LEBRA FREE ELECTRON LASER AS A RADIATION SOURCE FOR PHOTOCHEMICAL REACTIONS IN LIVING ORGANISMS

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Abstract

The Laboratory for Electron Beam Research and Application (LEBRA) free-electron laser (FEL) irradiation system has been developed and improved to irradiate single cells at tunable wavelengths ranging from 350 to 6500 nm. The objectives of this research are to determine the unique characteristics of LEBRA-FELs in order to evaluate their ability to control photoreactions in living organisms. The authors examined a well-known photoreaction in lettuce seed germination, which is promoted by red light and inhibited by far-red light. The LEBRA-FELs, centered at 660 nm (average irradiation energy: 20 μJ/pulse) with a half-bandwidth of 8 nm, and at 740 nm (average irradiation energy: 40 μJ/pulse) with a half-bandwidth of 16 nm, could promote and inhibit lettuce seed germination, respectively. This treatment was effective when using natural density filters to reduce radiation energy of the 660 and 740 nm FELs to as low as about 0.05 μJ/pulse (10 min irradiation) and about 0.63 μJ/pulse (10 min irradiation), respectively. The LEBRA-FEL, therefore, promises to be an attractive tool for the non-invasive analysis of photochemical reactions in living organisms.

INTRODUCTION

The radiation sources commonly applied to plants are commercially available lamps developed for human lighting applications (e.g., fluorescent, metal halide, high-pressure sodium, incandescent, light-emitting diode, and laser diode lights). In contrast, free-electron lasers (FELs) such as the one developed by the Laboratory for Electron Beam Research and Application (LEBRA) produce high-energy, tunable pulsed radiation [1, 2]. The advantages of the LEBRA-FEL compared with other lasers [3] are that its peak intensity ranges from 0.9 to 6.5 μm [4] through the use of silver-coated copper mirrors in the FEL resonator [2], that it can generate higher harmonics by means of non-linear optical crystals, and that it can emit wavelengths from 0.35 to 6.5 μm encompassing both the visible (VIS) and infrared (IR) regions. Previously, we established a microscopic irradiation technique for delivering VIS-FEL light to single cells through a tapered glass rod (<10 μm diameter) [5]. However, it is still unclear whether LEBRA-FELs can produce sufficient radiant energy at wavelengths effective for triggering photochemical reactions in living organisms. The aim of this study was to evaluate the effectiveness of LEBRA-FELs through lettuce seed germination tests [6-11]. Results showed promotion by red light (660 nm) and inhibition by far-red light (740 nm), indicating that LEBRA-FELs can be used to control lettuce seed germination and are thus promising as a potent radiation source for photochemical investigations in living organisms.

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MATERIALS AND METHODS

Lettuce Seeds

Lettuce seeds (*Lactuca sativa* L.) from the Red Wave cultivar (a leaf lettuce) were obtained from a commercial supplier (Sakata Seed Co., Yokohama, Japan) and used within the recommended periods. In preliminary germination experiments, the seeds used here exhibited several advantageous characteristics for the evaluation of the LEBRA-FEL as a radiation source for investigating photoreactions in living organisms. Almost no seeds germinate when kept in darkness at 26.5 °C for at least 20-24 h, and average values for induction and inhibition of germination are both over 95±2% upon irradiation with red light and far-red light, respectively. These characteristics were re-examined simply by a 10 min exposure to 660 nm LED light and 735 nm LED light at 26.5 °C in darkness. Both the LEDs were installed in a Photo Germinating Seeds Apparatus (GR-8; Shimadzu Rika Co., Tokyo, Japan).

Setup of FEL Irradiation System

A FEL microscopic irradiation system was set up on an optical bench at the user's laboratory of LEBRA (Figure 1A) as previously published [5]; briefly, an irradiation section of quartz fiber (0.6 mm diameter: Figure 1B) was installed on a fiber holder (H-7; Narishige Group, Tokyo, Japan), which could be readily manipulated by two micromanipulators (MN-153 and MMO-220A; Narishige Group).

Light Sources

According to previous research [6-11], red light promotes germination of lettuce seeds whereas far-red light inhibits it. Therefore, we chose 660 nm red light as a candidate wavelength for the LEBRA-FEL promotion light source, and 740 nm far-red light for the inhibition light source. The tip of the quartz fiber could focus radiation on the surface of the sample seed, which had been inserted into a capillary (Figure 1B), and allowed for accurate direction of the radiation to the targeted sample. The FEL radiation energy could be reduced by means of combined neutral density (ND) glass filters (Kenko Tokina Co. Ltd., Tokyo, Japan), by which the FEL

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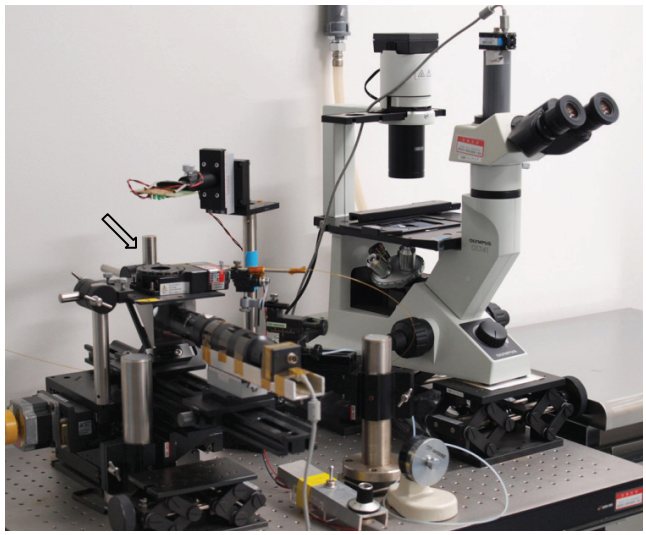

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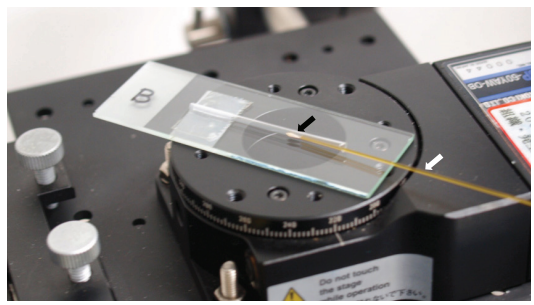

B

Figure 1: A, LEBRA-FEL micro-irradiation system. B, Enlarged view of irradiation apparatus (view from top) indicated by the arrow in A. A lettuce seed (filled arrow) was introduced to a capillary, and irradiated by a given wavelength of FEL through a quartz fiber (open arrow). Green LEDs were placed 8 cm above the level of the θ-axis rotation stage (Sigma-Koki Co. Ltd., Tokyo, Japan), and used as safety lighting sources. If reduction of FEL radiation energy was necessary, natural density (ND) filters were placed over the entrance side of the FEL light source (position not shown).

radiation energy was attenuated by a factor of at least 1/800. The FEL radiation energy at the tip of the quartz fiber was measured by a power meter (FieldMax$_{II}$-TOP; Coherent, Inc., Portland, OR).

Imbibition of Seeds

Before the experiments, the lettuce seeds were sown on two layers of washed filter paper (No. 2; Toyo Roshi Co., Ltd., Tokyo, Japan) and Kimwipe paper (Kimberly-Clark Co., Tokyo, Japan), covered with 2 ml distilled water in a 5 cm glass Petri dish, and imbibed for 1 h at 26.5 ± 0.5 °C in darkness. Ikuma and Thimann [12] estimated the maximum induction of germination to be about 1.5 h at 25 °C in darkness, which was identified when the water content of the seed increased by roughly 40% above their air-dry weight [12]. To determine when the lettuce seeds used here had reached this state, the seeds were allowed to imbibe at 26.5 ± 0.5 °C for various periods of time, and any increase in fresh weight was measured. The whole seeds did not complete water uptake within 1 h of imbibition, but after 50 min of imbibition, the weight of the seeds had reached 40% above air-dry weight.

Pre-Exposure for FEL Irradiation

Previous reports have revealed that LED light sources promote and inhibit lettuce seed germination [13, 14] in the same manner as fluorescence lights [15, 16], sodium light illuminators [17], and incandescent lights [18], among others. We used LED lights in place of FEL lights for convenience to deplete the active material [19, 20] formed during storage of the seeds [21]. To deplete the red light-formed active material, the seeds were exposed to far-red LED light, and then to deplete the reversed active material, seeds were exposed to red LED light [20]. In practice, after a given time of imbibition (usually 1 h), the glass Petri dish was immediately transferred to the chamber (6 × 6 cm, 3 cm deep) of the Photo Germinating Seeds Apparatus, which was used here for pre-exposure to red or far-red light at 26.5 °C. This apparatus is designed specifically for lettuce seed germination tests: it possesses a row of small, divided sections with built-in light sources and each section can be separately illuminated by 735, 660, 505, or 470 nm LEDs. The pre-exposure LED light conditions were chosen based on the manufacturer's instructions.

FEL Irradiation Experiments and Germination Tests

Each seed exposed to red or far-red LED light was removed from the Petri dish with fine forceps, and immediately introduced into a capillary made from a Pasteur pipette (see Figure 1B). Prior to irradiation experiments, Pasteur pipettes had been cut to fit the diameter of the lettuce seeds (about 1.4 mm in wet form), individually fixed on glass slides, and filled with about 100 μL distilled water (see Figure 1B). During the experiment, the surface of the seed coat was manipulated under a dissection microscope so that a given wavelength from the LEBRA-FEL irradiated a section of the seed hypocotyl near the micropyle (a small opening in the ovule wall). Care was taken that the FEL irradiation experiments did not exceed 2 h [21, 22], including the times for imbibition and pre-exposure with LED light (usually 10 min).

A previous report [12] indicated that the post-induction phase starts with a highly oxidative reaction that immediately follows photoinduction. To achieve continuing incubation under oxidative conditions, the

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seed, immediately following irradiation, was moved from the capillary to a 7 cm Petri dish for cultivation, which had been prepared with 4% agar in distilled water. Each Petri dish thus sown was covered with a piece of aluminum foil and incubated at 26.5 ± 0.5 °C in a Climatic Test Chamber (Atmos Chamber MTH-2200; Sanyo, Osaka, Japan). The germination processes were recorded and evaluated after 20–24 h incubation under a dissection microscope. All handling was performed in darkness or carried out under green LED light (4 units; 520 nm light, half-bandwidth; 30 nm).

Evaluation

Except for observing and counting germinations, all other operations were carried out in darkness or under green LED light as above. In control experiments, the lettuce seed germination processes were determined and divided into five developmental stages after 20–24 h incubation (Figure 2). The established standard developmental stages in lettuce seeds were used for evaluation of the FEL irradiation experiments.

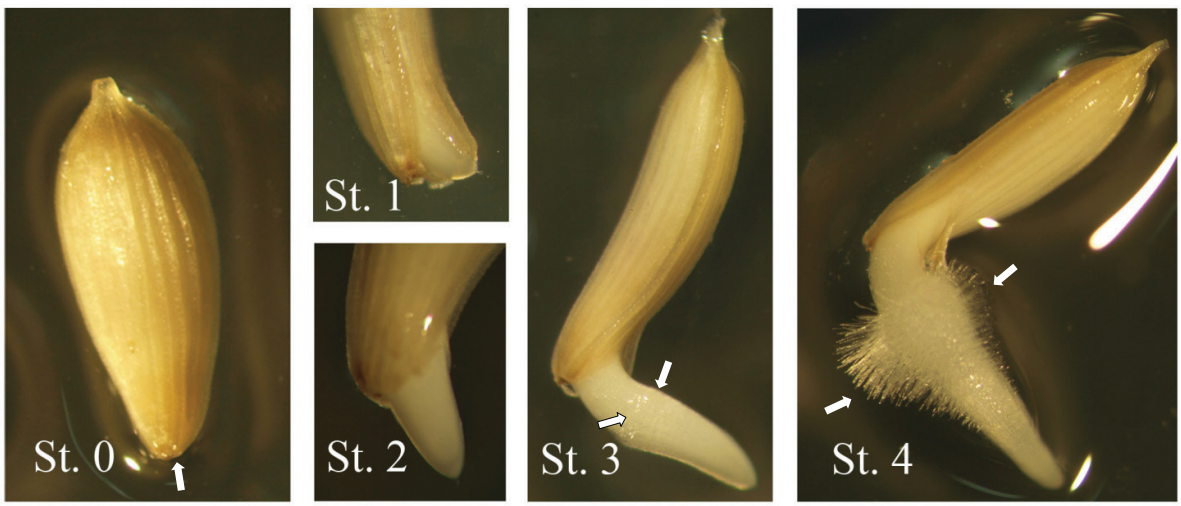

Figure 2: Developmental stages (St.) of lettuce seeds and definitions of the germination processes. St. 0, no germination (arrow indicates part of the seed irradiated by FEL). St. 1, initial rupture of the seed coat (testa). St. 2, endosperm rupture and radical emergence. St. 3, initial appearance of air roots (arrows) and growth of the radical. St. 4, further elongation of air roots (arrows) and also radical elongation. Scale bar = 1.0 mm.

RESULTS AND DISCUSSION

Characteristics of LEBRA-FELs

Figures 3A and 3B show the spectral energy distribution of 660 nm red light and 740 nm far-red light, respectively. Such tunable FELs have sharp peak emissions with typical spectral bands; the bandwidths at half energy are 8 nm and 16 nm for the 660 and 740 nm LEBRA-FELs, respectively. These values vary between daily experiments. The characteristics of the two wavelengths are summarized in Table 1, in which the FEL radiation energy values have been converted into photosynthetic photon flux density (PPFD) values.

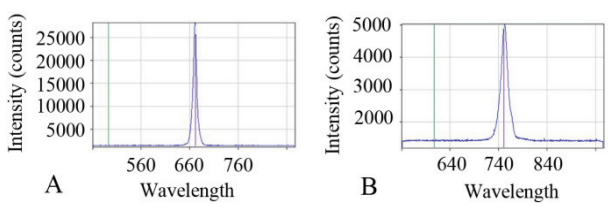

Figure 3: Spectral energy distribution of the LEBRA-FELs with peak emission at 660 nm (A) and 740 nm (B).

Figure 4 shows a typical irradiation experiment using the tip of the quartz fiber (0.6 mm diameter) to directly irradiate the tip of the hypocotyl, which is the probable photoreceptor in lettuce seeds [22], with 660 nm red FEL light.

Table 1: Characteristics of 660 nm and 740 nm LEBRA-FELs Used in This Study

Wave-length, nm	Energy[(1)], μJ/pulse	Half bandwidth, nm	Photosynthetic Photon Density Flux[(2)], μmol/m²/s
660	10-30	8	776
740	20-60	16	1740

[(1)] FEL radiation energy at the tip of quartz fiber was measured with a power meter.

[(2)] The conversion from FEL radiation energy (J/pulse) to PPFD is based on the numbers of photons in a certain waveband incident per unit time (s) on a unit area (m^2) divided by the Avogadro constant (6.022×10^{23} mol^{-1}). In practice, the radiation energy of 660 and 740 nm were taken as the average: 20 and 40 μJ/pulse, respectively.

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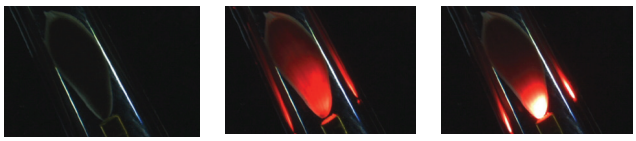

A B C

Figure 4: Lettuce seed irradiated by 660 nm FEL through a quartz fiber (diameter, 0.6 mm). A, control experiment. B and C, irradiation experiments. Note that the surface of the seed coat (B) and the part of the embryonic axis centered on the micropyle (C) were irradiated, respectively.

Evaluations of LEBRA-FELs

Table 2 summarizes the outcome of the lettuce seed germination experiments where seeds were irradiated by LEBRA-FELs. It is evident that 660 nm red light from the LEBRA-FEL promotes seed germination, and 740 nm far-red light showed the opposite effect, as previously reported [6-11]. The reversibility between promotion and inhibition by red and far-red FELs can be repeatedly demonstrated (see Experiments 4 and 5 in Table 2). These results are also in complete agreement with previously reported findings [23]. Hence, we can deduce an important characteristic of LEBRA-FELs from our lettuce seed germination tests: that they can be used as a potential radiation source for controlling photoreactions in lettuce seeds.

Table 2: Effect of LEBRA-FELs on Lettuce Seed Germination

No. of Experiment	Treatment with FEL[(1)] (Irradiation Time, min)[(2)]	Germination[(3)]
1	In darkness, control	None
2	660 nm (10 min)	Promotion
3	740 nm (5 min)	Inhibition
4	660 nm (10 min), then 740 nm (5 min)	Inhibition
5	740 nm (5 min), then 660 nm (10 min)	Promotion

[(1)] Average radiation energies were calculated to be approximately 20 μJ/pulse for the 660 nm FEL and approximately 40 μJ/pulse for the 740 nm FEL. Note that the seeds in experiments 4 and 5 were irradiated in sequence.
[(2)] Although the saturating dose of both lights was reached at a short exposure (within 1 min) as found in the case of fluorescence lights [23], most of the experiments were irradiated for 10 or 5 min using 660 nm FEL or 740 nm FEL, respectively, in order to secure the full effects.
[(3)] After incubation for 20-24 h at 26.5 ± 0.5 °C in darkness, germination state was determined as either promotion or inhibition, which can also refer to the developmental stages mentioned elsewhere: Promotion, St.1 to St. 4; Inhibition, St. 0.

Table 3: Effect of LEBRA-FEL Reduced by ND filters on Lettuce Seed Germination

660 nm Irradiation		Reduction of FEL Radiation Energy[(1)] by ND Filter	740 nm Irradiation	
St. 0	St. 1~St. 4		St. 0	St. 1~St.4
0	12	No Filter	11	0
0	3	4^{-1}	3	1
ND		8^{-1}	5	2
0	3	32^{-1}	10	6
1	14	64^{-1}	6	3
0	9	128^{-1}	0	3
2	5	256^{-1}	ND	
3	5	400^{-1}	ND	
6	0	800^{-1}	ND	

[(1)] Average radiation energies were calculated to be approximately 20 μJ/pulse for the 660 nm FEL and approximately 40 μJ/pulse for the 740 nm FEL.
[(2)] All seeds used here were imbibed at 26.5 ± 0.5 °C in darkness and then, prior to irradiation experiments, exposed to 10 min of 735 nm LED light or 660 nm LED light, both of which were used to deplete naturally occurring active materials which might impact lettuce seed germination. The effects of the irradiation experiments are denoted by numbers of seeds counted and evaluated by lettuce seed developmental stages.
ND: No Data

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The FEL radiation energy, if necessary, can be reduced by use of ND filters as mentioned above. Table 3 shows the results of these experiments, quantified as the number of individual lettuce seeds in each developmental stage (St. 0, St. 1, St. 2, St. 3, and St. 4). It can be concluded that LEBRA-FELs have proved to be effective lighting sources for analyzing pure photochemical reactions without hindering other innate factors of plant lettuce seeds. By combination with ND filters, the radiation energy of the 660 nm FEL was diminished to roughly 1/400th of its original energy (20 μJ/pulse at the tip of the quartz fiber) and was still effective in promoting lettuce seed germination. However, when the radiation energy of the 740 nm FEL was reduced to roughly 1/128th of its original energy (40 μJ/pulse at the tip of the quartz fiber); the radiation energy was ineffective at inhibiting germination.

Lastly, considering several key characteristics of the LEBRA-FELs such as high radiation energy, high pulse wavelengths, narrow spectral half-width, and tunable wavelengths from VIS to IR (350 nm to 6500 nm), it is therefore expected to be a useful tool in investigating photochemical reactions in plants as well as in other living organisms.

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