Using violet laser-induced chlorophyll fluorescence emission spectra for crop yield assessment of cowpea (*Vigna unguiculata (L) Walp*) varieties

INSTITUTE OF PHYSICS PUBLISHING MEASUREMENT SCIENCE AND TECHNOLOGY
Meas. Sci. Technol. **15** (2004) 1255–1265 PII: S0957-0233(04)66417-4

Using violet laser-induced chlorophyll fluorescence emission spectra for crop yield assessment of cowpea (*Vigna unguiculata (L) Walp*) varieties

Benjamin Anderson[1], **Paul K Buah-Bassuah**[1] **and Jonathan P Tetteh**[2]

[1] Laser and Fibre Optics Centre, Department of Physics, University of Cape Coast, Cape Coast, Ghana
[2] Department of Crop Science, School of Agriculture, University of Cape Coast, Cape Coast, Ghana

E-mail: lafoc@ghana.com

Received 18 July 2003, in final form 7 January 2004
Published 28 May 2004
Online at stacks.iop.org/MST/15/1255
DOI: 10.1088/0957-0233/15/7/005

Abstract
The use of violet laser-induced chlorophyll fluorescence (LICF) emission spectra to monitor the growth of five varieties of cowpea in the University of Cape Coast Botanical Garden is presented. Radiation from a continuous-wave violet laser diode emitting at 396 nm through a fibre is closely incident on *in vivo* leaves of cowpea to excite chlorophyll fluorescence, which is detected by an integrated spectrometer with CCD readout. The chlorophyll fluorescence spectra with peaks at 683 and 731 nm were used for growth monitoring of the cowpea plants over three weeks and analysed using Gaussian spectral functions with curve fitted parameters to determine the peak positions, area under the spectral curve and the intensity ratio F683/F731. The variation in the intensity ratio of the chlorophyll bands showed sensitive changes indicating the photosynthetic activity of the cowpea varieties. A discussion of the fluorescence result as compared to conventional assessment is presented with regard to discrimination between the cowpea varieties in terms of crop yield performance.

Keywords: chlorophyll fluorescence spectra, violet laser diode spectrometer, cowpea varieties yield

1. Introduction

Mutation breeding of plants by ionizing radiation and chemical mutagens to increase genetic variability is a long known scientific procedure. The application of gamma rays and x-rays as radiation on plant material such as seeds, pollen, whole plants, buds, callus tissue from culture is found to be the preferred method. Such plant breeding methods tend to improve crop yield, disease resistance, food quality, drought resistance and adaptability of the plant. The problems arise when a large number of such plants are to be screened by individuals in terms of performance with regard to the above-stated parameters.

Normally, the screening of such plant varieties relies mostly on observing the plants at maturity, or harvest time, when information is retrieved on the size, number and weight of the seeds. However, the situation can be improved when the growth process of such plants is monitored in order to obtain certain parameters to predict the crop yield before harvest time, to aid in the selection of the best variety. It has been observed in recent times that ultraviolet light-induced chlorophyll fluorescence is a good method for plant

monitoring in agricultural and plant sciences applications [1]. Based on the ratio of chlorophyll fluorescence emission spectra intensities at 683 and 731 nm, F683/F731, and other significant intensity ratios, it has been established this technique can discriminate between normal and stress conditions in vegetation [2–5]. From these measurements, it has also been observed that an increase of the ratio F690/F740 is caused by a lower chlorophyll content and/or decline in photosynthesis [6].

Recently, laser-induced chlorophyll fluorescence detection of plants using a pulsed laser emitting at 355 nm as an excitation source has been used to monitor the growth process of two samples of lettuce till harvest time. The system, which is bulky and expensive for field measurement, makes use of a pulse-gated high speed detector system and an intensified CCD detector for suppression of ambient light [7]. The results from this set-up using fluorescence peak ratio intensity to predict the growth process seem very innovative and promising. However, the use of a steady-state system using a continuous-wave violet laser diode emitting at 396 nm and with a compact integrated spectrometer with CCD read out has simplified the field measurements [8]. The violet diode laser system (VDLS) is less expensive, portable, easy to operate and convenient for spectral assessment. This system (VDLS) yields spectral resolution of about 5 nm which is good enough for such environmental recordings. In the present work, the use of the VDLS to excite intact leaves chlorophyll fluorescence from five varieties of cowpea (*Vigna unguiculata (L) Walp*) during their growing process is reported. The analysis of the fluorescence spectra using peakfit software was performed to distinguish among these cowpea varieties with regard to their crop yield, and was compared with conventional yield assessment methods.

The essence and relevance of such investigations tend to address two major issues. Firstly, in Ghana most of the cowpea varieties grown by the local farmers are prostrate, photosensitive and late maturing. With the frequent dry spells being experienced in Ghana, late maturing varieties are unable to utilize the low moisture levels for higher grain yield. To solve these problems pertaining to the low yields from cowpeas, the Crop Research Institute (CRI), Ghana Grains Development Project (GGDP), Agricultural Science Institutions and other sectors of the Ministry of Food and Agriculture, have been working very hard to come out with new cowpea varieties that tolerate or would be resistant to the factors contributing to the low yield of cowpeas. Currently, cowpea varieties named 'Ayiyi' and 'Bengpla' have been released by CRI and recommended by GGDP. The School of Agriculture, University of Cape Coast has also developed some new lines (UCC-Early and V12-W) which are being evaluated for release. The International Institute of Tropical Agriculture (IITA), Ibadan, has also introduced some new lines into the country to be tested for release.

Secondly, various problems pertaining to their adaptability and cultural practices required for optimum utilization of the growing season for good harvest in comparison with the agronomic performances of five varieties of cowpea in the Cape Coast district, are being investigated by applying violet laser-induced chlorophyll fluorescence and conventional methods to predict crop yield before harvest time.

2. Materials and methods

2.1. Cowpea (Vigna unguiculata (L) Walp)

The cowpea is a legume and has a considerable variation in shape, size, colour and texture. The usual colour of seed coat ranges from white, red, brown, purple and variously mottled to black [9]. In Ghana, for example, wide arrays of legumes such as cowpea, soybean, pigeon pea, groundnut and bambara groundnut are produced. The cowpea, which is popularly known as bean, is preferred on account of its short life cycle, fodder use and quality.

The cowpea is normally grown from dry seeds, which are either planted in rows or scattered. It tolerates soil conditions from sandy soils to heavy expandable clays for growth but prefers loamy soils for optimum seed yield. The cowpea grows in slightly acidic to neutral soils but is not tolerant of alkaline soils. The crop does not tolerate water logging, but thrives best under drier conditions [10]. In the coastal savanna vegetation zones, planting of cowpeas is done in April–May to mature in August and, for the minor season, September–October to mature in January. Cowpeas are warm-weather plants that can withstand considerable drought and moderate amount of shade but are injured by slight frost [11].

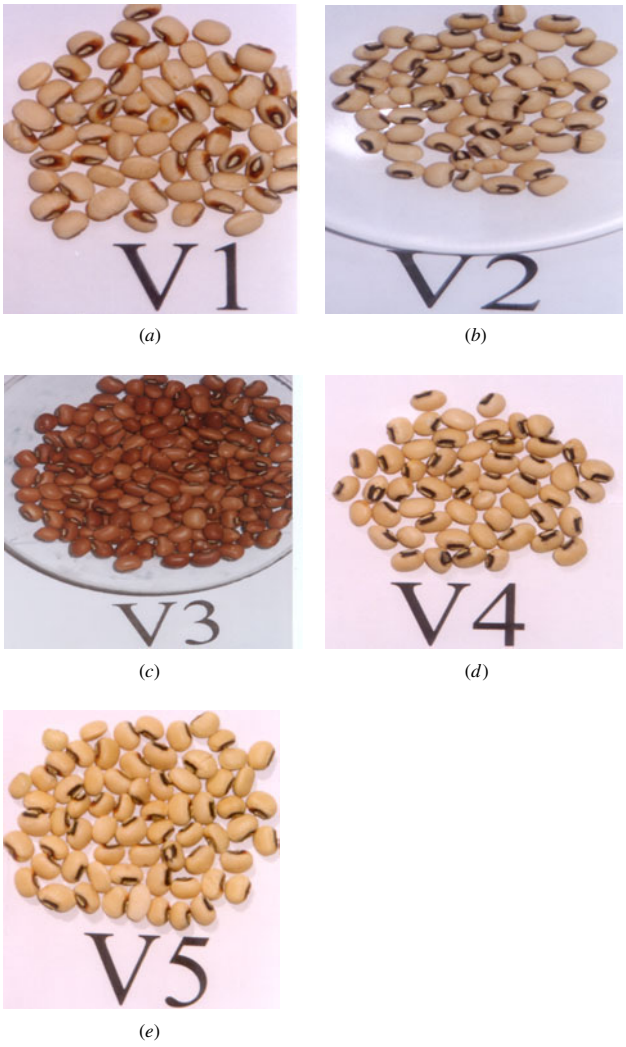
Apart from the groundnut, the cowpea is the second most important legume in the tropics followed by the bambara groundnut. [12]. The crop is rich in protein, iron, starch, calcium, phosphorus and vitamin B, which makes it excellent food when eaten even in small amounts [13]. The cowpea has the ability to fix atmospheric nitrogen into soil in symbiosis with the rhizobium bacteria species [14].

Field experiments already performed reveal that regarding nitrogen fixation in tropical grain legumes, the cowpea has high nitrogen-fixation ability, which ranges from 70–350 kg ha^{-1} of nitrogen [15, 16].

The main components of seed yield in cowpea are positively correlated with the number of pods per plant, the number of seeds per pod, seed size (100 or 1000 seed wt). Characters like days to flowering, days to maturity, pod length, peduncle length, which are secondary characters, are also found to have an effect on seed yield.

2.2. Plant growth

Five varieties of cowpea, namely 'Ayiyi' (V1) and Bengpla' (V4) (from the Crop Research Institute (Kumasi, Ghana)) 'V12-W' (V2) and 'UCC-Early' (V3) (from University of Cape Coast (Cape Coast, Ghana)) and 'IT87D-611-3' (V5) (from the International Institute for Tropical Agriculture (IITA) (Ibadan, Nigeria)) were grown in the Botanical Garden of the Department of Botany, Faculty of Science, University of Cape Coast, Ghana during the 2001 rainy season (May–July). All the varieties used for this investigation (V1, V2, V4 and V5) have white seed coat except V3 which has light brown seed coat. In addition, the varieties V2, V4 and V5 have black eyes whiles V1 and V3 have brown eyes as distinguishing features and are shown in figure 1. The seed sizes of these varieties which are measured in g/1000 seeds at 12% moisture content are 181.1, 129.0, 126.5 and 133.3 for V1, V2, V3, V4 and V5 respectively. Seed size is an important yield component in cowpea production for conventional method. All the varieties



(*a*) (*b*) (*c*) (*d*) (*e*)

Figure 1. Pictures of cowpea (*Vigna unguiculata (L) Walp*) varieties used for the investigation and assessment in the Botanical Garden of the University of Cape Coast, Ghana. V1 is 'Ayiyi,' V2 is 'V12-W', V3 is 'UCC Early', V4 is 'Bengpla' and V5 is 'IT87D-611–3'. The distinguishing features of the varieties such as the coat colour and the eye are depicted in the picture in each variety.

(This figure is in colour only in the electronic version)

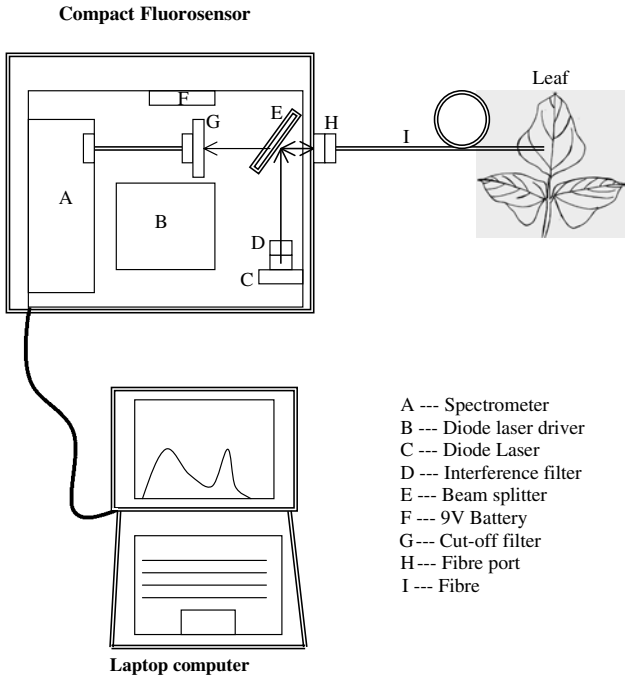


Figure 2. Experimental set-up for the detection of chlorophyll fluorescence from *in vivo* leaf using the violet diode laser fluorosensor.

are early maturing (they mature within 65 to 75 days after sowing), and have semi-erect growth habit.

For uniformity, seeds were sown in 15 l buckets that were filled with equal amounts of sandy loam soil to about 6 cm from the top. The soil was collected from a fallow site at the University of Cape Coast farm. The chemical properties of the soil were as follows: pH = 6.4, nitrogen = 0.09%, organic carbon = 0.6%, phosphorus = 63.16 ppm, potassium = 0.54 cmol kg and calcium = 3.72 cmol kg. The properties of this soil indicated that it was relatively fertile to support cowpea production without any fertilizer application on local farmers' field [29].

In this investigation, 50 plants were used for the measurements. In each of the 25 pots were grown 2 plants. Each variety was given 5 pots and thus 10 plants were obtained finally for each variety. Four seeds were sown per pot and later thinned to two plants per pot after germination. Germination began 3 days after sowing and by the sixth day, germination was completed, for all the five cowpea varieties. The 25 pots were divided into five groups, with each group containing each of the five varieties. Watering was done once a week to supplement moisture deficit. They were placed on flat ground, equally spaced and in an open space for uniform sunlight.

In the sixth week, the measurements of the laser-induced fluorescence were taken on each plant in each pot. Measurements were taken on the middle leaflet of the fourth trifoliate leaf from the shoot apex of the main stem as shown in figure 2 in the sixth, seventh and eighth weeks after sowing. Growth and yield parameters such as leaf area, shoot dry weight, peduncles per plant, pods per plant, seeds per plant and seed dry weight were also taken after the eighth week, and were used to compare with the readings of the spectra.

2.3. Violet laser-induced chlorophyll fluorescence system and measurements

Figure 2 shows the experimental arrangement for the detection of the chlorophyll fluorescence from the *in vivo* plant leaves. The system (VDLS) was developed and assembled with the assistance of [8]. The violet diode laser emitting at 396 nm at an output power of 3 mW (Nichia NLHV500) was placed in a tube that has a lens (Geltech C230TM-A) for collimation of the laser output radiation. The output beam is 'cleaned up' for broadband spontaneous emission using a narrowband interference filter (CVI F25-400-4-0.5) and is focused by a fibre-port lens assembly (Optics for Research PAF-SMA-6-NUV-Z) into a 600 μm core diameter fused silica step index multimode optical fibre via a dichroic beam splitter (CVI) placed in front of the spectrometer (Ocean Optics S2000). This USB2000 miniature fibre optics spectrometer directly

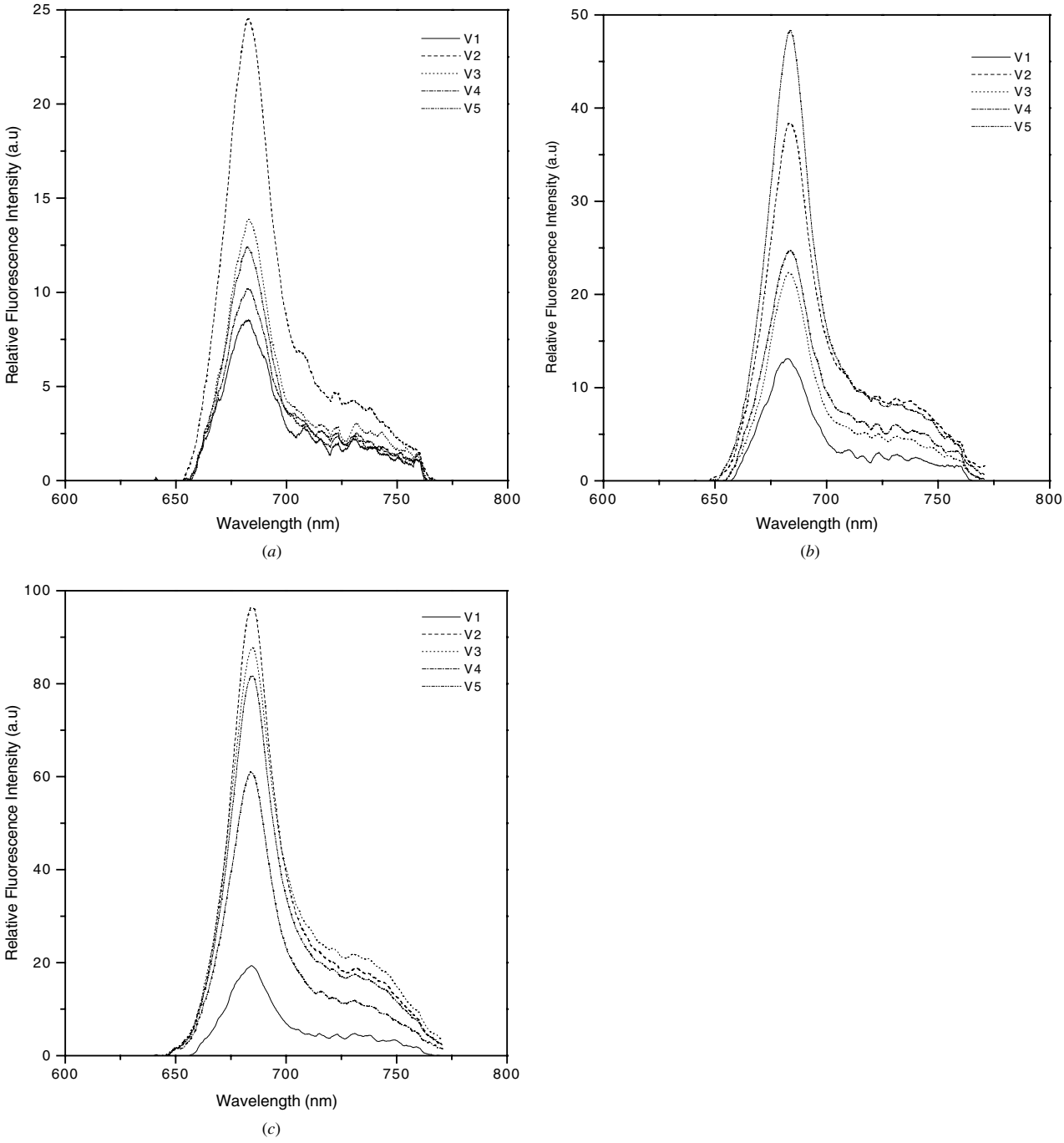


Figure 3. Fluorescence spectra for the five crop varieties of cowpea with label code (V1, V2, V3, V4 and V5) taken in the three measuring weeks. (*a*) Sixth measuring week; (*b*) seventh measuring week; (*c*) eighth measuring week; V1—solid line, V2—dashed line, V3—dotted line, V4—dashed-dotted line, V5—dashed-double dotted line.

plugs into the USB port of a laptop PC, from which it draws its power, thus eliminating any A/D interface. Another 1 m, 600 μm fused silica core step index multimode optical fibre with 660 μm cladding diameter of Tefzel jacket was coupled to the output of the laser beam by a SMA connector fixed on the side of the VDLS.

The output end of the fibre was supported by a fibre holder and kept in contact with the upper leaf surface while the lower side of the leaf was sheltered with an aluminium plate for background cover to avoid collection of ambient light coming through the back of the leaf. The use of a fibre holder shades off the laser light intensity incident on the leaf from the ambient light since the irradiated area was selected by the fibre diameter. The use of the single optical fibre facilitates the measurement of fluorescence from leaves attached to the various cowpea plants, enabling quick sampling on different cowpea varieties.

In order to obtain a flat intensity response over the full spectrum, using a linear CCD array detector that is responsive from 200–1100 nm and integrated with the spectrometer, a calibration was performed using a 200 W calibrated quartz tungsten/halogen lamp (Oriel 63355) with a known spectral

profile. The reference spectrum was recorded through the optical fibre.

Since the same optical fibre is used to transmit light to the sample under investigation and also to collect the excited light for detection, fluorescence from the fibre itself constitutes a background that needs to be corrected. This was accomplished by sending the light to the aluminium plate without the sample to obtain a background spectrum and then subtracting from the spectra collected from samples.

The laser light induced on the leaf under investigation comes from the violet diode laser of 1.2 mW output power. The chlorophyll fluorescence emission is Stokes-shifted with regard to the excitation wavelength and is returned through the same fibre after passing through the spectrometer which is equipped with a slit and a grating of 600 lines/mm. However, the elastically backscattered diode laser light is effectively blocked by a Schott GG420 coloured glass filter placed behind the dichroic beam splitter. The grating in the spectrometer disperses the light and a spectral region of about 330–1000 nm is captured on the 2048 element linear CCD array detector. A spectral resolution of about 5 nm is reached with the integration time of the CCD being set to 1 s. Fluorescence in the 600–800 nm spectral range was recorded from the samples after allowing 3 s for reaching steady state of fluorescence. All the measurements were conducted at a temperature of 29 °C on all the other varieties of the cowpea in the field. The sensitivity of the system was checked at weekly intervals, for the sample (leaves), using a standard fluorescent material (quinine sulphate) of known concentration and wavelength, which serves as calibration for the system to maintain reproducible recordings.

3. Results and analysis

The results of the measured chlorophyll fluorescence intensity as a function of the wavelength for the five varieties of cowpea during the sixth, seventh and eighth weeks after sowing are shown in figures 3(*a*), (*b*) and (*c*), respectively. Each spectrum is the average of 10 leaves of different plants of the same variety.

When the *t*-test was considered on each raw fluorescence spectra for the evaluation of standard errors for the peak centre and peak amplitude at 95% confidence levels, it was found that the determination of the peaks was acceptable with minimum standard error but the determination of the band area became easier with limited standard error when a Gaussian model fit was made on them, thus smoothing and averaging the randomness on the profile of the curve as well as getting two separate fit curves for area ratio evaluation. In addition, other parameters such as full wave half maximum (FWHM) and coefficient of correlation (r^2) values were determined.

Chlorophyll a in green vegetation is effectively excited in the blue spectral region yielding a dual-band spectral profile with peaks at about 683 nm and 731 nm [1].

3.1. Curve and curve assessment fitting of fluorescence spectra of cowpea varieties.

The PeakFit (4.11 version, Jandel Scientific, Germany) software was used to smooth the LICF spectra with

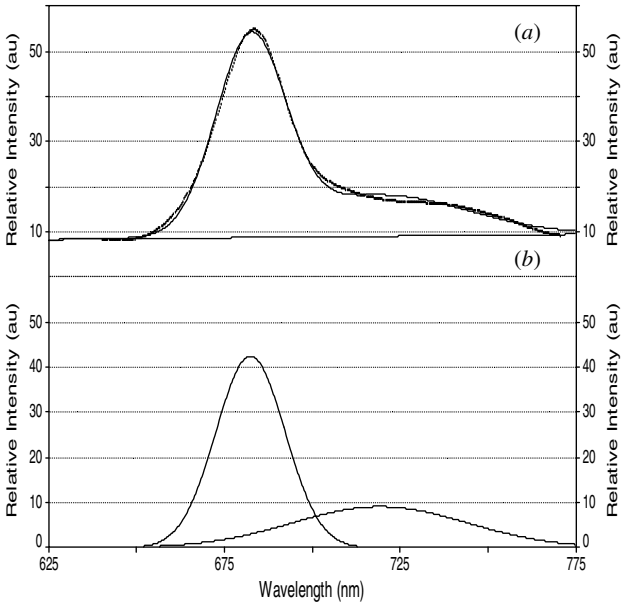


Figure 4. The raw chlorophyll fluorescence spectra for the cowpea variety V1 which is fitted with a Gaussian spectral curve of the sixth measuring week. The solid line is for the line shape of the raw experimental spectra chlorophyll fluorescence data. (*b*) The solid line is the deconvoluted Gaussian curve fit for the red and the far-red bands; chlorophyll fluorescence signal for cowpea variety label code V1 in the seventh measuring week.

Loess function. The software uses a Marquardt–Levenberg algorithm for iterative non-linear curve fitting with a combination of Gaussian spectral functions to analyse the spectra. It finds the true absolute minimum value of the sum of squared deviations (the value of χ^2) by the iterative process. The value of the coefficient of correlation (r^2) and the residual pattern determine the quality of fit [18, 19].

The Gaussian spectral function, which was simpler and gave a reasonable matching fit of the spectral data with the determination of good *t*-test, standard errors for peak amplitude, peak centre and FWHM were chosen for further analysis of the spectra.

Typical Gaussian bands resulting from the curve fit analysis of the measured spectra for a variety V1 of the cowpea plant is shown in figure 4. The constituent bands were found to centre around 683 nm and 731 nm. The choice of the Gaussian bands was found to be an accurate fit of the measured spectra with good r^2 values (0.99 ± 0.01). The curve fitted parameters such as peak centre, peak amplitude, full width at half intensity maximum (FWHM) and the area under each Gaussian curve for the plants were measured as shown in table 1.

The original raw spectral curve (LICF spectra) and the fitted LICF spectra were then compared as raw and treated data sets, respectively, using the *t*-test with a significant level of 5%. There was an observation of zero difference between the group means of the two data and that the *p* value ranged from 0.53 to 0.85 with a *t*-test value within the range −0.53–0.63 as shown in table 1, indicating no significant difference between the original and fitted curves in both amplitude and area. The standard error deviations in table 1 did not differ on particular plants with different week measurements but indicated variation due to sampling of LICF from leaves

Table 1. Results of curve fitting on the LICF spectra of five cowpea varieties. Parameters such as the spectral peak intensity, peak centre, Gaussian curve area (cm^2), full wave half maximum (FWHM) and correlation coefficient (r^2) for the five varieties of cowpea for the three measuring weeks (week 6, week 7, week 8). PI_R and PI_{FR} indicate red peak intensity and far-red peak intensity, respectively. The errors of measurements at *t*-test values have been indicated.

		Peak intensity (au)	Peak centre (nm)	Guassian area (cm^2)	FWHM (nm)	Fit standard error	r^2 value	*t* value[a]	*p* value[a]
V1-WK 6	$P1_R$	7.96 ± 0.14	681.75 ± 0.13	250.11 ± 2.50	29.52 ± 0.30	0.15	0.99	−0.31	0.76
	$P1_{FR}$	2.24 ± 0.13	730.27 ± 0.98	124.82 ± 1.25	54.66 ± 0.55				
V1-WK 7	$P2_R$	11.74 ± 0.17	682.42 ± 0.08	340.3 ± 3.40	27.23 ± 0.27	0.15	0.99	−0.32	0.63
	$P2_{FR}$	3.06 ± 0.16	727.59 ± 0.93	191.45 ± 1.91	61.88 ± 0.62				
V1-WK 8	$P3_R$	16.85 ± 0.18	683.53 ± 0.09	510.81 ± 5.11	28.49 ± 0.28	0.16	0.99	−0.30	0.81
	$P3_{FR}$	4.10 ± 0.16	730.04 ± 0.34	240.82 ± 2.41	52.18 ± 0.52				
V2-WK 6	$P1_R$	21.97 ± 0.14	682.64 ± 0.12	684.25 ± 6.84	29.27 ± 0.29	0.19	0.99	0.19	0.85
	$P1_{FR}$	4.85 ± 0.13	731.82 ± 0.67	260.05 ± 2.60	52.52 ± 0.53				
V2-WK 7	$P2_R$	38.61 ± 0.15	683.53 ± 0.10	1032.65 ± 10.33	24.93 ± 0.25	0.21	0.98	0.20	0.84
	$P2_{FR}$	7.33 ± 0.21	730.93 ± 0.61	349.41 ± 3.49	46.29 ± 0.46				
V2-WK 8	$P3_R$	92.12 ± 0.15	684.64 ± 0.08	2593.84 ± 25.94	22.70 ± 0.23	0.20	0.99	0.57	0.58
	$P3_{FR}$	13.94 ± 0.30	729.82 ± 0.35	763.16 ± 7.63	49.41 ± 0.49				
V3-WK 6	$P1_R$	13.09 ± 0.20	683.09 ± 0.10	353.53 ± 3.54	25.37 ± 0.25	0.19	0.98	0.63	0.53
	$P1_{FR}$	3.09 ± 0.06	733.83 ± 0.37	162.02 ± 1.62	51.63 ± 0.52				
V3-WK 7	$P2_R$	21.06 ± 0.36	683.31 ± 0.11	584.81 ± 5.85	24.48 ± 0.24	0.20	0.98	0.53	0.60
	$P2_{FR}$	4.55 ± 0.29	732.05 ± 0.62	258.82 ± 2.59	56.53 ± 0.57				
V3-WK 8	$P3_R$	66.18 ± 0.17	683.98 ± 0.11	1890.82 ± 18.91	24.48 ± 0.24	0.21	0.980	0.53	0.6
	$P3_{FR}$	9.33 ± 0.26	732.27 ± 0.40	419.17 ± 4.19	40.50 ± 0.41				
V4-WK 6	$P1_R$	9.39 ± 0.18	682.42 ± 0.12	296.4 ± 2.96	26.80 ± 0.27	0.13	1.00	−0.53	0.60
	$P1_{FR}$	2.24 ± 0.14	728.93 ± 0.37	134.48 ± 1.34	56.53 ± 0.57				
V4-WK 7	$P2_R$	22.12 ± 0.14	683.31 ± 0.10	628.81 ± 6.29	26.71 ± 0.27	0.15	0.99	−0.62	0.55
	$P2_{FR}$	5.45 ± 0.28	730.28 ± 0.42	335.49 ± 3.35	64.54 ± 0.65				
V4-WK 8	$P3_R$	55.27 ± 0.19	683.75 ± 0.09	1713.06 ± 17.13	25.820 ± 0.26	0.16	0.99	−0.57	0.58
	$P3_{FR}$	9.82 ± 0.45	733.16 ± 0.46	469.78 ± 4.70	41.39 ± 0.41				
V5-WK 6	$P1_R$	11.74 ± 0.14	682.42 ± 0.05	358.75 ± 3.59	25.88 ± 0.26	0.20	0.98	0.22	0.83
	$P1_{FR}$	2.58 ± 0.16	732.94 ± 0.26	146.48 ± 1.46	53.86 ± 0.54				
V5-WK 7	$P2_R$	44.24 ± 0.19	683.53 ± 0.08	1328.32 ± 13.28	25.37 ± 0.25	0.15	0.99	0.20	0.84
	$P2_{FR}$	8.30 ± 0.29	734.94 ± 0.43	313.68 ± 3.14	40.50 ± 0.41				
V5-WK 8	$P3_R$	80.73 ± 0.52	684.42 ± 0.11	2659.17 ± 26.59	27.60 ± 0.28	0.21	0.98	0.22	0.83
	$P3_{FR}$	12.72 ± 0.56	732.72 ± 0.59	867.42 ± 8.67	43.18 ± 0.43				

[a] Significant level $\alpha = 0.05$ and $t_{\alpha/2} = 1.96$.

belonging to plants of different cowpea varieties. This is evident with the Gaussian area at week 8, where the confidence intervals are broader with fixed peak centre and amplitude of the chlorophyll bands. However, the r^2 values ranged between 0.98 and 1.00.

From table 1, the shifts of red peak and far-red peaks as the weeks advanced were ±2 nm and ±3 nm respectively. The FWHM values that show the light intensity band of the spectra from the leaves depict some consistency in the signal from the measurement as the weeks progressed with a small variation of ±3 nm.

3.2. Growth monitoring process with LICF and conventional assessment method

The red and far-red fluorescence maxima observed in the emission spectra have been identified as due to chlorophyll [20, 21]. Moreover, the relative intensity of the fluorescence peaks at 683 nm and 731 nm gives information on the chlorophyll pigment [22, 23] of the cowpea plant and is related to plant growth with regards to photosynthesis. Chlorophyll a in green vegetation is effectively excited in the blue and red spectral regions yielding a dual-band spectral profile with peak centres around 683 nm and 731 nm. From [7], the first peak is situated at a wavelength where the chlorophyll pigments still absorb light. This means that when the chlorophyll content in a leaf increases the first peak cannot rise at the same rate as the second one. Thus, the intensity ratio is related to the chlorophyll content and can be used for chlorophyll concentration assessment [24]. The fluorescence ratios, that is, red fluorescence (RF)/far-red fluorescence (FRF) calculated from the peak intensities (PIR) and Gaussian area (AR) of the Gaussian curves, are shown in table 2 along with their average ratios, that is, the average peak intensity ratio (APIR) and average area ratio (AAR) for the three measuring weeks (week 6, week 7, week 8).

The PIR changes are attributed to the response of the chlorophyll pigments and are shown in figure 5(*a*). Regarding such Gaussian fits, it was observed that for a constant spectral shape in terms of its FWHM as deduced from table 1, the areas of integrated Gaussians are proportional to their heights such that the intensity of the chlorophyll fluorescence could be deduced using the area under the two Gaussian bands of the spectra. Thus, the ratio of the peaks and that of the areas give additional information on the variability of the growth pattern for each cowpea variety.

The relative intensity of the fluorescence and the Gaussian integrated area derived from the fitted spectra shows variation

Table 2. The LICF ratio data; fluorescence peak intensity ratio (PIR), average fluorescence peak intensity ratio (APIR), Gaussian curve area ratios (AR) and average Gaussian curve area ratios (AAR) and the agronomic performance parameters (APP); number of seeds per plant (NS), number of peduncles per plant (NP), weight of seeds per plant (WS) and leaf area (LA) obtained using the conventional agricultural methods, are presented with their standard errors.

		Cowpea varieties				
		V1	V2	V3	V4	V5
Chlorophyll fluorescence ratios						
	WK 6	3.55 ± 0.27	4.53 ± 0.15	4.24 ± 0.15	4.19 ± 0.34	4.55 ± 0.34
Peak intensity ratio (PIR)	WK 7	3.84 ± 0.26	5.27 ± 0.17	4.63 ± 0.37	4.06 ± 0.23	5.33 ± 0.21
	WK 8	4.11 ± 0.20	6.61 ± 0.15	7.09 ± 0.22	5.63 ± 0.28	6.35 ± 0.32
Average PIR (APIR)		3.81 ± 0.24	5.54 ± 0.16	5.32 ± 0.25	4.64 ± 0.28	5.42 ± 0.29
	WK 6	2.00 ± 0.04	2.63 ± 0.05	2.18 ± 0.04	2.20 ± 0.04	2.45 ± 0.05
Area ratio (AR)	WK 7	1.78 ± 0.04	2.96 ± 0.06	2.26 ± 0.05	1.87 ± 0.04	4.23 ± 0.08
	WK 8	2.12 ± 0.04	3.40 ± 0.07	4.51 ± 0.09	3.65 ± 0.07	3.06 ± 0.06
Average AR (AAR)		1.96 ± 0.12	3.00 ± 0.18	2.98 ± 0.18	2.57 ± 0.15	3.30 ± 0.20
Agronomic performance parameters (APP)						
Leaf area (LA)/mm^2		44.8 ± 0.45	45.80 ± 0.46	45.20 ± 0.45	58.60 ± 0.59	47.00 ± 0.47
No of peduncles (NP)/plant		13.80 ± 0.14	11.60 ± 0.12	13.60 ± 0.14	4.60 ± 0.05	13.40 ± 0.13
No of seeds (NS)/plant		63.60 ± 0.64	61.80 ± 0.62	63.00 ± 0.63	34.00 ± 0.34	50.00 ± 0.50
Weight of seeds (WS)/g		12.10 ± 0.12	8.60 ± 0.09	8.80 ± 0.09	4.60 ± 0.05	6.60 ± 0.07

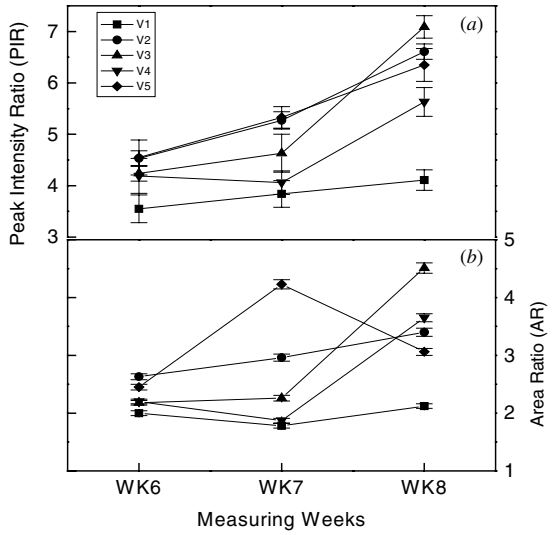


Figure 5. Variation of the five cowpea varieties for the three measuring weeks (week 6, week 7, week 8). V1–V5 are the label codes of the cowpea varieties; ▲ shows the growth pattern of variety V1 as the leaves advanced in age; ▼ shows the growth variation of V2; ♦ shows the growth pattern of V3; ● shows the growth pattern of V4; ■ indicates the growth pattern of V5. (*a*) Peak intensity ratio (PIR) variation for the cowpea varieties plotted against the measuring weeks. PIR increases as the plants advance in age. (*b*) A plot of area ratio (AR) for the cowpea varieties versus the measuring weeks. The AR gradually increases with varieties V1, V2, V3 and V4 except V5 that sharply reduces after the seventh week.

in the plant variety from V1 to V5 as shown in table 1. As the plant aged from week 6 to week 8, the PIR and AR as well as APIR and AAR as shown in table 2 clearly depict the differences among the varieties of cowpea. The data on AR indicated that the chlorophyll fluorescence of V1 was relatively lower than the rest, followed by V4 while V2, V3 and V5 have almost the same values. Figure 5(*b*) shows the graphical weekly representation of the AR of the measuring weeks versus the crop varieties. The behaviour of V5 at the eighth week looked quite odd because it is the variety which was introduced from International Institute of Tropical Agriculture, Nigeria to a new environment, Ghana. It, however, reduced in area at the eighth week close to maturity. Though V4 was attacked by insects, the pattern of the area and the peak intensity increased towards the maturity period as the spectra depicted.

The LICF results which are based on the assessment of photosynthetic capacity of each variety can thus enable the plant breeder to use such results as indicators in identifying and selecting individual plants at an early stage of growth from large population size. The growth and yield parameters from the conventional method for this study were leaf area (LA), number of seeds (NS), weight of seeds (WS) and number of peduncles per plant (NP). These parameters were measured and tabulated as part of table 2. From table 2, V4 has the largest LA value of 58.0 cm^2 whilst V1 had the least value of 44.8 cm^2 but the differences were significant at $p = 0.05$.

It was again observed that the highest number of peduncles per plant, seeds per plant and seed dry weight per plant was observed for V1 while V4 recorded the lowest values for those traits, an observation that may be assigned to the insect pest damage.

The conventional assessment method, which is also based on the assessment of photosynthetic capacity, is indicative of the biological yield potential at the later stages of growth (harvest time). Both LICF spectra and the conventional method preclude other agronomic parameters such as adaptability, disease resistance and consumer acceptability which are also important indicators and parameters for plant variety selection.

Figure 6 shows bar graphs of vegetative and economic yield parameters using the conventional method in relation to the LICF spectra deductions. The APIR as well as the AAR for each plant variety increases gradually from V1 to V2 and V3 and then decreases for V4 and rises again V5. Such variation confirms various growth processes dictated by the chlorophyll

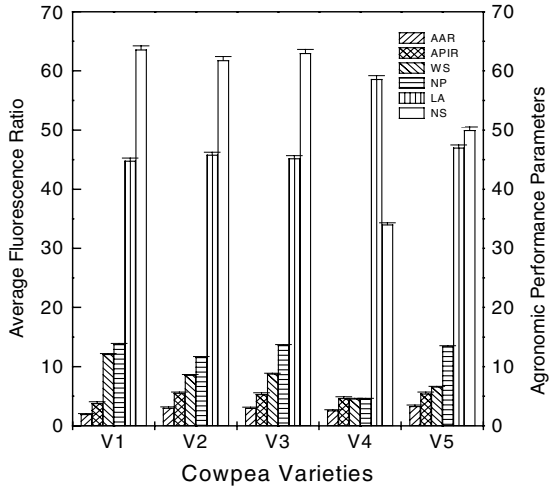


Figure 6. Bar graph of both conventional methods for the agronomic performance parameters (APP) and laser-induced chlorophyll fluorescence LICF for the fluorescence intensity ratios plotted against each cowpea variety with label code (V1, V2, V3, V4 and V5). Error bars are shown on each of the bar graphs. APP were WS, NP, LA and NS while LICF were APIR and AAR as the other parameters for assessing plant growth.

fluorescence. The conventional method has LA and NP as the vegetative yield parameters while WS and NS represent grain or economic yield for the agronomic performance parameters (APP). V1 gave more seeds followed by V2 and V3 which are varieties bred in Cape Coast. This observation showed the same relation for NP as well as WS. The standard errors of the data are shown by the error bars on the plot. It could be observed from the graph that the results from both the conventional assessment method and LICF methods are well comparable.

These observations indicate that APIR and AAR are useful parameters to discriminate among different crop varieties and consequently predict the crop yield of the cowpea varieties.

4. Discussion

For all the parameters for the varieties as deduced from tables 1 and 2, there were changes in the relative chlorophyll fluorescence intensity values and a noticeable shift in the peak and area positions especially in the far-red bands. The chlorophyll inside the palisade tissue at the upper surface of the leaf absorbs the violet laser beam and the chlorophyll subsequently emits fluorescence in the red and the far-red region. From the inverse relationship that exists between observable chlorophyll fluorescence intensities and photosynthetic efficiency [25], it can be deduced that the chlorophyll fluorescence intensities from the red band showed high photosynthetic efficiency by showing the least photosynthetic efficiency in sixth week of measurement.

The utilization of the Gaussian bands was found to give a very good fit of the chlorophyll fluorescence spectra as clearly shown from the r^2 values reported in table 1 as well as from the residual pattern (not shown) and the prediction and confidence intervals of 95% for the fit. From a statistical point of view, the parameters of the Gaussian curves carried all the information contained in the spectra. As the weeks advanced from week 6 and week 7 to week 8 the chlorophyll fluorescence intensity ratio increased tremendously for varieties V2, V3, V4, V5 as compared to V1 as shown in figure 5(*a*).

These observations, however, changed in the seventh week for V2, V3 and V4. V1 maintained its photosynthetic efficiency with low chlorophyll fluorescence intensity. The chlorophyll fluorescence spectra, for the seventh and eighth weeks, showed that the spectra were not influenced by re-absorption. Each chlorophyll fluorescence spectrum showed the spectral differences in its internal features with regards to biochemical or morphological differences in the cowpea varieties.

From the PIR and AR, these cowpea varieties in general showed very low chlorophyll fluorescence in the sixth week because of their low values. The chlorophyll fluorescence emission spectra for the seventh week exhibited a higher intensity than in the sixth week in each of the varieties. This may be because the plants lose their chlorophyll content as the weeks advance, giving high fluorescence spectra in the red and far-red bands, thus increasing their peak ratios and area ratios. The PIRs, which are largely affected by the chlorophyll content, were not constant with the plant development [26]. Thus a contribution due to differences in the amount of chlorophyll pigment as the weeks advanced cannot be overruled, though the chlorophyll concentration was not directly measured. But then intensity or concentration of the chlorophyll can be deduced from the area under the Gaussian spectral curve. On the other hand, when the PIR becomes larger with decreasing chlorophyll amount, the red band is partially re-absorbed by the absorption band of the chlorophyll as they overlap.

According to Subhash [27], when photosynthesis declines *in vivo* leaves fluorescence intensity ratio of the chlorophyll bands increases, this in a sense suggests that the increase in the PIR was due to the decline in photosynthetic activity of the leaves and that the leaves began their senescence around the sixth week.

Each laser-induced chlorophyll spectrum changed its wavelength peak corresponding to each variety, especially in the far-red band. The shift in the peak wavelength of about 3 nm, with a decrease in wavelength, and the increase in the peak intensity ratio confirm the fact that there was a decline in the chlorophyll content and possibly in the photosynthetic efficiency due to chlorophyll pigment breakdown. But this was not the case for V4, which showed an appreciable increase in the peak wavelength of about 2 nm and a decrease in peak intensity ratio suggesting that there was substantial increase in the chlorophyll content and/or photosynthetic efficiency during the seventh week of measurement.

The general increase in the peak intensities as well as the peak intensity ratios of V2, V3 and V4, with V3 being exceptionally high, in the eighth week of measurements suggests that there was total chlorophyll pigment breakdown giving very low chlorophyll content. This sharp increase suggests that the varieties have high rate of chlorophyll degradation and therefore high photosynthesis declination rate.

It is reasonable to consider that the origin of the laser-induced chlorophyll fluorescence spectra was the organic constituents and specifically the genetic make-up of leaves. To confirm this assertion, the spectra bands of red and far-red were considered in terms of peak intensity wavelength.

Figure 5(*a*) demonstrates the relationship between the laser-induced chlorophyll fluorescence spectra of peak intensity ratio versus the crop varieties. In the eighth week, all the varieties generally showed an increase in their peak intensity wavelengths in the red band, which may be attributed to the family characteristics of the cowpea plants, but V1 gave a gradual linear unique characteristic in the spectra. The peak intensity ratio was exceptionally very low as compared to the other varieties. The increases in the chlorophyll fluorescence as the plants grew, were due to chlorophyll pigment decomposition that indicated a decrease in the photosynthetic efficiency. The low and relatively constant increase in the chlorophyll fluorescence intensity as observed in the variety V1 makes it more photosynthetically efficient than the other varieties. This means that for V1, a greater quantity of the incident light energy was actually used photochemically and only a small quantity was given off as fluorescence.

These results suggest that chlorophyll fluorescence peak intensity and area ratio might be used to predict, before harvest time, the variety that can give higher performance values and a better yield. The area ratio deduced from the spectral curve of the red and far-red bands gave the peak intensity of chlorophyll fluorescence emitted from each variety. From figures 5(*a*) and (*b*), it appears that the fluorescence spectral change for each cowpea variety is an early indicator to understand the growth process as well as the possible mechanism of the photosynthetic apparatus.

On the basis of leaf area per plant alone (table 2 and figure 6), one would expect V4 to out perform all other varieties, since the higher the leaf area, the greater the capacity to intercept light for photosynthesis. This was not the case due to the high incidence of insect pest damage on this variety. Nevertheless, higher leaf area alone is not adequate to predict the yields of a variety. It needs to be combined with higher photosynthetic efficiency per unit leaf area in order to achieve higher yields.

The peduncles are the growth structures that bear flowers which later develop into pods. The number of peduncles observed for the various varieties followed the same pattern; V1 having more peduncles than the others while V4 gave the least number of peduncles. Plants with high numbers of peduncles therefore have the potential of producing more flowers and pods than those with fewer numbers of peduncles. Not all the pods on the plant produce seeds. Again the number of seeds produced by a plant depends on the availability to assimilate or the photosynthetic capacity of the plant. Plants with high photosynthetic capacity tend to produce more seeds per pod, and give higher seed yield. It implies therefore that V1, which gave the highest number of seeds per plant and seed dry weight per plant, also has the highest photosynthetic capacity among the other varieties. The seeds of V1 were found to be significantly larger than the rest ($p < 0.01$) of the varieties. It was able to synthesize sufficient assimilate to produce and retain more seeds that grow to maturity than did the other varieties. It also implies that even though V1 had the lowest leaf area per plant, the few leaves present were more efficient in their photosynthetic ability resulting in a higher net yield in assimilate than the other varieties.

From these observations in relation to the agronomic performance parameters (APP), the fluorescence spectra analysis of APIR and AAR is found to be reliable in such a prediction process using the peaks and areas under the Gaussian spectral bands. This prediction has been tested on the reliability of data by considering the *t*-test of such spectra as well as the evaluation of the standard error of the fitted spectral curve. The chlorophyll fluorescence peak and area ratios F683/731 are characteristic of the cowpea plant showing differences in the spectra as the leaf aged from week 6 to week 8. Since there are marked differences among the cowpea varieties in the course of the plant growth, one tends to notice that the peaks and areas under the spectral curves are related and give some indication on the chlorophyll concentration as the leaf ages. This is an important indication to use to assess the photosynthetic activity of the plant at the early stages of growth.

Figure 6 therefore compares the chlorophyll fluorescence in the photosynthetic activity in both methods (LICF and APP). From the bar graphs in LICF and APP, the results obtained, however, show much correlation and agreement of growth pattern as well as crop yield prediction. While LICF does not give quantity of seed weight, other parameters such as APP do, but one sees it as an advantage for the early prediction without any variance in results between the two methods.

With regards to figure 5 the PIR and AR graphs indicate that as the leaf grows up to week 6 differences among the varieties are minimal until week 7, where observations show that greater differences in chlorophyll production in the photosynthetic activity among the cowpea variety growth give much confidence in the prediction at the early stages to distinguish good crop yields among the cowpea varieties. From these two graphs (figures 5 and 6), the results of these methods depicted that variety V1 gave a high yield in the environment while V2 and V3 have similar yield capabilities. The effect of insect attack on V4 suppressed the variety from giving its maximum yield while V5 did not perform well in the new environment. V5 is the variety introduced from Nigeria, IITA.

Such a comparison shows that early prediction of agronomic performance of cowpea varieties with LICF is possible. It can be observed that it would not be necessary to wait to harvest time before knowing the best variety. Thus the plant growth based on both vegetative and grain yield could be predicted well using the peak intensity ratios as well as the area ratio derived from the data of LICF.

5. Conclusion

The laser-induced chlorophyll fluorescence (LICF) spectra of five cowpea varieties have been examined with a portable continuous violet diode laser fluorosensor. The growth pattern of the varieties relates to the spectra of the chlorophyll fluorescence intensity peak and area ratios of red to far-red bands for three week measurements before harvest time. The ratio of the two chlorophyll peaks and areas (683/731) showed that both the vegetative and grain yields of the plants can be deduced from the chlorophyll fluorescence.

It was found that the peaks determined from the raw data spectra with the curve fit and the curve fitted spectra did not deviate from the mean using the *t*-test statistic. However, for sensitivity the Gaussian curve fitted areas were reliable as the

paired data of raw and curve fitted *t*-test resulted within a range −0.53–0.63 showing some spectral data of the variation of the confidence intervals which become broader with fixed peak centre and amplitude of the chlorophyll bands in different weeks. The standard error of these data appears minimal.

In addition, the curve fitting of LICF spectra using an iterative process by linear combination of Gaussian spectral functions gave curve fitted parameters that enabled further deduction of indicators for early stage prediction. This was achieved by the deconvolution of the red and far-red bands of LICF spectra by curve fitting and results did not change the original data but helped to differentiate the effects of chlorophyll in each variety as the cowpea aged with respect to the use of both peaks and areas to follow the fluorescence spectral changes of the growth pattern. In this way the use of raw data alone per se, for the analysis, can only give the spectral peak position and ratio without deviations but excludes the use of other parameters for further analysis of the area under the curve.

The measurements focused on the use of intensity variation of the red–far-red fluorescence ratio which indicated the variation of chlorophyll fluorescence content involved in the growth process of the cowpea varieties. The study has shown that changes in the ratio of red fluorescence to far-red fluorescence for peak positions and Gaussian curve areas give a clear picture for monitoring the growing process of the mutants by observing the chlorophyll fluorescence spectra of each variety and a means of selecting the variety with better crop yield. It thus confirms the result of the use of a second harmonic Nd:YAG laser for observing plant growth processes up to harvest time [7], which has been equally achieved qualitatively with a less expensive and portable continuous violet diode laser in the field.

However, this method gives a dynamic signal, related to the higher reaction to photosynthesis, and helps to infer some practical application for monitoring the growth process of the plants. The LICF results of PIR and AR of the crop varieties showed some covariation to conventional method parameters such as NP and NS as well as the leaf area (LA) values. As a consequence, the screening of such plant varieties with LICF parameters for crop yield assessment seems feasible. The relative fluorescence ratio that indicates assessment of crop yield could be deduced from week 6 and week 7 before maturity and harvest time with such a method [3, 7]. The LICF method could be used to monitor plant growth and estimate green leaf area index that also gives an insight into vegetation yield [28].

It also shows that the prediction of the LICF method applied at close range to the leaves gives possibilities to assess qualitatively the growth process of the different varieties and subsequent screening of the cowpea varieties. It is therefore an effective tool that can be used to screen various varieties of particular species during the plant breeding process. In the study, it was found out that V1 was the variety that gave a higher yield and thus a better contributed mutant which has been found elsewhere to have high consumer preference [29]. It is being suggested that the use of the LICF method for such non-destructive measurement and screening of mutants has a great potential for qualitative crop yield detection before harvest time. It was also found in this study that the cowpea varieties were friendly and adaptable in the environment where growth process was done. The LICF method does not extend the study to the prediction of disease-resistant varieties. The results of LICF and APP compare well and tend to make the LICF method an important screening apparatus for plant breeders.

Acknowledgments

The authors acknowledge Sune Svanberg and Sara Palsson of Lund Institute of Technology for their assistance in directing us to assemble the violet laser diode fluorosensor under the IPPS (Uppsala University) project for Africa in Lund, Sweden. They also acknowledge the collaboration of Lennart Hasselgren, the Head of the IPPS programme in Sweden for the financial support. The Laser and Fibre Optics Centre (LAFOC) is grateful to the International Centre for Theoretical Physics, ICTP, Office of External Activities Division for financially supporting the activities and training of graduate students and research at LAFOC. We are also grateful to Third World Academy of Sciences (TWAS), Trieste for assisting LAFOC with a research grant on this project. We are most grateful to Dr B K Gordor, Head of Mathematics and Statistics Department of University of Cape Coast for his invaluable assistance.

References

[1] Cerovic Z G, Samson G, Morales F, Tremblay N and Moya I 1999 Ultraviolet-induced fluorescence for plant monitoring: present state and prospects *Agronomie* **19** 543–78

[2] Chappelle E W, Wood F M Jr, McMurtrey J E III and Newcomb W 1984 Laser-induced fluorescence of green plants: I. A technique for the remote detection of plant stress and species differentiation *Appl. Opt.* **23** 134–8

[3] Saito Y, Kanoh M, Hatake K-I, Kawahara T D and Nomura A 1998 Investigation of laser-induced fluorescence of several natural leaves of application to lidar vegetation monitoring *Appl. Opt.* **37** 431–7

[4] Subhash N and Mohanan C N 1995 Remote detection of nutrient stress in groundnut plants by deconvolution of laser-induced fluorescence spectra *Proc. Int. Geoscience and Remote Sensing Symp. (Firenze)* vol 3 p 2323–5

[5] Lichtenthaler H K 1990 Applications of chlorophyll fluorescence in stress physiology and remote sensing *Applications of Remote Sensing in Agriculture* ed M Steven and J A Clark (London: Butterworths Scientific) pp 287–305

[6] Karlsson T 1992 Laser-induced fluorescence of intact plants, LRAP-130 Lund pp 1–28

[7] Takeuchi A, Saito Y, Kanoh M, Kawahara T D, Nomura A, Ishizawa H, Matsuzawa T and Komatsu K 2002 Laser-induced fluorescence detection of plant and optimal harvest time of agricultural products (lettuce) *Appl. Eng. Agric.* **18** 361–6

[8] Gustafsson U, Palsson S and Svanberg S 2000 Compact fiber optic fluorosensor using a continuous-wave violet diode laser and an integrated spectrometer *Rev. Sci. Instrum.* **71** 3004–6

[9] Cobley L S and Stele W M 1976 The legumes *Introduction to the Botany of Tropical Crops* (London: Longmans Green) pp 91–6

[10] Asamoa G K 1973 Soils of the proposed farm sites of the University of Cape Coast *Soil Research Institute Technical Report* 68

[11] Wolfe T K and Kipps M S 1959 *Production of Field Crops* 5th edn (New York: McGraw-Hill) pp 371–5
[12] Aryeetey A N 1971 Increasing cowpea production in Ghana *The Ghana Farmer* **15** 51–5
[13] FAO (Food and Agricultural Organization of the United Nations) 1988 Traditional Food Plant, FAO, Rome *FAO Food and Nutrition Paper* 42
[14] Purseglove J W 1968 *Tropical Crops. Dicotyledons* vol 1 (London: Longman) p 332
[15] Ayanaba A 1979 Biological nitrogen fixation in Africa *Proc. GIAM-V (Bankok)* pp 45–51
[16] Doku E V 1970 Variability in local exotic varieties of cowpea (*Vigna unquiculata (L) Walp*) in Ghana *Ghana J. Agric. Sci.* **3** 139–43
[17] Peak fit (4.11 version) 1997 *Handbook and Users Manual* (Germany: Jandel Scientific) pp 2–11, 8–1
[18] Marquardt D W 1963 An algorithm for least-squares estimation of non-linear parameters *J. Soc. Indust. Appl. Math.* **11** 431–41
[19] Subhash H, Agati G, Fusi F, Mazzinghi P and Lercadi B 1993 Significance of curve fit analysis of laser induced fluorescence in vegetation remote sensing *Proc. Lasers'93 Conference (Nevada)* pp 113 –7
[20] Krause G H and Weiss E 1991 Chlorophyll fluorescence and photosynthesis: the basics *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **42** 313 –49
[21] Broglia M 1993 Blue-green laser-induced fluorescence from intact leaves; activities light sensitivity and sub cellular origins *Appl. Opt.* **32** 334–8
[22] Lichtenthaler H K (ed) 1988 *Application of Chlorophyll Fluorescence* (Dordrecht: Kluwer)
[23] Lichtenthaler H K, Buschmann C, Rinderle U and Schnuck G 1986 Application of chlorophyll fluorescence in ecophysiology *Radiat. Environ. Biophys.* **25** 297–308
[24] Lichtenthaler H K and Rinderle V 1988 The role of chlorophyll fluorescence in the detection of stress conditions in plants *CRC Crit. Rev. Anal. Chem.* **19** (Suppl 1) 29–85
[25] Rosema A, Snell J F H, Zahn H, Buurmeijer W F and Van Hove L W A 1998 The relation between laser-induced chlorophyll fluorescence and photosynthesis *Remote Sens. Environ.* **65** 143–54
[26] Zaro-Tejada P T, Miller J R, Mohammed G H and Noland T L 2000 Chlorophyll fluorescence effects on vegetation apparent reflectance: 1. Leaf-level measurement and model simulation *Remote Sens. Environ.* **74** 582–95
[27] Subhash N 1994 Detection of vegetation stress from laser-induced fluorescence signatures *LAMP Seminar, ICTP (Trieste)* pp 1–13
[28] Ford Denison R and Russotti R 1997 Field estimates of green leaf area index using laser-induced chlorophyll fluorescence *Field Crops Res.* **51** 231–40
[29] Afful B 2001 Evaluation of the agronomic performance of eight cowpea (*Vigna unguiculata (L) Walp*) varieties in the Cape Coast District *BSc Agric. Thesis* University of Cape Coast, pp 1–94