Swept source optical coherence tomography for *in vivo* growth monitoring of *capsicum annuum* seeds treated with different *NaCl* concentrations

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ABSTRACT

In this study, Optical coherence tomography (OCT) is demonstrated as a plausible optical tool for *in vivo* detection of plant seeds and its morphological changes during growth. The experiment was carried out on *Capsicum annuum* seeds that were treated with different molar concentrations of *NaCl* to investigate the most optimal concentration for the seed growth. The monitoring process was carried out for 9 consecutive days. The *in vivo* 2D OCT images of the treated seeds were obtained and compared with seeds that were grown with sterile distilled water. The obtained results confirm the feasibility of using OCT for the proposed application. Normalized A-scan analysis method is utilized for supporting the concluded results.

Keywords: Optical coherence tomography, *in vivo* monitoring, *Capsicum annuum* seeds, germination, sodium chloride (*NaCl*),

1. INTRODUCTION

Germination of seed which is, the process of plant growing that is contained within the seed, is influenced by various factors from both internal and the external conditions. These factors include temperature of the soil, water content availability of oxygen and the availability of light and dark conditions, soil salinity concentrations, pH and other chemicals that contributes to the nature of the soil. Only the appropriate content of soli salinity will aid in germination. An excess amount of salinity will result in soil degradation or in inhibition of germination process in seed [1]. Thus, choosing the right amount of salt salinity for seed is a major concern for farmers in order to produce maximum yield.

Until now analyzing or estimating the germination growth stage, or to study the effect or inhibition level on germination within the seed have been done by various methods like sectioning of seeds and viewing them through microscope, histological studies, SEM (Scanning Electron Microscope) imaging, MRI (Magnetic Resonance Imaging) and X-radiography. All these methods are either destructive methods which disable the possibility of continuous monitoring of the same seed or comparatively less in resolution to optical coherence tomography (OCT). In this study, we implemented and demonstrated the usefulness of optical coherence tomography (OCT) as an alternative and a real-time imaging method for seed growth monitoring. OCT is an *in vivo*, non-contact, non-destructive real-time imaging method that was first introduced in the year 1991 [2]. OCT has started to be profoundly used for diagnostics in medical imaging and research studies that requires high resolution imaging of samples along with thickness in the range of few millimeters. Being capable of providing high resolution images that are on par with histological images, the application of OCT is diversely spread in many fields which include ophthalmology, otorhinolaryngology, dermatology, blood flow measurements, defect inspection and thickness measurements in electronics devices like LEDs and LCDs, and also in agriculture [3].

Through this study, we have demonstrated and shown the broadened possibilities of using OCT as a tool for *in vivo* growth analysis of primed seeds. The 2D OCT images of seeds showing the morphological changes by growth within the seeds are shown and the obtained results are supported by normalized A-scan analysis

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2. MATERIALS AND METHODS

2.1 Plant preparation

To assure homogeneity and viability of the samples used for experiment, the *C. annuum* plant seeds that were utilized for experiments were collected from the department of Microorganism, Kyungpook National University, Daegu, South Korea. All the obtained seeds had an initial average weight of around 1.5 g. prior to the experiment. The seed samples were washed with distilled water and dried at room temperature (27 °C). During the entire experimental period of the OCT imaging was carried out in a controlled environment maintained with a temperature of 27 ± 3 °C, and with a humidity of 75 to 80 %. The total experimental duration lasted for 9 consecutive days. In total 75 *C. annuum* seeds were used for experiments. These 75 seeds were then separated into 5 groups containing 15 seeds per each group. Petri dishes base was surfaced with tissue papers which act as container base for germinating the seeds. Individual solutions of sterile distilled water (SDW), 0.1 M *NaCl* (molar concentration of sodium chloride), 0.2 M *NaCl*, 0.3 M *NaCl* and 0.4 M *NaCl* were filled in the individual petri dishes respectively, thus making it four different primed seed conditions. The solutions were filled to a level slightly above the halfway through the petri dishes to ensure complete immersion of the seed samples in their individual solutions. All the dried seeds were placed in each petri dish in such a way that they were equally spaced from each other within the petri dish. All the petri dishes were marked with a unique name for identification, and all the petri dishes were separated by a minimum distance of 30 cm from each other. The *C. annuum* seeds that were primed with sterile distilled water (SDW) were utilized. The seeds used for histology were primed in SDW for 1 day. The samples used for histology were fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.05M sodium cacodylate buffer solution for 24 hours. The samples were washed and dehydrated. The dehydrated samples were embedded in a Spurr's resin after being infiltrated with propylene oxide. Finally, the samples were sectioned using an Ultra microtome (MT-7000, RMC, Tucson, AZ, USA). The sectioned samples were stained with 2% methylene blue solution to be observed under a light microscope (BX50, Olympus, Tokyo, Japan).

2.2 OCT system setup

The A custom built swept source optical coherence tomography (SS-OCT) is used for OCT imaging of samples during the experiment. A schematic diagram of the SS-OCT system that was used is shown in Figure 1 (a). The SS-OCT build was driven by a high-speed, broad bandwidth swept laser source (AXP50125-6, Axsun Technology, USA) with a center wavelength of 1310 nm and a full width half maximum (FWHM) of 110 nm. The sweeping rate of the source is 50 kHz, with an average output power of 20 mW. The laser output from the source is connected to a fiber coupler of ratio 80:20. The 20 % of the output arm of the coupler is then connected to the input arm of a circulator, and the output arm of the circulator is connected to the reference arm setup. Similarly, the 80 % output from the coupler arm is also connected to the input arm of another circulator. The output arm of this circulator is connected to a sample arm setup. The interfered signal is then collected from the output arms of the 50:50 ratio coupler and connected to the positive and negative ports of a balanced photodetector (PDB430C, Thorlabs Inc, USA). The signal obtained from the photodetector is digitized using a digitizer (ATS9462, Alazar Technologies Inc, Canada). The axial and lateral resolutions in air for the built system are 6.8 μm and 14.6 μm, respectively. A software based data processing technique was designed for constructing the 2D OCT image. OCT imaging of the seed was maintained at the center of the seed, and the expected radicle emergence point was faced forward while imaging. Also, the seeds were marked with a marker to indicate the position (region of interest), where it was scanned using OCT, and multiple 2D OCT scans were acquired close to the aforementioned marker, which enabled to match the 2D OCT image of the exact similar imaging position with the data obtained on earlier days.

To analyze the obtained 2D images, a Matlab based software program was used for detecting the intensity peaks in depth direction. The built program searched for intensity peaks within a given window size (example, 60 A-scans). The algorithm detects for maximum intensity in each A-scan signal sequentially. All the peak positions of A-scans within the window are rearranged while matching the peak intensity index in the A-scans to flatten the image. The index positions with high intensity is rearranged and matched linearly to obtain a flattened image. It is rearranged so that first intensity peak is obtained from the each A-line of 2D image and it is plotted at the beginning plot. Thus, the absence of intensity owing to air region can be considered negligible and has no negative effect on the plotted A-scans. Subsequently, all the rearranged and flattened A-scan lines are summed up and averaged in order to obtain an averaged A-scan profile, then it was divided by the maximum value to obtain a normalized A-scan intensity plot for each 2D OCT image.

A comparison between a 2D SS-OCT image and a histologically sectioned image of *C. annuum* seeds, which was obtained at the initial sterile distilled water (SDW) priming stage (day 1) is shown in figure 1. Figure 1 (B) is a 2D SS-

OCT image of the primed seed, and figure 2 (C) is an enlarged histology sectioned image of the same seed. By comparative analysis, it is notably seen that the 2D OCT image shows a similar growth pattern of seed structures in testa, endosperm, and cotyledon. As per seed growth the cotyledon and the embryo growth can be observed in 2D OCT images, which are shown in the figures in later results section.

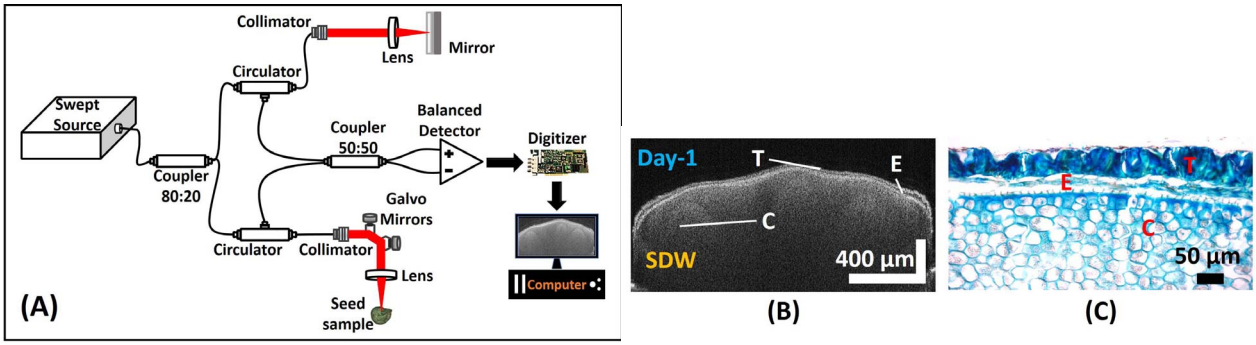

Figure 1. schematic diagram of SS-OCT setup, (A) Schematic diagram of the SS-OCT system, (B) 2D SS-OCT image of *C. annuum* seed primed in sterile distilled water, (C) Histology image of same seed.

3. RESULTS

Figure 2 shows the SS-OCT images taken on consecutive days of seeds that were soaked in different solution concentration. In figure 2, group (A) seeds were primed in sterile distilled water (SDW), group (B) seeds were primed in 0.1 M *NaCl*, group (C) seeds were primed in 0.2 M *NaCl*, group (D) seeds were primed in 0.3 M *NaCl,* and group (E) seeds were primed in 0.4 M *NaCl.*

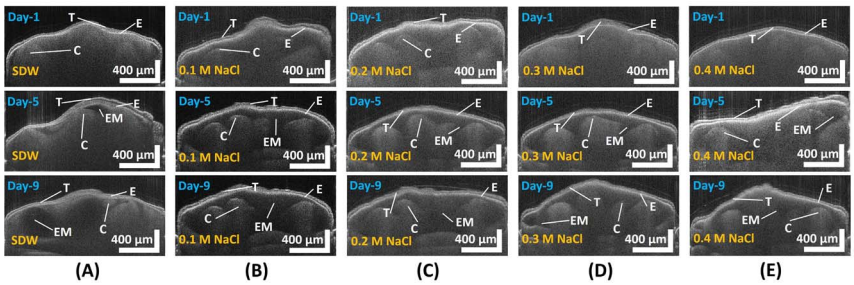

Figure 2. 2D SS-OCT monitoring images of *C. annuum* seeds primed in different solutions, Group (A) seeds were primed in sterile distilled water (SDW), group (B) seeds were primed in 0.1 M *NaCl*, group (C) seeds were primed in 0.2 M *NaCl*, group (D) seeds were primed in 0.3 M *NaCl,* and group (E) seeds were primed in 0.4 M *NaCl.*

In group (A) SDW seeds, it can be observably seen that on Day-1 the three major distinct layers of seeds are visible, the testa (seed coating), cotyledon, and endosperm. And as of from Day-5, the notable traces of collective vacuum like deposition begins to appears beneath the endosperm layer, this is due to the development of embryo. As the seed grows the embryo becomes clearer and the cotyledon layer forms into a multiple lobe like structures, this is visible in images Day-7 also, and the seed starts to show more structural details as the seed germination increases. On Day-9 the embryo is seen on most parts of the seed also the cotyledon looks less in area in comparison to earlier days of seed growth images. On Day-9 the seeds look visibly well germinated. In group (B), monitoring images of seed primed in 0.1M *NaCl* salt solution the Day-1image seems mostly similar structural development of seed as visible in Day-1 of SDW solution. But, not much noticeable difference was seen in Day-7 and Day-9 images. Also, the appearance of embryo is not as prominently seen as in Day-9 of SDW seed. In group (C), monitoring images of seed primed in 0.2 M *NaCl* solution, unlike in Day-1 images of SDW primed and 0.1 M *NaCl* primed seed, the Day-1 image of 0.2 M *NaCl* seed image begins to shows barely visible bottom structure in seed. And the images of Day-5 show fairly similar changes in internal morphological structures as in image of Day-5 of 0.1 M *NaCl.* The intensities of structures in Day-9 of 0.2 M *NaCl* are slightly higher in comparison to Day-9 image of 0.1 M *NaCl* and SDW solutions. This can seemingly be the effect of slower germination rate induced by 0.2 M *NaCl* solution on seed growth. In group (C), 0.3 M *NaCl* primed seeds, it can be noted that the embryo development is not much prominently visible in Day-1 and Day-3. The embryo development in

seed can be seen from Day-5 images and following. In Day-9 image the bottom part of the seed structures commences to become visible, which had started to become visible on earlier day images of other solution. In group (C), 0.4 M *NaCl* primed seeds, it can be observed that the visibility of embryo development begins on Day-5. The visibility of bottom structures of the seed in Day-9 image is even lesser in comparison to 0.3 M *NaCl* primed seed images. Also, very less noticeable internal morphological changes were observed in Day-9 image when compared to Day-7 image of 0.4 M *NaCl* primed seed.

Figure 3 shows the OCT images of seeds primed in all solutions that were imaged on Day-9. And the right image plot is the respective normalized A-scan plot of the seeds which were taken within the red rectangular box regions in the OCT images. As aforementioned, these normalized A-scans were plotted by normalizing 60 consecutive A-scan signals in each image. Due to maximum growth of embryo in SDW primed seed, the red plot shows the least intensity in signal. The blue plot shows second least in intensity showing its respective growth in internal morphology in seed. The 0.2 M *NaCl*, 0.3 M *NaCl*, and 0.4 M *NaCl* primed seeds represented by the plots green, black and pink respectively, shows growth of very less in comparison, due to inhibition in growth of seed after reaching a certain level. At the end of green plot the raise in intensity is due to the strong scattering of light from the bottom structures of the seed. The least germination was observed in 0.4 M *NaCl* and 0.3 M *NaCl* primed seeds

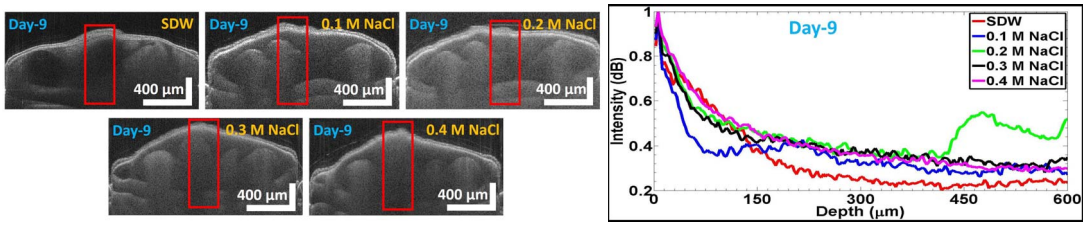

Figure 3. Comparative A-scan analysis of seeds primed in different solutions on Day-9, Red box region indicated the region of interest used for A-scan analysis. Graph in right side is the normalized A-scan analysis plot.

4. CONCLUSION

We have demonstrated the usefulness of OCT as a tool for continuous monitoring of *in vivo* morphological changes in seeds and how it can be used for seed growth analysis and to detect the state of germination inhibition in seeds. Internal morphological changes in seed as it grew were observed. Seeds primed with SDW shows prominent growth, and seeds primed with 0.1 M *NaCl* and 0.2 M *NaCl* shows slower but constant growth in comparison. However, the seeds primed with 0.3 M *NaCl* and 0.4 M *NaCl* showed initial morphological changes within the seed, but after which no noticeable changes were observed. The results of this study show the potential applications of OCT for agronomical studies related to seed growth. By employing further studies in seed germination assisted with OCT imaging, may possibly help farmers to choose appropriate conditions to aid faster seed germination, resulting in quicker harvest.

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