = C_i - \frac{A_{Vcmax}}{g_m}$

$$g_m = \frac{A_{measured}(\alpha \times \beta \times PAR \times \Phi_{PSII} - 4 \times (A_{measured} + R_d))}{\alpha \times \beta \times PAR \times \Phi_{PSII}(C_i - \Gamma^*) - 4(C_i + 2\Gamma^*)(A_{measured} + R_d)}$$

Rate of net CO₂ assimilation determined by the RUBP limited state (A_{jmax}) (Bellasio, Beerling and Griffiths, 2016)

$$A_{jmax} = \frac{(C_c - \Gamma^*)}{(4C_c + 8\Gamma^*)} \times \frac{\sigma \times PAR + J_{max} - \sqrt{(\sigma \times PAR + J_{max})^2 - 4\theta \times \sigma \times PAR \times J_{max}}}{2\theta} - R_d$$

$$A_j = \frac{J(C_c - \Gamma^*)}{(4C_c + 8\Gamma^*)} - R_d$$

Variables was fitted concurrently to ambient O₂ A/Ci curves after constraining equations with values from Ethier and Livingston (2004)

The processes of photosynthesis and transpiration are computed from the differences in CO₂ and H₂O between in-chamber conditions and pre chamber conditions. LI-COR Bioscience's LI-6400XT photosynthesis gas exchange system is used to non-destructively measure amount of CO₂ flux indicated by NIR absorbed in leaf in the Leaf Chamber Fluorometer (LCF) which indirectly indicates of photosynthesis activity of the plant under steady-state conditions. The instrument also measures physiological, climatic and environmental parameters. The change in the CO₂ concentration of the air flowing across the LCF is indicator of photosynthetic carbon assimilation in leaf by wheat crop. An Infrared Gas Analyzer (IRGA) consists of reference and sample CO₂ chambers such that reference chamber gives the actual concentration of CO₂ in air and sample chamber contains the amount of CO₂ present inside the 2 cm² leaf area enclosed in the chamber. The LCF generates rectangular pulses of actinic light on to leaf area consisting RGB-Red (630nm) for measuring and saturation flashes, Blue (470nm), Far red (740nm) Light Source using Pulse-Amplitude Modulation (PAM) of both dark and light adapted leaf samples.

The mass balance of CO₂ in an open system is given by

$$sa = u_e c_e - u_o c_o$$

c_e and c_o are entering and outgoing mole fractions (mol CO₂ mol air⁻¹) of carbon dioxide

$$sa = u_e c_e - (u_e + sE) c_o$$

$$\text{where } c_e = C_r / 10^6$$

$$c_o = C_s / 10^6$$

$$a = A / 10^6 \text{ Where } C_r \text{ and } C_s \text{ are sample and reference CO}_2 \text{ concentrations (}\mu\text{mol CO}_2 \text{ (mol air)}^{-1}\text{)}$$

$$A = \text{net assimilation rate, } \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$$

$$a = \text{net assimilation rate, mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$$

$$A = \frac{F(C_r - C_s)}{100S} - C_s E$$

$$A = \frac{F(C_r - C_s - C_s (\frac{W_s - W_r}{1000 - W_s}))}{100 S}$$

$$A = \frac{F(C_r - C_s (\frac{1000 - W_r}{1000 - W_s}))}{100 S}$$

where F is air flow rate ($\mu\text{mol s}^{-1}$) and A based on the water mole fractions of the sample (W_s) and reference (W_r) in terms of mmol H₂O per mol of air, as well as leaf area (S) in cm².

Other measured parameters include minimal fluorescence (F_o), fluorescence under saturating light conditions (F_m), fluorescence under non-saturating light conditions (F), maximal fluorescence during a saturating light flash (F_m'), and minimal fluorescence of a light adapted leaf (F_o'), and calculated parameters include $F_v (=F_m - F_o)$, Maximum quantum yield (F_v/F_m), efficiency of PS II (F_v'/F_m'), effective quantum yield (Φ_{PSII}), Photochemical quenching of fluorescence (qP), Non-photochemical quenching of fluorescence (qN), Non-Photochemical Quenching (NPQ) and Electron transport rate (J). Another parameter maximum carboxylation rate ($V_{c \max}$) may be obtained from measured A/Ci curves fitted the FvCB model (K. Yang et al., 2018). Chlorophyll fluorescence parameters of dark-adapted leaves were obtained before dawn (1:00–3:00 h local time), while levels of gas exchange and chlorophyll fluorescence of light-adapted leaves were obtained at midday (11:00–13:00 h local time). The leaves obtained from each site were used to determine assimilation and fluorescence levels, and to ensure that field measurements were coincident with remote sensing observations. Leaf chamber conditions were set constant for all measurements conducted: incident photosynthetic photon flux density (PPFD) on the leaves was set to 1500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, CO₂ levels in the analyzer were held constant at 370 $\mu\text{mol CO}_2 \text{mol}^{-1}$ air. Temperature, air pressure and relative humidity were maintained at ambient levels. Air flow rate through the leaf chamber was maintained at 500 $\mu\text{mol s}^{-1}$ (Zarco-Tejada, Catalina, González, & Martín, 2013).

At Photosystem level, $SIF_{PS}(\lambda) = PAR \times fAPAR \times \Phi_{SIF}(\lambda)$

$$SIF_{total} = \Phi_{SIF} * \beta * \varepsilon * APAR_{green}$$

At Canopy level, $SIF_{obs} = APAR \times \Phi_{SIF} \times f_{esc}$

$$f_{esc} \approx \frac{NIR_v}{fAPAR}$$

$$f_{esc} = \frac{\pi \times SIF_{obs}}{SIF_{total}} \text{ (Z. Liu \& Porcar-castell, 2021)}$$

where f_{esc} is the escape fraction of whole canopy far-red SIF emissions; NIR_v is the product of NIR reflectance and the NDVI ; fAPAR is fraction of absorbed photosynthetically active radiation.

$$fAPAR = \frac{APAR}{PAR_i}$$

APAR calculated as a difference between incoming PAR (PAR_i) and a sum of PAR reflected from the canopy and PAR transmitted through the canopy.

$$fAPAR_{green} = \frac{PAR_i - (PAR_r + (APAR_{EB} + APAR_{CB}))}{PAR_i}$$

$$fAPAR_{chl} = k \times fAPAR_{green}$$

$$fAPAR_{chl} = 0.516 WDRVI + 0.726$$

$$APAR_{chl} = PAR \times fAPAR_{chl}$$

$$Ja = \Phi_{PSII} * \beta * APAR_{green}$$

$$VPD = 0.611 \times e^{\frac{17.27 \times T_a}{T_a + 237}} \times (1 - R_h)$$

$$\frac{Ja}{SIF} \sim \frac{\Phi_{PSII}}{\Phi_{SIF}}$$

In low light and when stress is not persistent, Photochemical Quenching mainly regulates Φ_F . As a result, Φ_F and the photochemistry operating quantum yield in PSII (Φ_P) have an inverse relationship. When radiation increases or stress occurs, the light-saturation of the carbon reactions of photosynthesis occurs, leading to activation of NPQ mechanisms. In these cases, both Φ_P and Φ_F decrease in proportion due to NPQ. (Z. Liu & Porcar-castell, 2021).

NPQ, a parameter that estimates the light-induced photo protection through thermal dissipation of energy

$$SIF = \frac{1 - \Phi_{PSII maz}}{(1 + k_{DF}) \times [(1 + NPQ) \times (1 - \Phi_{PSII maz}) + qL \times \Phi_{PSII maz}]} \times PAR \times \alpha \times \beta$$

$$SIF = \frac{1 - \Phi_{PSII maz}}{(1 + k_{DF}) \times [(1 + NPQ) \times (1 - \Phi_{PSII maz}) + qL \times \Phi_{PSII maz}]} \times PAR \times \varepsilon \times \alpha \times \beta$$

$$\Phi_{SIF} = \frac{1 - \Phi_{PSII maz}}{(1 + k_{DF}) \times [(1 + NPQ) \times (1 - \Phi_{PSII maz}) + qL \times \Phi_{PSII maz}]}$$

Where q_L is fraction of open PSII reaction centres. NPQ is non-photochemical quenching. $\Phi_{PSII\ max}$, $(F_m - F_o)/F_m$ is maximum quantum efficiency of PSII. PAR is the light intensity ($\mu\text{mol PPFD m}^{-2} \text{ s}^{-1}$); The leaf absorptance, designated as α , has a value of 0.84. Meanwhile, β represents the portion of light assigned to PSII equal to 0.5 (Björkman & Demmig (1987); Schreiber, 2004; von Caemmerer, 2000); and k_{DF} is k_D/k_F , with k_D and k_F representing the rate constants of constitutive thermal dissipation and fluorescence, respectively, assuming that k_{DF} is 10 (Pfündel, 1998) (Shi et al., 2022).

The primary source of fluorescence emissions in the red and far-red bands is PSII and PSI, respectively. Under herbicide stress, there is a significant increase in the relative SIF intensities at 688 nm and a decrease in the relative SIF intensities at 760 nm. As a result, the Fr688/760 ratio has proven to be an effective and sensitive method for quickly identifying herbicide damage in winter wheat. (Liangyun Liu, Zhao, & Guan, 2013). It's important to note that the SIF at 760 nm is the result of a combination of two photosystems (I and II), and it can be significantly impacted by water stress (Leizhen Liu et al., 2019).

$$\Phi_{PSII\ maz} = \frac{F_m - F_o}{F_m}$$

$$\Phi_{PSII} = \frac{q_L \Phi_{PSII\ maz}}{(1 + NPQ)(1 - \Phi_{PSII\ maz}) + q_L \Phi_{PSII\ maz}}$$

$$q_L = \frac{F'_m - F_s}{F'_m - F_o} \times \frac{F'_o}{F_s}$$

$$F'_o = \frac{F_o}{\Phi_{PSII\ maz} + \frac{F_o}{F'_m}}$$

$$NPQ = \frac{F_m}{F'_m} - 1$$

Electron transport Rate

$$J_{SIF} = PAR \times \alpha \times \beta - (1 + NPQ) \times SIF \times (1 + k_{DF})$$

$$J_{SIF} = PAR \times \alpha \times \beta - \frac{(1 + NPQ) \times SIF \times (1 + k_{DF})}{\varepsilon}$$

$$J_{SIF} = \frac{q_L \Phi_{PSII\ maz} (1 + k_{DF})}{(1 - \Phi_{PSII\ maz})\varepsilon} \times SIF$$

Or

$$J = \frac{q_L \Phi_{PSII\ maz}}{(1 + NPQ)(1 - \Phi_{PSII\ maz}) + q_L \Phi_{PSII\ maz}} \times PAR \times \alpha \times \beta$$

V_{cmax} , J_{max} , Γ^* and R_d were fitted from the response curves of A_n/C_i measured with Li-6400-XT by using methods of fitting A– C_i curves to estimate photosynthetic parameters of the FvCB model have been used (Ethier & Livingston, 2004).

Using model of (Farquhar, von Caemmerer, & Berry, 1980b), net CO_2 assimilation rate of a leaf is given as :

$$A_n = \min[A_{Vcmax}, A_j]$$

The rate of net CO_2 assimilation determined by the Rubisco limited state (A_{Vcmax})

$$A_{Vcmax} = \frac{V_{cmax} \times (C_c - \Gamma^*)}{(C_c + K_{CO})} - R_d$$

In the Rubisco limited phase, the response of net assimilation A to C_c is described using the FvCB (Farquhar et al., 1980) equation defined by $A_{Vcmax} = \frac{V_{cmax} \times (C_c - \Gamma^*)}{(C_c + K_C(1 + O/K_O))} - R_d$

Where V_{cmax} is the maximum velocity of Rubisco for carboxylation; R_d is Day respiration rate; K_C is the Michaelis constant of Rubisco for carbon dioxide; K_O is the inhibition constant (usually taken to be the Michaelis constant) of Rubisco for oxygen. Γ^* is the chloroplastic CO_2 photo compensation point; K_{co} is the composite parameter for the Michaelis–Metten constants of RUBP carboxylation and oxygenation, which was assumed to be constant at $807.73 \mu\text{mol mol}^{-1}$ at 25°C (Moualeu-Ngangue et al., 2017)

$$\Gamma^* = \frac{0.5 O}{S_{c/o}}$$

$$S_{c/o} = \frac{V_{c,max}K_O}{V_{o,max}K_C}$$

Where S_{co} Rubisco's relative specificity for CO_2 as opposed to O_2 ; and O is O_2 concentration in the media.

The chloroplast CO_2 concentration (C_c)

$$C_c = C_i - \frac{A_{Vcmax}}{g_m}$$

$$g_m = \frac{A_{measured}(\alpha \times \beta \times PAR \times \Phi_{PSII} - 4 \times (A_{measured} + R_d))}{\alpha \times \beta \times PAR \times \Phi_{PSII}(C_i - \Gamma^*) - 4(C_i + 2\Gamma^*)(A_{measured} + R_d)}$$

Rate of net CO_2 assimilation determined by the RUBP limited state (A_{jmax}) (Bellasio, Beerling, & Griffiths, 2016)

$$A_{jmax} = \frac{(C_c - \Gamma^*)}{(4C_c + 8 \times \Gamma^*)} \times \frac{\sigma \times PAR + J_{max} - \sqrt{(\sigma \times PAR + J_{max})^2 - 4\theta \times \sigma \times PAR \times J_{max}}}{2\theta} - R_d$$

$$A_j = \frac{J(C_c - \Gamma^*)}{(4C_c + 8\Gamma^*)} - R_d$$

Rate of net CO₂ assimilation simulated (A_{SIF})

$$A_{SIF} = \frac{(C_c - \Gamma^*)}{(4C_c + 8 \times \Gamma^*)} \times \{ \alpha \times \beta \times PAR - (1 + NPQ) \times SIF \times (1 + k_{DF}) \} - R_d$$

Where σ is set to 0.3 (Long et al., 1993); θ is fixed at 0.9 (Medlyn et al., 2002)

Results

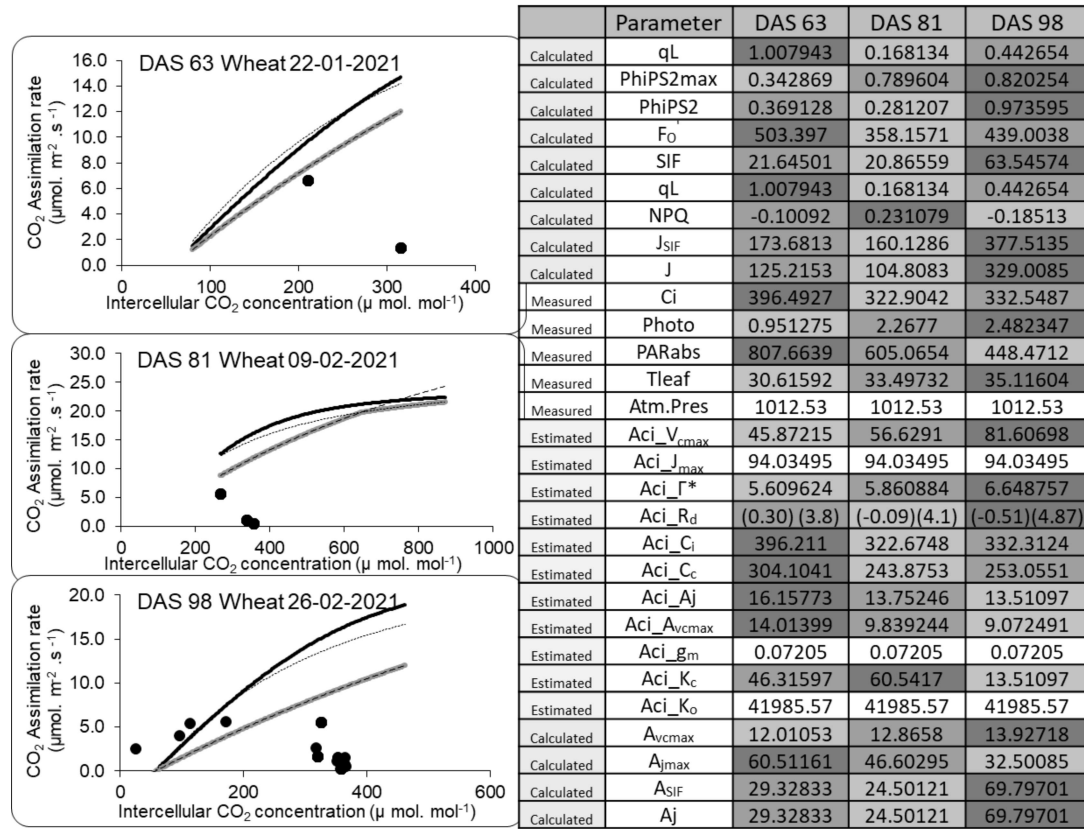
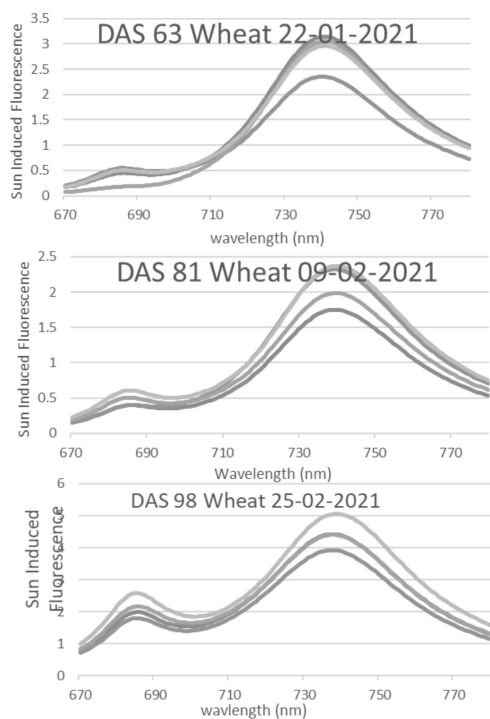


Figure 3: Retrieved Fluorescence Spectrum



Sun Induced Fluorescence Spectrum
From spectroscopic measurements

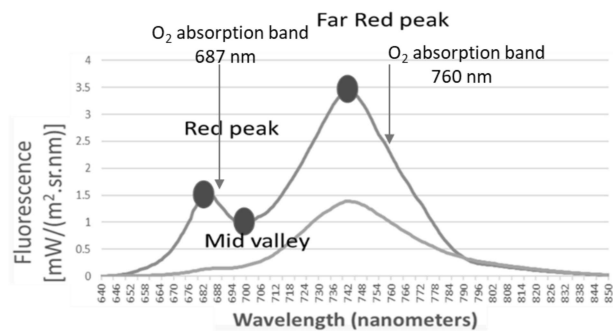
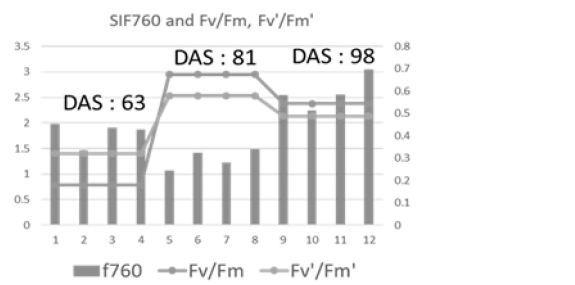
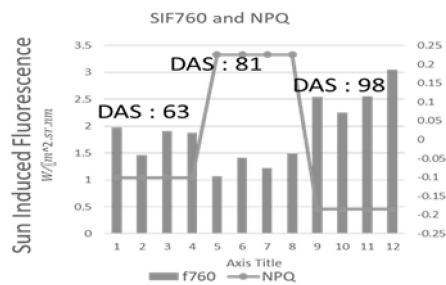
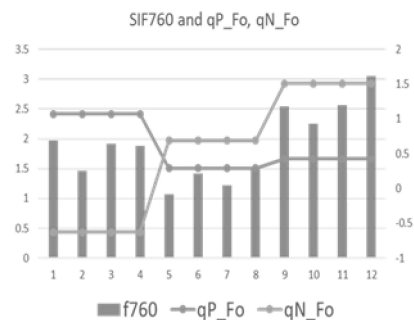


Figure 4: Fluorescence variation with respect to photosynthetic responses



Photosynthesis parameters associated with plant stress	
$F_v/F_m = (F_m - F_o)/F_m$	It is determined in dark adapted leaves and it is a measure of the maximum efficiency of PSII when all centers are open. This ratio is a sensitive indicator of plant photosynthetic performance
F_v'/F_m'	It describes energy dissipation. It is an estimate of the PSII quantum efficiency if all PSII reaction centers are in the open state.
$NPQ = (F_m - F_m')/F_m'$	NPQ is linearly related to heat dissipation and varies on a scale from 0 until infinity even if in a typical plants value ranges between 0.5 and 3.5 at light saturation level.
$qP_Fo = (F_m' - F_s)/(F_m' - F_o)$	Photochemical quenching includes photosynthesis and photorespiration, and tends to be greatest in low light, since that's where leaves use light most efficiently
$qN_Fo = (F_m - F_m')/(F_m - F_o)$	Non Photochemical quenching of fluorescence that includes mechanisms such as heat dissipation. qN is highest when light intensities are high, perhaps reflecting a plant protection mechanism to avoid over-energization of the thylakoid membrane.



It is clear that carboxylation capacity (V_{cmax}), chloroplastic CO_2 photo compensation point (Γ^*) increased with growth stage. The net CO_2 assimilation determined by the RUBP (A_j) is more affected by mild water stress unlike response of net assimilation in Rubisco limited phase (A_{vcmax}). SIF obtained from calculation of parameters show decrease during mild water stress.

Conclusion

The ground experiment convey that during mild to moderate water stress in wheat crop at vegetative stage (81 DAS) the F_v'/F_m' & F_v/F_m showed increase with high dissipation of heat (NPQ). Under mild to moderate water stress and low and moderate light intensity, on DAS:81, the F_v'/F_m' , F_v/F_m showed increase and higher heat dissipation (NPQ). This also reported with lower SIF760 values. With onset of water shortage, the increase in Φ_{PSII} was caused by an increase in F_v'/F_m' and by a decrease in indicated increased photorespiration since photosynthesis was hardly affected by water heterogeneity of Φ_{PSII} . The increased F_v'/F_m' , F_v/F_m reflects increased quantum efficiency (Φ_{PSII}) can be seen as an acclimation process to avoid an over- excitation of PSII under more severe drought conditions. The Non-photochemical quenching parameter (q_N or q_N_{Fo}) increased from DAS 63 to DAS 98 indicating senescence and non photochemical pigments composition increase over time. The ground experiments and modelling over multiple crops under different stress level will help to generate signal for SIF for low to moderate and moderate to high stress. These study will also promise the better usage of the FLEX data over Indian agriculture

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