Study of Water and Nutrient Stresses in Plants by Laser Induced Fluorescence Spectra

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Abstract

A plant interacts with atmosphere in several ways involving different parts in different processes. The leaves of the plant interact with light and prepare the food required by the plants. The rate of photosynthesis is determined by the intensity of incident radiation, the temperature in the neighborhood of the plant, the water contents and the density of the pigments present in plant leaves. The excess or deficient quantity of these parameters has direct influence on the photosynthesis rate. The excess or deficient quantity of the parameters responsible for the plant metabolism may be called as stress. It is very important to detect the stresses because they have direct influence on the plant health.We have studied combined effect of water and nutrient stresses in the plant Ocimum basilicum (Lamiaceae) by using laser induced fluorescence spectra.

Key words: Photosynthesis, He-Ne laser, photomultiplier tube, Ocimum basilicum (Lamiaceae)

Introduction

It is necessary to analyze the physiological state of plants growing over wide surfaces such as agricultural areas, forest and oceans. For biochemical analysis a collection of plants sample is necessary and it is also essential to know about the early detection of stress condition so that proper precaution may be taken and the plants may be saved from the unwanted damage. The study of growth physiology of plants would help in the technological development of tissue culture. There are many ways of detection of stresses in plants. One of the powerful techniques to detect the stress is the laser induced fluorescence kinetic method.[(1-3)]

Sample preparation

We selected the plants Ocimum basilicum (Lamiaceae) for performing experiment to study the fluorescence kinetics. The plants are collected from the green house of the campus of Amravati University, Amravati. The plants were developed under the condition of natural light and temperatures. Plants were well watered and regularly nourished and developed thoroughly in the washed river sand condition. After one month of the planting in the green house the plants were transferred in the open top chamber so that they get fresh air. A leaf is detached from each of the plants and immediately transferred to the laboratory for performing the experiment related to fluorescence.The flow of water and minerals from roots of the plants get stopped and the process of photosynthesis is also terminated and hence the plant leaf is under combined water, nutrient stress.

Experimental Set Up

For the study of the effect of short term combined effect of water and nutrients stress in the plant leaves, the experimental arrangement for recording fluorescence kinetics and fluorescence spectra are shown in fig.1 A He-Ne laser delivering 2 mw output power at 632.8 nm was used as an excitation source. Line filter is used for avoiding interference of other wavelengths with the laser radiation, which is reflected from the mirror and then the beam passes through beam splitter and was focused by a lens on the entrance end of the optical fiber. An aluminium tube was fixed at the output end of the fiber to maintain a constant leaf area illumination. The light source illuminants 0.4 – 0.5cm^2 area on the leaf and the observations are recorded at normal angle of incidence at a distance 0.1 cm from the leaf surface. The laser induced chlorophyll fluorescence spectra were analyzed using a jobin – Yvon H10DUV scanning monochromator. Fluorescence emission collected by the optical fiber was focused onto the entrance slit of the monochromator and detected by Hamamamstu R928 photomultiplier tube. The entrance and exit slits of the monochromator were 0.5 mm wide each that gives a spectral resolution of 2 nm with 1200 grooves/mm holographic grating.

For recording the fluorescence kinetics adjust fixed wavelength say 685 nm from the scanner. The leaf is placed in the dark so that it gets protected from any stray light. Beam is incident at one position of any one surface of the leaf immediately after re-drawing from the dark. The observations were immediately made after the beam was incident upon the leaf. This gives fluorescence maximum. After getting maximum fluorescence in the beginning, the

intensity of the fluorescence goes on decreasing consistently as the time passes. After 5-6 minutes it gets stabilized and steady state fluorescence is obtained. After getting the steady state fluorescence adjust range of wavelength 650 nm and 750 nm from the scanning monochromator for recording fluorescence spectrum. Same procedure is repeated for the observation of fluorescence kinetics at 730 nm and fluorescence spectrum is recorded for the same range. The same experiment is repeated for different timings for getting the information in the stressed condition. [4-5]

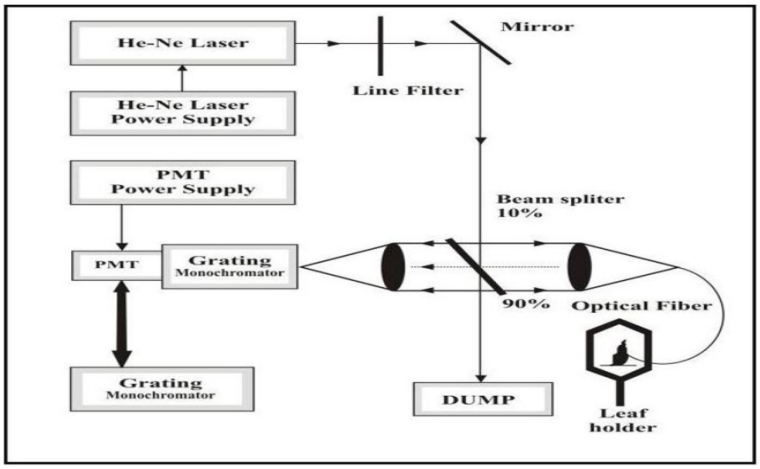

Figure. 1 Experimental arrangement of He-Ne laser based system for recording LIF spectra of plant leaves.

Results and Discussion

The experiment is carried out by He-Ne laser as an excitation source. Here the study of combined water and nutrient stress has been carried out by recording the fluorescence kinetics and fluorescence spectra of leaves. The fluorescence spectra and fluorescence kinetics of adaxial and abaxial surfaces are recorded at different timing by keeping plant leaf as it is in darkness immediately after detaching from the plant. When leaf is detached from the plant it will be under combined water and nutrient stress. The shapes of He-Ne laser induced chlorophyll fluorescence emission spectra are characterized by two maxima at about 685 and 730nm. The spectra delivered by both the surfaces of the leaf are recorded. Fluorescence kinetics at 685 nm and 730 nm and the fluorescence spectrum after immediately achieving steady state fluorescence. It is seen from the Fig. 2 that the intensity of fluorescence kinetics at 685 nm is more than that at 730 nm. It is observed that the steady state fluorescence condition reaches within about 5 min. After reaching steady state also the fluorescence spectrum exhibits the peaks at 685 and 730 nm. Peak

height of the fluorescence spectrum of the fluorescence kinetics and fluorescence spectra after 20, 40, 60, 80 and 100 hours emitted by the lower surface are recorded. The comparison of the spectra clearly shows that the shape of the fluorescence kinetics changes with time. As the stress increases it will take more time for reaching steady state fluorescence. The observations clearly indicate that the time required for achieving steady state condition that could be used to measure the stress factor. It is obvious that by recording the time for reaching steady state fluorescence, we may remotely detect the stress stage condition of whole plant. [6-10]

Conclusion

In our study of short term combined water and nutrient stress it is concluded that peak height at 685 and 730 nm in the fluorescence spectra are the indicators of stress factors. As the shape of fluorescence kinetics takes horizontal form, it is said to be in the stress condition. The observations also shows that the steady state fluorescence is suitable tool for nutrients related stress in plants. The results also show that the laser induced fluorescence technique is very useful in vegetation. Further, it also shows that the laser induced fluorescence technique is very useful in vegetation remote sensing experiments.

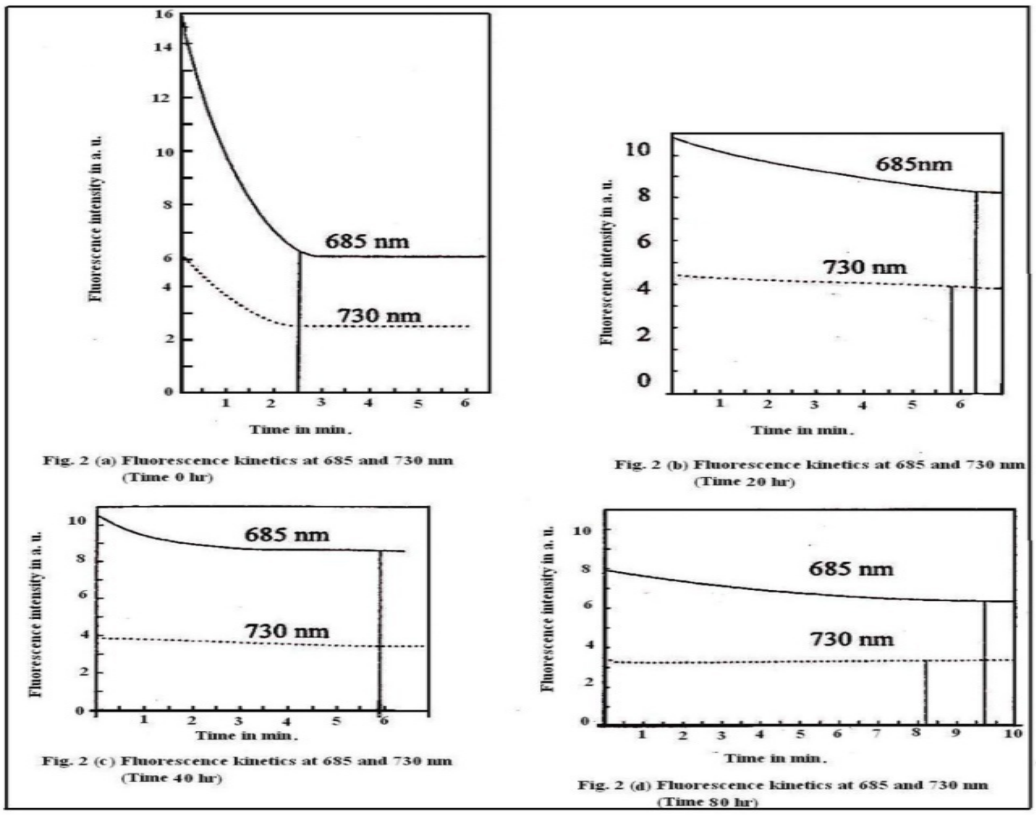

Figure. 2 Comparative Characteristics of fluorescence kinetics at 685 and 730 nm upper surface of leaf.

References

1. Subhash N. and Motianan c., " Remote detection of Nutrient stress in Groundnut plants by Deconvolution of laser induced Fluorescence spectra", IEEE, pp2323-2325, 1995.
2. Subhash N. et al. " Air pollution stress detection in plants by laser induced chlorophyll fluorescence." project report submitted to state council of science, technology and Enviorment, government of Kerala, Thiruvanthapuram, 2003.
3. Marinella Broglia, "Blue Green laser induced Fluorescence from intact leaves Actinic light sensitivity and subcelluar origin" , Applied optics, vol. 1, 32, no. 3, 1993.
4. Chappelle E. W. et al. " Laser induced Fluorescence green plants". Applied optics, 23, 134-138 1984.
5. Chappelle E. W. et al. " Laser may help in Remote assignment of vegetation " , Laser focus, 25(6), 123-132, 1989.
6. Subhash N. and Mohanan C., "Curve fit Analysis of chlorophyll fluorescence spectra": Application of Nutrient Stress Protection in sunflower Remot sens. Enviorn, 60, 347-350, 1997.
7. Lichtenthaler H. K.. "Laser induced chlorophyll fluorescence and blue fluorescence of green vegetation", proceedings 10^{th} EP Sel symposium Toulouse, 1990.
8. Burtoskova H. et al., "The arrangement of chloroplasts in cells Influences the Reabsorption of chlorophyll fluorescence emission", The effect of Desiccation on the chlorophyll Fluorescence Spectra of Rhizominium punctatum leaves, vol.62 no. 2-3, 251-260, 1999.
9. Stresser R. J., Schwarz b., Eggenberg P., "Fluorescence Routine tests to describe the behavior of a plant in its environment. Application of chlorophyll fluorescence." Kluwer Acad. Publisher's pp. 181-187, 1988.
10. Chappelle E. W. et al., "Laser induced blue fluorescence in vegetation", CH2825-8/90, pp. 1919-1922, 1990.