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# LAMP
# SERIES REPORT

(Laser, Atomic and Molecular Physics)

## DETECTION OF VEGETATION STRESS FROM LASER-INDUCED FLUORESCENCE SIGNATURES

N. Subhash

MIRAMARE-TRIESTE

International Atomic Energy Agency
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## DETECTION OF VEGETATION STRESS FROM LASER-INDUCED FLUORESCENCE SIGNATURES[1]

N. Subhash [2]
International Centre for Theoretical Physics, Trieste, Italy.

MIRAMARE - TRIESTE
June 1995

[1]This work was presented as a LAMP seminar at the International Centre for Theoretical Physics, Trieste, Italy, 14 October 1994.

[2]Permanent address: Optics and Laser Laboratory, Centre for Earth Science Studies, Trivandrum - 695 031, India. FAX: 91-471-442280

## Preface

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**ICTP LASER GROUP**
**P.O. BOX 586**
**34100 Trieste**
**Italy**
**Phone: +39+40+2240322**
**Fax: +39+40+2240443**
**email: *passarel@ictp.trieste.it***

## ABSTRACT

The *in vivo* laser-induced fluorescence (LIF) signatures of UV irradiated Salvia splendens plants were measured using an Optical Multichannel Analyser (OMA) system with Nitrogen laser excitation. The LIF spectra which consisted of the blue-green and the red chlorophyll bands were analysed with a non-linear iterative procedure using Gaussian spectral functions. The fluorescence intensity ratios of the various bands obtained from curve fitted parameters were found to be more sensitive to changes in the photosynthetic activity of the plant. The variation in the intensity ratio for the chlorophyll bands for nutrient stressed sunflower, cotton and groundnut plants as well as the nutrient and water stressed rice plants are also presented. It is observed that vegetation stress not only changes the fluorescence intensity ratios and the vitality index of the plant but also changes the peak position of the emission bands, in some cases. It is also seen that analysis of the fluorescence spectra in vegetation remote sensing applications would require a deconvolution procedure to evaluate the exact contribution of each band in the total spectra.




## 1. Introduction

Laser-induced fluorescence (LIF) has shown great potential for remote detection of vegetation stress[1-4]. LIF studies of vegetation are directed towards exploring the possibility of using lasers as a remote means of measuring vegetation characteristics such as plant vigour[5], as affected by various stress factors such as drought, nutrient deficiency, acid rain, etc., plant type identification and forest biomass estimation. In LIF studies the spectral characteristics of the fluorescence signal can be used to make inferences regarding health and identity of plants[6]. In forest volume or biomass estimation studies lasers are used as LIDAR systems to supply canopy/tree-height data that can be used to determine the volume or weight of wood on the ground.

Terrestrial vegetation gets its green colour from the photosynthetic pigments, chlorophyll *a/b*. The yellow carotenoids, which contain xanthophylls and β - carotene, are localised together with the chlorophyll in the photosynthetic biomembaranes called thylakoids. Within the membranes they are organised in different chlorophyll-carotenoid-protein complexes which are the plant organs for photosynthesis and carbohydrate formation. Inside the leaf the photosynthetic pigments are bound to the chloroplasts. The epidermis of leaves is free of chlorophylls and carotenoids and is mostly colourless. Photosynthesis takes place in the plant mesophyll, inside the chloroplasts under the influence of light. The actual reaction consists of a complicated reduction-oxidation chain involving a large number of intermediary components. The final result is the conversion of carbon dioxide and water into glucose and oxygen.

In plants the laser beam induces photosynthesis in the same way as the visible portion of the solar radiation induces photosynthesis. The primary absorber of energy during photosynthesis is chlorophyll *a/b* proteins. Both chlorophyll *a* and *b* show absorption maxima in the blue and red regions and minimum in the green-yellow region. The absorbed energy migrates to the reaction centers where photosynthetic quantum conversion takes place. Under optimum conditions, the majority of energy is used for photochemistry to drive

photosynthesis while, a small portion of the absorbed energy is emitted as heat and as fluorescence.

$$E_{absorbed} = E_{photochemistry} + E_{heat} + E_{fluorescence}$$

The magnitude of chlorophyll fluorescence is a function of the photosynthetic efficiency. The more efficient the photosynthesis is, the less will be the fluorescence emission. In a functioning photosynthetic apparatus the fluorescence emission is about 2-5% of absorbed energy and this depends on the chloroplast type, age, physiological state of the leaf and the intensity of light. This inverse relationship between the *in vivo* chlorophyll fluorescence and photosynthesis can be used to study and detect stress in plants.

Chlorophyll *a* is the only pigment and source for the red chlorophyll fluorescence of leaves because the accessory pigments, chlorophyll *b* and carotenoids transfer their absorbed energy to the chlorophyll *a*. There are two photosynthetic processes which contain chlorophyll *a* in their reaction centres, but red chlorophyll fluorescence primarily comes from the reaction centre of Photosystem II (PS 2). Chlorophyll *a* in PS 1 shows no fluorescence at room temperature. Chlorophyll *b* shows fluorescence only after separation and not *in vivo* in the leaf or the chloroplast. The yellow carotenoids also do not exhibit any fluorescence.

Fluorescence when excited by blue light comes from the outer mesophyll layers because of the strong absorption by chlorophyll and carotenoids. When excited by red light, only chlorophyll absorb light, and not the carotenoids, and hence the red light penetrates deeper into the mesophyll layers. As a consequence, the reabsorption of the emitted 690 nm fluorescence then proceeds at a higher rate than with blue excitation thereby reducing the intensity of the 690 nm band when excited by red light[7].

It is advantageous to use lasers to excite photosynthesis and induce fluorescence from intact plant leaves since the photosynthetic efficiency is more and there is no interference from ambient light. A UV laser such as nitrogen laser, is most appropriate for the study because it can excite fluorescence not only from



chlorophyll *a/b* but also from other plant pigments which are specific to a particular plant type.

LIF signatures of plants have two components. They are the laser-induced red chlorophyll fluorescence with maxima near 690 nm and 730 nm and the laser-induced blue-green fluorescence having a maxima near 450 nm with a shoulder at around 520 nm[1,7-9]. The *in vivo* red fluorescence is inversely related to photosynthetic activity of plants. When the photosynthesis declines, the red fluorescence intensity as well as the fluorescence intensity ratio (FIR) of the chlorophyll bands, F690/F730 increases, for both red and UV laser excitation. In the past few years the FIR of the chlorophyll bands F690/F730 has been investigated in detail by many groups and is now established as a stress indicator of plants[5,10-11] and has been utilised in active remote sensing of plants from a ground operated fluorescence LIDAR system[2].

The fluorescence kinetics of leaves, first described by Kautsky and Hirsh[12], was widely analysed during the last 15 years for studying plant physiology. This kinetics is usually measured on predarkened leaves and consists of a fast fluorescence rise to a maximum intensity level ($f_{max}$) followed by a slow fluorescence decay ($f_d$) to a steady state value ($f_s$) in 3-5 min. The fluorescence decrease ratio, $R_{fd} = f_d/f_s$, is an indicator of the potential photosynthetic activity of the leaf. It also signals the intactness of the photosynthetic apparatus and hence can be considered as a vitality index of the plant[3,5].

The stress adaptation index related to two $R_{fd}$ values simultaneously measured in the two wavelength bands at 690 nm and 730 nm is represented by the following equation[5]:

$$A_p = 1 - (1 + R_{fd}\,730) / (1 + R_{fd}\,690)$$

The value of $A_p$ is an indicator of how the leaves reorganise the structure of the photosynthetic apparatus for best adaptation to the stress conditions. Green photosynthetically active leaves show $A_p$ values of about 0.2-0.3, while senescent leaves[5] or plants undergoing severe stress have values below 0.15. Usually plants with lower $A_p$ values suffer from stress earlier than those with

higher $A_p$ value.

In this paper, the effect of UV irradiation in the LIF spectral signatures of Salvia splendens plants, carried out at the Instituto di Elettronica Quantistica (IEQ-CNR), Italy, and the results of nutrient stress on groundnut, cotton, sunflower and rice plants, carried out at the Centre for Earth Science Studies (CESS), India, are presented.

## 2. Experimental Details

The fluorescence spectra from intact leaves of plants are recorded using the set up shown in Fig. 1. A pulsed Nitrogen laser (PRA International Model LN103) operating at 337.1 nm was used for measuring the vis-near-ir spectra[13-14]. This laser was operated at 10 Hz and externally triggered by pulses from the Optical Multichannel Analyser (OMA) system (EG&G PAR Model 1460). The laser beam was coupled to a 600 μm fused silica optical fiber by a suitable lens. Proper coupling of the beam on to the fiber was achieved by mounting all the optical components on good quality optical mounts and translation stages and fixing them on optical rails (Eailing Optics). The mirrors and lenses were aligned to obtain a Gaussian intensity profile at the out put end of the fiber which is kept in contact with the leaf. This type of arrangement for excitation facilitates easy and quick measurement of fluorescence from the intact plant leaf. Laser light intensity was controlled using a set of neutral density filters. A laser power meter (Ophir Optics Model PD2A) monitors the laser intensity through the coupling dichroic beam splitter. The power meter was interfaced with the OMA console and shot-to-shot laser energy was acquired together with each spectrum, allowing precise correction for laser fluctuations.

Fluorescence was collected by the same optical fiber and focused on to the entrance slit of the Monochromator (Jobin -Yvon model HRP, f= 600 mm). A Schott WG360 filter was used to avoid backscattered beam from the leaf surface entering the Monochromator. The LIF spectra was detected by a 1024-channel intensified diode array detector (EG&G PAR Model 1421) cooled to 10°C and processed in the OMA system. Using a 150 lines/mm grating and an entrance slit width of 100 μm, a resolution of 1.1 nm is easily achieved. The OMA



system was calibrated in wavelength in the desired spectral region with a neon spectral lamp by a linear fit. The LIF spectra were acquired spearately in the 400-600 nm and 600-800 nm sampling intervals. The fluorescence spectra were also corrected for detector response as function of wavelength by using a National Institute of Standards and Technology traceable calibrated tungsten source and presented as relative fluorescence intensities (RFI) in arbitrary units.

The OMA system can acquire a complete spectrum in about 10 ms facilitating fast measurement of *in vivo* fluorescence from different points on the intact leaf. The high sensitivity of the system provides excitation of the spectra with low laser power (0.4 μW at the fiber tip) to avoid inducing fluorescence kinetics. Under these conditions the fluorescence spectra relates to the ground state initial fluorescence $F_o$ when all the electron acceptors are open.

## 3. Results and discussion

A. UV irradiation stress in Salvia splendens plants

*i). Plant growth and LIF spectral analysis*

The Salvia splendens plants, cv. Primco, were grown under controlled conditions in a green house in Pisa, Italy. UV stress was induced by a pair of low pressure Hg fluorescent lamps (Phillips, TL 40 W/12) fitted in the greenhouse at a height of about 1 meter from the canopy. Total UV irradiation was about 38 $kJm^{-2}$ of UV-A and 70 $kJm^{-2}$ of UV-B in addition to natural light. The UV light dose was equally distributed for 1 week during a 12 hours light period, with cyclic irradiation of 30 sec every 10 min. A set of plants was kept out of UV light for control. The plants were 6 weeks old at the time of measurement.

The fluorescence spectrum for both control and UV treated plants are shown in Fig. 2. Each spectrum is the average on at least 30 leaves of different plants. The leaves under investigation were of the same development stage so that the maturity of the control and stressed groups remained the same. As previously reported[1,6,8], the emission bands are centered at about 450, 685 and 730 nm, with

a shoulder at about 530 nm. In the UV treated plants the intensity of the 685 and 730 nm bands is higher than in control. There occurs a reduction in intensity for the blue band at 450 nm with UV light stress as suggested by Lichtenthaler and Stober[8], while the 530 nm band seems unaffected by UV treatment.

It is well known that the 690 and 730 nm bands are due to chlorophyll *a/b* of the reaction centers and of the light harvesting antenna[7]. On the other hand, many plant pigments and plant products such as cinnamic acid, caffeic acid, chlorogenic acid and other phenols, as well as more complex structures like flavonoids, anthocyanins and stilbenes, present in the large vacuoles of the epidermal layer have been proposed as candidates responsible for the blue and green bands[15-16]. Lichtenthaler and Stober[8] has suggested that flavonols present in the epidermis has absorption bands below and above 400 nm and they can not only absorb UV light but also the emitted blue fluorescence. Many stress factors such as nitrogen deficiency, high light stress, etc., increase the level of flavonols in the epidermal cells. Flavonols can therefore reduce the blue fluorescence intensity by taking off UV light from blue fluorescing substances and by reabsorbing emitted blue fluorescence thereby leading to a decrease in the blue-green fluorescence intensity with stress. In addition the photolysis of pigments absorbing at 337 nm and emitting in the blue region can decrease the intensity of the 450 nm band in UV treated plants . Since the UV absorbing pigments are mostly present in the epidermal layer, their role is primarily in protecting the leaf mesophyll from the environment. Their photolysis, therefore, augments the penetration of the 337 nm excitation into the mesophyll layers, increasing the excitation of deeper chlorophyll, thereby resulting in more intense red and near-ir fluorescence bands[14].

Fig. 3 shows the effect of UV treatment with 458 nm Argon ion laser excitation. This spectrum is the average of 20 measurements on different leaves of the control plant and 32 measurements on different leaves of the treated plant. It can be seen that there is not much change in the LIF spectra. This is an indication that UV treatment did not change the concentration of chlorophyll, which would affect the F690/F730 ratio. But the change in the spectra with Nitrogen laser excitation must be attributed to difference in light penetration through the leaf or different energy transfer process among pigments.



*ii) Curve fitting of fluorescence spectra*

The LIF spectra were analysed using the Marquardt-Levenberg algorithm[17] for iterative non-linear curve fitting using a combination of Gaussian spectral functions. The program finds the true absolute minimum value of the sum of squared deviations (the value of $\chi^2$) by the iterative process. The values of the correlation coefficient ($r^2$) and $\chi^2$ determines the quality of fit[14].

Typical Gaussian bands resulting from the curve fit analysis of the measured spectra for the control plants of Salvia is shown in Fig. 4. The constituent bands are found to be centred at 451.3, 536.2, 683.5 and 729.6 nm. Choice of four Gaussian bands were found to give an accurate fit of the measured spectra as can be seen from the values of the correlation coefficient which were higher than 0.97 and the sum of squared deviations lower than $5.10^{-8}$. Table I gives the curve fitting parameters such as the peak centre, full width at half intensity maximum (FWHM) and the area under each Gaussian curve for both control and treated plants.

It appears that the effect of UV treatment on the peak position of the chlorophyll bands is minimal. However, there is a shift of about 2.5 nm towards the longer wavelengths for the blue fluorescence band, while for the green band the shift is about 9 nm towards the shorter wavelengths. These shift in the peak position of the bands were confirmed by repeating the curve fitting with the peak position of the various bands locked to the curve fitted peak center values of the control plants. In this case the $r^2$ and $\chi^2$ values of the fit were worse, with broader prediction and confidence intervals. It is reported[15] that the epidermis contributes only very little to the green fluorescence near 525 nm and this emission may be from the mesophyll layers. The function of the epidermis is to act as a barrier for the UV irradiation from reaching the mesophyll layers. Thus the change in the position of the blue-green bands with UV irradiation can be attributed to alteration of the UV absorbing fluorescence pigments.

The fluorescence intensity ratios calculated from the amplitudes and areas of the Gaussian curves are shown in Tab. II along with the ratios determined from the measured spectral intensities. It is worth noticing that the chlorophyll

fluorescence ratio F690/F730 does not show much change with Gaussian fitting and is almost unaffected by the UV treatment. This shows that for the UV dosage given, the leaf chlorophyll concentration remains constant. Marked differences are instead observed for the other fluorescence intensity ratios where the 450 and 530 nm bands are involved. This is because of the almost complete overlap of the 530 nm emission to the tail of the 450 nm band, which makes the calculation of its contribution with respect to other bands very approximate. The correct evaluation of the various fluorescence intensity ratios must therefore be done on the four Gaussian bands obtained by curve fitting.

The effect of a particular stress on plants may also appear of different magnitude as a consequence of the data processing methodology used. A comparison of the four fluorescence bands in UV treated plants with respect to their controls for different data processing methods are shown in Fig. 5. It can be seen that the peak area and peak amplitude values of the Gaussian spectra are much more sensitive than the measured fluorescence as stress indicators.

B. Nutrient deficiencies in oil yielding plants

The experimental set-up was similar to the one described in Fig. 1, except for the usage of a He-Ne laser for excitation and a Jobin Yvon H10 DUV scanning monochromator with a Hamamatsu R928 photomultiplier for signal detection. The plants for the study are grown under controlled conditions in a green house at CESS, India, based on the Tamil Nadu Agricultural University Package for each crop. White river sand free of minerals is taken in earthen pots and the plants are grown using appropriate salts as nutrient source. The plants were divided into four groups, withholding one nutrient, nitrogen(N), phosphorous(P) or potassium(K), from the control group containing all essential nutrients for growth. The potted plants were placed in trays containing the nutrient solution and water for 3 hours, on alternate days, so that water and nutrient requirements are met simultaneously.

The measurements were made on TMV-2 (I) and CO-2 (II) varieties of groundnut, MCU-9 variety of cotton and Morden variety of sunflower after 45 days of growth. The monochromator is set at 690 nm and the Kautsky induction



kinetics is recorded on predarkened leaves for 5 minutes. Afterwards the steady state LIF spectrum is recorded and the induction kinetics is recorded again at 730 nm on another point of the same leaf. Spectral fittings performed using two Gaussian functions was found to give a good fit of the data as evidenced by the $\chi^2$ and $r^2$ values.

Fig. 6. gives typical fluorescence spectra with the deconvoluted Gaussian components for the control plants of cotton, sunflower and groundnut(I and II). The dots represent digitized spectral data points, the solid line is the fitted curve, the constituent bands are shown by the thin dotted line, and the thin straight line at the bottom represents the background intensity. There are marked differences between the apparent contribution of the 690 and 730 nm bands in the measured fluorescence spectra and their true contribution as seen from curve fitting. The peak position of the bands are shown in Table III along with F690/F730 ratios obtained from LIF spectral intensity, Gaussian band intensity and curve area. The chlorophyll content of leaves measured using a Agrophotometer are also shown in Table III and all data represent the mean of 4-5 measurements on different plants belonging to each group. For sunflower plants there is a notable shift in the red and near-ir band position towards blue region with nutrient stress[18]. In the case of nitrogen deficient sunflower plants the shift of the near-ir band is about 10 nm. For other plant groups of cotton and groundnut there is no appreciable shift in the position of these bands with treatment.

The change in the FIR determined from both the curve fitted amplitudes and Gaussian curve areas provide additional information on the effect of stress on these plants. In the case of Groundnut I and II plants there is an increase in the F690/F730 ratio with treatment, with maximum increase noticed for the nitrogen deficient group[19]. The cotton plants do not seem to be very much affected by stress. In the case of sunflower, the F690/F730 ratio shows marked increase for the nitrogen deficient group while there are notable changes in the amplitude and area ratios for the phosphorous and potassium group. It is possible that the shift in maxima of the chlorophyll bands of sunflower along with changes in the FIR can serve as indicators of nutrient related stress. It can also be seen that there is an overall improvement in the sensitivity of determining F690/F730 ratio with curve fitting for all the plant groups studied as in the case of Salvia plants.

Table IV gives the mean $R_{fd}$ values determined from the induction kinetics at 690 and 730 nm and the stress adaptation index $A_p$ evaluated using these $R_{fd}$ values typically for groundnut I plants. The values shown are mean of measurements on 4-5 plants of each group and they show how best these plants can adapt to the external stress condition.

Among groundnut plants on which the LIF measurements were made, only the nitrogen deficient plants showed visible symptoms, such as yellowing of leaves followed by stunted growth, while P and K deficient groups showed no symptoms of damage. In sunflower, N deficient plants showed retarded growth, while K deficient plants showed similar symptoms only from the third week. However it was also possible to detect changes in fluorescence signature from plants which showed no visible symptoms of stress. In a recent study on rice plants[20-21], while visible deficiency symptoms appeared only in N, P, and K groups, the FIR F690/F725 determined from a He-Ne laser excited spectra showed notable changes, as can be seen from Fig. 7, in plant groups belonging to Ca, Mg, Mn, Fe and Si deficiency where there were no visible symptoms of stress. Similarly the impact of water stress can also be detected in rice plants from the changes in the $R_{fd}$ values[22]. Fig. 8 shows the vitality index derived from the induction kinetics for different varieties of rice plants subjected to 48 hours of water stress. Table V shows the effect of prolonged water stress in a particular rice variety (2: Kunnappan). The $R_{fd}$ values at 690 nm were measured on intact plant leaves after withholding watering on 35-40 days old potted rice plants and each value represents the average of 10 measurements. Since there are marked changes in $R_{fd}$ values with water stress, it is possible to identify varieties suitable for drought prone environments. As can be seen from these studies, the most important advantage of the LIF technique in vegetation studies is that it can sense changes in the photosynthetic activity of plants due to vegetation stress before the appearance of visible symptoms so that timely remedial measures are possible.

## 4. Conclusions

Short term and long term stress not only increases the chlorophyll fluorescence ratio, but also changes the intensity ratio of the blue-green fluorescence to the red fluorescence. We have investigated and found that the changes in these ratios with UV irradiation stress is significant. It is noticed that the sensitivity of these ratios gets improved if contribution from the constituent bands are taken into consideration for evaluation of the ratios; which is possible by curve fitting of the LIF spectra using a linear combination of Gaussian spectral functions. In addition it is seen that changes in the position of these bands and changes in the FIR obtained from curve fitting have great potential for utilisation as indicators of stress in plants.

Remote sensing of the fluorescence signatures of terrestrial vegetation is useful in getting an accurate picture of plant health. However, it is necessary to concentrate on parameters that can be sensed in a short time using airborne systems. Since the measurement time for induction kinetics is large, it is not suited for this purpose. Other possible alternatives are the measurement of fluorescence intensity ratios and fluorescence life time with short laser pulse excitation. Our studies have shown that laser-induced remote sensing of the fluorescence parameters of vegetation, such as the fluorescence intensity ratio relating to the blue-green bands, require a deconvolution procedure to correctly evaluate the contribution of each band in the total spectra[23]. Consequently, in remote sensing applications the leaf fluorescence spectra must be recorded with high spectral resolution as these signatures contain physiological information about plant health and this will complement information obtained from passive reflectance measurements. Thus a combination of LIF and reflectance measurements may prove to be a very accurate method in future vegetation remote sensing applications.

## Acknowledgements

The author is thankful to the Commission of the European Communities, Brussels, for the 'Marie Curie' award for this study at the Instituto di Elettronica Quantistica, Consiglio Nazionale delle Ricerche, Firenze, Italy and to the CSIR, New Delhi, India, for project funding at the CESS. He is also thankful for the support during his visit to the ICTP under the Associateship scheme.

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**Table I**
**Results of curve fitting with four Gaussian functions on the fluorescence spectra of Salvia plants**

| Band | Peak Centre $\lambda_p$ (nm) | | Gaussian Curve Area | | FWHM ($cm^{-1}$) | |
|---|---|---|---|---|---|---|
| | Control | UV | Control | UV | Control | UV |
| Blue | 451.3 | 453.8 | 4.03 | 3.38 | 4718 | 4181 |
| Green | 536.2 | 526.9 | 0.27 | 0.46 | 2280 | 2696 |
| Red | 683.5 | 684.2 | 0.16 | 0.24 | 541 | 575 |
| Near-IR | 729.6 | 730.7 | 0.34 | 0.45 | 1142 | 1084 |

**Table II**
**Fluorescence intensity ratios (FIR) from spectral intensity and curve fitted spectral components**

| Ratio | Spectral Intensity | | Peak Amplitude | | Peak Area | |
|---|---|---|---|---|---|---|
| | Control | UV | Control | UV | Control | UV |
| F685/F730 | 1.17 | 1.22 | 0.95 | 1.00 | 0.46 | 0.54 |
| F450/F530 | 2.45 | 2.25 | 8.23 | 5.13 | 15.16 | 7.38 |
| F450/F730 | 2.72 | 2.00 | 3.15 | 2.06 | 11.77 | 7.48 |
| F530/F730 | 1.11 | 0.89 | 0.38 | 0.40 | 0.78 | 1.01 |

**Table III**

**Results of curve fitting on the LIF spectra of cotton, sunflower and groundnut plants**

| Plant type | Red Band (nm) | Near-ir Band (nm) | Spectral F690/F730 ratio | Curve fitted F690/F730 ratio | Gaussian curve area F690/F730 ratio | Chlorophyll content (ppm/unit leaf area) |
|---|---|---|---|---|---|---|
| Groundnut I(C) | 687.8 | 730.3 | 1.41 | 1.15 | 0.52 | 1.62 |
| Groundnut I(-N) | 687.3 | 724.6 | 1.72 | 1.31 | 0.51 | 1.43 |
| Groundut I(-P) | 689.2 | 730.6 | 1.48 | 1.20 | 0.58 | 1.49 |
| Groundnut I(-K) | 689.0 | 731.1 | 1.52 | 1.22 | 0.58 | 1.25 |
| Groundnut II(C) | 685.1 | 727.0 | 1.58 | 1.27 | 0.61 | 1.89 |
| Groundnut II(-N) | 684.2 | 722.9 | 1.84 | 1.46 | 0.63 | 1.93 |
| Groundnut II(-P) | 684.2 | 725.6 | 1.50 | 1.23 | 0.54 | 1.85 |
| Groundnut II(-K) | 688.8 | 731.1 | 1.34 | 1.28 | 0.63 | 2.25 |
| Cotton (C) | 688.2 | 729.0 | 1.55 | 1.26 | 0.52 | 1.71 |
| Cotton (-N) | 686.4 | 727.0 | 1.44 | 1.15 | 0.57 | 1.73 |
| Cotton (-P) | 688.5 | 729.2 | 1.42 | 1.11 | 0.50 | 1.65 |
| Cotton (-K) | 690.3 | 732.0 | 1.37 | 1.07 | 0.55 | 1.39 |
| Sunflower (C) | 688.2 | 725.4 | 1.75 | 1.19 | 0.27 | 1.87 |
| Sunflower (-N) | 686.3 | 716.4 | 2.13 | 1.31 | 0.73 | 2.13 |
| Sunflower (-P) | 687.1 | 723.8 | 1.96 | 1.25 | 0.32 | 1.69 |
| Sunflower (-K) | 687.5 | 722.2 | 2.01 | 1.27 | 0.56 | 1.36 |



**Table IV**

**$R_{fd}$ values and stress adaptation index $A_p$ for groundnut plants**

| Groundnut I plant group | $R_{fd}$ (690 nm) | $R_{fd}$ (730 nm) | Stress adaptation index ($A_p$) |
|---|---|---|---|
| Control | 1.152 | 1.063 | 0.041 |
| -N | 1.450 | 0.967 | 0.196 |
| -P | 1.723 | 1.592 | 0.048 |
| -K | 0.640 | 0.636 | 0.002 |

**Table V**

**Change in $R_{fd}$ at 690 nm with prolonged water stress for rice**

| Time (Hrs) | Lower Surface | | | | Upper surface | | | |
|---|---|---|---|---|---|---|---|---|
| | $f_{max}$ | $f_d$ | $f_s$ | $R_{fd}$ | $f_{max}$ | $f_d$ | $f_s$ | $R_{fd}$ |
| 00 | 7.87 | 6.68 | 1.34 | 4.87 | 6.87 | 5.67 | 1.22 | 4.63 |
| 48 | 10.88 | 8.55 | 2.35 | 3.63 | 9.53 | 7.33 | 2.20 | 3.30 |
| 96 | 11.18 | 8.67 | 2.52 | 3.46 | 10.28 | 7.83 | 2.45 | 3.19 |
| 144 | 9.12 | 6.92 | 2.20 | 3.17 | 7.46 | 5.40 | 1.87 | 3.10 |

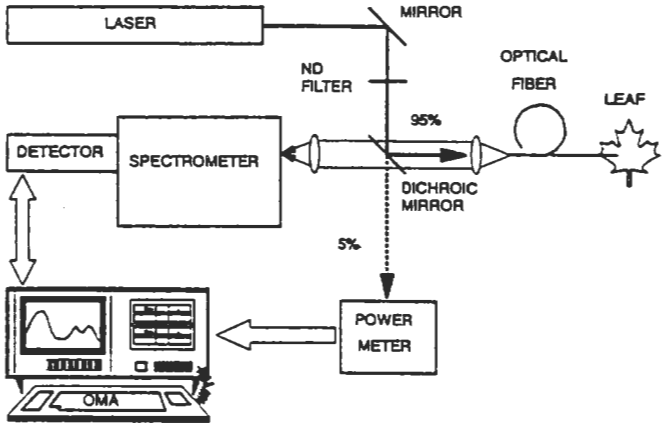



Fig. 1. Experimental arrangement for *in vivo* laser-induced fluorescence measurement from intact plant leaves

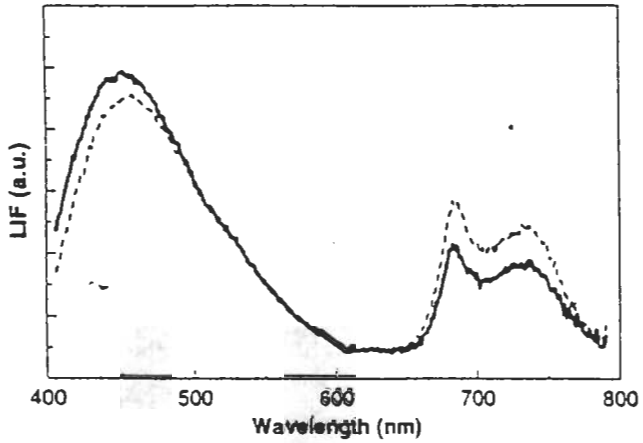



Fig. 2. Fluorescence spectra of control (solid line) and UV irradiated (dashed line) Salvia plants



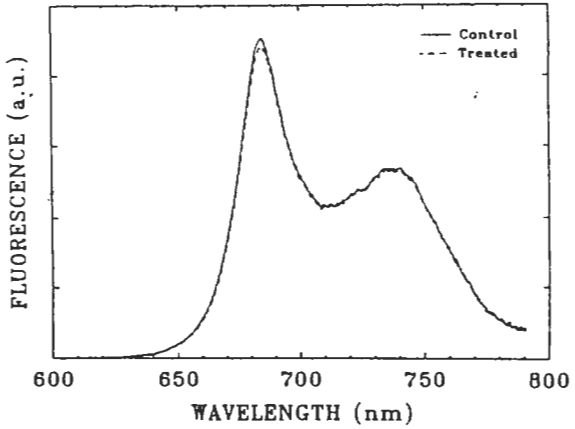



Fig. 3. LIF spectra for treated and control plants with 458 nm Argon ion laser excitation

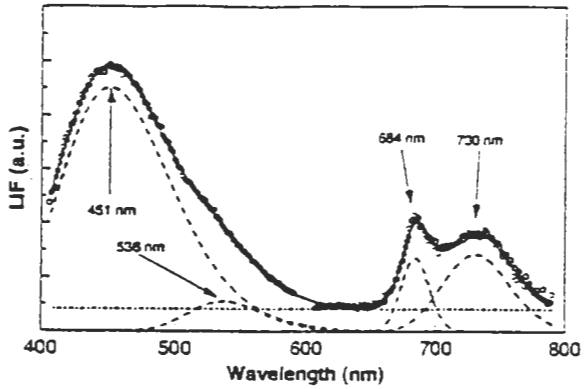



Fig. 4. Lineshape deconvolution of the spectra of control plants. Circles are the experimental points; dotted lines are the component peaks; dot-dashed line is the fitted constant background and the solid line is the resulting lineshape

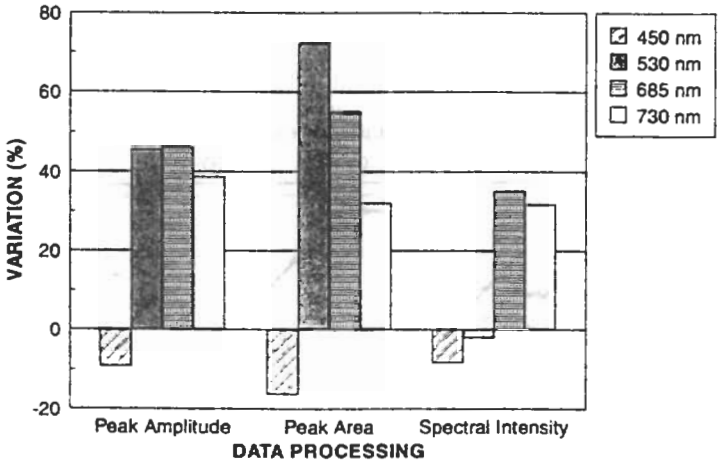



Fig. 5. Variation of the fluorescence intensity of the four bands of Salvia from measured spectral intensity, Gaussian peak amplitudes and Gaussian curve areas



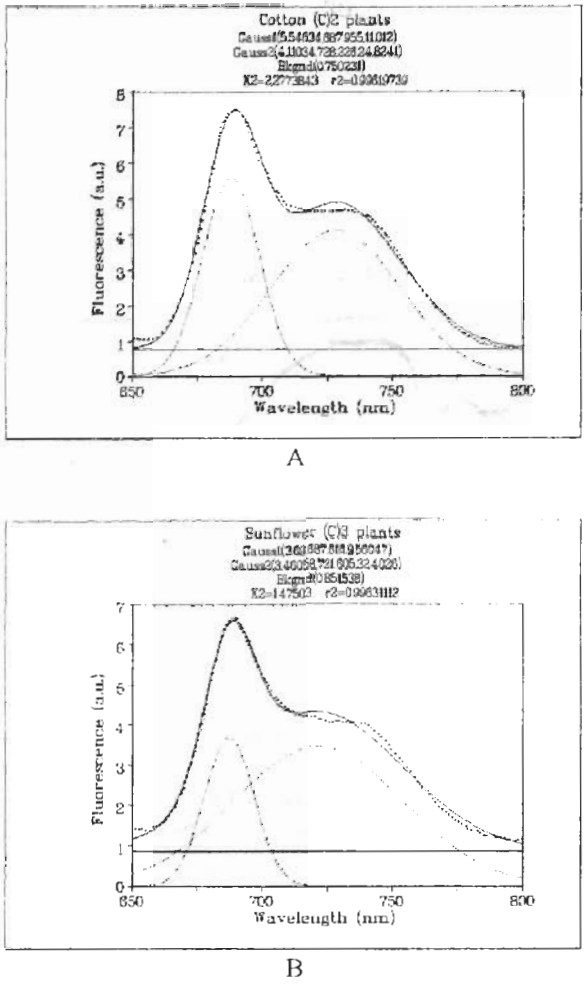
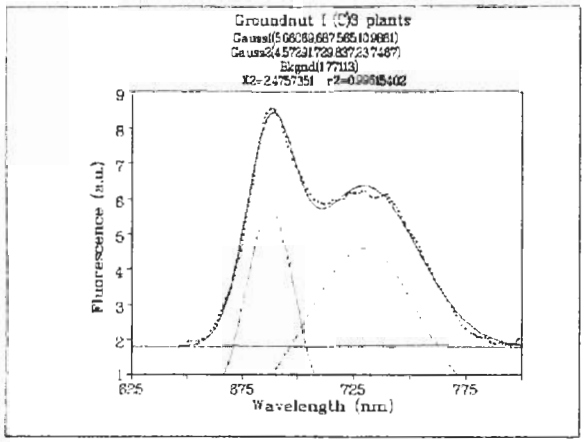
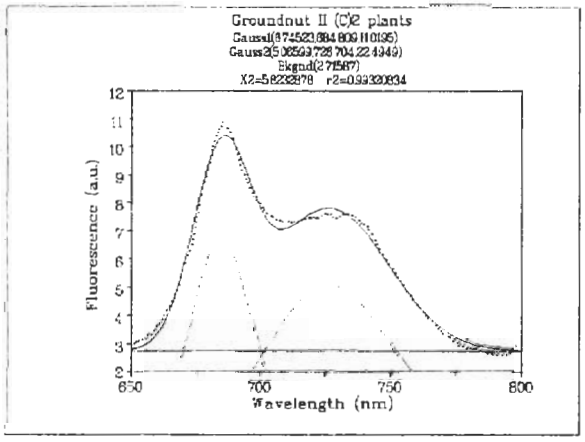



Fig. 6. Curve fit LIF spectra for typical control plants of A: Cotton, and B: Sunflower.




C




D

Fig. 6. Curve fit LIF spectra for typical control plants of C: Groundnut (I) and D: Groundnut (II) varieties



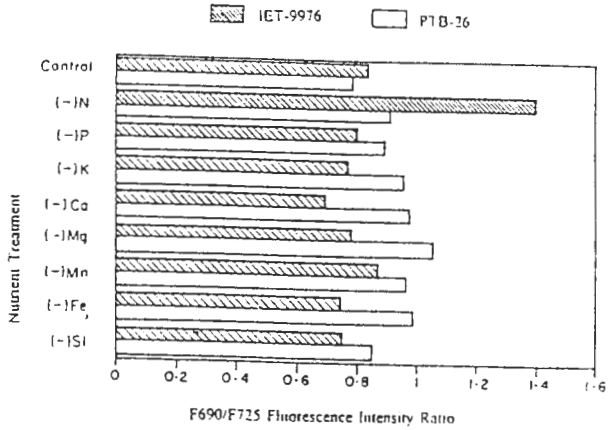



Fig. 7. F690/F730 variation with nutrient treatment for IET-9976 and PTB-26 rice variety

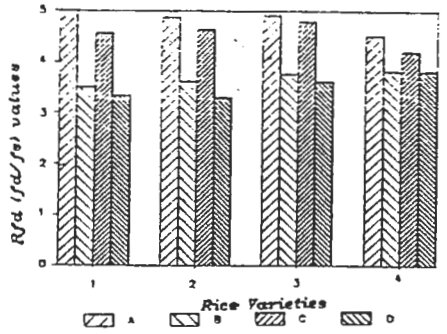



Fig. 8. $R_{fd}$ value prior to and after 48 Hrs. of water stress on Upper (U) and Lower (L) leaves of different rice varieties. A: Control (L), B: Stressed (L), C: Control (U) and D: Stressed (U).