A Method for EEG Fluctuation Processing
-Application to Fertilizer Difference Analysis in Vegetable Ingestion-

Takashi Ajiro†, Koichiro Shimomura†, Hirobumi Yamamoto† and Kenichi Kamijo†

†Plant Regulation Research Center, Toyo University, 1-1-1 Izumino, Itakura, Gunma, 374-0193, Japan

Summary
The electroencephalogram (EEG) is frequently used for studying psychological influences, by transforming original voltage fluctuations with the Fast Fourier Transform (FFT) or deciphering the waveform directly. While internal behavior of the brain remains difficult to evaluate, using FFT, powers of α and β waves (μV) can be analyzed by various sub-methods to evaluate the relaxed or stressed state of the brain, since these waves exhibit specific characteristics related to brain state. In the present study, we measured and analyzed EEG fluctuations while examinees ate Komatsuna, also known as the Japanese Mustard Spinach. Komatsuna plants were grown in three different and fertilizers at two densities, chemical, organic, and organic + chemical (each ×1 and ×2 density). We first applied analysis of variance (ANOVA) and relational voltage graphs that use relational values from initial values, to the formatted data. The formatted data are generated from the EEG data, by our specialized program that is developed in the previous work. Subsequently, we applied a detailed analysis that makes precise graphs with "interactions" of ANOVA. Results indicate that α and β waves have significant differences when eating Komatsuna grown using different fertilizers. Furthermore, some interactions between defined factors, including the kinds of fertilizers, showed significant differences. Thus, the fertilizers used for growing Komatsuna could influence EEGs in our experimental conditions.

Key words:
ANOVA, SOC, EEG, vegetable ingestion, fertilizer, Komatsuna, algorithm, analysis program

1. Introduction

There are many methods for measuring brain activity, including the electroencephalogram (EEG), magneto-encephalogram (MEG), and magnetic resonance imaging (MRI). In particular, the EEG is frequently used since the devices have a long history, are relatively easy to use, and comparatively inexpensive. Since EEG responses reflect fluctuations in the electrical activity of the human brain (in other words, psychological influences), many studies have investigated the effects of sensory inputs, such as sound, smell, sight and taste, on EEG activity [1-6]. Wave groups defined by representative values between frequency bands, such as θ, α, β, and δ waves, are often used in research. More specifically, α and β waves are frequently used to evaluate effects of relaxation. For instance, there are studies that analyze by these waves to evaluate mental states in working [7-8]. We also use α and β waves (including sub-α waves) in our research for evaluating the relaxed or stressed state of the brain in eating.

To date, little, if any, research has been conducted on EEG measurement and analysis during the ingestion of vegetables. Analysis of EEGs while drinking is similar to our work [9-10]; however, our work involves chewing solid foods. We analyze the EEG data using the original composite method, which is the analysis of variance (ANOVA) using statistical software and fluctuation graphs. The ANOVA examines significant variances of defined factors and their interactions, and the graphs compare defined levels in the factors (basic analysis). Furthermore, the fluctuation graphs for the significant interactions represent the levels of the factors related to these interactions (detailed analysis). In our previous work, we had executed two preliminary experiments that measure and analyze EEGs in Komatsuna ingestion [11]. In the present study, we have conducted a full experiment with a sufficient number of examinees, using the previous established method and the detailed analysis.

We measured EEG fluctuations in five pairs (a pair consists of a male and female examinee) of examinees as they ate Komatsuna, and analyzed the resultant data. The Komatsuna eaten during the experiment were grown in three kinds of fertilizers at two different densities, chemical, organic, and organic + chemical (each ×1 and ×2 density). Komatsuna have been cultivated since the Edo Period in what is today Komatsugawa, Japan. The komatsuna vegetable was allegedly named as a tribute to the prefectures in which the plant was originally cultivated, although it is now cultivated in Tokyo and neighboring prefectures. The appearance of two fascicles of Komatsuna is shown in Fig. 1. During the experiments, examinees were instructed to eat every part of the plant, which is cut and mixed. Previously, we researched the vegetable's benefits, such as antioxidative activity and vitamin content. We found that the same kind of vegetables have each delicate difference taste depending on the use of organic fertilizer or chemical fertilizer, by

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using a flavor recognition machine (TS-5000Z, Intelligent Sensor Technology Inc.)[12].

Results indicate that α and β waves during ingestion of Komatsuna have significant differences depending on the fertilizers used. We also found that some interactions between the defined factors, including the kinds of fertilizers, have significant differences. Thus, we conclude that the fertilizers used for growing Komatsuna could influence the EEG in our experimental conditions.

Herein, the preparation for our experiment, for instance the measurement device and overview of ANOVA theory, is described in Section 2. The experiment plan that includes our cooking method, experiment schedule, and measurement procedure is described in Section 3. We present the results with basic analysis in Section 4, and present the results of detailed analysis in Section 5. Finally, we present our conclusions and future works in Section 6.

Fig. 1 Two fascicles of Komatsuna (green vegetables)

2. Preparation

2.1 EEG Measurements

1) Simple EEG measurement device: A simple device called the "Brain Builder Unit" was used for EEG measurements [13]. A photograph of this device, including the electrodes, is shown in Fig. 2. The two electrodes of the headband contact the skin on the examinee's forehead, and the other electrode is a lead that clips onto the left ear lobe. The measurement device connects to a PC running a Windows operating system (Windows 98 or later) through a serial port and is controlled using the Mind Sensor II software [13]. A screenshot of the software is shown in Fig. 3. The software communicates with the measurement device via the serial port and both captures and displays EEG data in real time. The system measures EEG data from the right and left brain separately, and the measured data series is written to an ".fft" file that has an internal CSV format. The data visualization function of the software is shown in Fig. 3. Real-time raw voltage fluctuations (unit: μV) and spectra that indicate the power of frequencies by Fast Fourier Transform (FFT) are displayed during the EEG measurement.

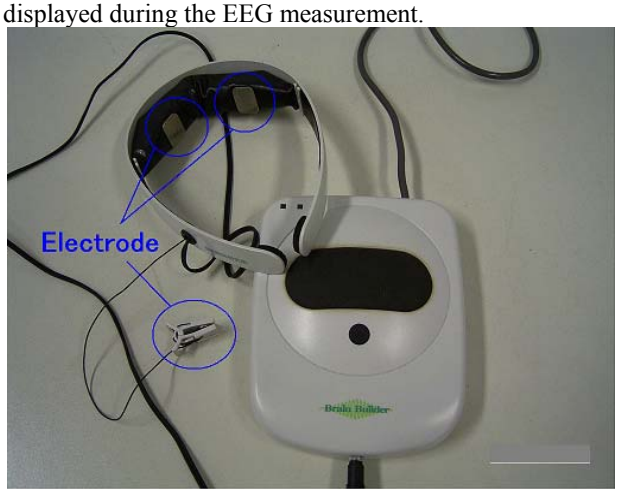

Fig. 2 The Brain Builder Unit with three electrodes

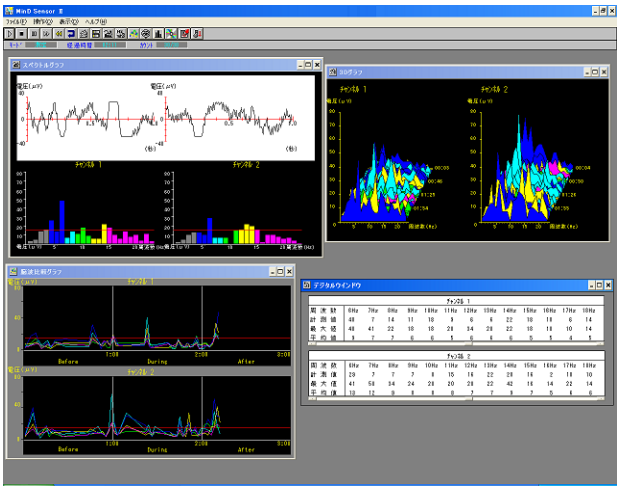

Fig. 3 Appearance of Mind Sensor II software (in Japanese)

2) Definitions of EEG frequency groups: In general, the categorized spectrum defined in an EEG frequency grouping is used in the EEG analysis instead of the raw electrical fluctuation or its spectrum, even though definitions of EEG frequency groupings vary according to researchers. Some definitions of these frequency groupings have been presented in books on EEG [14-15]. We used the definitions provided in the Mind Sensor II software manual, since they are valid definitions and are

confined to our research [13]. The definitions of EEG frequency groupings used in our experiments are listed in Table. 1.

The δ wave-group (hereafter, a wave-group is called a "wave") appears during deep sleep; however, we are unable to use this wave since electrical noise accompanies the EEG measurement. We are also unable to use the θ wave since the examinees are awake. The frequency band of the α wave is separated into three groupings, since these frequencies are the most important for examining effects of relaxation. Moreover, fluctuations in these frequencies occur only in awake individuals. The β wave appears during the attentive state, and we use it in comparison with the α waves. Although the general boundary of the β wave is considered to be 40 Hz, our definition is 23 Hz due to the limitations of the measurement device.

Table 1 EEG types and corresponding mental states

EEG type		frequency	mental state	
δ		1 ~ 3Hz	deep sleep	
θ		4 ~ 6Hz	light sleep, meditation	
α	slow α	7 ~ 8Hz	relaxing with depressed consciousness	relaxing, creativity uplifting
	mid α	9 ~ 11Hz	relaxing with concentration	
	fast α	12 ~ 14Hz	concentrating with stress	
β		15 ~ (23Hz)	attention, concentration	

2.2 ANOVA Software for Analysis Variance

For the ANOVA calculations, captured EEG data in the ".fft" file are processed and input into JUSE-QCAS Version 7 software [16]. This software supports many statistical operations, including the ANOVA function. A screenshot of the data-editing mode in this software is shown in Fig. 4. In this editing mode, the user can input data into cells that accept integer values, real values, and characters as text labels. The system recognizes the columns of these cells as two kinds of variables: "quality variables" and "quantity variables". The quality variables include the "levels" of "factors", and the quantity variables include the analysis data. In Fig. 4, "C3"–"C5" are quantity columns and "N6"–"N7" are quality columns. The system analyzes quality values (measurement data) according to the factors and allocations of the quantity values (levels of factors). As a result, the software generates a table of ANOVA data (called the ANOVA table).

Fig. 4 Screenshot of JUSE-QCAS Version 7 (in Japanese)

2.3 Experimental Environments

Our experimental environment and measuring equipment are shown in Fig. 5. The experimental room is enclosed in a tent, as shown in the top left photo. The inside of the tent area is shown in the top right photo. The equipment used for the experiment is shown in the bottom photo. The Brain Builder Unit is connected to the serial port of the PC, and the monitor displays the functions of the Mind Sensor II software. The lotion is used for the electrodes of the Brain Builder Unit, since these must contact the skin of the examinee. The CD player plays music before starting the first experiment phase ("before" phase), and the experimenters need only to push the start button to play the specified background music (BGM). Examinees listen to the BGM using a noise-cancelling headphone.

Fig. 5 Experimental equipment for EEG measurements

2.4 Basic Concept of ANOVA

The ANOVA statistical analysis method is based on the dependencies of factors related to the movement of the measurement values. In this method, independent factors are called "main effects," and factors generated by mixing independent factors are called "interactions." The main factors are denoted as "A", "B", "C" and the interactions are denoted as "A × B", "B × C", "A × C". The calculation results are called "p-values" (probability values) and are determined using a function of this method called an "assay." The result of the assay is represented by "**" if the p-value is less than 0.01, or by "*" if the p-value is less than 0.05. The p-value indicates the reliability of the significance. As an example, a p-value of 0.5 indicates statistical singular values of 5% that are included in the numerous measured values. In other words, it indicates 95% reliability of the analysis results.

The primary concept of this theory is to express measurement data by the sum of the squares that includes all factors, errors of measurement and total accident errors. The formulation is called "structure expression." The structure expression of a five-way layout of analysis of variance is defined in Fig. 6 (our results used this expression). The variance meanings of this formulation are as follows: "y" is measurement data, "a", "b", "c", "d", "e" are the level values of factors, "i", "j", "k", "l", "m", "n" are identical suffixes, "ε" is the total accidental error, and "μ" is the error of measurement. The theory of the analysis of variance is explained in Refs. [17-19].

$$y_{ijklmn} = \mu + a_i + b_j + (ab)_{ij} + c_k + (ac)_{ik} + (bc)_{jk} + (abc)_{ijk} + d_l + (ad)_{il} + (bd)_{jl} + (abd)_{ijl} + (cd)_{kl} + (acd)_{ikl} + (bcd)_{jkl} + e_m + (ae)_{im} + (be)_{jm} + (abe)_{ijm} + (ce)_{km} + (ace)_{ikm} + (bce)_{jkm} + (de)_{lm} + (ade)_{ilm} + (bde)_{jlm} + (cde)_{klm} + (abcde)_{ijklm} + \varepsilon_{ijklmn}$$

Fig. 6 Structure expression of five-way layout

3. Experiment Plan

3.1 Komatsuna Cooking Method

Sample Komatsuna are prepared using a precise cooking method, and then eaten by the examinees. The definition of the cooking method from our previous experiments, "Ex. 1" and "Ex. 2", has some ambiguities [11]. For instance, the food cutting interval was unclear. For this experiment, the precise cooking method is defined below to reduce the error in the sample foods.

1) Clean 2–2.5 fascicles of Komatsuna (as one set) using tap water, and wipe them off their wet by papers completely.
2) Adjust the weight of the set of Komatsuna to 90 g, cutting and discarding small or bad stems.
3) Add 1g of NaCl to 1ℓ of tap water. Bring the water to a boil using a gas heater.
4) Boil the set of Komatsuna for 30 seconds.
5) Squeeze moisture from the boiled Komatsuna until they are 90% of their initial weight."?]
6) Lay the boiled Komatsuna onto the cutting board lengthwise, and cut them at 10-cm intervals.
7) Place the cut Komatsuna in a plastic container, and mix leaves and stems using a spoon. Steps 6 and 7 are shown in Figure 7.
8) Put the corresponding sticker with the encoded kind to the plastic container.

Fig. 7. Boiled Komatsuna being cut and stored

3.2 Layout of factors and levels for ANOVA

In this section, we explain the measurement conditions defined by five factors and their corresponding levels, since these definitions are needed for ANOVA as described in Section 2.4. The ANOVA method assays significant variances of "main effects" that mean effects of individual factors, and "interactions" that mean interactive effects between multiple factors.

The meanings of the factors are: "A" is a kind of fertilizer used in growing Komatsuna (3), "B" is a sex of the examinee (2), "C" is the right or left brain (2), "D" is the before or during or after phase (3), and "E" is the density of the fertilizer used (2), where the values in parentheses are the number of levels. The meanings of the factors and levels are defined in Table 2, which also allocate them to alphabetical labels and numbers for ANOVA. In "fertilizer", "org + ch" is a mixture of organic and chemical fertilizers. In "density", the levels indicate

the densities of the fertilizers; for instance, "×2" means a fertilizer with twice the normal density.

Table 2 The five-way layout of the experiment plan for ANOVA

factor		level		
label	name	1	2	3
A	fertilizer	organic	chemical	org + ch
B	sex	male	female	
C	brain	right	left	
D	phase	before	during	after
E	density	x1	x2	

3.3 Experiment Orders for Examinees

The experiment orders were manually shuffled using the random number generation function "RAND" in Excel 2003 to cancel the historical effect error depending on a measurement sequence. The shuffled experiment schedule, which describes measurement orders for examinees, is shown in Table 3. Smaller numbers of "[ ]" on the "order" item indicate an earlier start for measuring EEG, and times on "start time" indicate start times of individuals that have the identical numbers of "[ ]". The experiment for one examinee consists of two parts, the "first half" and "later half". The letters "a"–"e" indicate encoded kinds that are defined and shown in Table 4, which codes the combinations of the kind and density. "name_x.fft" on "original name" defines the codes by the part of "x". Definitions of the translation rules to rewrite the encoded filenames to the intelligible them to avoid inputting error are also shown in the table.

Table 3 Experiment plan for the EEG measurements

	pair 1 (male)	pair 1 (female)	pair 2 (male)	pair 2 (female)	pair 3 (male)	pair 3 (female)	pair 4 (male)	pair 4 (female)	pair 5 (male)	pair 5 (female)
name										
filename	hagi_x	suzu_x	sai_x	sakai_x	hane_x	shino_x	saya_x	satou_x	some_x	taka_x
first half	a d e	f a c	e a f	e c d	e d a	c f e	a d f	d e c	a d e	c b f
latter half	b f c	b e d	b d c	b a f	c b f	d b a	b c e	a f b	b f c	e a d
order	[2]	[1]	[2]	[1]	[3]	[3]	[4]	[4]	[5]	[5]
start time	13:25	13:00			13:50		14:15		14:35	

Ota lab, group 1
Ota lab, group 2
Kamijo lab (experimenter)
etc.

Table 4 Filename translation table

combination of kind and density	original name	translated name
organic x1	name_e.fft	name_1y.fft
organic x2	name_f.fft	name_2y.fft
chemical x1	name_c.fft	name_1k.fft
chemical x2	name_a.fft	name_2k.fft
ch+org x1	name_b.fft	name_1w.fft
ch+org x2	name_d.fft	name_2w.fft

3.2 Experiment Condition

The experiment sequence is defined as follows: "Listening to BGM (before phase) → Eating pieces of Komatsuna (during phase) → Listening to BGM (after phase)". Examinees listen to the BGM during the "before" phase to stabilize their psychological state. Accordingly, the BGM must be mellifluous music played at low volume. We selected a suitable music for our experimental conditions. The experiments are executed according to the their orders defined in Table 3; for instance, "pair 1 female (first half), pair 2 female (first half), pair 1 female (later half), pair 2 female (later half), pair 1 male (first half)...". Both the "examinees" and "experimenters" are blinded to the kinds of Komatsuna being eaten (double-blind experiment). Details of the experimental conditions are as follows.

- Examinees put on the Brain Builder Unit electrodes and the noise-cancelling headphones (Quiet Comfort 3) connected to the CD player.
- "Sekai no shasou kara" ("From Train Windows in the World") is used as the BGM to cancel the environmental noise. Examinees listen to the BGM at a low volume (the volume level is 6 on our CD player).
- All measurements are conducted in the tent-enclosed space, which includes a table for eating the samples. Examinees are instructed to sit down quietly with their eyes open, to avoid α-wave noise.
- The layout of the experiment table is shown in Fig. 8. The edible sample is placed on a sheet of paper resting atop a cup. An alarm timer is located at the corner of the table.
- Five pairs of examinees participated in the experiment, with one pair comprising a male and female examinee. These pairs are defined in Table 3.
- The experiment sequence is "before → during → after". In the "before" and "after" phases, examinees sit quietly for 20 seconds. In the "during" phase, which lasts 20 seconds, examinees eat a sample of Komatsuna.
- Examinees masticate and swallow a sample of Komatsuna in the "during" phase. They are instructed to masticate more than 10 times before swallowing.
- Examinees are instructed to individually eat the sample since being fed by another person is a rare action that may generate an invalid EEG measurement due to the psychological effect.

Fig. 8. The layout on the experiment table

4. Basic Analysis and Results

4.1 EEG Processing Method for Analyzable data Generation

1) Manual EEG processing method: The measurement data of the experiment are synthesized by our EEG processing method, and the analyzable data and the basic graph data are subsequently generated. The manual EEG processing method has been presented in our previous paper [11], and is a basic method for our analysis process. EEG data from the Brain Builder Unit were manually processed prior to the development of our automated method. The descriptions of the EEG data items in the ".fft" Excel file are shown in Table 5. The file is renamed using the extension ".csv" before being opened. The manual processing algorithm in Excel is as follows.

1. Sort the all data by values in columns "A" by brain sides to split them.
2. Insert three row spaces into the bounds between brain sides. Insert three row spaces into the bounds between phases (before, during, after).
3. Sum values in each phase per frequency column, using Excel function "SUM". Enter the sums into the empty cells under the data sequences.
4. Select maximum values in each phase per wave group column, using Excel function "MAX". Enter the maximum values into the empty cells under the cells containing the sums.
5. Create the orthogonal table including all combinations for ANOVA in a new sheet.
6. Enter the maximum values into the large cells located in rows outside the data area.
7. Arrange the maximum values into the new sheet according to the format of the orthogonal table.

*The format of the orthogonal table is used for all combinations of data, and is defined in our previous paper [11].

Table 5 The descriptions of the EEG data items in the ".fft" Excel file

cell labels	meanings of data	wave type
A	right brain = 1, left brain = 2	
B	measurement time from start up (s)	
C ~ F	voltages of 0 ~ 3Hz (μV)	noise mainly
G ~ I	voltages of 4 ~ 6Hz (μV)	θ
J ~ K	voltages of 7 ~ 8Hz (μV)	slow α
L ~ N	voltages of 9 ~ 11Hz (μV)	mid α
O ~ Q	voltages of 12 ~ 14Hz (μV)	fast α
R ~ Z	voltages of 15 ~ 23Hz (μV)	β
AA ~ AD	etc.	

2) Overview of automation program process: We devised an automation algorithm for computer processing to eliminate the need for complicated manual processing. The algorithm differs greatly from the manual process, since the automated processing does not require use of Excel. Instead, the algorithm is implemented by programs in a development environment, such as Microsoft Visual C++ or GCC. An overview of this algorithm is described as follows.

#####File Input and ANOVA Data Generation Routine#####
1. Read key inputs on the console window (number of kinds, number of pairs, number of iterations, "before" time, "during" time, "after" time).
2. Read key inputs as a filename, and open this file.
3. Add a piece of data into the array, reading the data piece for one column. This process is looped until all data readings from the file have been finished.
4. Average the values of the temporary array when every single phase of "before", "during", and "after" have finished.
5. If the averaging of all phases is finished, select the maximum value of the average values per each EEG frequency.
6. Store these data in the data array.
7. Return to step 2 if files including unprocessed data exist.
8. Open a new file named "data.csv" in writing mode.
9. Write the data of the array to the file.
10. Close "data.csv".

##########Graph Data Generation Routine##########
11. Open a new file named "gr.csv" in writing mode.
12. Average the values of the data array per the "before", "during", and "after" phases.
13. Store these data in the graph array as absolute graph data.
14. Subtract the values of "during" and "after" from the values of "before".
15. Store these data in the graph array as relative graph data.
16. Write the data of the graph array to the file.
17. Close "gr.csv".

*The memory for the data array and the graph array is allocated on start up (static allocation).

The implementation of the algorithm is presented in our previous paper [11]. The results of our present experiment in this section are analyzed using this system. A screenshot of a program executing our algorithm is shown in Fig. 8.

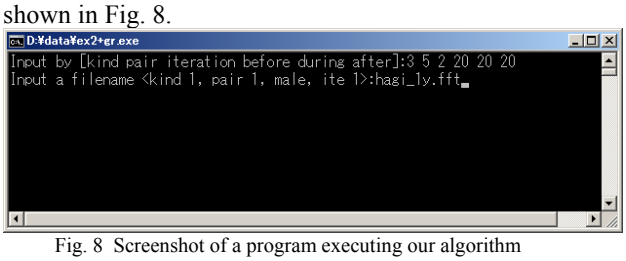

Fig. 8 Screenshot of a program executing our algorithm

3) Making relative data for ANOVA: The automation program generates absolute data, but the

ANOVA in the experiment requires relative data. Thus, relative data were manually generated using the following formula. More specifically, the data are generated by subtracting the "before" value from the value on the sheet "original". We calculated the relative data on the sheet "relative", copying the formula using the Excel function "auto fill". By "relative reference" of the Excel function, cell references in the formula are rewritten depending on the relative position when it is put on a cell.

```
=IF($D9=2,original!F9-original!F5,IF($D9=3,origina
l!F9-original!F1,0))
```

4.2 The Results of ANOVA

In these experimental results, the p-values of the factors and the interactions less than 0.2 were added (mixed) to the whole accident error value (error), using the "auto pooling" function of JUSE-QCAS. The pooling condition is constrained by the relation of each main effect and its dependant interactions, according to the ANOVA theory. Thus, the function decides whether the pooling can occur, and automatically calculates. Results of ANOVA that include slow α, mid α, fast α, and β waves in the experiment are shown in Table 6. The most important α wave is the slow α wave, since it indicates a deep relaxed state of the examinees according to the definition in Table 1. Similarly, the β wave is important for evaluating the active or stressed state of the examinees.

For the slow α, the main effect "A" has a significance of 99% reliability, and indicates that the difference in kind of fertilizer used for growing the Komatsuna results in a different brain state. Furthermore, the interactions "A × D" and "A × B × D" each have a significance of 95% reliability, and indicate that male and female examinees have different brain states depending on combinations of phases and kinds. The "A × B × D" is the most noteworthy interaction, since complex interactions take precedence over simple interactions or main effects on the ANOVA theory. Similarly, for the β wave, the main effect "A" and the interaction "A × B × E" each have a significance of 99% reliability, and indicate that male and female examinees have different brain states depending on combinations of phases, kinds and densities.

Table 6 The ANOVA results from the basic analysis

slow α

Factor	Sum of squares	Freedom degree	Unbiased variance	F0	Assay	P-value (upside)
A	177.297	2	88.649	7.262	**	0.002
B	22.215	1	22.215	1.82		0.186
AB	67.191	2	33.596	2.752		0.078
C	6.387	1	6.387	0.523		0.474
BC	44.333	1	44.333	3.632		0.065
D	6923.215	2	3461.608	283.589	**	0
AD	143.878	4	35.969	2.947	*	0.034
BD	31.274	2	15.637	1.281		0.291
ABD	173.759	4	43.44	3.559	*	0.016
CD	30.762	2	15.381	1.26		0.297
E	9.09	1	9.09	0.745		0.394
AE	78.011	2	39.006	3.196		0.053
BE	38.025	1	38.025	3.115		0.087
CE	155.558	1	155.558	12.744	**	0.001
BCE	61.132	1	61.132	5.008	*	0.032
DE	4.635	2	2.317	0.19		0.828
ADE	125.083	4	31.271	2.562		0.056
BDE	116.03	2	58.015	4.753	*	0.015
CDE	91.692	2	45.846	3.756	*	0.034
error	415.019	34	12.206	0.328		1
measurement error	10723.12	288	37.233			
total	19437.71	359				

mid α

Factor	Sum of squares	Freedom degree	Unbiased variance	F0	Assay	P-value (upside)
A	1.632	2	0.816	0.231		0.795
B	12.594	1	12.594	3.561		0.065
AB	4.555	2	2.277	0.644		0.53
C	7.734	1	7.734	2.187		0.146
AC	22.883	2	11.442	3.235	*	0.048
BC	20.43	1	20.43	5.777	*	0.02
D	2550.767	2	1275.383	360.625	**	0
BD	19.135	2	9.568	2.705		0.077
E	0.007	1	0.007	0.002		0.965
AE	10.774	2	5.387	1.523		0.228
BE	28.627	1	28.627	8.095	**	0.007
ABE	71.884	2	35.942	10.163	**	0
DE	4.682	2	2.341	0.662		0.521
BDE	30.527	2	15.264	4.316	*	0.019
error	169.756	48	3.537	0.398		1
measurement error	2559.436	288	8.887			
total	5515.423	359				

fast α

Factor	Sum of squares	Freedom degree	Unbiased variance	F0	Assay	P-value (upside)
A	59.331	2	29.665	3.458	*	0.04
B	4.822	1	4.822	0.562		0.457
AB	54.699	2	27.35	3.188		0.051
C	18.106	1	18.106	2.111		0.153
AC	90.773	2	45.386	5.291	**	0.009
D	3704.334	2	1852.167	215.93	**	0
AD	33.009	4	8.252	0.962		0.438
CD	9.246	2	4.623	0.539		0.587
ACD	57.146	4	14.286	1.666		0.175
E	0.055	1	0.055	0.006		0.936
AE	45.085	2	22.543	2.628		0.084
BE	0.036	1	0.036	0.004		0.949
ABE	43.761	2	21.88	2.551		0.09
CE	33.978	1	33.978	3.961		0.053
error	377.416	44	8.578	0.605		0.977
measurement error	4082.47	288	14.175			
total	8614.266	359				

β

Factor	Sum of squares	Freedom degree	Unbiased variance	F0	Assay	P-value (upside)
A	69.25	2	34.625	5.225	**	0.008
B	39.185	1	39.185	5.913	*	0.018
AB	36.006	2	18.003	2.717		0.075
D	4080.316	2	2040.158	307.859	**	0
E	8.915	1	8.915	1.345		0.251
AE	18.027	2	9.013	1.36		0.265
BE	3.225	1	3.225	0.487		0.488
ABE	134.483	2	67.241	10.147	**	0
DE	34.287	2	17.143	2.587		0.084
error	371.108	56	6.627	0.46		1
measurement error	4144.875	288	14.392			
total	8939.676	359				

For the mid α and fast α waves, some interactions also have significances, but the results of these waves are less notable in our evaluation since they have intermediate characteristics between slow α and β waves. Both significances of "A × C" indicate that the difference in brain side results in different brain states depending on kinds of fertilizer. However, the difference of fertilizer is thought to only weakly influence the mid α from the insignificance of "A".

4.3 The Fluctuation Graphs

The results of ANOVA are unable to represent and compare EEG fluctuations depending on the difference of level. The fluctuation graphs represent brain state changes in the waves per kind of fertilizer, which are plotted per phase (on the x-axis). Our generation program provides a function to generate graph data with the fixed pattern only, and other factors except kinds and phases are averaged. Thus, the basic results of fluctuation graphs do not reflect the ANOVA results. The purpose of basic analysis is to see effects of kinds, thus, more details are discussed in the detailed analysis.

The results of fluctuation graphs are shown in Fig. 10, and four types of graphs are represented. In the slow α wave, the electric voltages (μV) for "organic" fertilizer are higher than others in the "during" and "after" phases. Furthermore, in the β wave, "organic" is lower than "chemical" in these phases. This indicates that examinees eating Komatsuna grown with "organic" fertilizer are in a deeper relaxed state than when eating those grown with "chemical" fertilizer. Most graphs of "org + ch" are of lower power than other graphs of fertilizers. In particular, "org + ch" could reflect low brain activity since both the slow α and β waves are lower than others except the slow α wave in "after".

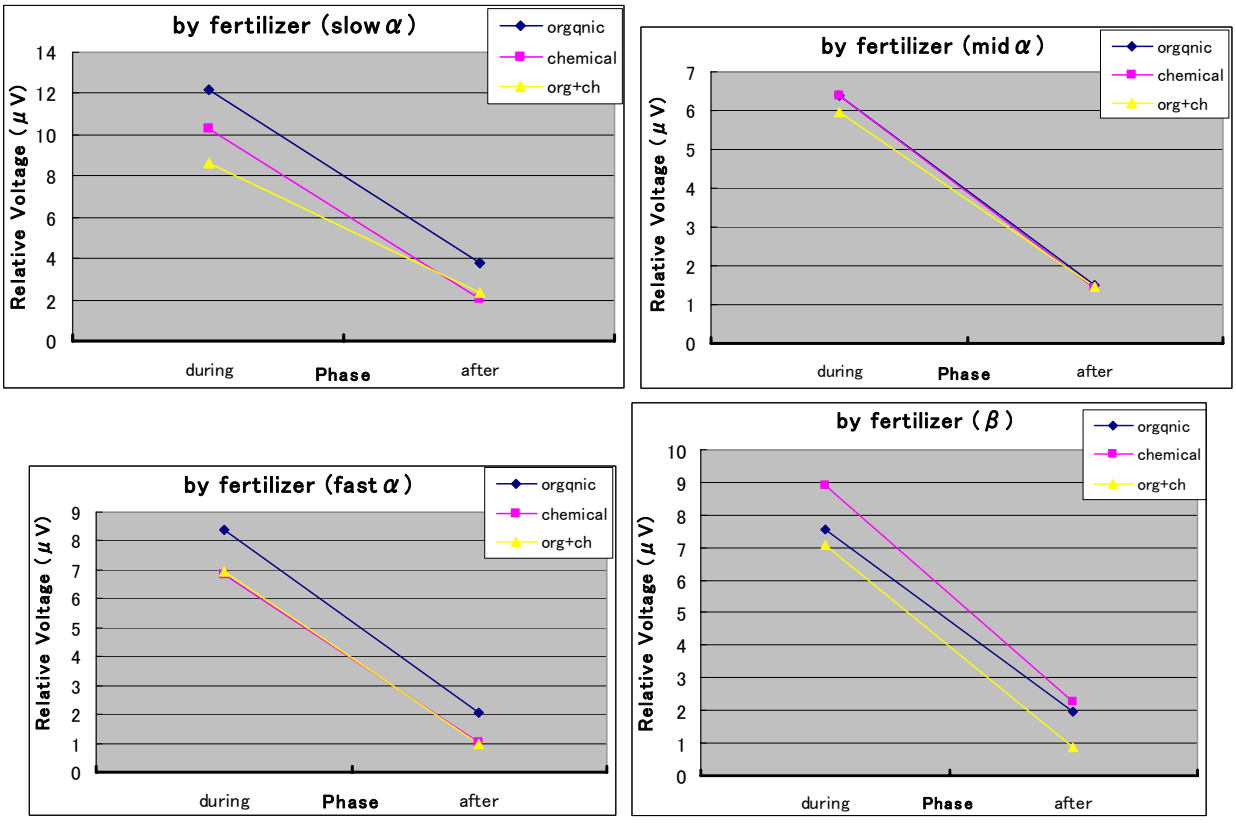

Fig. 10 The fluctuation graphs from the basic analysis

4.4 The Composite Analysis of α wave

1) The ANOVA result: The most important wave of the three α waves is the slow α. Therefore, we define the optimal wave named "smf α", combining the three α waves. We generated ANOVA data of smf α using the formulation "=MAX(F9,F84,F159)", which means that the datum of the smf α wave is generated using the highest voltage of the data of the three α waves.

The ANOVA results of smf α waves are shown in Table 7. These results are similar to the results of slow α, and "A × D" and "A × B × D" also have significances with 95% reliability. The result indicates that the smf α

fluctuation depends on combinations of sexes, phases and kinds. The difference in fertilizer could influence brain state strongly, since "A" has a significance with 99% reliability.

2) The fluctuation graph: The composite graph of the three α waves is calculated using "smf α = max (slow α, mid α, fast α)". This graph represents a comparison of relaxation levels by averaging the values of the three waves. While, the results are not precise, since this calculation process is simply the average of three waves, it does provide an overview of the difference in fertilizers.

The fluctuation graph of smf α by fertilizer difference is shown in Fig. 11. In the "during" and "after" phases, the graph points of Komatsuna by "organic" are higher voltages than other kinds of Komatsuna. This result indicates that Komatsuna grown with "organic" fertilizer could put examinees in a deeper relaxed state than others when eaten. Similar results were seen in the graph analysis of the slow α and β waves. Thus, in Fig. 10, the smf α graph is nearly identical to the slow α graph, since the lower voltages of the other three waves are discarded by the smf α formula.

Table 7 The ANOVA result of smf α wave

smf α

Factor	Sum of squares	Freedom degree	Unbiased variance	F0	Assay	P-value (upside)
A	184.013	2	92.007	7.783	**	0.002
B	1.435	1	1.435	0.121		0.73
AB	35.467	2	17.733	1.5		0.238
C	25.687	1	25.687	2.173		0.15
AC	31.692	2	15.846	1.34		0.276
BC	37.82	1	37.82	3.199		0.083
D	7387.309	2	3693.654	312.448	**	0
AD	130.847	4	32.712	2.767	*	0.044
BD	0.717	2	0.359	0.03		0.97
ABD	132.439	4	33.11	2.801	*	0.042
CD	45.823	2	22.911	1.938		0.161
E	5.91	1	5.91	0.5		0.485
AE	57.156	2	28.578	2.417		0.105
BE	18.437	1	18.437	1.56		0.221
ABE	46.77	2	23.385	1.978		0.155
CE	114.499	1	114.499	9.686	**	0.004
ACE	55.216	2	27.608	2.335		0.113
BCE	38.624	1	38.624	3.267		0.08
DE	3.695	2	1.847	0.156		0.856
BDE	73.435	2	36.717	3.106		0.059
CDE	67.671	2	33.835	2.862		0.072
error	378.293	32	11.822	0.349		1
measurement error	9756.812	288	33.878			
total	18629.77	359				

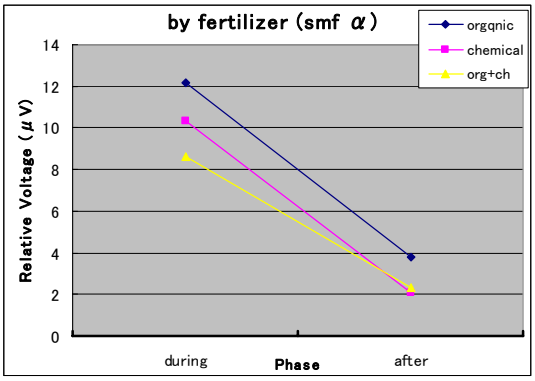

Fig. 11 Fluctuation graph of smf α wave

5. Detailed Analysis

5.1 Basic Concept and Methodology

In the previous section, the fluctuation graphs of the basic analysis only used the fixed graph layout, which consisted of phase (x axis) and relative voltage (y axis). This fixed graph, however, does not reflect the ANOVA results; thus, we introduce the concept of "detailed analysis". The detailed analysis represents the difference of levels depending on interactions using the ANOVA results. Consequently, fluctuation graphs of significant interactions are represented (only interactions including kinds). We made fluctuation graphs manually using the data from the basic analysis, and the following formulas are samples of graph plotted data. A detailed graph point is calculated, selecting and adding needed values on the sheet "relative". Unneeded values are eliminated as zero by equal evaluations and multiple calculations, and finally the sum is averaged. These values are more precise than values obtained using the basic analysis, since the basic analysis makes the relative graphs from the absolute graphs.

["organic - during" on the graph "smf α, A-D"]

{=SUM((relative!$A$309:$A$380=1)*(relative!$D$309:$D$380=2)*(relative!$F$309:$J$380)/5)/SUM((relative!$A$309:$A$380=1)*(relative!$D$309:$D$380=2))}

["chemical 1x - male after" on the graph "β, AE-BD"]

{=SUM((relative!$A$309:$A$380=2)*(relative!$B$309:$B$380=1)*(relative!$D$309:$D$380=3)*(relative!$E$309:$E$380=1)*(relative!$F$309:$J$380)/5)/SUM((relative!$A$309:$A$380=2)*(relative!$B$309:$B$380=1)*(relative!$D$309:$D$380=3)*(relative!$E$309:$E$380=1))}

A screenshot of the detailed analysis calculation is shown in Fig. 12. The blue lines indicate selected data by sex "male" and phases, and the red lines indicate selected data by sex "female" and phases. The left side of the orthogonal table (the rows of "kind", "sex", "brain", "phase", and "density") means data allocations used to ANOVA originally. The detailed analysis uses the part of the table to select data with the above formulas.

5.2 The Detailed Fluctuation Graphs

The fluctuation graphs from the results of the detailed analysis are shown in Fig. 13 and Fig. 14. The variations of the detailed graphs are based on the ANOVA results, and only the graphs for the significant interactions are represented. In the "β, AE-BD" graphs, male and female indicate differences in all phases. In particular, the graphs

of "organic ×2" are each in reverse order, which indicates that the male examinees are more stressed by "organic x2" than the female examinees. However, the female examinees are more stressed by "chemical ×2" than the male examinees. Moreover, the density effects per sex are different, and the power of density "organic ×2" is the lowest in female and it is the highest in male. In the "smf α, A-BD" graphs, both male and female examinees react similarly, with the highest voltage by "organic" in the "during" phase. No notable changes are seen in the graphs of reactions from "org + ch", with low or middle values shown for both the α and β waves. It could indicate inactive state of examinee's brain, thus, they could not feel any stress and relax compared to other kinds.

As general results of the detailed analysis, the fluctuations of smf α and β indicate that Komatsuna grown with organic fertilizer make the examinees relaxed than the one grown with chemical fertilizer in "during". However, in the β wave, the male and female examinees could have different feeling depending on the density, from the graphs of "β, AE-BD male" and "β, AE-BD female".

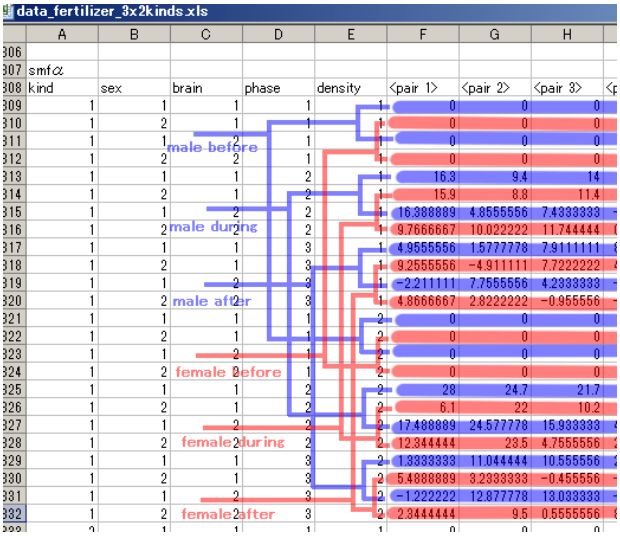

Fig. 12 Calculation of detailed data in Excel 2003.

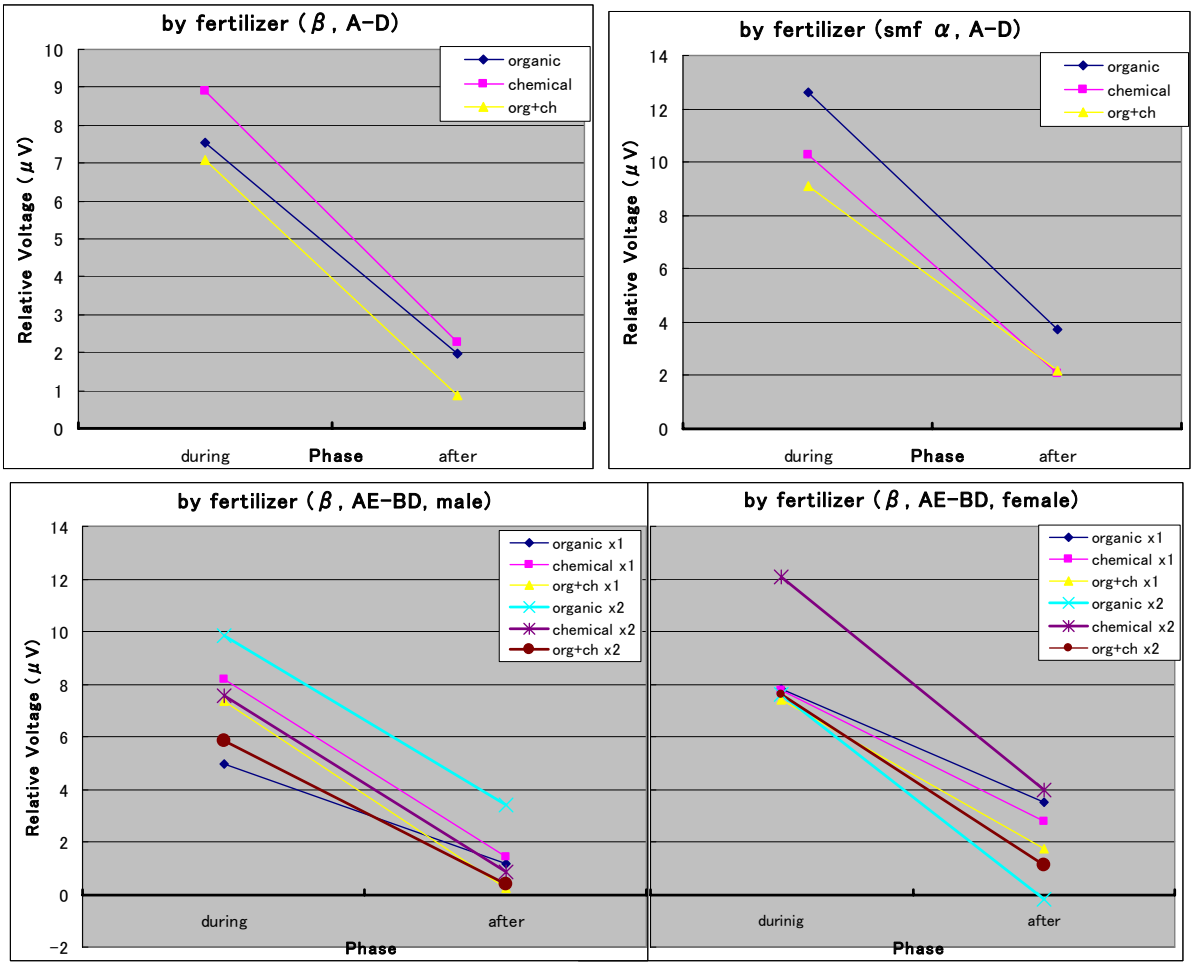

Fig. 13 The fluctuation graphs by the detailed analysis (1)

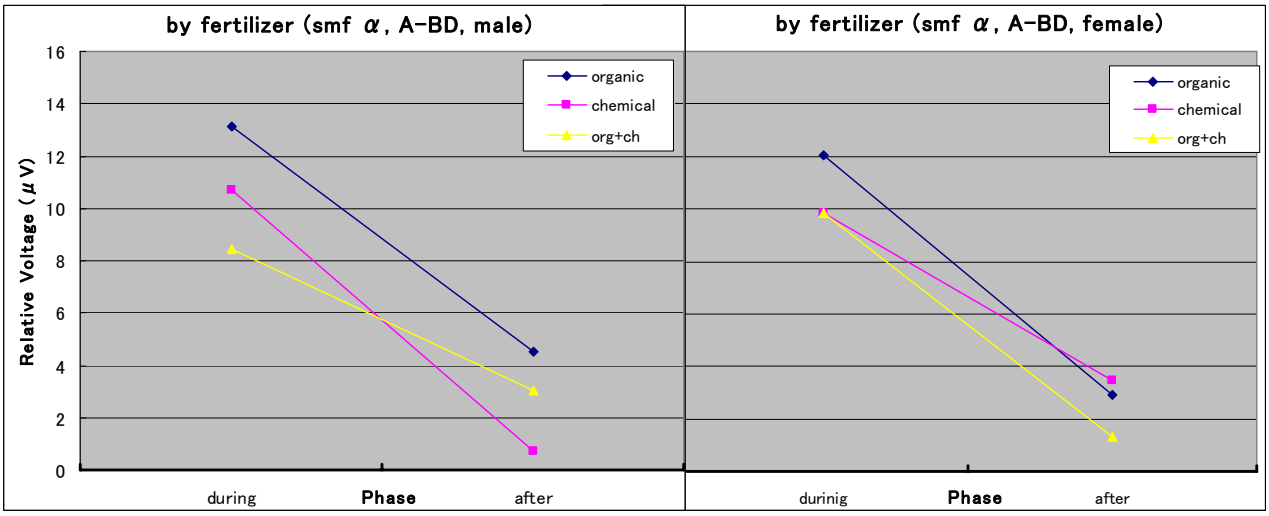

Fig. 14 The fluctuation graphs by the detailed analysis (2)

6. Conclusion and Future Works

We measured EEG fluctuations during ingestion of Komatsuna with Brain Builder Unit. And we analyzed the fluctuations by ANOVA and fluctuation graphs (basic analysis), using our analysis program, JUSE-QCAS and Excel 2003. We found that α and β waves during ingestion of Komatsuna have significant differences depending on the fertilizer used to grow them. In particular, eating Komatsuna grown using organic fertilizer appeared to make examinees more relaxed than eating plants grown using other fertilizers, since the α wave in the "during" phase showed the highest voltage. Furthermore, we also found that some interactions between the defined factors, including the fertilizers, have significant differences (detailed analysis). Notably, males and females show differences in the changes in the α and β waves, as indicated by the interactions of ANOVA and by the detailed graphs. The ingestion reflecting the significances of the interactions are represented in the detailed graphs. We conclude that the fertilizers used for growing Komatsuna could influence EEG in our experimental conditions.

In future works, we will first analyze EEGs of examinees eating Komatsuna grown under other conditions, such as the use of different pesticides, different atmospheric temperatures, or hours of sunlight. Finally, we will design an automation algorithm for the detailed analysis described in Section 5, and will develop a program based on this algorithm to reduce workload and expedite analysis.

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Takashi Ajiro was born in 1980. He received his Ph.D. degree in engineering from Toyo University in 2008. He is now a research assistant at Plant Regulation Research Center, Toyo University from 2008. His main research interests are information science and engineering, especially models of computation, programming languages, and visual language environments.

Koichiro Shimomura was born in 1951. He received his Ph.D. degree in Pharmacy in 1981 from Kyushu University. He joined National Institute of Health Sciences. He is now a professor at Faculty of Life Sciences, Toyo University from 2000. His research interest is mainly antioxidative compounds produced by plants. He is a member of Pharmaceutical Society of Japan, Japan Society for Bioscience, Biotechnology, and Agrochemistry and Japanese Society for Plant Cell and Molecular Biology.

Hirobumi Yamamoto was born in 1960. He received his Ph.D. degree in Pharmacy in 1989 from Kyoto University. He was an assistant professor in Faculty of Pharmaceutical Sciences, Nagasaki University. He is now a professor at Faculty of Life Sciences, Toyo University from 2003. His research interests are biochemistry and metabolic engineering in plant. He is a member of Pharmaceutical Society of Japan and Japanese Society for Plant Cell and Molecular Biology.

Kenichi Kamijo was born in 1949. He received his Ph.D. degree in Geophysics from Kyoto University in 1994. He is now a professor at Faculty of Life Sciences, Toyo University. His research interests include complex systems in informatics, geoinformatics and bioinformatics. He is a member of IEICE, JSAI, Meteorological Society of Japan and Geodetic Society of Japan.