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生きている植物の葉面電位計測システムの開発

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キーワード：生体計測、生体応答、葉面電位、周波数応答特性、環境応答

１．はじめに

植物の最適な栽培管理にとって、変動する生育環境に対する植物の生体応答の計測は重要である。生体応答のひとつである葉面電位応答は、光合成や水分生理などに関係する植物の生理活性を総合的に表象すると考えられている。従来、葉面電位の計測システムとして、針電極等を用いた計測システムが開発されている。

しかしながら、従来の計測方法は植物に対して侵襲的であるため、長期の安定した計測が難しいといった問題がある。本研究は、このような問題を鑑みてなされたものであり、長期計測を安定して実施できる葉面電位計測システムを開発するとともに、明暗の光周期刺激に対する葉面電位変動の周波数応答特性を求めることを目的とする。

２．実験方法

本研究では、葉面電位計測システムを①電極②電圧増幅器③低域周波数フィルタ④レコーディングシステムから構成した。実験に用いた供試植物は黄金花月（ベンケイソウ科）であり、鉢植えの黄金花月をチャンバー（温度２３度、明期の光強度２５０００Lx）に設置した。明暗の光周期は、実験区として１２時間周期（６時間明期－６時間暗期)、２時間周期、４０分周期、１６分周期、及び２分周期の 5 区を設定した。なお、明期と暗期の時間比率は、すべて１：１である。これらの光周期に対する葉面電位応答を１週間連続して計測した。

３．結果と考察

本計測システムでは、電極として皿電極（心電図用電極）を用いたため、計測部位である葉表面を傷つけなかった。このため、長期間（１週間程度）の計測を安定して実施可能であった。

葉面電位は光点灯直後に、まず上下に大きく変動し、そして約４時間後には光点灯前の電位レベルに戻った(図１)。また、葉面電位は、光周期に応じて周期的に変動した。

時系列データとして得られた各実験区のそれぞれの計測結果を FFT 解析したところ、葉面電位変動のピーク周波数は各実験区における光周期に一致した。また、光周期を入力信号、葉面電位変動を出力信号とし、入力信号と出力信号との振幅比（ゲイン）を FFT 解析の結果から求めた。ゲインは、４０分周期と１６分周期の光周期区において他の周期区よりも高かった（表１）。

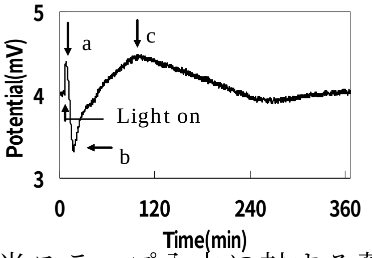

図 1 光ステップ入力に対する葉面電位応答

Illumination On-Off Interval	Peak Frequency (Hz)	Gain(dB)
60min-60min	0.000137	-4.6
20min-20min	0.000407	0.6
8min-8min	0.001057	2.1
1min-1min	0.008300	-11

表１　各実験区のピーク周波数とゲイン

MEASURING THE LEAF ELECTRICITY OF LIVING PLANT

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Abstract: Frequency response of Cactus leaf electricity to oscillated illumination stimuli was measured. For this purpose, a sensing system enabling to long term and stable measurement was developed. The illuminations were 1minute light – on and 1minute light – off, 8minutes light – on and 8minutes light – off, 20minutes light – on and 20minutes light – off, and 60 minutes light – on and 60 minutes light – off. The result showed that the response was similar to that of a low pass filter.

Keyword: Plant, Light, Response, Electrical behavior, Sensor systems.

1.　INTRODUCTION

Speaking Plant Approach (SPA) is an attractive method for investigating what the plant needs under variations in its environment changes. The SPA requires the collection of in-situ data from a living plant.

Therefore understanding more about living plants with modeling or system identification is encouraged. (Morimoto, *et* al., 1988)

Leaf surface electricity, as bioelectricity, generally rooted in physiological activity. (Kano, *et* al., 1988). Kano and Yamaguchi (Kano and Yamaguchi, 1996) reported correlation between freshness and electric potential voltage on plant surface. Leaf electricity is composed of AC and DC ingredients. DC ingredient especially fluctuated when the illumination was changed. DC was suggested to have a relation to photosynthesis activity. (Utamaru *et* al., 1999).

There were many reports about the measurement of the bioelectricity of plants by using fine needle electrodes, which tended to injure or destroy plant tissues and cells. This kind of measurement is not available for investigating long term behavior regarding to plant activities.

The objectives of this paper are:

(1) Measuring of leaf electricity on intact plant by developing a sensing system to enable a long term stable measurement.
(2) Detecting electric variations or fluctuations by oscillating illumination stimuli.

２. EXPERIMENTAL MATERIALS AND METHOD

The experiment was aiming at getting the response data of leaf electricity as output signal to illumination interval changes. Experimental system is shown in Fig. 1.

2.1 Test Plant

The test plant was Crassula, a kind of cactus whose photosynthesis type is generally CAM. Crassula leaves was tough, flat and smooth. This made it easy to be fairly pasting of sensing devises on the leaf surface. The test plant in a flowerpot was kept in a biophotochamber.

2.2 Biophotochamber.

Light intensity level of fluorescent lamps in chamber was 25000 lux. Temperature was kept at 23 degree Celsius.

2.3 Measuring System

Leaf electricity; The system set-up was composed of four parts; sensor devices to detect the electric potential on a leaf surface, an amplifier to magnify output from the sensors, a low pass filter for both reducing noise levels and cutting high frequency domain. Final component was pc-card recorders with a lap top computer to storage the data in.

The sensor devices were assembled from three electrodes. The two electrodes were put on sensing points. The distance between the two electrodes was about 1cm on the same leaf surface. The last one was put on the stem nearby ground surface as an electric reference point. Electric potential on leaf surface detected the two electrodes is measured referenced the stem point.

The Ag/AgCl vitrode disposal electrodes were plate type electrodes. They had no needles pricking the test plant. The electrode was placed on a solid gel plate. Ion concentration in the gel generated the electric potential to be detected. The gel plate mainly composed of water and acrylic glycerin enabled long-term measurement.

The instrumentation amplifier, an IC chip with a high common-mode-rejection-ratio of 120 dB for effectively reducing noise levels, was used. Resistance connected to IC chip could change the gain of amplifier. In this experiment, no resistance was observed in order to reduce noise levels as much as possible. Then, the gain resulted in one. The amplifier worked to change the high impedance of test plant to a lower level.

Changeable resistance and ceramic condenser worked as a low pass filter to avoid unexpected electro magnetic noise such as commercial radio wave, 50 Hz in Tokyo, and pass the leaf electricity signal. Cut off frequency (f_c) was obtained by the equation:

$$f_c = 1/ 2\pi \mathrm{RC} \qquad (1)$$

In this experiment, f_c was 1.6 Hz by a 100kΩ resistance and a 1μF condenser.

Frequency response of this sensing system had a good behavior in both low frequency and cutting high frequency domains over about 1 Hz. The noise after passing the filter and Imperfection of white noise used as test input signal made roughness of the Gain as shown in Fig. 2.

Environment; Illumination timing was measured by a photo sensor. This was made of solar battery panel. When the light was on, it generated 1 mV signals, and when the light was off, it become zero. Temperature in the biophotochamber was recorded by a thermo-recorder.

2.4 Experimental Plot

Illumination intervals were provided as follows:

(1) 6 hours light – on and 60 hours light – off.
(2) 1 minute light – on and 1 minute light – off.
(3) 8 minutes light – on and 8 minutes light – off.
(4) 20 minutes light – on and 20 minutes light – off.
(5) 60 minutes light – on and 60 minutes light – off.
Each experiment was repeated several times.

4. RESULTS AND DISCUSSION

FFT analyses was applied to the time series data with hamming window. Bias trends were estimated by least square method and then eliminated.

Leaf electricity obtained tended to show typical three stages; non-steady and fluctuated pattern of electricity for the first measurement stage, followed by the steady state stage of a continued crisp pattern, and the attenuating stage as the pattern was damped into a flat level. In the steady sate stage, repeatability of a typical pattern was confirmed in the leaf electricity, which can be provided for the data analysis. The period of each experiment was consequently available up to 5days.

4.1 Response to Stepwise Illumination

Transient response to a stepwise illumination continuing 6 hours was observed as shown in Fig. 3. The three peaks (a, b, c in Fig. 3) were typical futures with good repeatability. When the light was turned on, the leaf electricity rapidly moved to peak a in about 3 minutes and to peak b in about 15 minutes. Then it recovered and got to peak c in 120 minutes. A level of peak c was almost equal to that of peak a. after peak c, the electric potential moderately decreased to the former level observed at illumination start in 300 minutes. A ratio of the time intervals between peaks a to band peaks b to c was about 1 : 8.

A magnitude of potential difference between peaks a to b and peaks b to c was equal to that between peaks b to c.

A result of FFT analysis on the data in Fig. 3 is shown in Fig. 4. The figure indicates that frequency response of leaf electricity was similar to that of a low pass filter, especially it has relatively higher gain between 0.0006Hz and 0.001 Hz.

4.2 Response to Cyclic Illumination

Leaf electricity under variable illumination cycles was shown in figure. 5. Fluctuations in leaf electricity were similar to a sinusoidal wave for 1-1 min ON-OFF interval, 8-8min interval and 20-20 min interval, while for 60-60min interval, leaf electricity varied along a long-term wave pattern with short-term spike.

Only in 8-8min interval, leaf electricity showed minus. It meant that potential of one sensing point which inputted to amplifier as negative voltage were larger than another potential which inputted as positive voltage. Then the potential difference between the two points was outputted negative.

When the illumination was changed light-ON to light-OFF, leaf potential difference became a little larger in 2 minutes, while 10 seconds under 1-1min illumination interval. Then the potential difference became smaller in 6 minutes. After this, leaf potential difference became larger again, under 20-20min interval and 60-60min intervals.

When the illumination was changed light-OFF to light-ON, the potential difference became larger under 8-8 min and 1-1min intervals. Under 20-20 min interval, the potential difference also became lager and continued to be large in 5 minutes. In the 60-60 min interval, the behavior was similar to stepwise response.

The results of FFT analysis are shown in Fig. 6. Clear Peaks appeared corresponding to illumination interval frequency. An envelope curves of peaks slope down to high frequency. The slope of leaf electricity was sharper than that of illumination.

Characteristics of frequency response to cyclic illumination are shown in Table 1. Peak frequencies correspond to illumination intervals. A level of gain indicated that the ratio of the amplitude of leaf electricity for peak frequency to that of illumination interval.

The numerical values of gain don’t make sense. Because the amplitudes of leaf electricity and illumination depends on sensor settings. But the size variation of gain over different frequencies is significant.

8-8min and 20-20min intervals (0.000977Hz and 0.000407 Hz) showed higher gain than other intervals. This result fitted to Fig.4, roughly.

5.CONCLUSION

Leaf electricity on intact plant was observed with a sensing system enabling a long term and stable measurement

Frequency response of leaf electricity to illumination interval was similar to that of a low pass filter.

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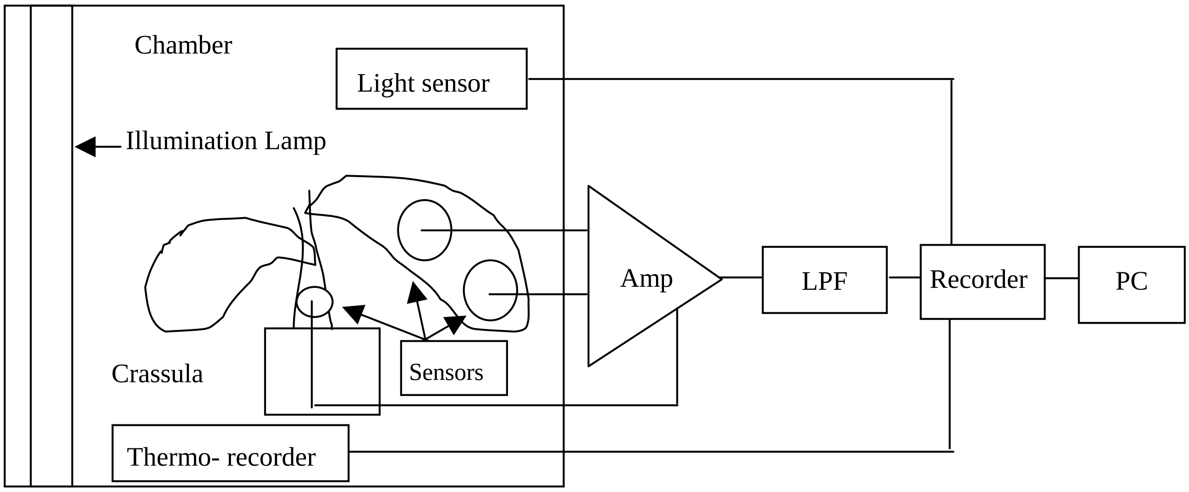


Fig. 1. Experimental system.

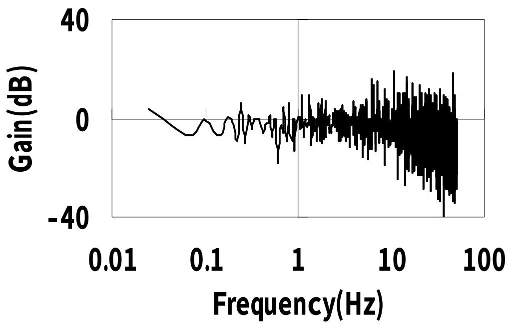


Fig.2. Gain of the sensing system.

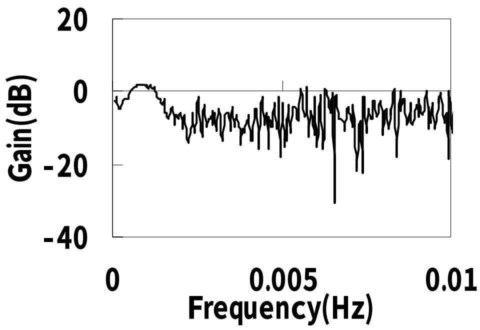


Fig. 4. Frequency response of the leaf electricity.

Table 1 Response to cyclic illumination

Illumination On-Off Interval	Peak Frequency (Hz)	Gain(dB)
60min-60min	0.000137	-4.6
20min-20min	0.000407	0.6
8min-8min	0.001057	2.1
1min-1min	0.008300	-11

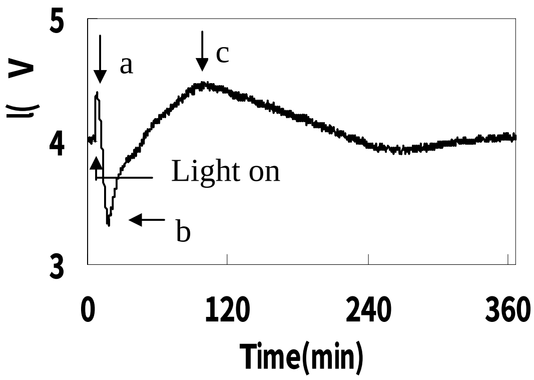
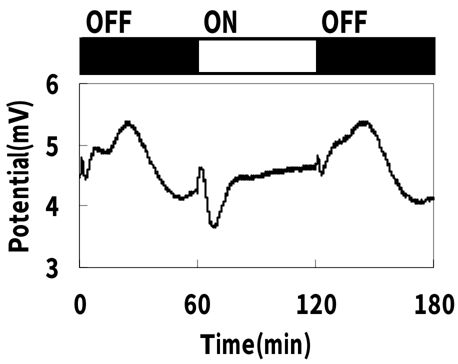
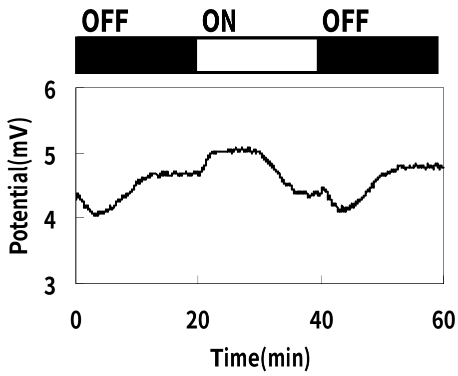


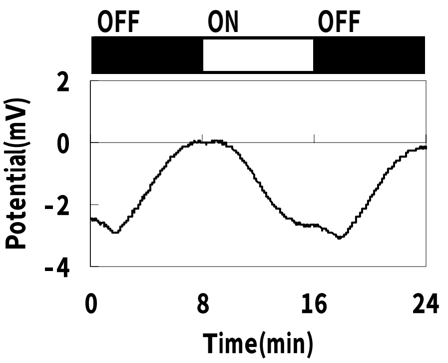
Fig. 3. Response to stepwise illumination.



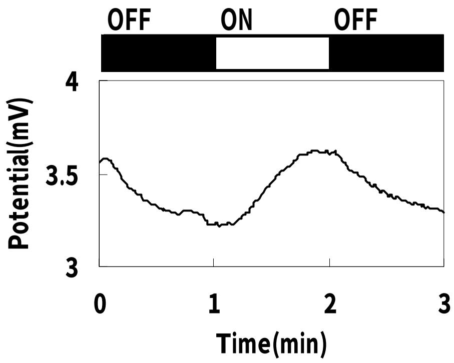
(a) 60-60 min interval



(b) 20-20 min interval

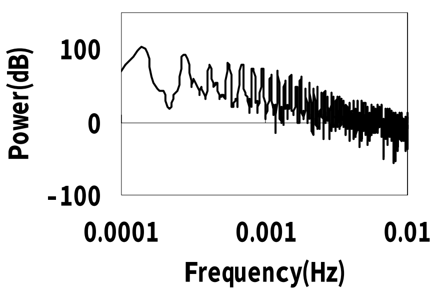


(c) 8-8 min interval

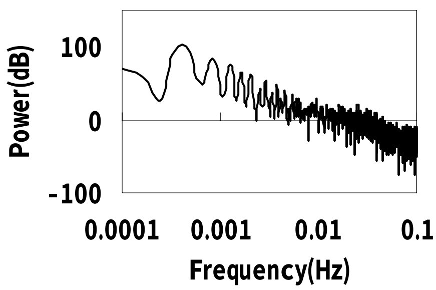


(d) 1-1min interval

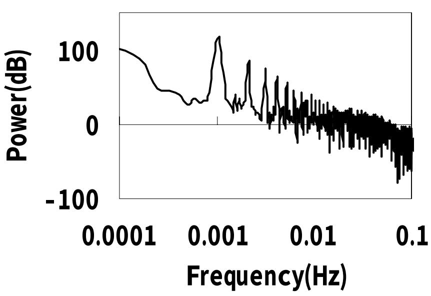
Fig. 5. Leaf electric potential under variable illumination cycles.



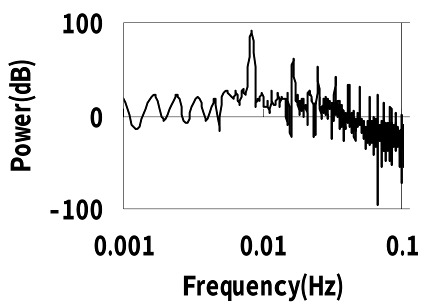
(a) 60-60 min interval



(b) 20-20 min interval



(c) 8-8min interval



(d) 1-1 min interval

Fig. 6. Power spectrum of leaf potential under variable illumination cycles