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Monitoring the Development of Cotton (*Gossypium barbadense* L.) using Emission Spectra of Chlorophyll Fluorescence

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Abstract: This work was proposed to monitor the growth and development of cotton (*Gossypium barbadense L.*) under different environmental conditions using emission spectra of chlorophyll a fluorescence from the intact leaves. The emitted fluorescence signal by cotton plants was measured using spectrometer/graphic and data analysis software. Cotton was grown in the summer season under sunlight exposure i.e. summer conditions, whereas in winter conditions the plants were grown in a shaded area. Both groups of plants were irrigated by tap water. Winter conditions exhibit environmental stress of temperature and light intensity. Monochromatic blue light of 450 nm was used as an excitation source to induce the chlorophyll fluorescence emission at 685 nm and 733 nm. The peak intensity ratio (P.I.R.), which is the ratio between maximum emitted light intensities at 685 nm and 733 nm respectively (I_F685/I_F733), and the area ratio (A.R.) which is the ratio between the areas under the curves of the two emission lines were used for monitoring the development of cotton, from the germination until the flowering. The results revealed that the summer conditions gave a faster seed germination rate and the best growth and development of cotton, while the stressing winter conditions expressed in delayed seed germination, poor growth that led to failure of flowering and immaturity. The peak intensity ratio and area ratio for the summer and winter conditions were found to follow linear relationships. The line slopes of the peak intensity ratio and area ratios were 1.36 and 0.46 and the intercepts were 0.04 and 0.04 respectively.

Keywords: Cotton; Summer and winter conditions; Fluorescence; Chlorophyll a; I_F685/I_F733.

Introduction

Monitoring of plant development by spectroscopic detection of electromagnetic radiation is a powerful, noncontact and nondestructive method. Plant tissues absorb the energy of the electromagnetic radiation in the visible region by the photosynthetic pigments (chlorophyll a, b, and carotenoids). This energy is used for the photosynthetic processes [1, 2]. Chlorophyll a (*Chl* a) molecules are arranged into two groups of pigments known as photosystem I (PSI) and photosystem II (PSII). Each photosystem has antennae chlorophyll molecules embedding a reaction center (RC). When an antennae chlorophyll molecule absorbs photons, it transfers this energy to another nearby one until reaching the reaction center chlorophyll molecules [2, 3]. Part of the absorbed energy is lost during the migration from the pigment antennae to the reaction centers and can be dissipated by a variety of non-photochemical

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processes. Such processes include the dissipation of heat and emission of small but diagnostically significant amount of the absorbed radiation. This emission which occurs in the red and far-red regions is termed as chlorophyll fluorescence i.e. I_F685 and I_F733 respectively. This fluorescence signal (I_F), is therefore, determined by the rate of constants of these competing reactions and by the fraction of open reaction center as only those which can contribute to the photochemical de-excitation. Any exciting light (laser or laser emitting diode) capable of inducing Chlorophyll a fluorescence can be used for plant development monitoring in agricultural and plant science applications.

The shape of the fluorescence emission spectrum of leaves depends on the wavelength of the excitation light [4] and the environmental conditions of the measurements. Incident ultra violet or blue light is absorbed by carotenoids and by the chlorophyll of the chloroplast already at the upper part of the leaf mesophyll i.e. palisade and spongy tissues which are responsible of the photosynthetic activity in plants. The major part of the blue excited chlorophyll has to cover a short distance before it finally leaves the leaf at the epidermis and the chlorophyll fluorescence is only slightly reabsorbed by *in situ* chlorophylls. However, in the case of red light, which is only absorbed by Chlorophyll a, a substantial part of the excitation light penetrates deeper into the leaf mesophyll. This will generate more reabsorption of the red light chlorophyll fluorescence [5]. It has been observed in recent studies [6, 7] that Chlorophyll a fluorescence obtained by ultra violet, violet and blue light excitation sources is a good method for plant monitoring. The absolute emission signal of leaves can vary from sample to sample due to leaves' heterogeneity [8, 9] and other small differences such as the excitation and sensing angles of the fluorescence, and the roughness and scattering properties of the leaf surface. Thus, the absolute fluorescence I_F685/ or I_F733 usually varies to a large degree than the fluorescence ratio I_F 685/ I_F 733 [1]. The fluorescence ratio, therefore, represents a more accurate tool for measuring the different changes in quantities of the fluorescence characteristics of leaves.

In the present work, the use of the blue or laser emitting diode (LED) to excite intact leaves Chlorophyll a from cotton crop during its development is reported. The analysis of the fluorescence spectra using peak intensity ratio and area ratio of I_F 685 and I_F 733 from the Gaussian curves fitting were performed to distinguish between cotton growing in summer conditions with equal exposure to sunlight, and cotton growing in winter in a shaded area. This technique has been established to discriminate between normal and stressing conditions in vegetation [10, 11]. The importance of such investigations is that, cotton is an important economical crop in the Sudan; a good portion of cooking oil is extracted from its seeds, and its flowers are used in weaving, textile and for various medical purposes.

Materials and method

Cotton is a warm weather fiber plant, which is grown normally from dry seeds, that is planted either in rows or scattered. Seeds are known to grow in different types of soils (sand or clay) but for optimum seed yield, heavy clay soils are preferred. Crops grow better under regular watering, but they don't suffer much otherwise.

Cotton seeds, brought from Gezira Scheme, were divided into two groups; one was grown in the summer conditions, directly exposed to sunlight, while the other was grown in the winter conditions, in a shaded place. Seasonality in this study is based on the sowing time of cotton seeds. Generally, agricultural activities in the Sudan are performed in two rotations which begin during late summer and early winter. In the Gezira Scheme, Sudan, sowing of cotton begins in June-July to mature in December. It should be noted that germination is considered as the most important stage in the life of cotton plants. The experiments were carried out at the Botanical Garden of the Faculty of Science, Al Neelain University, Khartoum, Sudan (altitude: $15^{\circ}\,29'$ and $15^{\circ}\,37'$ *N* and longitude: $32^{\circ}\,33'$ and $32^{\circ}\,34'$ *E*). Temperature in Khartoum has a very wide range, between 46°-20° C in summer and 40°-7° C in winter. The maximum temperature is reached in May-June and the minimum in January. The light intensity, measured as average sunshine/day, varies

between 8.1-9.6 hrs/day in summer season and between 9.7-10.3 hrs/day in winter. The area received 194 mm during 2006 in the form of erratic and inconsistent rains that occurred during late summer (July-September) [12]. The first seed group was grown during the 2006 rainy season (June), flowered in September and became fully matured in December of the same year. The second group was grown during December, 2006 (winter conditions). However, the plants of this group showed a poor growth rate and did not mature or flower.

Seeds were grown in eight pots that were filled with heavy clay soil to about 10 cm from the top. The average diameter of the pots is about 26 cm. The soil was collected from the bank of the Blue Nile near Khartoum. Sixteen plants were used for the measurements, two in each of the eight pots. Eight plants were grown and measured during the summer season. They were placed in four pots in an open space for equal exposure to sunlight. The other eight plants were placed in a shaded place and were similarly grown and measured during the winter season. To ensure successful germination, four seeds were put in each pot and after the seedling emergence; they were thinned to leave the two best plants. The pots in the summer and winter conditions were irrigated daily with tap water till the end of the experiments.

For the summer group, germination began five days after sowing and by the twelfth day, it was completed. But for the winter group, germination began four weeks after sowing due to low temperatures and was completed by the fifth week. The measurements of the fluorescence for the summer group started at the beginning of the third week after sowing, and are taken from different leaves on each plant in each pot. For the winter group, the measurements started at the beginning of the fifth week after sowing due to the slow rate of development of the plants. The measurements were taken in the same way as for the summer group i.e. from different points of the lower most and the upper most fully developed leaves and were then averaged.

A laser emitting diode (LED) emitting at 450 nm wavelength and output power of 60 μW was used as an excitation source. A compact software controlled spectrometer (USB2000/Origin 6.1, Ocean Optics/Origin Lab, Dunedin, USA/Northampton, USA) was used for recording the fluorescence signal emitted by the plants' intact leaf. The resolution of the spectrometer was 1.34 nm FWHM [13], and its detector covers the wavelength range from 350-1100 nm. The whole setup was coupled to a laptop computer for mobile use, to record field measurements. Recorded data were analyzed using MICROCAL ORIGIN 6.1 computer program. The software uses an algorithm curve fitting with a combination of Gaussian spectral functions to analyze the spectra.

Results and analysis:

The results of the measured chlorophyll a fluorescence intensity as a function of the wavelength for the summer group of cotton during the third to the eighth week after sowing are shown in Fig. 1A and Table 1 (A and B). For the winter group, the results were recorded during the fifth to the eighth weeks after sowing are shown in Fig. 1B and Table 2 (A and B). Each spectrum is the average of seven days, and each day spectrum is the average of 10 different leaves obtained from different plants of the same group.

The Gaussian fitting was performed as shown in Fig. 2, where the smoothing and the averaging of the randomness on the profile of the curve were done in addition to decomposing the peak into two overlapping peaks for area ratio evaluation. In addition, the full width at half maximum (FWHM), denoted as $\Delta\lambda$, was determined as shown in Tables 1 and 2.

The Chlorophyll a fluorescence spectra for the summer season cotton of the fifth measuring week with Gaussian curve fitting for I_F685 and I_F733 bands are shown in Fig. 2. The constituent bands were found to center around 685 nm and 732 nm. The evaluation of the standard errors for the wavelength at maximum peak (λ_{max}), fluorescence intensity peak amplitude (I_F) and the band area (A) indicated that the determination of the peaks is acceptable with minimum standard error. The parameters obtained for the two sets of plants, during the period of monitoring the development are listed in Table 1 for the summer conditions and in Table 2 for the winter conditions.

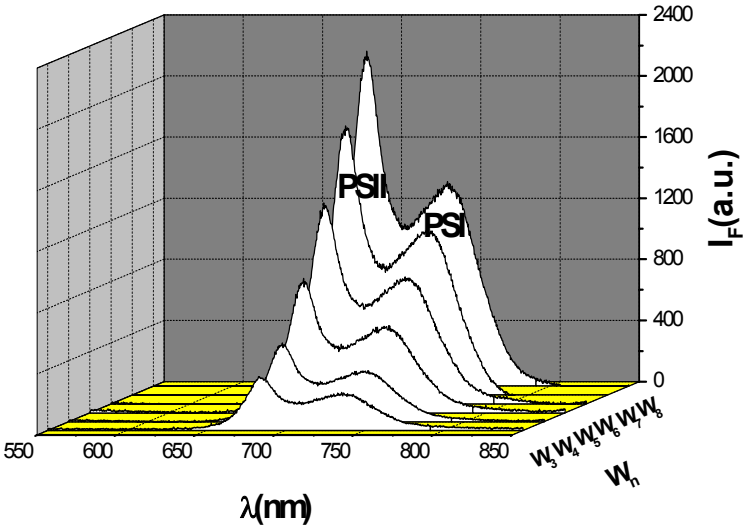

FIG. 1A

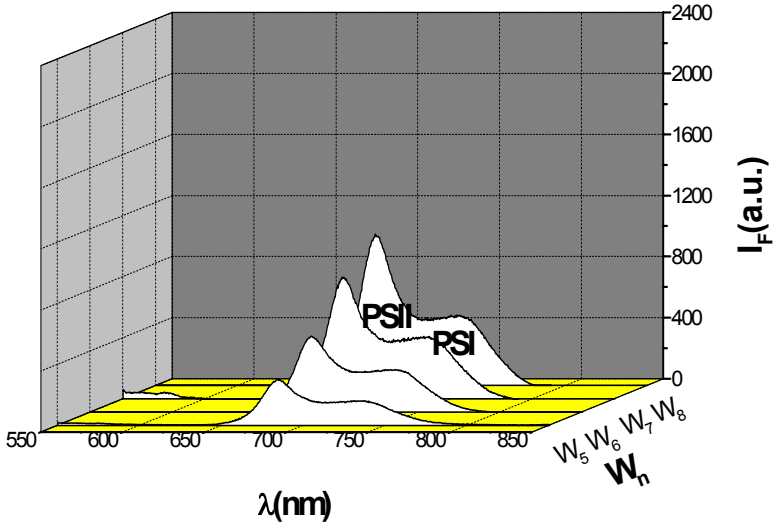

FIG. 1B

FIG. 1. Fluorescence spectra for the two groups of cotton: (A) under summer conditions; (B) under winter conditions; (W_n: Number of weeks)

TABLE 1A. Summer conditions. Parameters for I_F 685 band.

W_n	λ_{max}(nm)	$\Delta\lambda$ (nm)	A (m^2.)	I_F (a. u.)
W_3	685.2±0.5	23.1±0.3	0.90±3.2	353.4±0.5
W_4	685.3±0.2	22.7±0.4	1.34±2.4	497.8±0.3
W_5	685.1±0.1	22.5±0.3	2.10±5.4	849.1±0.6
W_6	685.1±0.8	21.7±0.6	3.43±4.3	1307.6±0.5
W_7	684.3±0.7	21.4±0.5	4.82±3.3	1735.1±0.3
W_8	684.1±0.6	21.1±0.3	6.15±4.6	2209.2±0.2

TABLE 1B. Summer conditions. Parameters for I_F 733 band.

W_n	λ_{max}(nm)	$\Delta\lambda$ (nm)	A (m^2)	I_F (a. u)
W_3	734.3±0.9	47.6±0.7	1.4±7.3	240.2±0.7
W_4	734.4±0.8	46.3±0.6	1.9±9.3	331.0±0.5
W_5	733.0±0.9	43.6±0.5	2.9±3.2	552.1±0.4
W_6	733.8±0.7	42.4±0.1	4. 4±2.4	828.1±0.3
W_7	733.8±0.8	42.9±0.4	5.8±4.3	1075.0±0.2
W_8	733.6±0.6	42.2±0.4	7.0±5.3	1343.8±0.1

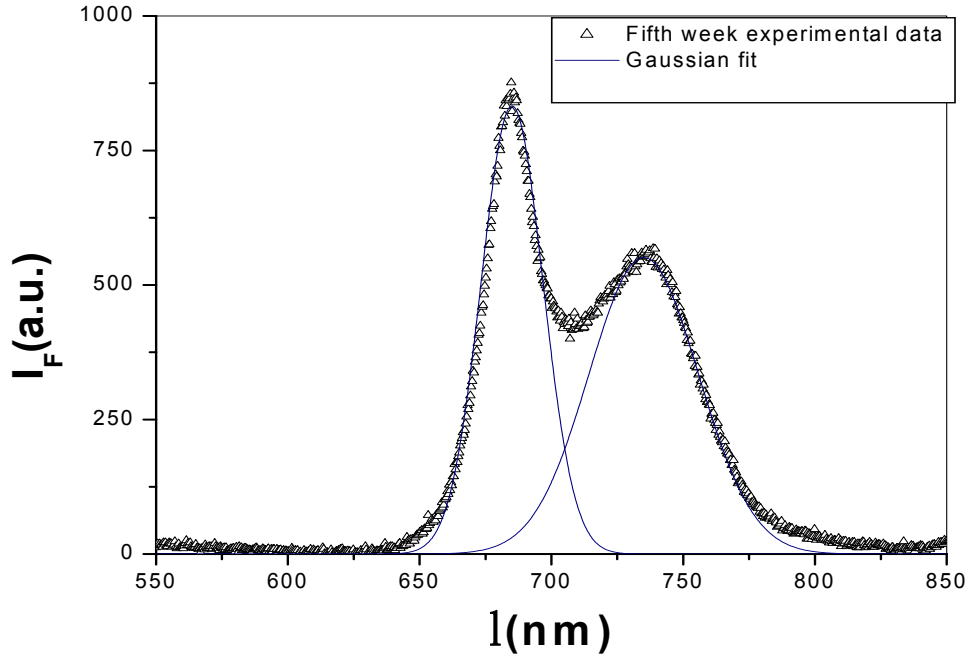

FIG. 2. Chlorophyll a fluorescence spectra for the summer condition cotton at the fifth measuring week with Gaussian curve fitting for I_F685/I_F733 bands.

TABLE 2A. Winter condition. Parameters for I_F 685 band.

W_n	λ_{max}(nm)	$\Delta\lambda$ (nm)	A (m^2)	I_F (a. u.)
W_5	685.5±0.4	22.8±0.3	0.58±5.0	245.4±0.4
W_6	685.5±0.2	22.5±0.5	1.2±5.5	459.3±0.4
W_7	685.3±0.7	22.1±0.5	1.8±4.0	638.5±0.3
W_8	684.8±0.4	21.6±0.7	2.3±4.4	813.3±0.2

TABLE 2B. Winter condition. Parameters for I_F 733 band.

W_n	λ_{max} (nm)	$\Delta\lambda$ (nm)	A (m^2)	I_F (a. u.)
W_5	732.2±0.8	44.1±0.1	0.87±8.8	166.6±0.5
W_6	731.4±0.5	44.0±0.4	1.58±4.7	298.4±0.6
W_7	731.4±0.4	42.8±0.9	2.33±7.4	403.4±0.2
W_8	730.7±0.2	41.7±0.8	2.81±4.3	508.0±0.2

$\lambda_{max:}$ Peak center; $\Delta\lambda$: FWHM; A: Gaussian area; $I_{F:}$ Fluorescence maximum intensity.

From Table 1 (A and B) for cotton growing in summer conditions, the shifts of λ_{max} for I_F685 and I_F733 bands as the weeks progressed were 1 nm towards shorter wavelengths and the values of $\Delta\lambda$ decreased with 2 nm and 5 nm, respectively. However, the Gaussian area and the fluorescence intensity increased as the weeks progressed. From Table 2 (A and B) for cotton in winter condition, the shift of λ_{max} of the I_F685 and I_F733 bands as the weeks progressed, were 1 nm and 2 nm towards the shorter wavelength, and the values of $\Delta\lambda$ decreased with approximately 1 nm and 3 nm, respectively. However, the Gaussian area and the fluorescence intensity increased. The I_F685 and I_F733 fluorescence intensities are known to be due to Chlorophyll a emission [14, 15]. The peak intensity ratio and the area ratio which are centered about 685 nm and 732 nm, respectively give information about the chlorophyll pigment and are related to plant growth with regard to photosynthesis [16, 17]. Photosystem II emits at a wavelength where the chlorophyll pigments still absorb light. This means that when the chlorophyll content in the leaf increases, photosystem II can not increase at the same rate as photosystem I. Thus the peak intensity ratio and the area ratio of photosystem II are

related to the chlorophyll content and can be used for evaluating the chlorophyll concentration [9]. The peak intensity ratio and also the area ratio against the measuring period for each spectrum of the two groups were calculated from the Gaussian fitting curves and are shown in Table 3 for all the period of measuring.

TABLE 3. Peak intensity ratio (P.I.R.) and area ratio (A.R.) (values are the mean of 10 determinations).

TABLE 3A. Summer conditions

W_n	P. I. R. (± 0.09)	A.R. (± 0.07)
W_3	1.47	0.64
W_4	1.50	0.69
W_5	1.54	0.73
W_6	1.58	0.78
W_7	1.61	0.83
W_8	1.64	0.88

TABLE 3B. Winter conditions

W_n	P.I.R. (± 0.04)	A.R. (± 0.04)
W_5	1.47	0.67
W_6	1.54	0.73
W_7	1.58	0.77
W_8	1.60	0.80

The changes or differences in the values during the measuring period in the two groups depend on Chlorophyll a concentration, thickness of the sample, light scattering properties, geometrical and other factors such as photochemical quenching which causes fluorescence decline by reduction-oxidation state of the first e^- acceptor molecules of PSII [4, 14]. Also it can be due to non-photochemical quenching which include environmental stress, known to induce a strong fluorescence quenching caused by the thylakoid damage [2]. The areas of integrated Gaussians are proportional to their heights such that the intensity of the Chlorophyll a fluorescence could be deduced using the area ratio [6]. Thus, the peak intensity ratio data and that of the area ratio give additional information for monitoring the plant's growth. The changes in the peak intensity ratio and the area ratio versus the measuring period are shown in Fig. 3.

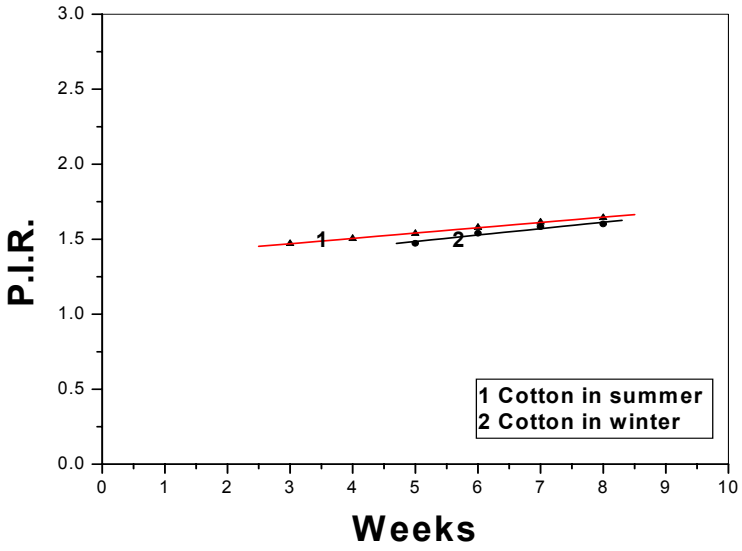

FIG. 3A. For summer conditions; the slope of the line (A) = 1.36 and the intercept of the lines (B) = 0.04; and for winter conditions A = 1.27; B = 0.04

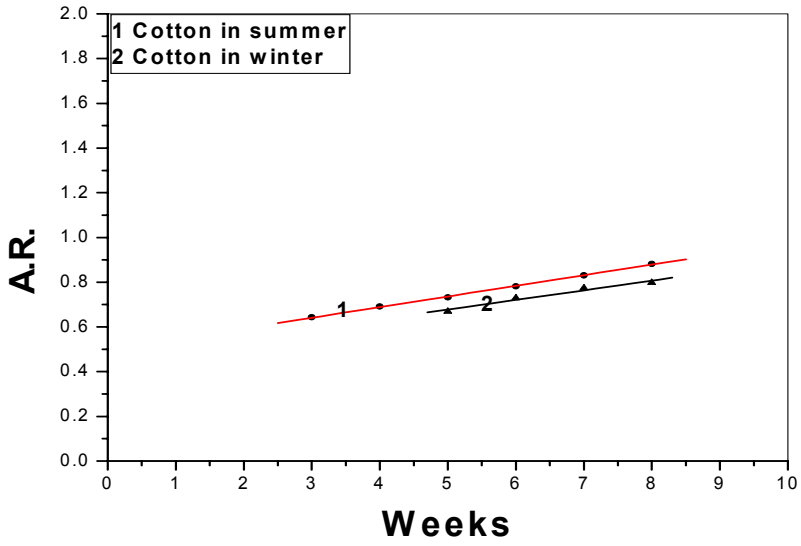

FIG. 3B. For summer conditions: A = 0.50; B = 0.05; and for winter conditions A = 0.46; B = 0.04

Discussion

Seed germination, flowering, fruiting and development of cotton is positively influenced by temperature, light intensity and soil moisture [18 - 20]. The results of the present study showed that cotton grown under sunlight summer conditions gave higher and faster germination rates and better growth performance and development compared to those grown under shaded winter conditions. Signs of stress were expressed on the latter cotton group by low growth rates, stunted plants, immaturity and failure of plants to form any flowers. Such results are in agreement with many authors for cotton [21 - 23] and other plants [24 - 26]. The adverse effects of environmental stress on the different plants have been demonstrated by those authors as a reduction in plants' heights, number of primary branches/plant, number of leaves/plant and dry matter production. Superiority of summer group over winter group is also related to the suitable conditions of temperature, light intensity and soil moisture that prevail during summer season. It is worth mentioning that the summer plants receive extra irrigation in the form of rain. As the weeks advanced from germination, summer and winter conditions as well as the peak intensity ratio and the area ratio showed a linear increase rate with time, but differ in slopes and intercepts. The slope of the summer group (1.36) is higher than the winter group (1.27), which shows that the 1st group expressed a better growth relative to the 2nd group. The lower slope could be an indication for the immaturity and failure of flowering in winter conditions. These results are in an agreement with [9] observations, which showed that the increase of Chlorophyll a concentrations increased the red and far-red fluorescence, however, at high chlorophyll a concentration, only the far-red fluorescence is increased. This can be explained by the fact that plants exhibit two peaks spectra: at 680 and 730 nm corresponding to photosystem II and photosystem I, respectively. The former pigment is known to contain roughly equal amounts of chlorophyll a and b whereas the latter pigment contains a higher ratio of chlorophyll a compared to be [27]. However, our results contradicts with the findings of [28] which showed that fluorescence ratio of I_F685 and I_F733 decreased with increasing chlorophyll content of developing leaves. In addition, Ref.[29] and Ref.[16] demonstrated that the Chlorophyll a fluorescence intensity does not usually depend on the Chlorophyll a concentration; but rather on the amount of the light energy absorbed by the leaves. The decrease of Chlorophyll a fluorescence intensity at the early stage of germination is attributed to the small amount of the light energy absorbed because of the small amount of the molecules. The constant increase in the Chlorophyll a fluorescence intensity signal in summer compared to that in winter suggests that Chlorophyll a concentration in summer plants is higher than in winter plants and may be attributed to the environmental stress that

causes thylakoid damage of the winter plants which usually reduces the fluorescence intensity [6]. Such situation may be explained as due to the relatively low temperatures and light intensity in the winter season for shaded plants.

The shift of λ_{max} towards the shorter wavelength and the decrease of $\Delta\lambda$ value as the weeks advanced; together with the increase of the peak intensity ratio or the area ratio confirm the fact that there was an increase in the Chlorophyll a concentration.

Conclusions

The development of cotton was monitored under summer and winter conditions using I_F685 and I_F733 spectra. All growth parameters of cotton grown under summer condition were higher than in winter conditions due to environmental stress. The peak intensity ratio and the area ratio of cotton were found to follow a linear relation for the two groups in the two seasons. The curve fitting of the spectra indicated similarities between the peak intensity ratio and the area ratio which reflects the exactness of the Gaussian curves. The ratio I_F685/I_F733 can be used to monitor the development of cotton and probably other plants. Future studies must put into account another way for the determination of Chlorophyll a content using SPAD chlorophyll meter or acetone extract and the chlorophyll activity with chlorophyll fluorescence induction kinetics or CO_2 fixation measurements.

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