POTENTIAL PHOTOCHEMICAL APPLICATIONS OF THE FREE ELECTRON LASER IRRADIATION TECHNIQUE IN LIVING ORGANISMS

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Abstract

The free electron lasers (FELs) of the Laboratory for Electron Beam Research and Application (LEBRA), Nihon University and of Kyoto University (KU) were combined to produce tunable FEL wavelengths from the visible to the mid-infrared region (0.4–20.0 μm). We have previously verified that visible light from the LEBRA-FEL can control the germination of lettuce seeds, a well-known photochemical reaction in plants. We found that red light (660 nm FEL) promotes the germination and far-red light (740 nm FEL) inhibits it. In this article, we further examine the photochemical effects on lettuce seed germination of various wavelengths from visible to mid-infrared generated by combining the two FELs. The red spectra of FEL ranging from 600 to 680 nm (activity peak: 660 nm) promoted germination at an activity level of over 40%, whereas the far-red spectra of FEL from 700 to 760 nm (activity peak: 740 nm) inhibited germination at a similar activity level. For the other wavelengths examined, we did not observe the promotion or inhibition of seed germination at an activity level of more than 40%. However, the unique characteristics of the combination of two FELs may prove to be a useful tool because of its high pulse radiation energy, narrow spectral half-bandwidths, and tunable wavelengths from visible to mid-infrared. It may allow the identification of novel photochemical reactions in living organisms.

INTRODUCTION

In 2001, the Laboratory for Electron Beam Research and Application (LEBRA) achieved the first lasing of 0.9–6.5 μm, in the near- and a small part of the mid-infrared regions of free electron lasers (FELs), in which higher harmonics were generated by using nonlinear optical crystals. Now, the FELs cover a wide range of wavelengths from visible to mid-infrared regions from 400 nm to 6.5 μm [1]. In 2012, the Institute of Advanced Energy in Kyoto University (KU), succeeded in producing a FEL (KU-FEL) that covered a large part of the mid-infrared region, and its high-energy and tunable wavelengths have recently been extended from 5 to 20 μm [2]. Following these breakthroughs, we have focused on using both these FELs for investigating photochemical reactions in living organisms. In 2013, we verified that the visible LEBRA-FELs can control the germination of lettuce seeds, a well-known photochemical reaction in plants [3, 4], and showed that red light (660 nm FEL) promotes germination, whereas far-red light (740 nm FEL) inhibits it [5]. We also found that the FEL treatment was still effective when using neutral density filters to reduce radiation energy at 660 nm and 740 nm to as low as about 0.05 μJ/pulse (10 min irradiation) and about 0.63 μJ/pulse (10 min irradiation), respectively [5].

The aim of this study is to determine the photochemical efficiency in lettuce seed germination tests of the tunable wavelengths of FEL from 400 nm to 20 μm, generated by combining the two FEL facilities at LEBRA and KU [1, 2]. The results showed that lettuce seed germination was promoted when the seeds were irradiated by the red FEL spectra from 600 to 680 nm and inhibited by the far-red FEL spectra from 700 to 760 nm. The activity levels that corresponded to the photochemical efficiency were more than 40% for the promotion and inhibition. For the more than 25 other FEL wavelengths examined, ranging from visible to mid-infrared regions, photochemical efficiencies of less than 40% activity was observed for promoting and inhibiting lettuce seed germination.

MATERIALS AND METHODS

Lettuce Seeds and Imbibition

Lettuce seeds (Lactuca sativa L.) from the Red Wave cultivar (a leaf lettuce) were obtained from a commercial supplier (Lot Nos. 546427 and 546430; Sakata Seed Co., Yokohama, Japan) and were used within the recommended period. Prior to irradiation experiments, the seeds were imbibed, as described in our previous report [5].

Setup of FEL Irradiation Systems

The setups of the LEBRA-FEL and KU-FEL irradiation systems are shown in Figure 1 (A-1 and B-1). The sample stages for the FEL irradiation experiments are shown in the magnified views (A-2 and B-2). In the sample stage, 5 or 6 lettuce seeds can be irradiated at once. A set of typical germination results at a wavelength of 690 nm is shown in Figure 2 (A and B).

Radiation Sources

We use the definitions in the Photonics Spectrum Reference Chart (Laurin Publishing, Pittsfield, MA) relating to ranges for the visible (400–750 nm), near-infrared (750 nm to 3 μm), and mid-infrared spectra (3-30

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ISBN 978-3-95450-133-5

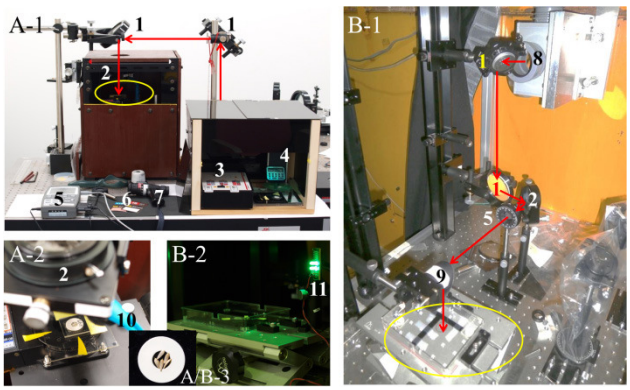

Figure 1: Setup of the two irradiation systems: LEBRA-FEL (A-1; view from front) and KU-FEL (B-1; view from top). Parts shown in yellow ovals in A-1 and B-1 are the sample irradiation stages, both of which are enlarged in A-2 and B-2, respectively. The inset (A/B-3) shows the hollow 6 mm in diameter where five lettuce seeds can be placed. Each path for the given wavelengths, indicated by red arrows, can irradiate the seeds on the sample stage. 1: flat mirror (Sigma-Koki); 2: lens (Sigma-Koki); 3: photo seed germination apparatus; 4: safety light-1; 5: laser power/energy meter (FieldMax$_{II}$-TOP); 6: detector cards (left: visible, right: infrared; Edmund Optics, Inc., Tokyo, Japan); 7: infrared conversion viewer (Newport Corp.); 8: output port of the FEL beam transport; 9: off-axis parabolic mirror (Sigma Koki);10: safety light-2; 11: safety light-3.

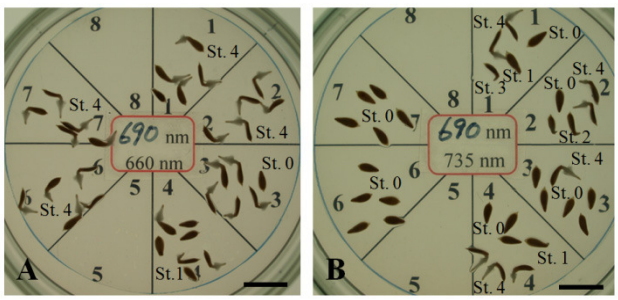

Figure 2: Examples of 690 nm FEL irradiation experiments. A: Inhibition experiment. B: Promotion experiment. Prior to 690 nm FEL irradiation, the seeds were depleted of active materials by exposure to LEDs. Agar plates were divided into eight blocks: No. 1–4 for irradiation experiments, No. 6 and 7 for controls. Five developmental stages (St. 0–4) defined previously [5] were used to evaluate the irradiation experiments. Scale bars = 10 mm

μm). The LEBRA-FEL can generate visible, near-infrared, and part of the mid-infrared spectra. The KU-FEL can produce a large part of the mid-infrared spectra. We collaborated closely on the specifications for the irradiation light sources, particularly for the longer wavelengths in the mid-infrared region. The typical beam parameters of the two FELs have been described previously [1, 2], in which the FEL radiation energies could be reduced by combined neutral-density glass filters (Kenko Tokina Co. Ltd., Tokyo, Japan) or a polarizing filter (WP25H-K, Thorlabs Inc, Newton, NJ). The irradiation energies used here are listed in Table 1 and typical half-bandwidths have been reported previously [5]: 8 nm for the 660 nm FEL, and 16 nm for the 735 nm FEL. The radiation power is measured by a power meter (FieldMax$_{II}$-TOP, Coherent, Inc., Portland, OR), a pyroelectric energy detector (818E-20-50S, Newport Corp., Irvine, CA), or a multi-function optical meter (1835-C, Newport Corp.).

Depletion of Active Materials in Seeds

After imbibition for a given time (usually 50 min [5, 6]), the seeds were depleted of the naturally occurring active materials that accumulate during seed storage [7]. To deplete the active material produced in response to red light, the seeds were exposed to far-red FEL. Then to deplete the active material produced in response to far-red light, the seeds were exposed to red FEL [5]. These characteristics were manipulated by a 10 min exposure to 660 or 735 nm light-emitting diode (LED) light, both of which were installed in a photo seed germinating apparatus (GR-8, Shimadzu Rika Co., Tokyo, Japan). This apparatus is designed specifically for lettuce seed germination tests: it contains a row of small, divided sections with built-in LED lighting sources and each section can be separately illuminated by 735, 660, 505, or 470 nm LEDs. To deplete the naturally occurring active materials, conditions for pre-exposure to LEDs were chosen based on the manufacturer's instructions. After a 50 min imbibition, the seeds were sown on two layers of washed filter paper in glass Petri dishes (4.5 × 4.5 cm, 2 cm deep), and were immediately transferred to the chambers (6 × 6 cm, 3 cm deep) of the photo seed germination apparatus. The seeds were pre-exposed for 10 min to red or far-red light at 25.0 °C. The seeds were then irradiated for 5 min at a given FEL wavelength to determine whether the wavelength can promote or inhibit germination of the lettuce seeds.

FEL Irradiation Experiments and Germination Tests

About thirty-five seeds were exposed to red (660 nm) or far-red (735 nm) LED light for at least 10 min in the photo seed germination apparatus, and were then removed one by one from the Petri dishes with fine forceps. The seeds were immediately placed in a sample irradiation glass stage (1.5 × 1.5 cm) with a small hollow 6 mm in a diameter, which was large enough to place 5-6 seeds in without touching (Figure 1; enlarged view of A/B-3). The FEL irradiation experiments did not exceed 2 h [5, 6], including time for imbibition, pre-exposure with LED (usually 10 min), and irradiation time (5 min). These FEL irradiation experiments were repeated three or four times. Controls were taken at the beginning and the end of the irradiation experiments. To achieve continuous incubation under oxidative conditions, immediately following irradiation, the seeds were moved from the sample stage to a 7 cm Petri dish, which had been prepared with 0.4% agar in distilled water. Unirradiated control seeds were

ISBN 978-3-95450-133-5

also transferred. Each Petri dish was shielded from light by wrapping in a double sheet of aluminum foil, and then incubated at 25.0 ± 0.5 °C in a climatic test chamber (Atmos Chamber MTH-2200; Sanyo, Osaka, Japan) or an incubator (SLC-25A, Mitsubishi Corp., Tokyo, Japan). The germination processes were recorded and evaluated within 20–24 h of incubation under a dissection microscope. All handling was performed in darkness or carried out under safety lights (white lights) covered with band pass filters (BPB-45 or BPN-50, Fujifilm Corp., Tokyo, Japan), as well as green LEDs (4 units; 520 nm spectrum, half-bandwidth; 30 nm). Either of the two kinds of band pass filters was fixed on the front of a white light (0.5 W-LED, Panasonic Co., Tokyo, Japan). The safety lights had no effect on the seed germination for up to 20 min irradiation. This means that under the safety lights, the seeds can be manipulated without being affected by the light during the operations (usually not exceeding 5 min). The installation of the safety lights is shown in Figure 1.

Evaluation

Except for observing and evaluating the state of germination, all other operations were carried out in darkness or under the safety lights. To evaluate the irradiation experiment results, the seed germination processes were determined and divided into five developmental stages (St. 0–4) within 20–24 h of incubation [5]. Figure 2 shows an example of the irradiation experiments at a given FEL wavelength. To evaluate effectiveness of the FEL irradiation experiments simply, we further divided the five developmental stages into two groups: one includes seeds at stages St. 0–2, and the other includes seeds at stages St. 3 and 4. This made evaluating the efficiency of FEL easier, because almost all the seeds normally develop to St. 4 within 20–24 h incubation at 25 °C in the dark. The seeds in the first group (St. 0–2) showed little or no activation in the promotion experiments, whereas the seeds in the second group (St. 3 and 4) showed activation in the promotion experiments. This was reversed for the inhibition experiments.

RESULTS AND DISCUSSION

One of the most important prerequisites is to switch the seeds into either of the two active states of germination, promotion or inhibition, so that the seeds fully respond to the photo-potential of a given wavelength of light. In the promotion experiments, the active materials produced by red light were depleted by irradiating some of the seeds with far-red light, and then the response of the seeds to various wavelengths of light was examined. In contrast, in the inhibition experiments, the active materials produced by far-red light were depleted by irradiating some of the seeds with red light, and then the response of the seeds to different wavelengths was examined. The reversibility of the active states of promotion and inhibition produced by red light and far-red light, respectively, was repeatedly demonstrated by far-red FEL and red FEL in our previous report [5]. Other studies have shown that LEDs can retrieve the reversibility of photoreactions in lettuce seed germination [8] in the same manner as fluorescent lights, sodium light illuminators, and incandescent lights. Therefore, instead of FEL irradiation, we prepared the two photosensitive states of lettuce seeds by exposing them to either red (promotion) or far-red (inhibition) LED light. Both the seed lots used here showed high photosensitive states: the activity levels of promotion or inhibition exceed 93%. This means that the seed were sufficient to determine whether the FEL spectra, ranging from visible to mid-infrared, affect the seeds.

Efficiencies of Visible Spectra

The results of the irradiation experiments at visible wavelengths are summarized in Table 1. The active wavelengths for promoting seed germination are 600–680 nm (>40% promoting activity) and those for inhibition are 700–780 nm (>40% inhibiting activity). The values of inhibition and promotion given in Table 1 are plotted to show the typical apparent patterns of the effectiveness of wavelengths for lettuce seed germination (Figure 3). Interestingly, there are two active peaks for inhibition, which resemble the typical color curve of red-absorbing materials, as reported by Hartmann and Mollwo [7], who used a xenon arc cinema projector as a lighting source. Furthermore, the wavelengths at 410 nm and 500 nm seemed to be effective in germination of the lettuce seeds, which are consistent with previous work [9] although further FEL experiments are required.

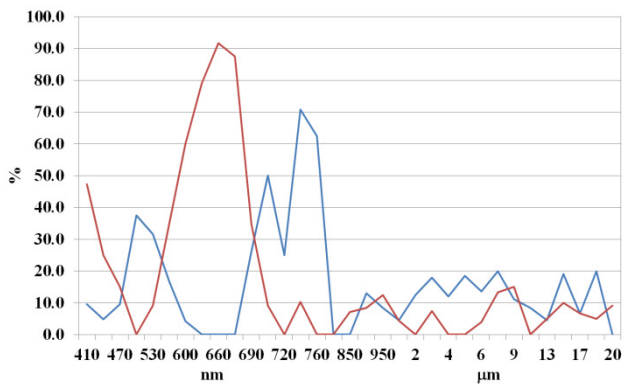

Figure 3: Action spectra for promotion (red line) and inhibition experiments (blue line) constructed from the data shown in Table 1. Activity levels over 40% are recognized as effective.

Efficiencies of Near- and Mid-Infrared Spectra

Based on reviews covering recent biological applications of infrared radiation sources [10], some studies of the biological and biomedical effects of infrared spectra have been published [10], and it is a promising treatment for certain medical and scientific fields [10]. We intend to use near- and mid-infrared radiation sources to identify the biological effects of radiation on cells, tissue, and small living organisms. Therefore, our initial work has focused on irradiating lettuce seeds, the germination

ISBN 978-3-95450-133-5

of which can be easily controlled by visible light exposure and can be evaluated within a day. Table 1 also summarize the efficiencies of near-infrared and mid-infrared irradiation, respectively. Even using high radiation energies, activities of over 20% for either promotion or inhibition were not detected. Under these conditions, heat effects are negligible because both LEBRA-FEL and KU-FEL use discontinuous pulsed spectra of 2 and 1 Hz, respectively, rather than continuous pulsed spectra. Even increasing the irradiation time from 5 min to 10 or 20 min for the 9 μm FEL and 20 μm FEL did not affect the spectra with over 20% activity (data not shown here).

Table 1: Effect of Visible, Near-Infrared and Mid-Infrared Spectra [(1)] on Lettuce Seed Germination

FEL μm	Inhibition		Promotion		Evaluation[(2)] %		Energy μJ/pulse
	St. 0-2	St. 3-4	St. 0-2	St. 3-4	Inhi-bition	Promo-tion	
0.41	2	19	10	9	9.5	47.4	11.7
0.44	1	20	15	5	4.8	25.0	12.5
0.47	2	19	17	3	9.5	15.0	12.5
0.50	6	10	17	0	37.5	0.0	9.5
0.53	6	13	20	2	31.6	9.1	18.4
0.56	3	15	13	7	16.7	35.0	23.7
0.60	1	23	10	15	4.2	60.0	3.2
0.63	0	17	5	19	0.0	79.2	3.2
0.66	0	22	2	22	0.0	91.7	7.8
0.68	0	17	2	14	0.0	87.5	3.3
0.69	6	17	15	8	26.1	34.8	5.2
0.70	11	11	20	2	50.0	9.1	9.2
0.72	4	12	17	0	25.0	0.0	11.1
0.74	17	7	26	3	70.8	10.3	17.7
0.76	10	6	16	0	62.5	0.0	10.7
0.80	0	16	17	0	0.0	0.0	19.4
0.85	0	15	13	1	0.0	7.1	22.3
0.90	3	20	22	2	13.0	8.3	14.0
0.95	2	22	21	3	8.3	12.5	4.8
1.0	1	21	22	1	4.5	4.3	10.5
2.0	3	21	24	0	12.5	0.0	175
3.0	5	23	25	2	17.9	7.4	600
4.0	3	22	26	0	12.0	0.0	650
5.0	5	22	30	0	18.5	0.0	700
6.0	3	19	24	1	13.6	4.0	325
7.0	3	12	13	2	20.0	13.3	319
9.0	2	16	17	3	11.1	15.0	350
11.0	1	11	14	0	8.3	0.0	308
13.0	1	21	19	1	4.5	5.0	350
15.0	4	17	18	2	19.0	10.0	350
17.0	1	14	14	1	6.7	6.7	304
19.0	4	16	19	1	20.0	5.0	350
20.0	0	20	20	2	0.0	9.1	300

[(1)] Wavelengths from 0.41 to 3 μm were generated by LEBRA-FEL and those from 7 to 20 μm were generated by KU-FEL.

[(2)] The effects of the irradiation experiments are denoted by numbers of seeds counted and evaluated by lettuce seed developmental stages [5], and expressed in percentages calculated by following formulas: Inhibition (%) = $a/(a + b) \times 100$, where a is the number of seeds at St. 0–2 and b is the number of seeds at St. 3–4. Promotion (%) = $c/(c + d) \times 100$, where c is the number of seeds at St. 0–2 and d is the number of seeds at St. 3–4.

Finally, we would like to propose here that new photochemical techniques with tunable wavelengths (0.4–20 μm) produced by combined use of LEBRA-FEL and KU-FEL (and other FELs in the future) may offer a useful tool for investigating photo-triggered biological systems, including the nervous system, in living organisms.

ACKNOWLEDGMENT

We thank Prof. T. Morii (Institute of Advanced Energy, Kyoto Univ.) and his staff (Dr. E. Nakada and Dr. S. Nakano) for helpful assistance. This work was supported by the "Joint Usage/Research Program on Zero-Emission Energy Research", Institute of Advanced Energy, Kyoto University (ZE25 and ZE26C-2).

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ISBN 978-3-95450-133-5