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Evaluating Abnormal Condition in Physiological Disorder Using a Fluctuation Characteristic of Plant Bioelectric Potential

植物生体電位の揺らぎ特性を用いた生育障害の評価

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Summary

For maintaining a steady food supply, the trend has been toward developing factory-like production methods in Japan. To date, there is no indicator that can predict growth status of crops. We have accordingly focused on the bioelectric potential inside the plant, which could be an indicator of plant growth. However a few researches have been conducted on its role in determining plant growth status. In this study, we aimed to use plant bioelectric potential as a means of determining growth conditions. Fluctuation characteristics in plant bioelectric potential frequency components should provide an indication of conditions between normal and inhibited growth. We discuss that different growth condition factors were identified using logistic regression analysis. This experiment yields that a discrimination rate of 86.6% was obtained using the calculated logistic regression model between these conditions.

キーワード：植物生体電位，生育評価，最大エントロピー法，揺らぎ特性，ロジスティック回帰分析

Keywords : plant bioelectric potential, evaluating growth condition, maximum entropy method, fluctuation characteristics, logistic regression analysis

1. Introduction

Recently, both the supply and safety of food have become increasing concerns, owing to environmental issues.1) At the same time, the decline in the agricultural population and the low food self-sufficiency ratio are also becoming an issue in Japan. According to estimates by the Ministry of Agriculture and Fisheries in 2010, Japan's self-sufficiency rate was 39% by its calories and the elderly make up 60% of the agricultural population. 1,2) The productivity per unit area in Japan is high compared with those in other countries, owing to the skill of seasoned farmers. 3) However, the impending retirement of these skilled farmers, approximately 62% of whom are over 65 years old, is a serious concern. Therefore, to maintain and improve self-sufficiency, the productivity of young and inexperienced farmers needs to increase.

The difference between skilled and inexperienced farmers is the ability to make considered judgments.4) Skilled farmers based on their experience can respond to the growth status of their crops. For example, they can diagnose plant diseases by visual inspection and determine the appropriate treatment. Making a sound decision about plant growth conditions requires much background knowledge regarding plants. Nowadays, evaluating the freshness and quality of crops has to depend on inspection by skilled practitioners, which necessitates determining optimal growth conditions

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Fig.1 Photograph of subject plant.

by objective evaluation. If standardized production can be conducted in the point of production using the objective evaluation, farmers become available for steady supply of food owing to solution of these issues. In this study, we discuss that information in plant is applied to the objective evaluation of growth conditions.

There are several methods available for measuring parameters necessary for growth, including nitrogen levels and dryness. [4] These are all invasive methods that require collecting part of the plant for analysis; therefore, it is difficult to use these methods while growing crops. For this reason, noninvasive means of evaluating growth such as chlorophyll fluorescence or 3D diagnostic imaging have become a part of agricultural research. [5] However, all these methods are useful only after the conditions have already deteriorated. They cannot be used to make predictions before the condition has deteriorated. The use of bioelectric potential as a noninvasive means of evaluating growth is being investigated. The plant bioelectric potential is the potential difference caused by variations in the concentrations of ions in the plant cell membranes. It has been shown to vary according to environmental conditions and the growth process.[6-9] However, little research has been conducted on the bioelectric potential as an indicator of crop growth.[10, 11] If the bioelectric potential inside the plant could provide direct information about the physiology and surrounding environment, this information could be applied to effectively manage the growth environment.

We have been studying the use of plant bioelectric potential as a means of determining growth state, using the already established chlorophyll fluorescence as a standard. Previously, we tested the suitability of bioelectric potential measurements under conditions that would definitely damage plant growth. The results showed that there was a difference in the frequency components of the bioelectric potential before and after spraying plants with herbicide, which means physiological disorder.[12]

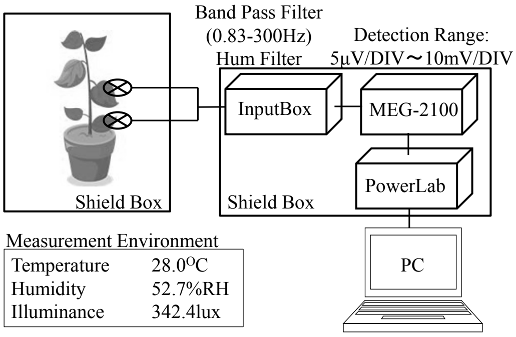

Fig.2 Measurement system in this experiment.

In this study, we evaluated bioelectric potential frequency components using chlorophyll fluorescence as a standard. A discriminatory model was formulated on the basis of the logistic regression analysis of frequency components calculated from fluctuation characteristics in the bioelectric potential as compared with an abnormal condition. A system of diagnosing growth based on the bioelectric potential inside the plant may aid in determining the growth state before this becomes visually apparent on the surface of the plant.

2. Experimental Methods

2.1 Experimental Plant

The easily cultivated ornamental plant pothos (*Epipremnum aureum*) was used for this study. It grows well over a wide range of temperature, has broad leaves, and is amenable to experimentation. Figure 1 shows a photograph of this plant. Six sample plants were used in the experiment and controlled in the same environment. After the initial measurements, herbicide was sprayed on each of the plants, and analysis was performed on the plants that had significantly different chlorophyll fluorescence values

2.2 Experimental System

A simplified diagram of the plant bioelectric potential measuring equipment is shown in Fig. 2. The recording electrode came from an electroencephalogram (EEG) (NE-117A, NIHONKODEN). The electrode was attached to the leaf surface of the plant using an electrically conducting paste (Z-401CE, NIHONKODEN). The ground was placed in the soil in the plant pot. Because the plant bioelectric potential is very small, the potential from the electrode was amplified by a differential amplifier (MEG-2100, NIHONKODEN) through an input box (JB-220J,

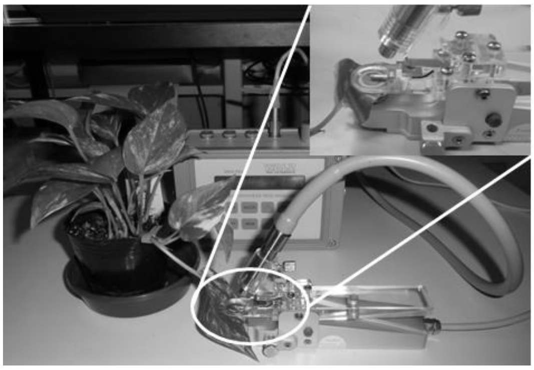

Fig.3 Photograph of measurement of chlorophyll fluorescence by MINI-PAM.

NIHONKODEN). A filter circuit was incorporated into the amplifier to remove noise. To remove noise from the utility power, a band-pass filter (0.08–300 Hz) and a HUM filter were used. The amplified potential was converted to a digital signal using an A/D converter and interfaced to a PC, which analyzed the data. The sampling frequency for the bioelectric potential was 100 Hz. The measurement time was 1 min and each plant was measured 16 times. The measurement period was 2 weeks. Because the bioelectric potential is so small, the effect of noise was a concern. All measuring equipment and plants were accordingly measured in a shield box. We blocked out light in the experiment room and experimented under the under the fluorescent light to remove sunshine affects among measurement time. The illuminance (lux) was approximately 340 lux in the time.

2.3 Experimental Agricultural Chemical herbicides

The herbicide used for the experiment was Nekosogi Quick Pro (Rainbow Pharmaceutical Co., Ltd., registration number 21593). The liquid herbicide (15 ml) was diluted 100 times, in accordance with the specifications. The amount of herbicide used was based on the volume of the pot, which was calculated from the internal diameter of the pot (9 cm; pot 3). The herbicide (1.17 ml) was sprayed twice onto the surface of the leaves on days 1 and 9 of measurement.

3. Methods of Analysis

3.1 Chlorophyll Fluorescence

Growth inhibition should become apparent 3–4 days after the herbicide application. This is the time required for the suppression of growth factors in the plant particularly vital amino acids and proteins. Chlorophyll fluorescence is used to measure by the noninvasive means of evaluating the photosynthesis. The chlorophyll fluorescence value is expressed as the plant growth conditions and the value of approximately 0.7 indicates that the plant is healthy. This response was taken as an indicator for evaluating growth.13) In this experiment, measurements were made before and for 1 week after herbicide application. The fluctuations in electrical potential were evaluated with reference to changes in the chlorophyll fluorescence value. The chlorophyll fluorescence was measured using a pulse-modulator-type chlorophyll fluorescence meter (MINI-PAM, WALZ). A photograph of this measurement procedure is shown in Fig. 3. Chlorophyll Y (*Fv*/*Fm*) was measured on the surface of the leaves to which the plate electrodes for electric potential measurement were attached. *F* refers to the fluorescence intensity (photon emission reaction rate). *Fv*/*Fm* is an index for chlorophyll fluorescence based on a typical saturation pulse measurement. It represents the largest quantum yield for the photosynthesis II chemical pathway, as calculated using eq. (1).

$$Fv / Fm = \frac{(Fm - F_0)}{Fm} \tag{1}$$

Here, F_0 is the instantaneous minimum fluorescence yield captured between small fluctuations. *Fm* is the maximum fluorescence yield. *Fv* is calculated from the difference between F_0 and *Fm*. Because of variations in the measurements, six replicates were recorded.

3.2 Fluctuation Characteristics in Plant Bioelectric Potential

In this section, we describe the analysis of data collected according to the method explained in §2.2. Time series data for bioelectric potentials can vary considerably owing to artificial stimuli such as the amount of light or direct contact.7, 10-12) The tiny and irregular wave forms from internal sources are accordingly difficult to detect.

Prior attempts to find a correlation between the plant bioelectric potential and the plant physiological response have been made by exposing the plant to different environmental pollutants.14-16) These studies showed a correlation between pollutant gases and bioelectric potential. After injection of pollutants, the power spectrum showed a reduction. In this present study, we also focused on frequency component analysis as a means of measuring correlations particularly the use of fluctuation characteristics in frequency components as a discrimination model. The fluctuation analysis method is shown in Fig. 4. First, the bioelectric potential signal frequencies are determined, and a

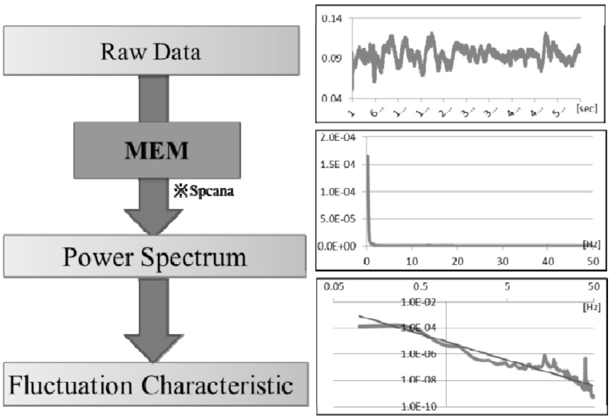

Fig.4 Analysis approach for fluctuation characteristics.

straight line is approximated using both logarithmic axes. The characteristic values are used as a means of formulating a model that can identify the slope and the tangent of the approximate straight line calculated. In the frequency analysis, we did not use conventional fast Fourier transformation (FFT), instead, we used the maximum entropy method (MEM).17) MEM for limited data provides a spectral estimate by maximizing the autocorrelation function entropy. The method was used by the Spcana which is the software for frequency analysis 18) in this study because it is suitable for time series data over a short time frame and for measurement with high noise levels.

3.2 Logistic Regression

Logistic regression is a method of analysis that models an explanatory sigmoid curve using explanatory variables to explain objective variables. The objective variables are represented by categorical variables. It is one method of multivariate analysis used to construct a model for the presence and absence of certain events, such as disease outbreaks, car accidents, or earthquakes.19, 20) Logistic regression analysis was chosen for its special features. The explanatory variable need not follow a normal distribution, and the method also allows the effect of the objective variable on each explanatory variable to be determined. These features make it useful for identifying different parameters. The state of the plant was fitted as an objective variable (see below), based on the slope and intercept of the explanatory variable.

- 0: Growth inhibited
- 1: Growth normal

The growth conditions were characterized using individual differences in chlorophyll fluorescence on the basis of a model derived from logistic regression analysis to generate new test data.

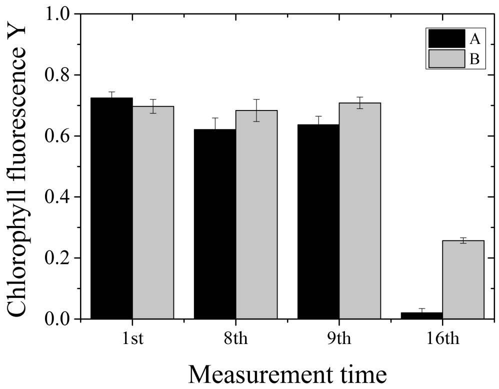

Fig.5 Chlorophyll fluorescence of subjects A and B as a function of measurement time.

4. Experimental Result

4.1 Chlorophyll Fluorescence

Figure 5 shows individual differences in chlorophyll fluorescence produced by the herbicide, and the data represents the mean and standard deviation of chlorophyll fluorescence. The first data set was recorded before application of herbicide, and the eighth was recorded 1 week after application. The ninth set of measurements was made before a second application of herbicide, and the sixteenth was recorded 1 week after the second application. There were two herbicide applications, and the data provide a comparison between 1 week before and after. For two of the six plants, the chlorophyll fluorescence after the second herbicide application fell to less than 0.4, and there was a significant difference between the ninth and sixteenth data points. (11) The chlorophyll fluorescence value decreased markedly on the sixteenth. The Surface color of leaves on the sixteenth turned from a green to a pale green or yellow. In contrast, there was not a significant difference among the other four subject plants. The fluctuation characteristics in the bioelectric potential in two plants, A and B, were evaluated.

4.2 Plant bioelectric Potential

The fluctuation characteristics in the two plants that showed a significant difference in chlorophyll fluorescence were calculated for each 4-day period. The distribution of these fluctuation characteristics is shown in Fig. 6. The vertical axis shows the slope and the horizontal axis shows the tangent in these characteristics. The data groups for the fluctuation characteristics on day 16 (marked with a ▼) tend to be grouped at the top left, compared with the points

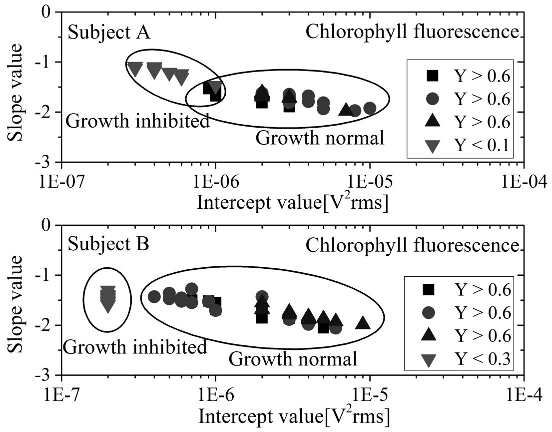

Fig.6 Scatter plots in fluctuation characteristics (top: subject A, bottom: subject B).

for the other 3 days (1st marked with a square, 8th marked with a circle and 9th marked with a triangle). The data for day 16 show the effect of growth inhibition, which caused the chlorophyll fluorescence to drop below the standard value of 0.4. In this study, we assumed that the data for 3 days (1st, 8th, 9th) is growth normal condition in which the values were above 0.6 and the data for day 16 is growth inhibited condition because of the half value of approximately 0.7 which is indicated in healthy condition.

Figure 7 shows the approximate the curves generated from the fluctuation characteristics over 4 days. The approximate curves from chlorophyll fluorescence values of 0.4 or less (dashed line) are different from the curves for which the chlorophyll fluorescence value exceeded 0.6.

The above results show that the sixteenth data set represents growth inhibition, whereas the sets for the other 3 days show normal growth. Linear discrimination is achieved by the use of logistic regression analysis.

4.3 Logistic Regression Analysis

The fluctuation characteristics of the two plants (A, B) described in the previous section were subjected to logistic regression analysis by first analyzing the data. Three explanatory variables were compared: the slope, the intercept, and both variables. The results are shown in Table 1-3. This table shows that the discrimination rate was higher when both variables, rather than the fluctuation characteristics, were used. The calculated logistic regression equation is shown below as eq. (2).

$$\log \frac{p}{1-p} = 1.130 x_{\mathrm{int}ercept} - 12.877 x_{slop} + 53.297 \quad (2)$$

Next, the test data were identified using Eq. (2), and the growth status of the test data (C, D, E, F) was determined.

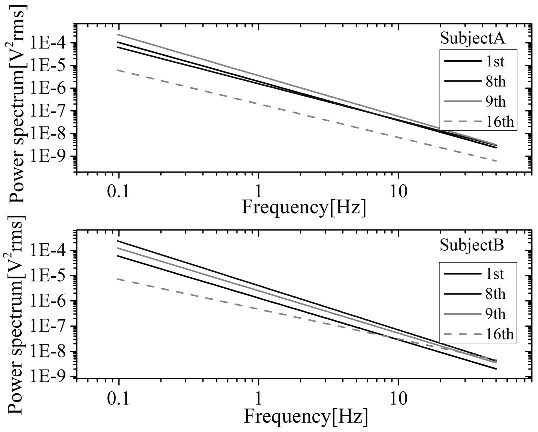

Fig.7 Approximate curve in fluctuation characteristics (top: subject A, bottom: subject B, both logarithmic axes).

The test data came from the test plants measured on the other days, where there were significant differences between chlorophyll fluorescence values. The three explanatory variables were compared and the result is shown in Table 4.

Table 4 shows that even for the test data, using both fluctuation variables as a discriminant increased the discrimination rate. Furthermore, for the test plant D1, the discrimination rate was low, regardless of the feature used. Table 5 shows the standard deviations for the fluctuation characteristics calculated from the bioelectric potentials. The standard deviations for both the slope and the intercept were 1.5 times greater in the healthy plants than in the unhealthy plants.

5. Discussion

The experimental results show that the tangent and the slope of the fluctuation characteristics for plants in a normal state of growth approximate a normal distribution. The tangent and the slope are reduced in the growth-inhibited plants. The herbicide created unfavorable growth conditions by inhibiting photosynthesis. During photosynthesis, the chlorophyll in chloroplasts extracts an electron from water by the addition of light. Blocking this function appears to be reflected in a reduction in bioelectric potential, reducing the power spectrum and the tangent. Random features similar to white noise are said to become strong as the slope of the fluctuation shows a decline.21) The results appear to show that the growth state of the plant has an effect on the fluctuation characteristics of the bioelectric potential, regardless of individual differences.

Table 1 Result of discrimination rate in the case of using the slope.

		Predicted condition		Discrimination rate
		0 : Growth inhibited	1 : Growth normal	
Correct condition	0 : Growth inhibited	20	10	66.7%
	1 : Growth normal	4	87	95.6%
Percentage of total				88.4%

Table 2 Result of discrimination rate in the case of using the intercept.

		Predicted condition		Discrimination rate
		0 : Growth inhibited	1 : Growth normal	
Correct condition	0 : Growth inhibited	27	3	90.0%
	1 : Growth normal	4	87	95.6%
Percentage of total				94.2%

Table 3 Result of discrimination rate in the case of using both slope and intercept.

		Predicted condition		Discrimination rate
		0 : Growth inhibited	1 : Growth normal	
Correct condition	0 : Growth inhibited	29	1	96.7%
	1 : Growth normal	2	89	97.8%
Percentage of total				97.5%

Table 4 Results of discrimination rate in relation to test dataset (subject: C, D, E, F).

		C1	C2	D1	D2	E1	E2	F1	F2	Average
Chlorophyll fluorescence Y		0.65	0	0.66	0.4	0.54	0.42	0.64	0.49	
Indification percentage	slop	100.0%	100.0%	12.5%	100.0%	100.0%	100.0%	75.0%	100.0%	85.9%
	intercept	100.0%	60.0%	50.0%	100.0%	100.0%	100.0%	68.8%	100.0%	84.8%
	slop & intercept	100.0%	80.0%	12.5%	100.0%	100.0%	100.0%	100.0%	100.0%	86.6%

Table 5 Standard deviation among fluctuation characteristics in test dataset.

Standard deviation	0 : Growth inhibited	1 : Growth normal
Slope	0.34	0.17
Intercept	3.91	2.56

The logistic regression analysis shows that using the slope and the tangent as explanatory variables provides the best means of discrimination, although the accuracy in discrimination was low for plant D1. A possible explanation is that the standard deviations of the fluctuation characteristics are larger for the normal growth state than for the growth-inhibited state. The chlorophyll fluorescence for plant D1 was 0.66, the highest value of all the test data. However, these experiments have shown that if the chlorophyll fluorescence value is 0.4 or lower, it may be possible to identify the growth state of the plant.

6. Conclusion

In this paper, we describe experiments on measuring the bioelectric potential of plants with inhibited growth as a way of determining their growth state and finding a means of evaluating plant growth. We formulated a model for discriminating growth state using the fluctuation characteristics from the bioelectric potential of growth-inhibited plants to improve our knowledge of how to evaluate plant growth. In this study, six plants were sprayed with herbicide, and those that showed a difference in the chlorophyll fluorescence values were subjected to logistic regression analysis to identify a discriminant function. We used a herbicide that inhibited photosynthesis as a standard way of determining growth state. Photosynthetic inhibition can be measured by chlorophyll fluorescence. Plants that showed a significant difference in the chlorophyll fluorescence values were subjected to frequency analysis of their bioelectric potential using MEM. Each coefficient in the fluctuation characteristics was treated as a feature to devise a discrimination model.

Logistic regression analysis showed that discrimination power was higher when both the slope and the intercept features were used together than when either was used alone. As a supplementary experiment, when the plants that had shown a significant difference in chlorophyll fluorescence were tested, using the already calculated discriminatory model, a discrimination rate of 86.6% was obtained. When the individual chlorophyll fluorescence values dropped to 0.4 or below, the growth state could be predicted to some extent.

In this study, we demonstrated that it is possible to distinguish two distinct growth states: healthy and unhealthy. However, it is necessary to evaluate more detailed gradations in plant health and to determine the length of time that growth inhibition has been occurring. Further experiments on finer gradations of plant growth state using a larger number of plants are required.

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References

1) M. Takatsuji: A Present Situation of the Completely-Controlled Plant Factory, J.SHITA., Vol.22, No.1, pp.2-7, 2010 (in Japanese).
2) M. Takatsuji: A Plant Factory, (Koudansha LTD., Tokyo) pp. 5-7, 1979 (in Japanese).
3) A. Shinjo: Agri-Informatics, IPS., Vol.51, No. 6, pp.635-641, 2010 (in Japanese).
4) M. Takatsuji: A Completely Controlled Plant Factory (Ohmsha Ltd., Tokyo), pp.23-43, 2007 (in Japanese).
5) X. Jin, S. Oshita, Y. Makino and Y. Kawagoe: Non-destructive Eestimation of Aerobic Plate Count Adhering to the Surface of Spinach Leaves by Spectroscopy, J.SHITA., Vol. 25, No.2, pp.102-108, 2013 (in Japanese).
6) Y. Miwa: Biological Information System in Plant, The Japan Society of Mechanical Engineers, Vol. 90, No.818, pp.76-82, 1987 (in Japanese).
7) T. Shimbo, M. Fujii, A. Sawada, T. Oyabu and H. Kimura: Response Characteristics of Plant Bioelectric Potential to Light Intensity Indoor and Outdoor, IEEJ Trans. SM., Vol.127, No.10, pp.425-430, 2007 (in Japanese).
8) K. Ando, Y. Hasegawa, H. Maekawa and T. Katsube: Analyzing Bioelectric Potential Response of Plants Related to Photosynthesis under Blinking Irradiation, IEICE Trans. Electron., Vol.91, No.12, pp.1905-1909, 2008.
9) H. Kobayashi, S. Kobayashi, K. Yamada and T. Yachi: The Cultivation of Rucola Using LED Lamps with Various R/B Rations, IEICE Technical Report. EE. Vol.106, No.494, pp.53-58, 2007 (in Japanese).
10) M. Kurita and K. Nakabayashi: Effect of Humidity on the Alternating Component of Leaf Electric Potential of Qing-geng-cai, J.SHITA., Vol.19, No.3, pp.117-122, 2007 (in Japanese).
11) K. Ando, Y. Hasegawa, T. Yaji, and H. Uchida: Study of Plant Bioelectric Potential Response due to Photosynthesis Reaction, IEEJ Trans. SM., Vol.131, No.9, pp.337-342, 2012 (in Japanese).
12) S. Shibata, F. Satou, H. Kimura and T. Oyabu: The Relationship between Chlorophyll Fluorescence Parameter (Fv/Fm) and Frequency Component of Plant Bioelectric Potential in Spraying Chemical Herbicides, IEEJ Trans. SM., Vol.132, No.6, pp.154-158, 2012 (in Japanese).
13) A. Tanaka, H. Usuki and K. Fukuda: Relationship between Vitality Estimated by Leaf SPAD Value and Diurnal Trends of Photosynthesis and Transpiration in Zelkova Serrata Makino Trees, Tree Health, Vol.13, No.1, pp12-16, 2009 (in Japanese).
14) Y. Hasegawa, S. Asada, T. Ka-tsube and T. Ikeguchi: Analysis of Bioelectrical Potential When Plant Purifies Air Pollution, IEICE Trans. Electron., Vol.E87-C, No.12, pp.2093-2098, 2004.
15) T. Oyabu, A. Sawada, K. Takenaka and B. Wolverton: Characteristics of Potted Plants for Removing Offensive Odors, Sensors and Actuators, Vol.89, pp.131-136, 2003.
16) S. Shibata, S. Lixin, H. Kimura and T. Oyabu: Characteristics of Plant Bioelectric Potential and Purification Function under LED Light, EICA., Vol.15, No.4, pp.65-71, 2011 (in Japanese).
17) T. Kojima, N. Hiroshi, Y. Yoshizaki and H. Soeda: Power Spectrum Analysis of Fish Heart Rate Variability Using Maximum Entropy Method, Japanese Society of Fisheries engineering, Vol.38, No.2, pp.145-150, 2001 (in Japanese).
18) M. Ozaki, T. Ohishi, T. Mori and Y. Kayaba: Sensory and Physical Evaluation for Surface Texture of Revetment Block in Small and Medium-sized Rivers, Technical Note of PWRI., Vol.4248, pp.59-64, 2012 (in Japanese).
19) K. Onishi, D. Ichikawa and H. Harada: A Factor of Affection Prognosis to Tonsillectomy on IGA Nephropathia, Japanese Society of Stomato-Pharyngology, Vol.19, No.1, pp.38, 2006 (in Japanese).

20) Raghavendra B.K and S.K Srivatsa: Evaluation of Logistic Regression and Neural Network Model with Sensitivity Analysis on Medical Datasets, IJCSS., Vol.5, No.5, pp.503-511, 2011.

21) M. Hirose and M. Adachi: Retrieval Characteristics of a Dynamical Associative Memory with 1/fα Fluctuations Generated by Adaptive Lattice Dynamics, IEICE Technical Report. NLP., Vol.109, No.269, pp.209-213, 2009 (in Japanese).