Sensors & Transducers, Vol. 171, Issue 5, May 2014, pp. 128-133

Sensors & Transducers

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Test and Control System for Chlorophyll Fluorescence Parameters Using LED as Excitation Source

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Received: 6 March 2014 Accepted: 7 April 2014 Published: 31 May 2014

Abstract: A new scheme on test and control system for chlorophyll fluorescence is presented in this work, which uses light-emitting diode (LED) excitation by means of measuring the fluorescence parameter φ_{psII}. The system takes programmable power supply as LEDs illumination drive power with high sensitivity and signal-to-noise ratio. MINIPAM is used to measure fluorescence parameter φ_{psII} and keeps communication with upper PC by serial port. The upper PC can control the power supply and process the data received from MINIPAM by software which is programmed in VB6. The results show that the system has a lot of advantages such as high accuracy and convenience. The effect of environmental factors on fluorescence parameters is analyzed comprehensively. It will be a practical measurement and control system for photosynthetic ability and have wide application foreground.

Keywords: chlorophyll fluorescence, light emitting diode (LED), programmable power supply, environmental factors.

1. Introduction

Solar energy is the most important energy source for life-actions on earth. Photosynthetic is the only way by which solar energy can be stored by plants. Extensive investigations have been conducted for improving the photosynthetic rate by controlling environment factors. There are three methods for quantifying photosynthetic rate by measuring the rates of CO_2 consumption, O_2 evolution and increment for leaves' dry matter [1].

The chloroplast is an organelle of plant cell. All photosynthetic processes (Iight absorption, charge separation, electron transport, CO2 fixation, and C4 pathway, etc.) are carried out in the chloroplast. Photosynthesis II (PSII) is regarded as the location for primary photochemical reactions, where the charge separation，water photo oxidation to evolve oxygen and to produce electrons and protons occur [2, 3]. The physiological states of chloroplasts would determine the ability of plant photosynthetic rate [4].

Fluorescence is radiated during the excited P*680 to P680 transition. For fluorescence, P*680 is created directly by light excitation and its origin is associated with photosynthesis [5]. The chlorophyll content is directly related to the plant physiological states and functions. Because existing a relationship between the fluorescence intensity and chlorophyll content within a limited range，the energy conversion in photosynthesis can be evaluated by quantifying fluorescence. Therefore, the researches on fluorescence provide one important method for expressing analysis of the plant photosynthetic ability [6]. Most commercially available instruments for measuring photosynthetic rate, such as the prevalent LI-6XXX series of photosynthetic system, are based on CO_2 consumption. The measurement is affected by environmental factors, such as light intensity, temperature, humidity and CO_2 concentration, etc. variations in these factors would cause substantial differences in the measurement results [7, 8].

In this paper, based on the parameters of fluorescence, a system for controlling and detecting photosynthetic ability is proposed. Comparing with traditional methods for measuring the fluorescence, the proposed system can quantify the plant fluorescence with less influence of the environment and control the parameters of fluorescence for users' wish. The current investigation has revealed that, there is a good reliability and convenience for the system to control the parameters of fluorescence.

2. Methodology

2.1. The Set-up of Experiment

Measurement of the photosynthetic efficiency can be derived from the minimum and maximum values: maximum quantum yield of PSII ($F_v/F_m=[F_m-F_0]/F_m$ requires dark -adapted leaves) and effective quantum yield of PSII ($\varphi_{\mathrm{psII}}=[F_{\mathrm{m}}'-F_s]/F_m'$, requires light-adapted leaves) [9, 10]. φ_{psII} provides an indication of the amount of energy used in photochemistry [11]. The system was made up of four parts: array of light-emitting diodes, programmable power supply, MINIPAM and upper PC. Fig. 1 shows the schematic of photosynthetic ability measurement system.

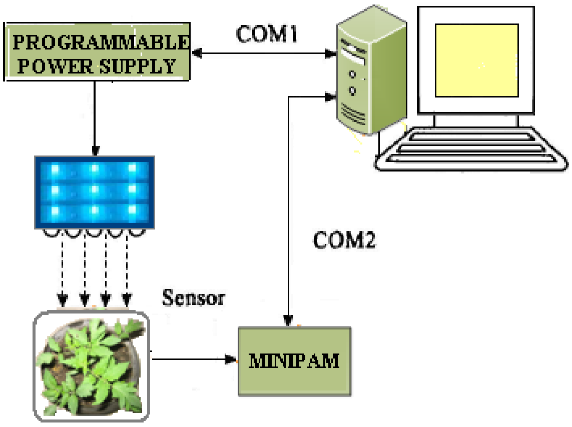

Fig. 1. Schematic of photosynthesis ability measurement system.

The image of the experiment carried out under laboratory conditions is shown in Fig. 2. The light which came from LEDs was considered measuring light MINIPAM was used to detect the parameters fluorescence. The output signal was collected by an upper PC and made out the results of current via man-made software. Then this current was sent to the programmable power supply via RS232 to control the luminous flux of LED.

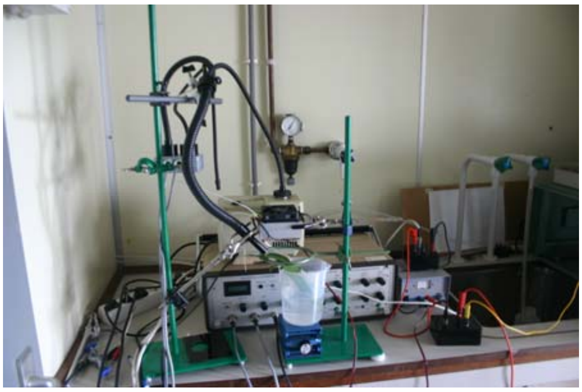

Fig. 2. Experiment under laboratory conditions.

2.2. Light-emitting Diode (LED)

The samples were irradiated by Luxeon 1 which was a kind of high flux light source (typical dominant wavelength: 625 nm, color: red, typical characteristics at 350 mA, junction temperature: 25 °C produced by Philip [12, 13].

The current used in this system ranges from 0 mA to 1 A. Fig. 3 shows the relationship curve between current and luminous flux of LED. It is noticed that as the current increases, the luminous flux of LED is linearly correlated. This experiment was carried out under laboratory conditions. This linear relationship between the ranges from 0 mA to 1 A can make the system more accuracy.

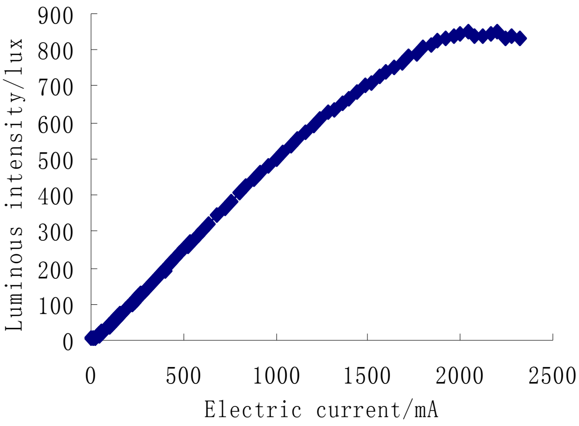

Fig. 3. Relationship curve between current and luminousflux of LED.

2.3. The Protocol between Power Supply and PC

Power supply can be connected to RS-232 interface by the DB9 plug on the back panel through the level switching circuit. The length of the frame is 26 bytes (compatible with FAB). The form is shown in Table 1.

Table 1. Frame format of power supply.

AAH.	Address	Command	Byte 4 to 25 are relevant information	Check

AAH occupies a byte. Address (01H in this system) occupies a byte. The 26th byte is check. It's the sum of the previous twenty-five bytes. Commands used in the system are as the followings:

80H set the upper limit of current, power and voltage level; 82 H controls the ON/OFF of the power supply. The current, voltage and power are all expressed in two bytes, with low byte in the front and the high byte behind. Examples:

(1) Set the parameters.

Max current 243 mA, max voltage 36 V, max power 108 W, voltage 24 V.

AA 00 80 F3 00 AO 8C 00 00 30 2A C0 5D 00 00 01 00…00 c2.

(2) Set control status.

PC control. Output ON: AA 01 82 03 00... 00 30

PC control. Output OFF: AA 01 82 02 00... 00 2F

2.4. Fluorescence Measurement

A RS-232 interface cable is provided to connect the MINIPAM to upper PC for operation under control-software. The MINIPAM can be operated by remote control from a PC terminal. For this purpose terminal-program must be installed and RS232 interface cable connected to the corresponding communication port. In custom applications it should be made sure that at last 50 ms elapse between two consecutively sent letters. The communication has lower priority than the measuring routines and at higher rates letters may get lost. For some commands the measuring program is transiently stopped. Hence, data transfer should not occur during measurements. The commands of MINIPAM are transferred in the form of ASCII finished by the byte of 0 DH (key of ENTER). For example, a command for operating MINIPAM to sent a saturation pulse. Firstly, the command of s is transferred to hex as 73 H. Secondly, the PC terminal sent 73 H to MINIPAM. Then the PC sent the finish byte 0 DH after 100 ms elapse.

The measuring principle of fluorescence parameters and steps is shown in Fig.4.

Firstly, the leaf after a thoroughly dark adaptation was illuminated under the test light, and the fluorescence parameters of Fo were gained after a short period of time (1 to 2 minutes). Then, a saturated pulse light was given and closed after a pulse in order to get the fluorescence parameters of Fm, and Fv/Fm was figured out which reflects the potential photochemical efficiency of PSII.

Secondly, the work light which can cause the effect of leaf photosynthesis is offered, after a few minutes when the leaf photosynthesis reaches a steady state, the fluorescence parameters of Fs is gained. At this time a saturated pulse light was given again and closed after a pulse to get the fluorescence parameters of Fm', then you can calculate the real quantum efficiency of PSII.

Thirdly, the work light was shut off, the far-red light was immediately opened and shut down after a few seconds, then get the fluorescence parameters of Fo', then you can calculate the coefficient of fluorescence photochemical quenching [14, 15].

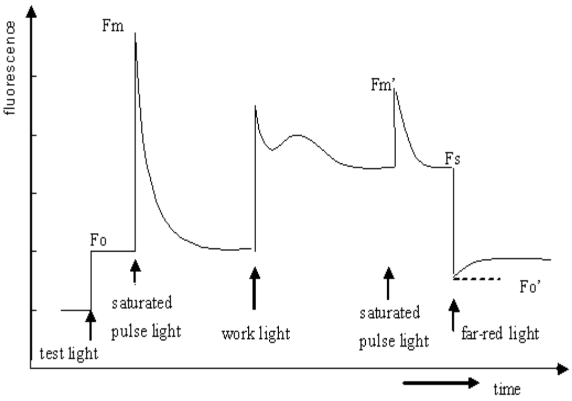

Fig.4. Measuring principle and steps of chlorophyll fluorescence parameters

3. Software

The software of traditional fluorescence detector and programmable power supply were supported by the producer. They were not changed for user's application easily. The system software is designed with VB6.

3.1. Set the Parameters

RS-232 communication should be set at first. The programmable power supply connected with PC by COM1. The MINIPAM connected with PC by COM2. Baud rate 9600. Then try to make a connection. If there is no connection, it would show a fault window. A lot of parameters can be set in the main window, such as the status of power supply, LED lighting time, measuring light intensity, saturation pulse intensity, saturation pulse width, current, wait time, desired φ_{psII}, error rate, etc.

3.2. Flow of the Software

The control status of power supply was set in Power On and PC Control. The current set in the software was transferred to the power supply in order to driving the LED array. Then set the parameters of MINIPAM, start saturation pulse, measure the result of yield after the waiting time that set by user.

In order to control the yield parameter timely, the software flow chart of auto-control model is shown In Fig. 5. Firstly, the desired yield parameter was put into the function Y=-0.0007 + 0.7748X, then transfer the first current X to the power supply to drive LED array. Secondly, the system operated MINIPAM to start a pulse of saturation, and collected the result of yield after delaying wait time.

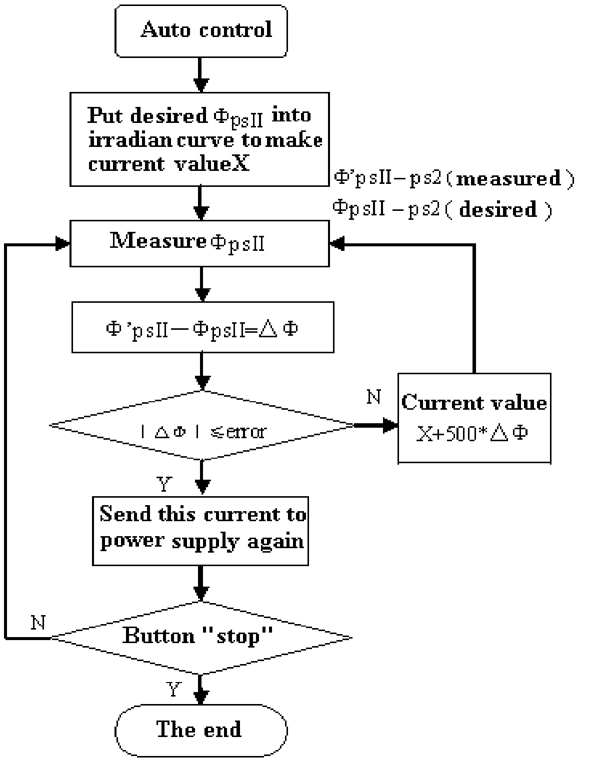

Fig. 5. Software flow chart of auto-control model.

Thirdly, φ'_{psII} -φ_{psII} =Δφ (φ'_{psII}: measured φ_{psII}, φ_{psII}: desired φ_{psII}). If the result of Δφ was bigger than the error rate set by the software, the Δφ would be put into the function X = X + 500 $\Delta\varphi_{psII}$ to make out the next current. If the result of Δφ was smaller than the error rate, current we used would continue to be sent to the power supply. The function we used in auto-control model made the φ_{psII} smooth rapidly.

4. Results and Discussion

Fig. 6 shows the relationship between φ_{psII} and current under laboratory conditions. The experiment was carried out under laboratory conditions (power time: 120 s, wait time: 2.5 s, sat pulse width: 1.4, ml-intensity: max, sat pulse intensity: max, temperature 25 °C). The increase of current was controlled by the upper PC software. As the irradiation intensity increases, the results of φ_{psII} were linearly correlated. The relationship curve shows that the system has high sensitivity and high accuracy.

Fig. 7 shows the interface of system under auto control model. The results of φ_{psII} were auto controlled by the upper PC software. The experiment was carried out under laboratory conditions (power time: 180 s, wait time: 2.5 s, sat. pulse width: 1.4, error rate: 0.01, ml-intensity: max, sat pulse intensity: max, desired φ'_{psII}: 0.6). The φ_{psII} curve under auto-control model shows that the system has high controllable ability and good reliability.

In all chlorophyll fluorescence parameters, the quantum yield can reflect the photosynthetic rate significantly. Multiple sets of physiological experiments showed that yield was affected by environmental factors significantly, especially temperature and light.

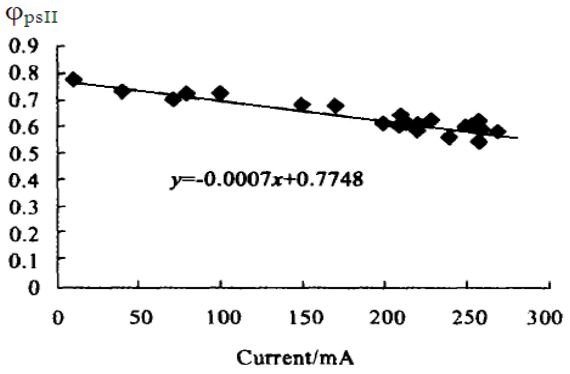

Fig. 6. Relationship between φ_{psII} and current under laboratory conditions.

In order to reveal the effects of environmental factors on the quantum yield, the temperature and light was changed in artificial methods, the curve of the yield under different temperature and illumination was shown in Fig. 8 [16-18].

As the temperature dropped from 15 °C to 0 °C, chlorophyll fluorescence parameters yield was declined, and the lower the temperature, the stronger the light, the greater the change in the magnitude of yield values, while it is kept stable level in other condition. It was demonstrated that photoinhibition occurred in leaves under low temperature [19, 20].

The experimental results also indicate that fluorescence parameters (especially yield and Fv/Fm) change significantly under low temperature stress. The relationship between fluorescence parameters and temperature is deduced. Effect of low temperature on photochemical efficiency (Fv/Fm) is shown in Fig. 9.

Thus it can be seen that low temperature stress caused damage to photosynthetic mechanism, in addition, photoinhibition occurred in the plant. As a result, the ratio of Fv/Fm is decreased obviously under low temperature stress.

Effects of low light on $\varphi_{PS\ II}$ in plant is shown in Fig. 10. It reflects the inactivation of PSII reaction center, reflecting the weakening of the PSII potential activity and solar energy conversion efficiency [21].

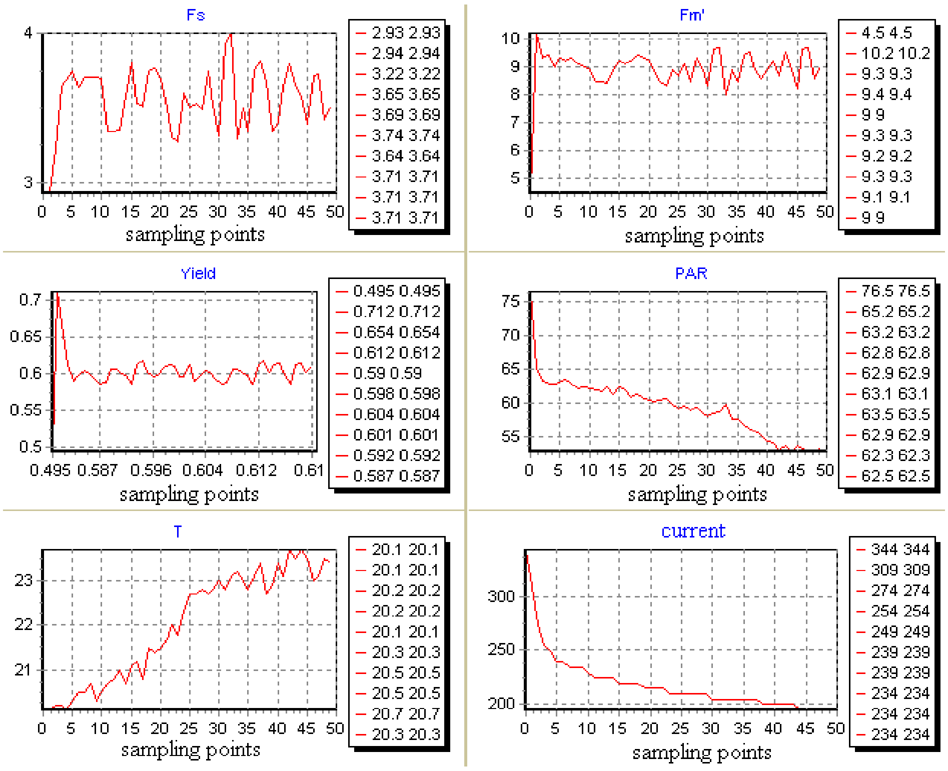

Fig. 7. Interface of system under auto-control model.

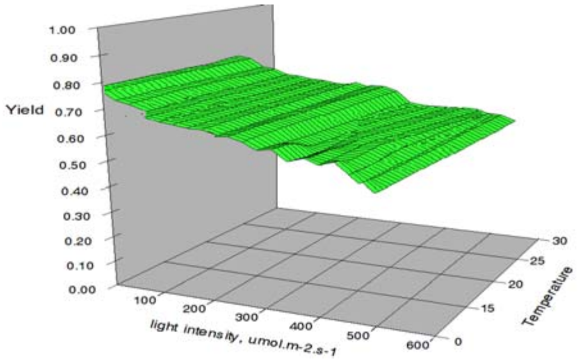

Fig. 8. Yield change under different temperature and light.

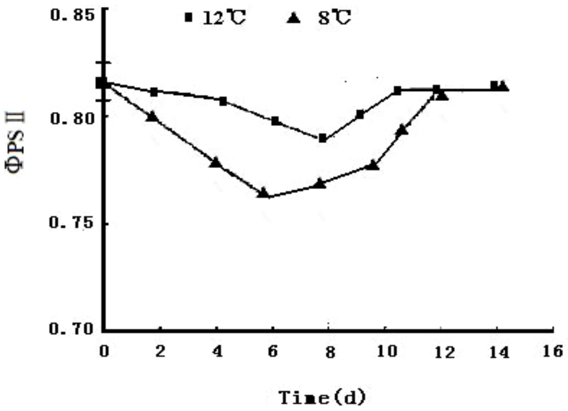

Fig. 10. Effects of low light on $\varphi_{PS\ II}$ of plant.

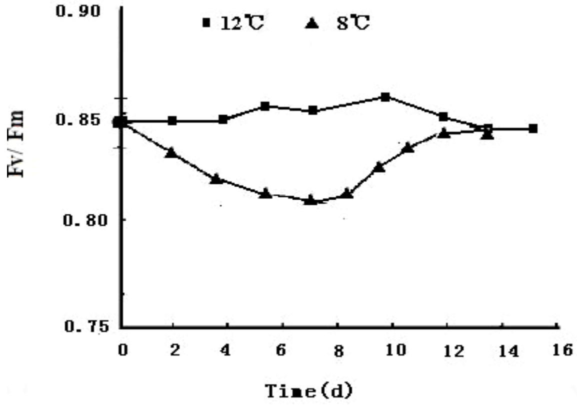

Fig. 9. Effects of low light on photochemical efficiency (Fv/Fm) in plant.

5. Conclusion

In this paper, a new test and control system for chlorophyll fluorescence was proposed, which using LED excitation by means of the fluorescence parameter φ_{psII}. The results indicated that, comparing with traditional method, the system can accurately measure the plant photosynthetic ability with minimal influence of the environment. The upper PC software can be operated easily for man-made experiment. Therefore it is likely that the system will be likely to provide a new approach for measuring the plant photosynthesis ability. In all chlorophyll fluorescence parameters, the quantum yield and F_v/F_m was affected by environmental factors significantly. It was demonstrated that photoinhibition occurred under low temperature stress, showing decrease of photosynthesis and quantum yield.

Acknowledgement

This work was financially supported by Liaoning Provincial Department of Education Project (No. L2012253).

The authors would like to acknowledge the Institute of Horticulture, Shenyang Agricultural University, China for their cooperation in performing these experiments in their greenhouse. The authors also wish to thank Dr. Jeremy Harbinson for his great support.

References

[1]. Shen Y. G., The important chemistry reaction on earth-photosynthesis, *Qinghua University Press,* Beijiing, 2000, pp. 84-85

[2]. Beer S. B., Vilenkin A., Weil M., Veste L., Eshel S., Measuring photosynthetic rates in seagrasses by pulse amplitude modulated (PAM) fluorometry, *Mar. Ecol. Prog. Ser,* 174, 1998, pp. 293-300.

[3]. Genty B., Briantais J. M., Baker N. R., The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence, *Biochimica Biophysia Acta*, 990, 1989, pp. 87-92.

[4]. Schreiber U., Bilger W., Neubauer C., Chlorophyll fluorescence as a non-destructive indicator for rapid assessment of in vivo photosynthesis, *Ecological Studies*, 1, 1994, pp. 49-70.

[5]. Hawes I., Sutherland D., Hanelt D., The use of pulse amplitude modulated fluorometry to determine fine scale temporal and spatial variation of in situ photosynthetic activity within an Isoetes-dorninated canopy, *Aquat Bot*, 77, 2003, p. 115.

[6]. Peter J. Ralph, Rolf Gademann, Rapid light Curves: A powerful tool to assess photosynthetic activity, *Aquatic Botany,* 82, 2005, pp. 222-237.

[7]. Li Bing-Qian, Bu Liang-Ji, Gan Xiong-Wen, Research on the relationship of the change in forward voltage with temperature of light emitting diode, *Acta Photonica Sinica*, 32, 2003, pp. 1349-1351.

[8]. Wang Cheng-long, Xing Da, Chen Qun, A novel method for measuring photosynthesis using delayed fluorescence of chloroplast, *Biosensors and Bioelectronics,* 20, 2004, pp. 454-459.

[9]. Gerjo J. Engbers, Influence of spectral light distribution and the photosynthetic acclimation to light of a tomato crop, *Wageningen University*, 2006.

[10]. Horton P., Hague A., Studies on the induction of chlorophyll fluorescence in isolated barley protoplasts. IV. Resolution of nonphotochernical quenching, *Biochim, Biophys. Acta,* 932, 1998, pp. 107-115.

[11]. Ralph P. J., Gademann R,. Larkum A. W. D., Spatial heterogeneity in active fluorescence and PSII activity of croal tissues, *Mar. Biol,* 2002, 141, pp. 539-646.

[12]. Lian M., Murthy H. N., Paek K., Effects of light emitting diodes (LEDs) on the in vitro induction and growth of bulblets of Lilium oriental hybrid ‘Pesaro’, *Scientia Horticulturae,* 94, 3–4, 2002, pp. 365-370.

[13]. Ilieva I., Ivanova T., Naydenov Y., et al., Plant experiments with light-emitting diode module in Svet space greenhouse, *Advances in Space Research,* 46, 7, 2010, pp. 840-845.

[14]. Kolber Z., Klimov D., Ananyev G., Rascher U., Berry J., Osmond B., Measuring photosynthetic parameters at a distance: laser induced fluorescence transient (LIFT) method for remote measurements of photosynthesis in terrestrial vegetation. *Photosynthesis Research*, 2005, 84 (1-3), pp.121-129.

[15]. Pang S. J., Jin Z. H., Sun J. Z., Gao S. Q., Temperature tolerance of young sporophytes from two populations of Laminaria japonica revealed by chlorophyll fluorescence measurements and short-term growth and survival performances in tank culture, *Aquaculture*, 2007, 262, 2-4, pp.493-503.

[16]. Hirotsu N., Makino A., Yokota S., Mae T., The photosynthetic properties of rice leaves treated with low temperature and high irradiance, *Plant Cell and Physiology*, 2005, 46 (8), pp.1377-1383.

[17]. Trouwborst G., Schapendonk A. H. C. M., Rappoldt K., et al., The effect of intracanopy lighting on cucumber fruit yield, *Model Analysis, Scientia Horticulturae,* 2011, 129 (2), pp.273-278.

[18]. Chen Q-J., Zhang F-M., Wang Y-J., Kurata K., The physiologic reaction of cucumber to low temperature and low light intensity. *Agricultural Sciences in China*, 2003, 2 (2), pp.200-205.

[19]. Houter N. C., Pons T. L., Gap size effects on photoinhibition in understorey saplings in tropical rainforest, *Plant Ecology*, 2005, 179, 1, pp.43-51.

[20]. Becker S., Graeve M., Bischof K., Photosynthesis and lipid composition of the Antarctic endemic rhodophyte Palmaria decipiens: effects of changing light and temperature levels, *Polar Biology,* 33, 7, 2010, pp. 945-955.

[21]. Nichol C. J., Rascher U., Matsubara S., Osmond B., Assessing photosynthetic efficiency in an experimental mangrove canopy using remote sensing and chlorophyll fluorescence, *Trees,* 2006, 20, 1, pp.9-15.

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