Ecotoxicology and Environmental Safety 137 (2017) 86–93

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Highly sensitive optical interferometric technique reveals stress-dependent instantaneous nanometric growth fluctuations of Chinese chive leaf under heavy metal stress

Kokge Thilini Kanchana Muthumali DeSilva[a,b], Uma Maheswari Rajagopalan[c], Hirofumi Kadono[a,*]

[a] Graduate School of Science and Engineering, Saitama University, 255 Shimo-okubo, Sakura-ku, Saitama-shi, Saitama 338-8570, Japan
[b] Department of Mechanical and Manufacturing Engineering, Faculty of Engineering, University of Ruhuna, Hapugala, Galle 80000, Sri Lanka
[c] Faculty of Food and Nutrition Science, Toyo University, 1-1-1 Izumino, Itakura-machi, Ora-gun, Gunma-ken 374-0113, Japan

ARTICLE INFO

Keywords:
Cadmium
Zinc
Growth fluctuations
Laser speckle
Optical interferometry
Plant growth

ABSTRACT

Plant growth apart from being a complex and highly dynamic is dependent on its immediate environment. Leaf expansion measurements using Statistical Interferometry Technique, a sensitive interferometric technique at nanometric accuracy and at sub-second levels revealed the presence of characteristic nanometric intrinsic fluctuations [Plant Biotechnology 31, 195 (2014)]. In this paper, we demonstrate that the nanometric intrinsic fluctuations are sensitive enough that they change under exposure of heavy metals, essential micronutrient zinc and non-essential element cadmium, at relatively low concentrations in the leaves of Chinese chive (*Allium tuberosum)*. The nanometric intrinsic fluctuations of leaves were observed for 4 h under three cadmium concentrations or two zinc concentrations. Results showed significant reduction of nanometric intrinsic fluctuations for all cadmium concentrations, and in contrast significant increase of nanometric intrinsic fluctuations for all zinc concentrations. There was significant reduction of nanometric intrinsic fluctuations for cadmium exposure of concentrations of 0.001 mM for even an hour, and significant increment of nanometric intrinsic fluctuations under 0.75 mM zinc from 1hr exposure. For comparison, antioxidative enzymes and metal uptake were also measured under 4hr exposure of cadmium or zinc. However, no significant changes could be seen in antioxidative enzymes within 4 h under the smaller concentration of 0.001 mM cadmium as seen for nanometric intrinsic fluctuations. The results imply that nanometric intrinsic fluctuations can be not only used as a measure for heavy metal stress but also it can be more sensitive to detect the toxic as well as positive effects of smaller amounts of heavy metal on plants at an early stage.

1. Introduction

Heavy metal contamination of water and soil is getting worldwide attention since it significantly affects on the reduction of crop production. Heavy metal toxicity and its effect on food chain has become the major health and environmental issue. The exposure to high concentration of heavy metal causes carcinogenic and teratogenic effects on human body. Cadmium (Cd) and zinc (Zn) are most common heavy metal contaminants (Lin and Aarts, 2012). Cd is highly toxic known to cause damage to all biological systems including humans. Some of the elements are essential for organisms at small concentrations. For example, Zn is a necessary micronutrient for plants as well as humans. It plays a significant role of metabolic processes in plants. However, under high concentrations of Zn roll out toxic effects on plant growth. The ministry of environment of Japan has announced the different permissible limits for Cd and Zn in water environment as 0.26 μM and 0.03 mM, respectively.

After absorbed by plants, Cd exhibits toxic effects on different cellular processes, proceeding to a plant growth reduction, leaf chlorosis increment, photosynthesis reduction, change of leaf color, and finally death (A. Singh and Prasad, 2014; S. Singh and Prasad, 2014). The significant decrease in photosynthesis trait of blackgram has

Abbreviations: ANSD, Average normalized standard deviation; CAT, Catalase; CCD, Charge-coupled device; H_2O_2, Hydrogen peroxide; ICP-AES, Inductively coupled plasma- atomic emission spectrometry; NIF, Nanometric intrinsic fluctuation; POD, Peroxidase; RER, Relative elongation rate; ROS, Reactive oxygen species; SD, Standard deviation; SIT, Statistical interferometry technique; SOD, Super oxide dismutase

* Corresponding author at: School of Science and Engineering, Saitama University, 255, Shimo-okubo, Sakura-ku, Saitama-shi, Saitama, 338-8570, Japan
E-mail address: kadono@mail.saitama-u.ac.jp (H. Kadono).

http://dx.doi.org/10.1016/j.ecoenv.2016.11.020
Received 12 July 2016; Received in revised form 23 November 2016; Accepted 25 November 2016
Available online 19 December 2016
0147-6513/

been observed under Cd treated conditions (Singh et al., 2008). The rate of root growth per day was decreased with increasing duration of the Cd treatment from 24 h to 72 h, and increasing Cd concentration from 10^{-4} to 10^{-2} M in garlic (Liu et al., 2004). The Cd accumulation significantly reduced biomass of root and shoot of barley (Boussama et al., 1999), and exposure to 25 µM Cd concentration, significantly reduced length of shoot and dry biomass of leaf of maize (Lagriffoul et al., 1998). Decreases in tomato plant height, root length, and volume were found to be significant under 1 µM and 10 µM Cd stress (Dong et al., 2005). The color of the leaves of maize appeared yellow-brown and some were broken after 10 days exposure to 0.1 mM of Cd (Wang et al., 2007).

Zn is a micronutrient needed for many enzyme activities and has been found to be beneficial to plant. Lower concentrations of Zn ranging from 0.075 to 0.75 mM (5–50 ppm) concentrations were shown to be enhancing length of root and shoot, and significantly enhance biomass of the sunflower (Jadia and Fulekar, 2008). Zn caused significant decrease of *Phaseolus vulgaris* L. of root and stem growth, but did not affect the leaf growth under 100 µM concentration (Chaoui et al., 1997). Low concentrations of Zn (up to 0.1 mM) stimulated growth of *Brassica juncea and Cajanus cajan* plant seedlings (Alia et al.al., 1995). Zn enhanced the growth of *Brassica juncea* seedling at 0.05 mM, but caused significant reduction of growth at 5 and 10 mM (Prasad et al., 1999).

Exposure to heavy metals increases the formation of reactive oxygen species (ROS) in plant tissues, causing oxidative stress in plant. Plant induces a series of reactions to control the level of ROS by antioxidative enzymes (Blokhina et al., 2003; DalCorso et al., 2008; Mishra et al., 2006; Sun et al., 2007; Zhang et al., 2005). ROS formation is an important controller of plant development, and is related to specific plant hormones regulating cell growth, and thus controlling the plant cell development and growth (Gapper and Dolan, 2006; Kim et al., 2009). The excessive production of ROS causes oxidative damage and finally cell death (Sharma et al., 2012).

To characterize the effects of heavy metal, conventional measurements such as biomass measurements, stomatal conductance measurements, height measurements, photosynthesis measurements, metal uptake measurements, and antioxidative enzyme analysis are commonly used (Jadia and Fulekar, 2008; Singh et al., 2008; Zhang et al., 2005). However, some of these techniques are post harvesting, destructive measurements, and need long time to obtain the results. Therefore, at present to our knowledge, there exists no method that can monitor the immediate response of plants.

In order to overcome the limitations and achieve the high accuracy in plant growth measurements, attempts were made to use an optical interferometer. In an optical interferometer, the coherent light reflected from an optically flat reference mirror and that reflected from a sample were superposed to make an interference pattern. In spite of its implementations in 1980's (Briers et al., 1977; Fox and Puffer, 1976; Fox and Puffer, 1977; Jiang and Staude, 1989), its usage was limited due to two major factors: First one was the complexity of implementation and the other was the scattering property of light from the scattering object surface and other deeper tissue structures. Plant is a highly scattering object, and the scattered light consists of the light components due to stationary structures and moving scatters, for example, organelles within the cells of a leaf. Unnecessary scattered coherent light progressive to generate a random pattern, known as speckles (Dainty, 1984), and such a speckles involve to reduce the accuracy of the interferometer.

On the other hand, Statistical Interferometry Technique (SIT), one of the novel interferometry techniques (Kadono and Toyooka, 1991; Kadono et al., 2001) developed in our lab, using a statistical property of laser speckle has been successfully applied on plant studies. SIT has been shown to be successful in finding the influence on growth behavior of fine roots of pine seedlings infected with ectomycorrhiza under ozone exposure at nano-scale (Rathnayake et al., 2007), measuring tiny root elongations of Japanese red pine seedlings infected with ectomycorrhiza (Rathnayake et al., 2008), investigating the effect of lightning state on the plant development (Kobayashi and Kadono, 2010), and measuring short-term and long-term growth related changes of plant leaves under ozone stress (Thilakarathne et al., 2014a, 2014b).

SIT enables to conduct a direct observation of short-term displacement or expansion of plants at accuracy, typically, in nanometer scale and in time scale of seconds. SIT provides a way to observe the immediate changes of plant growth that are happening at the cellular stage. Such changes happening at the scale of nanometer were referred to as nanometric intrinsic fluctuations (NIF) were found to be indicative of the physical well-being of the plant (Thilakarathne et al., 2014a, 2014b). In this study, we demonstrate the detection of the immediate influence of heavy metals, Cd and Zn, on Chinese chives through monitoring the intrinsic fluctuations.

Roots of Chinese chive (*Allium tuberosum*) were subjected to expose different cadmium chloride ($CdCl_2$) conditions, 0, 0.001, 0.01, and 0.1 mM or zinc nitrate hexahydrate ($Zn(NO_3)·6H_2O$) of concentrations, 0, 0.15, and 0.75 mM for 4 h. Instantaneous effects of Cd and Zn on leaf growth dynamics or growth rate were observed for 6 h through continuous measurements of in-plane displacements. Here, the growth rate was defined over 5.5 s. For comparison, oxidative stress markers such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), hydrogen peroxide (H_2O_2), and metal uptake were also measured to obtain information about physiological parameters.

2. Materials and method

2.1. Plant materials

Chinese chive is one of the popular vegetables in Japan and China and is cultivated in many places around the world. Chinese chive can re-sprout from roots and naturalize in scattered locations. Chinese chive is fresh top greens in the onion family of bulb vegetables. Its hollow, tubular leaf has smaller diameter than onions, but appear somewhat like onions. Chinese chive is very low in calories and is rich source of folates. At the same time, Chinese chive is also listed as invasive serious high impact environmental and/or agricultural weeds that spread rapidly and often create monocultures. In our study, we used 1–2 weeks old healthy Chinese chive leaves as samples. Plants were kept in a growth chamber to maintain the growth conditions (Conviron, Controlled Environmental Ltd, Canada). The conditions of growth chamber were maintained at an air temperature of 25 °C/20 °C, a light intensity of 260–350 µmol m^{-2} s^{-1}/0 µmol m^{-2} s^{-1}, and relative humidity of 55–65% following a 12 h/12 h cycle. The plants were regularly watered.

In the experiments, Chinese chive plants were removed from soil and cleaned using pure water. For the control measurements, roots of the samples were exposed to pure water and measurements were conducted for 6 h continuously. For Cd exposure measurements, Chinese chive roots were exposed to pure water for 2 h, and $CdCl_2$ (Wako pure chemical industries Ltd, Japan, contain not less than 95%, molecular weight 183.32 $gmol^{-1}$) solution was mixed with pure water, and Cd concentrations of the solutions were maintained to be 0.001, 0.01, and 0.1 mM. For Zn experiments, Chinese chive roots were exposed to pure water for 2 h, and $Zn(NO_3)·6H_2O$ (Wako pure chemical industries Ltd, Japan, contain not less than 99%, molecular weight 297.49 $gmol^{-1}$) solution was mixed with pure water, and Zn concentrations of solutions were maintained to be 0.15 and 0.75 mM.

2.2. SIT optical system

Fig. 1(a) and (b) show a schematic of the experimental system with the statistical interferometer and a photograph of the SIT system built on an optical bench, respectively. A He-Ne laser (GLG 5400, NEC Corporation, Japan, wavelength 633 nm) was used as a light source to