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Short Time Measurement of *R. sativus* Root Growth with Application of A.C. Electric Field

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Abstract: The effect of a.c. electric field on plant root growth has been investigated for application to the forestation in arid land. In previous studies, statistic analysis of root growth data has suggested possibility of electric growth enhancement in *R. sativus*. However, large deviation by the difference of individual plant and fluctuations in long term culture disturbed the clear demonstration of the electric effects. To avoid the difficulty of individual differences, magnified time lapse image capture system was constructed for root growth measurement. This system provides growth measurement within a few minutes, and enables comparison of growth rates with/without electric field in an experiment of single plant specimen, within a short period of time. Root growth measurement, with spatial resolution of 4 μm, was enabled with this system. Root growth rate was determined in a 5 minutes measurement with less than 10% standard deviation. Electric effect on root growth was also measured by this system. The results suggested the growth enhancement by the electric field of 100 - 300 V/m, and inhibition by the electric field of over 500 V/m.

Key Words: Electric field, Root growth, Time lapse image.

1. Introduction

Growing emission of carbon dioxide is one of the most important issue to be addressed by human society. Aside from the climate changes by global warming, increasing CO_2 concentration is a threat for human health and for the life of other species, such as seashells or coral. To mitigate the heavy burden of CO_2 emission, enhancement of the natural adsorption by green plant is a good option. In such a purpose, forestation of arid land is studied in places (Tanouchi *et al*., 2008). In the case of planting tree seedlings in arid land, survival rate is largely depending upon the water supply. Especially, utilization of underground water is crucial in arid region. Enhancing root growth and leading to the under ground water will contribute to plant survival.

Root growth enhancement by application of electric field has been investigated for some plant species (Brayman and Miller, 1986; Suzuki *et al*., 2002). In our previous study, root growth enhancement of *E. camaldulensis* (*Eucalyptus* tree) seedling was investigated (Hotta *et al*., 2012). It showed a tendency of root growth enhancement by the a.c. electric field of specific intensity. However, clear demonstration was difficult because individual differences of the plant were so diverse and fluctuation of growth rate will occur in a long term measurement. In previous studies, a root growth measurement session takes some days because root growth rate is so small. And it also requires many samples to average the individual difference. These experimental constrictions enforce the difficulty in discrimination of slight difference by an experimental parameter. To avoid such problems in plant culture and to clarify the effect of electric field on root growth, a short time growth measurement system was constructed in this study. For evaluation of the system, *R. sativus* (Radish) was used for test specimen. The root growth enhancement by a.c. electric field has been well established for *R. sativus* so far (Suzuki *et al*., 2001). Magnified images of a *R. sativus* root, growing in water culture vessel, was captured with interval of 1 minute. Root growth rate was analyzed from the difference between the images. Growth rate measurement, within some minutes, was demonstrated with this system. Effect of a.c. electric field on the growth of *R. sativus* root was measured for various field intensities. Appropriate time window for measurement of the growth rate with minimum deviation was also investigated.

2. Materials and Methods

2.1. Water culture of *R. sativus* seedling

The *R. sativus* seedlings were water cultured for optical observation of root growth. The inner dimensions of the culture vessel are 100 mm × 120 mm × 50 mm, which is made of 10 mm thick transparent acrylic plates. Only front side window was 2 mm thick (**Fig. 1**). 2 mm thick front compartment was separated by a perforated back plate from

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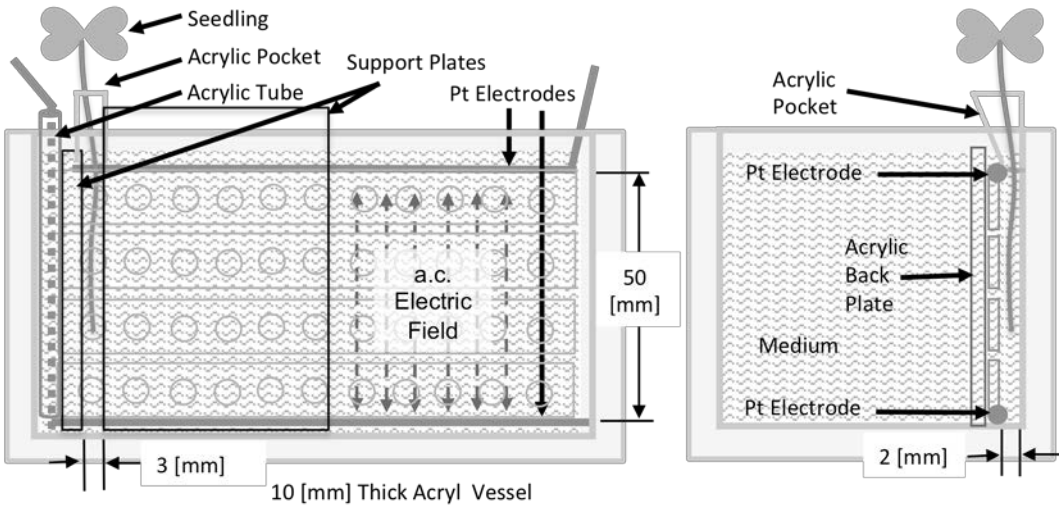


Fig. 1. The schematic of the culture vessel for electric field application in solution. Culture vessel is made by 10 mm thick acrylic resin plate. 2 mm gap of front compartment is separated by perforated back plate from bulk medium. Pt electrodes are installed on top and bottom of the front compartment. Plant seedling was placed in a acrylic pocket on top of the front compartment. The plant root is let to grow through a bottom hole of the pocket into the front compartment.

the bulk medium to guide the root grow straight. A small pocket was placed on top of the front compartment, having a bottom hole for the root to grow through. The bottom of the pocket was covered with a strip of cotton gauze sheet to prevent the seedling from falling down. The culture medium was Hyponex (HYPONeX Japan Co.), 10,000 times diluted with deionized water. The conductivity of the Hyponex solution was kept under 60 μS/cm by exchanging the medium to suppress the Joule heating. For the test specimen, *R. sativus* var. *longipinnatus* was purchased from Atariya Noen Co. Cultured plant was illuminated by Bio Fluorescent tube of NEC with illuminance of about 2400 lux. Room temperature and humidity were kept 25˚C and 60%, respectively, by air conditioner. During the growth rate measurement, all the system was placed in a closed room and illuminated by LED light with constant luminance.

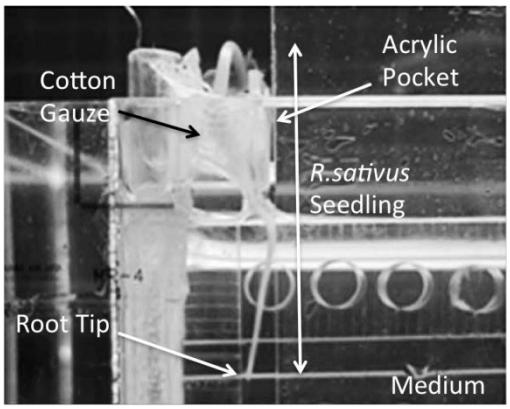


Fig. 2. *R. sativus* seedling in the water culture vessel. A seedling was placed in the acryl pocket on top of the culture vessel. The growing root tip of the seedling can be seen through transparent acrylic window. The root go down through a hole at the bottom of the pocket and grow along the narrow channel.

For the water culture, 35 seeds of *R. sativus* were sowed on a folded cotton gauze sheet dipped in deionized water for germination of the root. After 23 hours, one of 35 seedlings was picked up to transfer to the culture vessel. It was placed in the pocket of water culture vessel and let grown for 22 hours before the measurement. For accurate growth measurement, the front compartment was restricted to 3 mm width by 2 mm thick plates from both sides. The root was guided to grow straight along the narrow channel in the front compartment of the culture vessel to reduce the error by deviation in the image depth. The channel length was 50 mm and cross sectional dimensions of the channel were 3 mm × 2 mm. Photo image of the seedling in the culture vessel is shown in **Figure 2**.

2.2. Structure of magnified time lapse image capture system

The root growth was recorded by the magnified time lapse image capture system. The schematic of the system is shown in **Figure 3**. The image was captured by the Nikon1 J1 digital camera, through the single focus macro lens, Micro-Nikkor 105 mm F2.8, with 35 mm Microscopic Lens 24 × CM-3500 (Yoshida Industry Co., Ltd). The focal length behind the 105 mm lens was extended by 55 mm with the extension lings, PK-11, PK-12 and PK-13 for larger magnification. This lens system can project the image area of as small as 2 mm × 3 mm on the 8.8 mm × 13.2 mm size CMOS image sensor, with 3872 × 2592 pixel resolution. The aperture was fixed to 16 for larger focus depth, and shutter speed was 1/40 second for an image capture. The shutter was relayed automatically with programed intervals. Images were captured with 1 minutes interval for over 120 minutes.

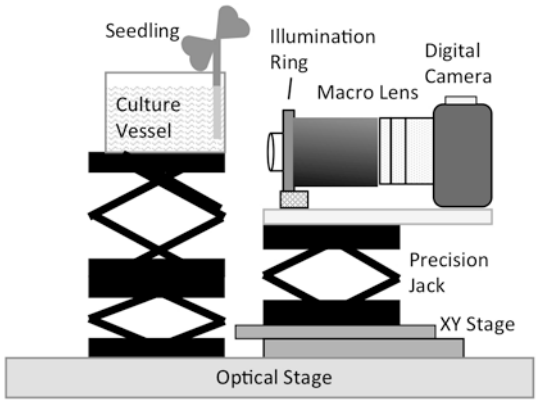


Fig. 3. Schematic of the Magnified time lapse image capture system. Culture vessel and optics were fixed on the optical stage. Focus and horizontal position of image was adjusted by the XY stage while the vertical position was adjusted by precision jack. The root of the specimen was illuminated by LED ring light around the close up lens.

Average root growth rate of *R. sativus* is around 0.5 mm/hour, so the root tip will be well within the image area of the system for 2 or 3 hours. ISO sensitivity was fixed to 800. A LED ring light was equipped around the close-up lens to illuminate the root specimen. The illuminance of the ring LED was ca. 3000 lx on the observation window.

The camera optics and the culture vessel were fixed on a optical stage to reduce the mechanical vibration. Large displacement in vertical axis, by the growth of root, was adjusted by the jacks under the vessel. Fine position in vertical and horizontal axis were adjusted by the precision lab jack and xy stage. Focusing to the root tip position was also enabled by the xy stage because of the narrow focusing range of the lens system, with the macro lens and the extension rings, needs fine adjestment.

Figure 4 shows a image of the root tip of a specimen, captured by the lens system. The seedling was inserted in a cotton gauze strip which was fixed on the bottom of the acrylic pocket on top of the culture vessel. The seedling was tightly connected with the cotton gauze by root hair, so the boundary point of root and stem was fixed to the vessel. The camera was aligned to focus on the root tip by precision stages, and both the vessel and the camera optics were fixed on a optical bench. Relative position of the root base to the image was fixed during the measurement, so the differences of root tip position in the successive time lapse images are the root growth within the shatter interval. The root length was measured by a image processing program, Image J (Rasband, 1997). The root length in a image was measured along the center of the thick white line, which is fitted to the curvature of the root. The ratio of actual length and pixel numbers was defined by measuring the pixel numbers of a image of a micro ruler for micro scope. The length/pixel conversion ratio was

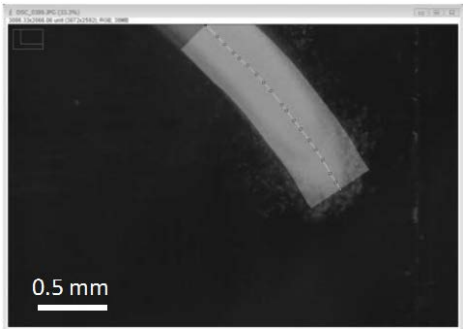


Fig. 4. A magnified image of a growing root of *R. sativus* seedling. Root length was measured by Image J image processing software. Thick white line is fitted to the curvature of the root image, and root length was measured along the center line.

used for calculation of root length by Image J.

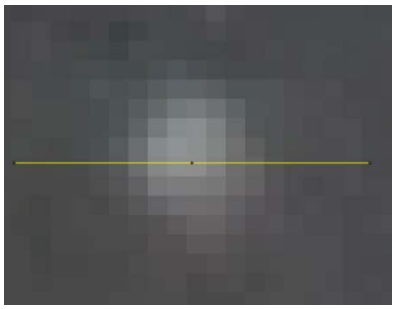
2.3. Application of electric field to the root of water cultured seedling

50 Hz a.c. electric field was applied to the root of seedlings by a pair of platinum wires, located on top and bottom of the front compartment of the culture vessel, 50 mm apart each other. The thickness of the compartment is 2 mm, and electric field is confined in the thin sheet of culture medium, so the field intensity can be well approximated by dividing the applied voltage by the electrode gap. 0 to 1500 V/m electric field, with 300 V/m interval, was applied to the culture medium with heating of less than 1˚C in the air conditioned environment for the low conductivity of the medium. Electric field was applied with the 5 minutes ON and OFF interval pairs. In preliminary experiments, shorter interval stimulation, such as 1 minute, failed to make effects within the interval time and electric field effect was not clearly correlated to the stimulation. Each session for one value of field intensity consists of 3 pairs of ON/OFF switching, which takes 30 minutes. 5 sessions of field application, with increasing field intensity of 100-500 V/m or 300-1500 V/m, are repeated for a seedling specimen. Including the final 5 minutes 0 V/m field interval, one experimental procedure takes 155 minutes. For continuous growth rate measurement, all the images, captured with 1 minute interval, were processed for growth rate measurement and evaluation of system resolution. However, in electric field application experiments, field application interval and response time seems to be larger than 5 minutes. And for slower growth rate of some specimen, only the images of 5 minutes interval were processed for data analysis.

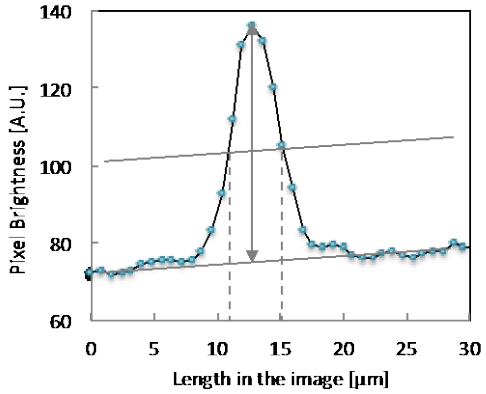
3. Results and Discussion

3.1. Resolution of the image capture optics

Figure 5 (a) shows a image of a 2 μm diameter latex bead (Fluoresbright microsphere Y2.0, Polyscience Inc.), taken by



(a) Optical image of latex bead, captured by the lens system. Brightness of the image was measured along the horizontal line.



(b) Brightness profile of the latex bead image. The full width at half maximum was measured as about 4 μm.

Fig. 5. Resolution of the magnified time lapse image capture system. For calibration of the resolution, images of latex micro bead with 2 μm diameter were captured and numerically analyzed.

the image capture system for calibration of optical resolution. Brightness profile of the picture along the horizontal line is also shown in Figure 5 **(b)**. Full width at the half maximum (FWHM) was 4.3 μm. This result suggests that the maximum resolution of less than 4 μm. The value of FWHM was twice larger than the diameter of the microsphere. Theoretical resolution of this system, calculated from the pixel number of image sensor and image area, is 0.77 μm for a pixel. However optical limit by interference of light, wave length dispersion of refractive index and optical aberrations of the lens system degraded the resolution.

3.2. Deviation of root growth rate in short and long term

The root growth curves of 3 *R. sativus* specimens, measured by this system over 135 minutes, are shown in **Figure 6**. The vertical axis is the root length diferrence from the start of observation. Root lengths of the 3 seedlings were measured with same condition. The lengths of the roots were measured at every 1 minute. The individual growth rates of 3 specimens were differed each other in 135 minutes measurement time, but range was within the 8-10 μm/min. The increase of root length even in 1 minute can be distinguished by this system. These results demonstrate the resolution and short time measurement capability of this system.

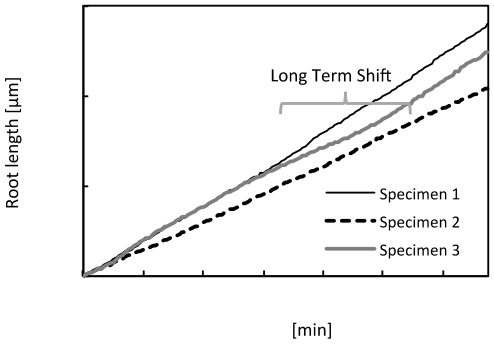


Fig. 6. Root growth of *R. sativus* without application of electric field. 3 specimens were cultured independently in the vessel and images of the root were captured over the range of 130 minutes with interval of 1minutes. Specimen 1 and 3 showed change of root growth rate around the 60-100 minutes after the start of the measurement.

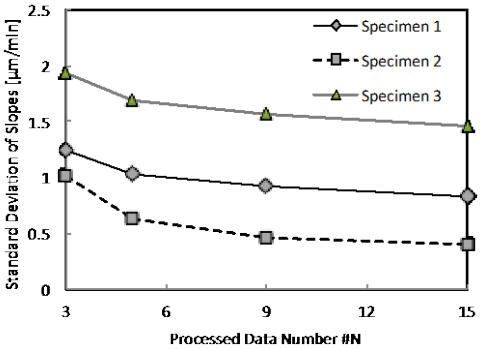


Fig. 7. Standard deviation of growth rate along the time course of growth measurement. Slope of growth curve was calculated for different numbers of data points, 3, 5, 9 and 15, over the total data of 130 minutes.

While the root growth of specimen 2 was linear to the time, the root growth rate of specimen 1 and 3 changed at around 60-100 minutes after the start of the measurement. The reason of these changes of growth rate is not determined. To evaluate the growth rate deviation from the linear relation, slope of auto regression line was calculated for moving window of 3, 5, 9 and 15 data over the time range of 10 to 125 minutes. The slope values were assumed as the root growth rate within the short time period. The fluctuation of this growth rate is represented by the standard deviation of these slope values over the 2 hours of observation time. The graphs in **Figure 7** show the standard deviations of these root growth rates for the data window length of 3 - 15 data (#N in the figure). One data correspond to 1 minute time span. The standard deviation of root growth rate is the highest in 3 minutes time window and decreases with the length of the time window. Standard deviation of each specimen decreased with the width of data window, as is predicted by theoretical consideration about the relation of sample number and deviation. However, decrease of standard deviation is not

significant for the time window lengths longer than 5, so minimum observation time window for this system was assured as 5 minutes.

On the other hand, base lines of the 3 graphs in Figure 7 differ each other, because of individual difference of each seed and long term shift of growth rate. As can be seen in the graphs in Figure 6, growth rate of specimen 1 and 3 shifted in time range of some 10 minutes at around 60 minutes from the start. These phenomena suggest the existence of long term response of a plant to some environmental change. But we are not sure what caused these shift in the experiment for now. At least, it can be concluded that root growth rate of a plant seedling can be changed in as short as some 10 minutes range. These results show that observation with interval of longer than some 10 minutes may fail to detect some plant response. However, deviation by measurement error is so large in 3 minutes. From these results, the optimum time window for root growth rate measurement is in the range of 5 to some 10 minutes.

3.3. The root growth under application of electric field

Figure 8 shows the root growth rate of one water cultured seedling under application of electric field. The applied field intensity is shown by the black rectangle column. The a.c. electric field of up to 1500 V/m was applied in the culture medium. Electric field was applied only in increasing order because higher field intensity may cause some irreversible change of plant tissue. With application of 300 V/m field, growth rate gradually increased from 3 µm/min to 7 µm/min in 30 minutes. The increasing curve didn't changed by ON and OFF of the field, so the response time of growth enhancement mechanism is speculated to be longer than 5 minutes. By application of higher intensity field, however, growth rate was reduced from 7 µm/min to 5-3 µm/min. This result suggests the existence of some mechanism of growth inhibition by the electric field of higher than 600 V/m. The response time of this mechanism seems to be shorter than 5 minutes because the growth rate was reduced by application of the field and slightly recovered by removal of the field within 5 minutes interval.

For more precise investigation of field intensity, root growth rate was also measured with application of lower intensity field, 0-500 V/m, as shown in **Figure 9**. Root growth rate was 9.5 µm/min from the start of observation. 100 V/m field seem to have little enhancing effect of root growth but it attained the maximum growth rate of 11 µm/min at 30 minutes, and the growth rate decreased by application of higher electric field. Over all growth rate was higher than 7 µm/min, This value is almost same as the maximum growth rate in Figure 8. It is possible that the maximum growth rate is limited by physiological reasons, and electric enhancement

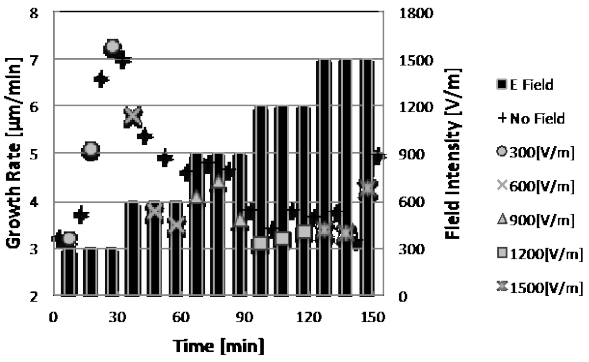


Fig. 8. Root growth rate under application of 0-1500 V/m a.c. electric field. 50 Hz a.c. electric field was applied on single seedling specimen repeatedly. The electric field was switched ON/OFF with 5 minutes interval. Open rectangle columns show the intensity of applied electric field, scaled in right axis. Root growth rate in the interval is shown by symbols. The + symbol corresponds to the 0 electric field interval. Other symbols correspond to the different field intensities.

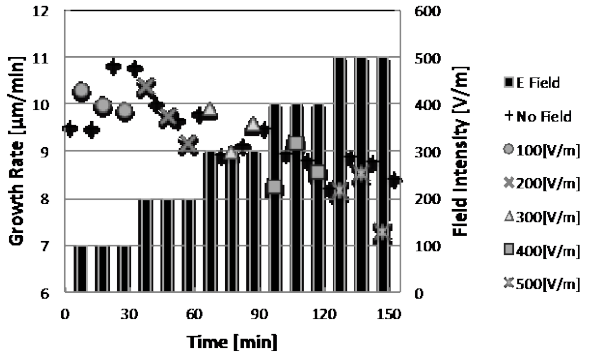


Fig. 9. Root growth rate under application of 0-500 V/m, 50 Hz a.c. electric field. 0-500 V/m electric field was applied to another single seedling specimen. Field intensity was reduced to 1/3 of the experiment in Figure 8 for precise investigation of filed intensity dependence. Over all growth rate is larger than that of the 300 V/m session in 0-1500 V/m experiment.

of growth rate from higher value is restrained within the maximum. This means the electrical growth enhancement may be applicable only for the seedling of lower growth rate. The difference of initial growth rate of individual seedlings might have disturbed the obvious change by electric field. The cause of individual difference is discussed later.

All the results suggest the existence of 2 different mechanism of electric effect on the root growth. One mechanism has suppressing effect on the root growth with short response time. This effect is negligible for the electric field intensity of lower than 600 V/m. Another mechanism has enhancing effect on the root growth with response time of longer than at least 5 minutes. This mechanism is effective within the range of 100-300 V/m. Maximum root growth rate of *R. sativus* seedling, within the experimental conditions, is about 11 µm/min and electric mechanism cannot override

this limitation. Growth rates of 3 specimens in Figure 6 were different, and even shifted in 2 hour experiment. This instability of growth rate disturbed the consistent experimental results. One possible reason of this instability is allelopathy of *R. sativus* seedlings. *R. sativus* seeds show lateral suppression in germination phase (Suzuki *et al*., 2002). Such a lateral control is generally observed in ecological systems. To investigate this assumption and have stable results in culture experiments, we need some modification of the culture vessel for multiple cultivation of seedlings.

The root growth suppression by electric field has short response time, and its mechanism may well be the electric break down of some biochemical signaling system on the cell membrane, such as ion channel or electrochemical receptor. On the other hand, electric root growth enhancement seem to have longer response time of some 10 minutes, which suggests the participation of biochemical process. This mechanism is still to be understood by the results in this study. One possible target of electric stimulation is some electric receptor on the cell membrane. Some plant species are known to have electric potential around it's root (Iwabuchi, 1989). This potential is speculated to be generated by the release of proton by H^+-ATPase (Suzuki *et al*., 2002). Proton concentration is also closely related with the growth control of plant root (Mentze *et al*., 1977; Toko *et al*., 1987). The function of the potential is not well established so far. But if the electric potential of proton gradient contributes to the growth control of root, weak electric field can cause some perturbation on the growth signal.

This speculation leads to the possibility of new electric stimulation scheme of plant root in arid land. Especially in salinated soil area, electric conductivity of bulk soil is rather high. Electric current will make largest voltage drop at the high resistance of root surface or cell membrane, where the plant may be most sensitive to the electric potential change. Electric stimulation, by installing stimulation electrode to the xylem of the plant and grounding the counter electrode to the soil, will enhance root growth in high conductivity soil area. Especially in this study, ion strength was restricted rather low to avoid the Joule loss heating by higher field intensity. The ion uptake is an important factor for the growth of *R. sativus* seedling and the lower ion strength might have suppressed the root growth. If the electric field of as weak as 100 V/m has a enhancing effect on the root growth rate, culture medium of higher ion strength can be available. Precise design of medium or soil conductivity, intensity and frequency of electric field will create a new possibility of this system.

4. Conclusion

The magnified time lapse image capture system was constructed for the study of root growth enhancement by a.c. electric field. The root growth enhancement of water cultured *R. sativus* seedling by application of electric field was suggested with this system.

References

Brayman A.A., Miller M.W. (1986): Induction of ELF transmembrane potentials in relation to power-frequency electric field bioeffects in a plant root model system. *Radiation. Environ. Biophys*., **25**: 141-149.

Hotta T., Suzuki S., Takahashi T. (2012): Investigation of The Ion Concentration Effect on Eucalyptus Root Growth in a.c. Electric Field. *Proc. BMEiCON2012*: 59-62.

Iwabuchi A., Yano M., Shimizu H. (1989): Development of Extracellular Electric Pattern Around Lepidium Roots. *Protoplasma*, **148**: 94-100.

Mentze J. *et al*. (1977): Auxin-induced H^+ Secretion in Helianthus and Its Implications. *Plant Physiol*., **60**: 509-512.

Rasband W.S. (1997-2014): *ImageJ*. U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih. gov/ij/.

Suzuki S., Maruta T., Nakamura Y. (2001): The Effects of Electric Field and lon Density on Root Growth. *J. Faculty Engng. Seikei Univ*., **38**(2): 1-8 (in Japanease).

Suzuki S., Maruta T., Nakamura Y. (2002): The Effect of Metal ion in The Medium on the Growth of Rahpanus sativus Seed and Lateral Inhibition. *IEEJ Trans. C*, **122**: 1686-1691 (in Japanease).

Tanouchi H., Aikawa S., Kawasaki S., Utsugi H., Hamano H., Saito M., Adrianne K., Kojima T., Yamada K. (2008): Examination of Biomas Production by Afforestation Technology in Arid Land of Low-Latitude Zone. *J. Echotech. Research*, **14**(3): 183-188.

Toko K., Iiyama S., Tanaka C., Hayashi K., Yamafuji K., Yamafuji K. (1987): Ralation of Growth Process to Spacila Patterns of Electric Potential and Enzyme aActivity in Bean Roots. *Biohyis. Chem*., **20**: 39-58.

Weisenseel M.H., Dorn A., Jaffe L.F. (1979): Natural H^+ current traverse growing roots and root hairs of Barley. *Plant Physiol*., **64**: 512-51.