音にさらされている葉の構造変化の光学的法による調査 Investigating structural changes in leaves under exposure to sound by optical techniques

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In recent years, it has been found that sound also has effect on plant growth and its yield with certain sound directing the seedling of corn toward the sound source and its ability in distinguishing stuttering of larvae from other sounds [1-2]. However, methods investigating the effects of sound either take a long time or destructive. Earlier, we reported the use laser biospeckle, in investigating the activities of an arugula plant (2 - 4 weeks old) under sounds of different frequencies of 0 Hz or control, 100 Hz, 1 kHz, 10 kHz [3]. Biospeckle activities were recorded for 20 sec at 15 fps following exposure to sounds for 1 min and the correlation parameter (r) characterizing the activity showed a clear difference in *r* between the control and

other frequencies. In order to correlate this with the actual structure of the leaf, in this study, we report the use of confocal microscopy under the same paradigm of exposing the plant to sound frequencies of 1 min. A total of fifteen plants had been exposed to sound for a min and the cross-sectional views of the leave sections had been observed with confocal microscope.

Figure 1 shows the paradigm of exposure to sound followed by sectioning the leaf for confocal study. After having the plant in darkness for 60 min, sound of a particular frequency (0.1,1,10 kHz) at 100dB was presented for a minute. Then leaves from

different plants have been taken out to be sliced and observed through a confocal microscope. A microgram of the sectioned

result of stomata is shown in figure 2. The diameters of the major and minor axis of stomata was were calculated. The ration of the stomata diameters under the presence and absence of sound was calculated and shown as a function of time in Fig.3. The vertical axis is the ratio of major and minor axis lengths at the first minute to those measured every 10 min from the same plant for a different leaf. As can be seen, there is a big decrease at the first minute in both major and minor axes lengths.

In order to determine whether the change is due to only light stimulus, control observation by confocal microscope was done under

complete darkness under the absence of sound. The figure is shown in Fig.3b. From Fig.3, within a minute, there is clear change in the stomatal widths.

We could confirm the changes in stomatal widths with sound stimulus by confocal microscope. However, confocal microscope is destructive as the leaf has to be sliced to view the changes. In future we propose to investigate the plant- sound interaction by optical coherence tomography. References:

1. Jung, J., Kim, S.-K., Kim, Y. J., Jeong, M.-J. and Ryu, C.-M., Front. Plant Sci., 9(25), (2018). 2.Gagliano, M., Mancuso, S. and Robert, D., Trends in plant Science 17(6), 323-325 (2012). 3.平井実,遠藤大樹, Uma Maheswari Rajagopalan,江目宏樹,河野貴裕,山田純, "レーザーバ イオスペックル法による音の植物活動への影響に関する評価",第80回応用物理学会秋季学術 講演会,北海道,2019年9月.



Figure 1 Paradigm used to study plant -sound interaction with sound present for 1min and the stomatal widths were measured every ten min by confocal microscope.



nomata Fig confocal microscope



Fig.3 Ratio of stomatal width under the presence and the absence of sound of both major and minor axes as a function of time with left (a) for sound and (b) no sound.