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Discovery of a Seasonal Dependence for a Bio-Reaction Rhythm in Cucumbers

Osamu Takagi*, Masamichi Sakamoto[2], Hideo Yoichi[1], Hideyuki Kokubo[1], Kimiko Kawano[1], Mikio Yamamoto[1]

[1]*Information and Research Center, International Research Institute (IRI), 1108-2 Sonno, Inage, Chiba 263-0051, Japan.*

[2]*Aquavision Academy, 1228-3 Tsubuura, Narita, Chiba 287-0236, Japan.*

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ABSTRACT

This paper investigated the bio-reaction rhythm in harvested cucumbers. To do this, we measured the concentrations of gas released from cut cucumber samples. We hypothesized that if periodicity in circadian variation is observed in the gas concentration change, it can be considered as the bio-reaction rhythm of the cucumber. The relation between the time of day when the cucumber was sectioned and the gas concentration was investigated. We obtained a periodic approximation curve for the gas concentration change and analyzed it to see whether gas concentration has periodicity. We discovered that the gas concentration released from the cut surface varied depending on the time of day when the cucumber was sectioned and that there was periodicity in the gas concentration variation. In addition, this periodicity varied with the season, with one cycle being 6 hours in summer and 24 hours in winter.

INTRODUCTION

Studying various bio-reactions of vegetables and fruits is important for establishing safety measures against pests when the vegetables and fruits are being grown, and for their safe distribution and storage after being harvested. Two interesting findings have been reported [1-4] on harvested vegetables and fruits, especially the rhythm of bio-reactions. (1) The biological functions of harvested vegetables and fruits lasted more than one week (cabbage, *Brassica oleracea* L. var. *capitata*; lettuce, *Lactuca sativa*; spinach, *Spinacia oleracea*; zucchini, *Cucurbita pepo* var. *cylindrica*; sweet potato, *Ipomoea batatas*; carrot, *Daucus carota subsp. Sativus*; and blueberry, *Cyanococcus*) [1-3]. While the biological function was maintained, artificially adjusting the time to irradiate vegetables and fruits with light changed the rhythm of the bio-reaction. (2) Bio-reactions of plants are generally thought to be influenced by circadian rhythms, but the rhythms of multiple bio-reactions with different periodicity were found to exist simultaneously *in vivo* for *Arabidopsis thaliana*, which is a plant of the *Brassicaceae* family (and a widely used model in plant research) and closely related to cabbage, broccoli, and cauliflower [4].

The purpose of this paper is to investigate the bio-reaction rhythm of harvested cucumbers. For this purpose, we measured the concentrations of gas (hexanol-hexanal series gas) released from cut cucumber samples. Characteristics, prerequisites and assumptions of our experiments were as follows. (1) Harvested edible cucumbers (*Cucumis sativus* 'white spine type' cucumber) were used. (2) The premise was taken that the bio-reaction rhythm of cucumber does not change before and after harvesting. (3) In the bio-reaction used, a cucumber was sectioned and gas was released after this cutting. (4) The relation between the time of day when the cucumber was sectioned and the concentrations of gas (hexanol-hexanal series gas) released during the period of more than 24 hours after the sectioning was investigated. (5) The assumptions were made that the degree of the bio-reaction of cucumber varies with time and this variation leads to variation of the amount of gas released from the cut cucumber. Further, the degree of bio-reaction of the cucumber varies with time (bio-reaction rhythm of cucumber), while the gas concentration varies with the sectioning time. These two events are synchronized with each other.

We measured the gas concentration to clarify the rhythmic characteristics of the cucumber bio-reaction. However, the gas released from the cut cucumber was not measured continuously over time. After sectioning the cucumber, we kept the sections in a sealed container for a certain period of time. Then, after the gas discharged from the cut surface reached an equilibrium state in the sealed container, the gas concentration was measured. That is, we determined the gas concentration based on the time when the cucumber was sectioned and we measured the degree of the bio-reaction at that time. Therefore, when periodicity was observed in the gas concentration change, the periodicity could be regarded as the bio-reaction rhythm. We analyzed whether the gas concentration was periodic by determining the periodic approximation curve from the measured data of the gas concentration corresponding to the cucumber sectioning time. As a result, we discovered that the gas concentration released from the cut surface was different depending on the sectioning time of the cucumber and that there was periodicity in the circadian variation between the sectioning time and the gas concentration. In addition, this periodicity varied depending on seasonal differences, with one cycle being 6 hours in summer and 24 hours in winter.

MATERIALS AND METHODS

After sectioning the cucumbers, we used the Simultaneous Calibration Technique (SCAT) [5] to measure the gas concentration released from the cut surface by the bioreaction. SCAT was a method we developed for detecting spatial characteristics using a bio-sensor (cucumber) that eliminates deviation of data caused by individual differences among cucumbers and change of environmental conditions. Therefore, SCAT can detect a weak effect that has some influence on the cucumber gas generation reaction system by which it is possible to detect the spatial characteristics that were considered difficult to detect by existing sensors [6-12].

In this paper, we did not use SCAT to detect spatial characteristics. We used it to clarify the change in gas concentration released from the cucumber section.

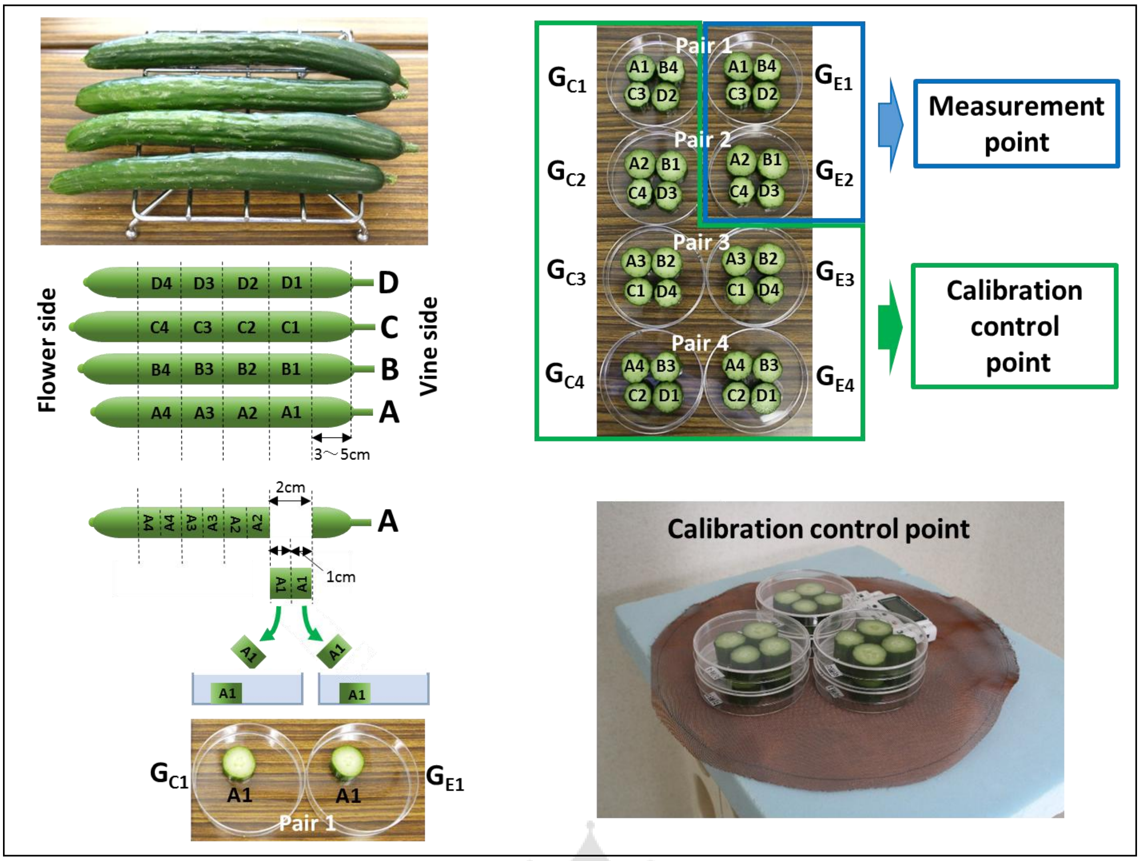

Figure 1 Sample preparation by SCAT

Fig. 1 shows how the cucumber section samples were prepared by SCAT. In one unit of SCAT experiments (the minimum experimental unit), four cucumbers and eight petri dishes were used. Four samples, each with a length of 2 cm, were cut from one cucumber. Next, the cut samples were further cut in half in the length direction and each of the eight half slices was placed in a separate petri dish to give four pairs of samples (pair 1 to pair 4). A total of four cucumber slices was placed in each petri dish, one from each of four cucumbers, to eliminate the influence of variance among individual cucumbers. Pairs 1 and 2 were designated as the main samples and pairs 3 and 4 were designated as control samples.

The surface of the cucumber slice between the pairs was the same cut surface. The main samples G_{E1} and G_{E2} were set at the measurement point. The main samples G_{C1}, G_{C2} and the control samples G_{E3}, G_{C3}, G_{E4}, G_{C4} were stacked two by two and were set at a calibration control point well separated from the measurement point. The cucumber section samples in covered Petri dishes were set at the measurement and the calibration control points for 30 minutes after preparation. After that, the cover lid was removed and the samples were placed in a polypropylene sealed container having a volume of 2.2 liters. Hexanol-hexanal series gas was released from the cut surface of the cucumber samples in the sealed container.

The main components of the mixed gas released from the cucumber have been found to be of 16 kinds [13-14]. Approximately 12 hours after sectioning, the released gas concentration reaches a maximum and then remains in equilibrium. The sealed container was kept at controlled room temperature (22 to 24 degrees Celsius) without direct sunlight for more than 24 hours. After storage, the gas concentration was measured by aspirating 300 mL of gas using a gas detector (GV-100: Gas Tech, Japan) and a gas detection tube (141 L: Gas Tech, Japan). The gas detecting tube measured the concentration of 2-hexanol gas. When 2-hexanol gas is measured, since the conversion factor is 3, the absolute value of the 2-hexanol gas concentration is determined by tripling the read value (ppm) of the detection tube. However, a mixture of 16 main types of gases is released from the cucumber sections and includes isomers of 2-hexanol and the like. In order to accurately determine the gas concentration released from the cucumber, the constitutional ratio of the 2-hexanol isomer and the conversion coefficient are required. However, at present, the conversion factor of the 2-hexanol isomer is unknown.

Our purpose here was not to determine the absolute value of the gas concentration released from cucumber but to clarify the characteristics of the relative change in the released gas concentration within a day. Therefore, in this paper, we used the read value (ppm) of the detection tube as if it were the gas concentration for our analysis.

All the cucumbers used in this experiment were edible cucumber (*Cucumis sativus* 'white spine type') which is generally available in Japanese markets. The experiments were conducted throughout the year from 2012 to 2016. The number of experiments was the same as the number of SCAT units, which totaled 1056 units. We used 4224 cucumbers. Of the 1056 units in total, 693 units were used in summer (12 hours or more of daylight), and 363 units were used in winter (less than 12 hours of daylight).

RESULTS

Fig. 2(a) shows the measured gas concentrations. The vertical axis is the gas concentration average $(G_{C1}+G_{C2}+G_{E3}+G_{C3}+G_{E4}+G_{C4})/6$ of the six samples set at the calibration control point. The horizontal axis is the time when the cucumber was sectioned (the time when the samples were set at the calibration control point) and it is indicated as a 24-hour clock. The yellow circles represent the gas concentration average $(G_{C1}+G_{C2}+G_{E3}+G_{C3}+G_{E4}+G_{C4})/6$ (n = 1056). The red squares represent average values of data within the 30-minute interval (n = 48) when dividing 24 hours into 30-minute intervals. The difference between the time when the cucumber was cut and the time when the samples were set at the calibration control point was 10 minutes or less, and we regarded them as almost the same time. The total average value of $(G_{C1}+G_{C2}+G_{E3}+G_{C3}+G_{E4}+G_{C4})/6$ was 332.2 ppm (n = 1056).

Fig. 2(b) is a histogram of the gas concentration average (n = 1056) shown in Fig. 2(a), and Fig. 2(c) is a graph of the normality test of gas concentration average. The red line in Fig. 2(c) is a straight line with a slope of 1, indicating that the data were substantially on a straight line. Assuming that the sample distribution followed the normal distribution as the null hypothesis, the Kolmogorov-Smirnov test had p = 0.0643. From this result, we judged the null hypothesis held even when the significance level was 5%, and the distribution of the gas concentration average could be regarded as a normal distribution.

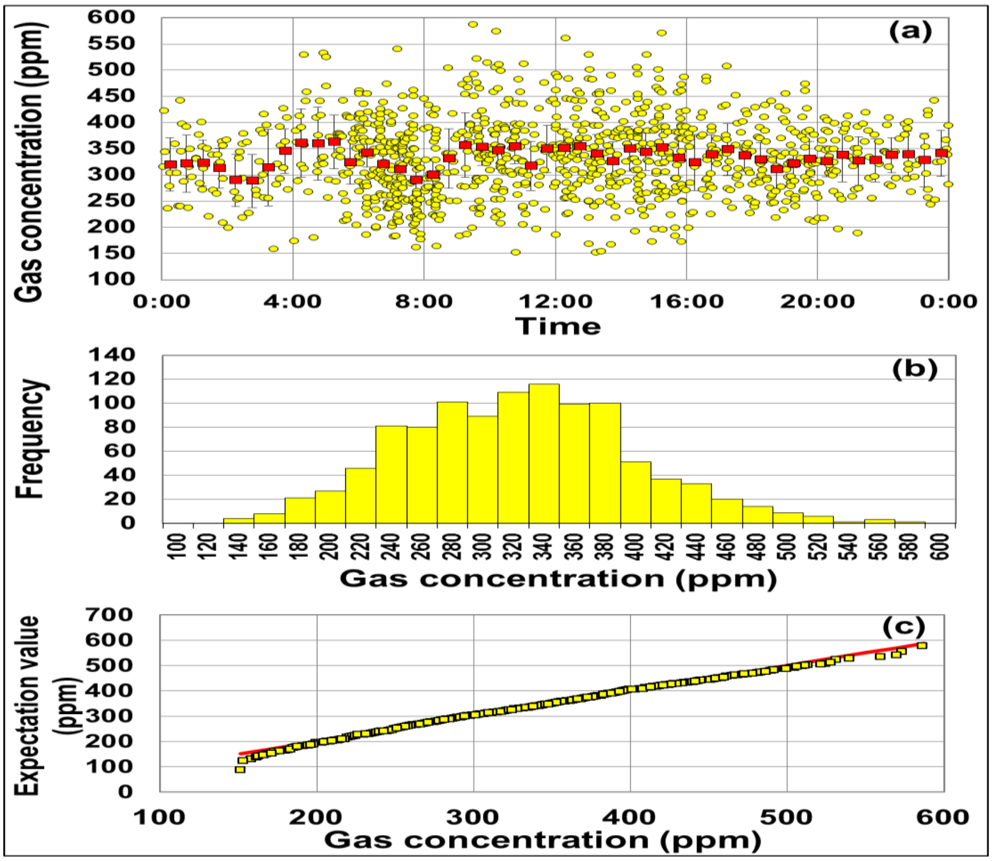

Figure 2 Measured gas concentration and normality of data

Fig. 3 plots the gas concentration average (shown in Fig. 2(a)) divided into summer data (n = 693) and winter data (n = 363). The vertical axis is the gas concentration average $(G_{C1}+G_{C2}+G_{E3}+G_{C3}+G_{E4}+G_{C4})/6$ of the six samples set at the calibration point. The horizontal axis is the time when the sample was set at the calibration control point, and it is indicated as a 24-hour clock. The orange circles are summer data (n = 693), and the blue circles are winter data (n = 363). The orange squares are average values of summer data within the 30-minute interval (n = 48) when dividing 24 hours into 30-minute intervals and the blue squares mean winter data average (n = 48). The error bars are 99% confidence intervals.

Summer data were data obtained when the length of daylight at the time of harvesting of cucumber was 12 hours or more. Winter data were data obtained when the length of daylight at the time of harvest was less than 12 hours. We assumed that the harvesting day was 4 days before the purchase date.

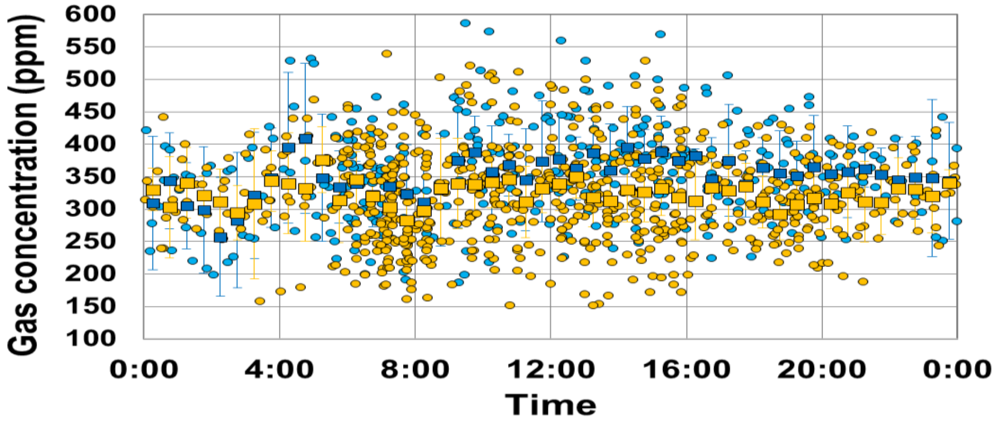

Figure 3 Gas concentration average: summer data and winter data

DATA ANALYSIS

The six sample dishes set at the calibration control point had the same environmental conditions from the time of cucumber sectioning to the gas concentration measurement, and we expected that the bio-reactions of the six were the same. Therefore, if there is a constant rhythm in the bio-reaction, we considered that periodicity appears in the gas concentration average shown in Fig. 2(a) and Fig. 3. In order to verify whether or not the gas concentration periodically changes, the periodic approximation curve of equation (1) is considered.

$$y = a + b\sin(2\pi xN) + c\cos(2\pi xN) = a + \sqrt{b^2 + c^2}\sin(2\pi xN + \varphi), \quad \varphi = \arcsin\left(\frac{c}{\sqrt{b^2 + c^2}}\right) \ldots (1)$$

Here, a, b and c are constants and π is the circumference ratio. X shows the time, but the time from 00:00 to 24:00 corresponds to a numerical value in the range of 0 to 1. The reason for this is that since the cucumber as a living body has a circadian rhythm basically, the change in the gas concentration released by the bio-reaction is considered to be in phase for each 24-hour period. N is the number of periods per 24 hours and N is an integer from 1 to 24. Equation (1) represents a periodic approximation curve in which one cycle is 24 hours when N = 1 and one cycle is 1 hour when N = 24. We calculate the periodic approximation curves of gas concentration average ($G_{C1}+G_{C2}+G_{E3}+G_{C3}+G_{E4}+G_{C4}$)/6 with respective values of N from 1 to 24 and fix the constants a, b and c. Thereafter, a correlation coefficient between the gas concentration average and the periodic approximation curve is calculated. As a result, if we judge that the correlation coefficient is significant, we can conclude that the period of the periodic approximation curve is the period of the gas concentration average and further, it is the period of the bio-reaction.

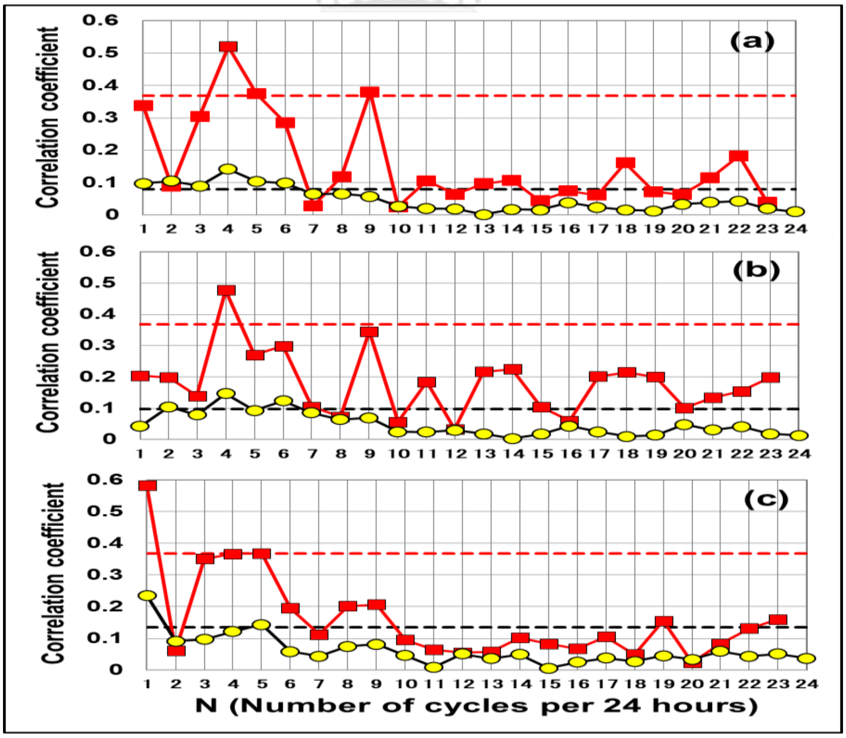

Figure 4 Correlation coefficient between gas concentration average and periodic approximation curve

Fig. 4(a) shows the analysis results of annual data. The correlation coefficients between the gas concentration average $(G_{C1}+G_{C2}+G_{E3}+G_{C3}+G_{E4}+G_{C4})/6$ (n = 1056; 48) in Fig. 2(a) and the periodic approximation curve are shown. The vertical axis is the correlation coefficient. The horizontal axis is the period (N) of the periodic approximation curve per 24 hours. The yellow circles are the result of the correlation coefficient between the gas concentration average $(G_{C1}+G_{C2}+G_{E3}+G_{C3}+G_{E4}+G_{C4})/6$ (n = 1056) and the periodic approximation curve. The black dotted line shows the criterion (0.0792) for judging whether or not the correlation coefficient is 1% significant when the number of data is 1056. The red squares are the results of correlation coefficients between the average of annual data (n = 48) in Fig. 2(a) and the periodic approximation curve. The red dotted line shows the criterion (0.3683) for judging whether or not the correlation coefficient is 1% significant when the data number is 48.

For n = 1056, significant correlation is obtained when the correlation coefficient is larger than 0.0792. From Fig. 4(a), the correlation coefficient is found to be significant when N = 1 to 6. For n = 48, significant correlation is obtained when the correlation coefficient is larger than 0.3683. As a result, when N is 4 and 5, significant correlation is obtained for both data numbers of n = 1056 and 48. Therefore, the annual data suggest that the gas concentration average and bio-reaction may have two periodicities of 6 hours (N = 4) and 4.8 hours (N = 5) per cycle.

Fig. 4(b) shows the analysis results of summer data. The correlation coefficients between the gas concentration average $(G_{C1}+G_{C2}+G_{E3}+G_{C3}+G_{E4}+G_{C4})/6$ (summer data, n = 693; 48) and the periodic approximation curve are shown. The vertical and horizontal axes are the same as in Fig. 4(a). The yellow circles are the result of the correlation coefficient between the gas concentration average $(G_{C1}+G_{C2}+G_{E3}+G_{C3}+G_{E4}+G_{C4})/6$ (summer data, n = 693) and the periodic approximation curve. The black dotted line shows the criterion (0.0977) for judging whether or not the correlation coefficient is 1% significant when the number of data is 693. The red squares are the results of correlation coefficients between the average of summer data (n = 48) and the periodic approximation curve. The red dotted line gives the same value (0.3683).

For N = 2, 4 and 6, this correlation coefficient is recognized as significant for summer data (n = 693). Additionally, for the average of summer data (n = 48) in Fig. 3, the correlation coefficient is recognized as significant for N = 4. As a result, when N = 4, significant correlation is obtained for both data numbers, n = 693 and 48. Therefore, from the summer data, it is suggested that the gas concentration average and the bio-reaction may have a periodicity of 6 hours (N = 4) per cycle.

Fig. 4(c) shows the analysis results of winter data. The correlation coefficients between the gas concentration average ($G_{C1}+G_{C2}+G_{E3}+G_{C3}+G_{E4}+G_{C4}$)/6 (winter data, n = 363; 48) and the periodic approximation curve are shown. The vertical and horizontal axes are the same as in Fig. 4(a). The yellow circles are the result of the correlation coefficient between the gas concentration average ($G_{C1}+G_{C2}+G_{E3}+G_{C3}+G_{E4}+G_{C4}$)/6 (winter data, n = 363) and the periodic approximation curve. The black dotted line shows the criterion (0.1350) for judging whether or not the correlation coefficient is 1% significant when the number of data is 363. The red squares are the results of correlation coefficients between the average of winter data (n = 48) and the periodic approximation curve. The red dotted line gives the same value (0.3683).

As a result, for N = 1 and 5 the correlation coefficient is recognized as significant. Additionally, for the average of winter data (n = 48) in Fig. 3, for N = 1 the correlation coefficient is recognized as significant. As a result, when N = 1, significant correlation is obtained for both data numbers, n = 363 and 48. Therefore, from the winter data, it is suggested that gas concentration average and bio-reaction may have a periodicity of 24 hours (N = 1) per cycle.

Our analysis of annual data suggested that the gas concentration released from the cucumber sections has two periodicities of 6 (N=4) and 4.8 (N=5) hours per cycle. However, as a result of examining the seasonal dependence in summer and winter, we discovered that the periodicity of the gas concentration varies depending on the season. Therefore, the periodicity of the annual data is considered to be the synthesis periodicity in summer and winter, and the bio-reaction rhythm of 6 hours and 24 hours in each cycle was verified.

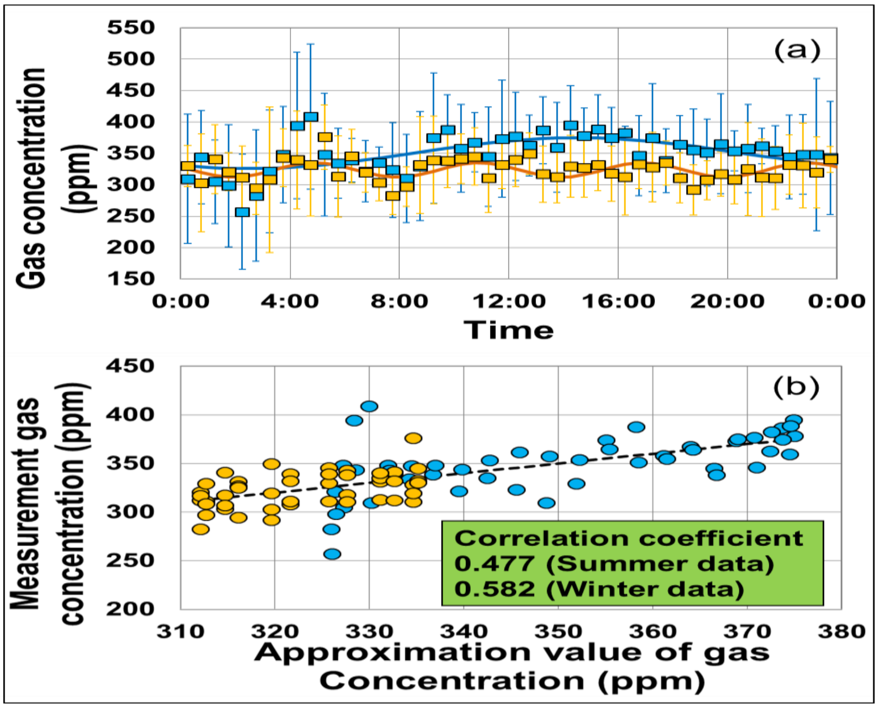

Figure 5 Periodic approximation curve and correlation diagram

Fig. 5(a) shows the gas concentration average (n = 48) and periodic approximation curves in summer and winter. The orange squares are gas concentration averages of summer data shown in Fig. 3. The orange curve is a periodic approximation curve (a = 323.64, b = - 10.58, c = 4.93) with one cycle of 6 hours. The blue squares are gas concentration averages of winter data shown in Fig. 3. The blue curve is a periodic approximation curve (a=350.48, b=-15.06, c=-19.36) with one cycle of 24 hours. The error bars are 99% confidence intervals.

Fig. 5(b) shows the correlation between the gas concentration average (n = 48) in summer and winter and the approximation value of the periodic approximation curve. The vertical axis is the measured gas concentration average, and the horizontal axis is the approximation values of periodic approximation curves for one cycle of 6 hours (summer) and 24 hours (winter). The summer data (orange circles) have the correlation coefficient of 0.477, while for the winter data (blue circles), the correlation coefficient is 0.582.

DISCUSSION AND CONCLUSION

We discovered that the gas concentration released from the cut surface of cucumber samples was different depending on their sectioning time and that there was periodicity in the circadian variation between the sectioning time and the gas concentration. As a result, we concluded that there was a seasonal dependence on the bio-reaction rhythm of cucumber, with one cycle being 6 hours in summer and 24 hours in winter.

During the winter season in Fig. 4(c), the correlation coefficients of N = 3, 4, and 5 (where N is the period of the periodic approximation curve per 24 hours) were close to a significant value. We considered this to be due to the gas concentration measured in the 6-hour period from 0 am to 6 am for N = 4 of the winter data in Fig. 5(a). In the winter, the gas concentration was rapidly increasing from 2 am to 5 am. This may qualitatively agree with the report [15] that plants predict the sunrise and begin preparation for photosynthesis before dawn in order to effectively utilize the light from the sun.

Further research on the seasonal dependence of the bio-reaction rhythm of plants may contribute to the development of effective pest control.

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