Effect of Zn toxicity on lentil seed germination through high-speed monitoring of Biospeckle Optical Coherence Tomography

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1. Introduction

Zinc (Zn) is an essential micronutrient for seed germination and plant growth. It plays an important role of metabolic processes. Nevertheless, like other heavy metals an excess of Zn accumulation causes a negative effect on seed germination. In our previous work the micronutrient effect of Zn was investigated, and enhancement of internal activity was observed [1]. In this study, a novel optical interference technique named as Biospeckle Optical Coherence Tomography (bOCT), which was developed by the authors [2], was proposed to monitor the toxic effect of Zn on lentil seed germination and seedling growth. OCT is a non-contact and non-destructive technique visualizing the makes use of the speckles observed in OCT to monitor internal structural changes in-vivo [3]. Moreover, it has become an urgent requirement to monitor the heavy metal effect on seed germination due to significant increment of heavy metal accumulation in farmland through mismanagement and the widespread usage. On the other hand, seed germination and dormancy have great significance on improvement of crop yield and quality requiring reliable seed screening process. Previous studies using bOCT revealed that biospeckle contrast could be affected by the biological activities within the plant depending on the healthiness of plants [4].

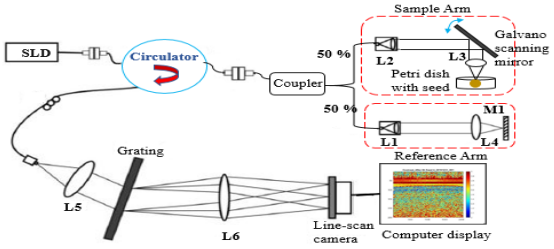

Fig.1 - Schematic of experimental system

2. Experiments and Discussion

Figure 1 shows the experimental system of OCT. In bOCT, from the acquired OCT structural images, the biospeckle contrast (C_b) which is defined as the ratio of standard deviation of the intensity at each pixel along the temporal axis of time to the mean value of pixels across total time of the scan was calculated according to the following equation.

$$C_b(x,y,t_i) = \frac{1}{< I_{OCT}(x,y,t_i) >}\left[\frac{1}{N}\sum_{j=1}^{N}\{I_{OCT}(x,y,t_{i+j}) - < I_{OCT}(x,y,t_i) >\}^2\right]^{\frac{1}{2}}$$

$$< I_{OCT}(x,y,t_i) >= \frac{1}{N}\sum_{j=1}^{N} I_{OCT}(x,y,t_{i+j})$$

Where, x, y represents the pixel coordinates, j is the scan number and N indicates the total number of scans. In the experiments, 100 mg/L Zn concentration was used to investigate toxic effects on the germination of the lentil seeds. For each sample, 6 seeds were placed on a filter paper in 9 cm Petri dishes. Thereafter, the Petri dishes were kept in a growth chamber at 27° C temperature, a relative humidity of >70% and a constant illumination of 4000 lux. The OCT observations were taken at 6h, 12h and 24h of exposure to Zn. A comparison of biospeckle contrast, during 24h of imbibition are shown in Fig.2. The bOCT images revealed that the clear reduction of internal activity as indicated by blue within the seed compared to control after exposures of Zn emphasizing the toxic effect of Zn on seed germination. The speckle contrast (mean of six ROIs) of each bOCT images was calculated and a significant difference was observed for average normalized contrast as shown in Fig.3.

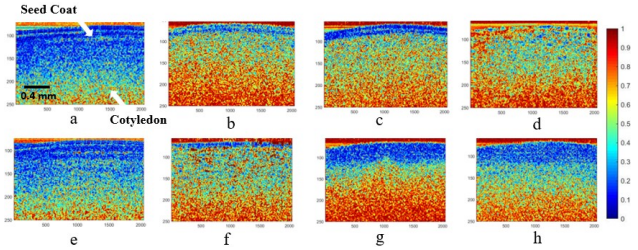

Fig.2. Comparison of bOCT images of lentil seed under Zn stress. before imbibition with out Zn (a); after 6 h of imbibition with out Zn (b); after 12 h of imbibition with out Zn (c); after 24 h of imbibition with out Zn (d); before imbibition with Zn (e); after 6 h of imbibition with Zn (f); after 12 h of imbibition with Zn (g); after 24 h of imbibition with Zn (h); Scale bar represents 0.4 mm.

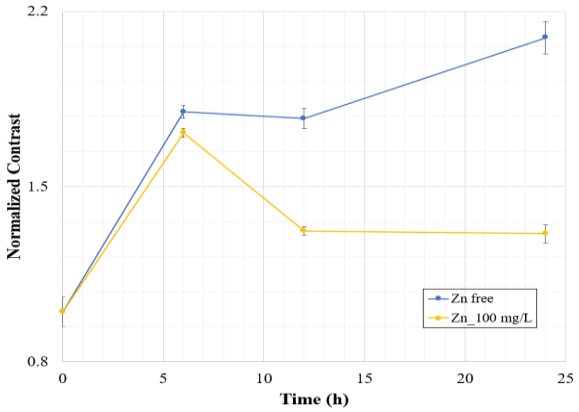

Fig.3. Averaged normalized contrast of bOCT image under Zn stress

3. Conclusion

In this study, we proposed the bOCT to monitor the toxic effect of Zn on lentil seed germination. The results show that the tested Zn concentration has negative influence on seed germination whereas clear reduction of the internal activity of seed was observed after 24h exposures of Zn during the germination. Hence, our results confirm that the negative impact of high Zn concentration on seed germination and the proposed method was able to observe that negative effect at an early stage. Currently we are working with synergic effect of microplastics and Zn on seed germination.

References

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