Journal of Rare Earths 35 (2017) 1273–1282

Contents lists available at ScienceDirect

Journal of Rare Earths

journal homepage: http://www.journals.elsevier.com/journal-of-rare-earths

The trivalent cerium-induced cell death and alteration of ion flux in sweetpotato [*Ipomoea batatas* (L.) Lam]☆

Jiaojiao Jiang [a, d], Jianzhong Hu [a, d], Zeyi Xie [a], Qinghe Cao [b], Daifu Ma [b], Yonghua Han [a, *], Zongyun Li [a, c, **]

[a] Institute of Integrative Plant Biology, School of Life Science, Jiangsu Normal University, Xuzhou 221116, China
[b] Xuzhou Institution of Agricultural Science, Xuzhou 221131, China
[c] Jiangsu Key Laboratory of Phylogenomics and Comparative Genomics, Jiangsu Normal University, Xuzhou 221116, China

ARTICLE INFO

Article history:
Received 13 March 2017
Received in revised form
27 June 2017
Accepted 27 June 2017
Available online 18 August 2017

Keywords:
Cerium
ROS
Caspase-1-like protease
Programmed cell death
Sweetpotato

ABSTRACT

The rare earth element cerium (Ce) in its several forms is extensively utilized in various fields, including nano-technology, agriculture, and the food industry. Due to its increasing unregulated usage, Ce is now a potential source of pollution and toxicity due to its excessive environmental accumulation. Unfortunately, analysis of the toxic effects of Ce in plants is still in its early stages. Herein, we investigated the effects of Ce^{3+} treatment on development-related indicators in sweetpotato. We found that a low concentration (10 mg/L) slightly improved oxidation resistance, while a high concentration (20–80 mg/L) negatively affected development and photosynthesis and triggered increases in reactive oxygen species (ROS) production, antioxidant enzyme activities, and malondialdehyde (MDA) content. Moreover, elevation and efflux of cytosolic Ca^{2+} and caspase-1-like activity were induced by high-concentration Ce^{3+} treatment. Finally, cell viability decreased as Ce^{3+} concentration increased. These results suggest that (1) a high Ce^{3+} concentration (20–80 mg/L) inhibits development and photosynthesis of sweetpotato and induces oxidative damage followed by lipid peroxidation in the root, (2) a caspase-1-like protease is induced by cytosolic Ca^{2+} and ROS overproduction to cause programmed cell death in the root, and (3) a high concentration of Ce^{3+} could trigger a hypothetical cell death pathway, wherein Ce^{3+} induces ROS production followed by cytosolic Ca^{2+} elevation, which activates caspase-1-like activity, which in turn leads to programmed cell death in the root of sweetpotato.

1. Introduction

When present in trace amounts, the 15 rare earth elements (REEs) have been widely reported to be potentially useful in improving growth and yield of certain crops. Consequently, REEs are occasionally added to specific fertilizers used in agriculture[1] and have been used as micro-fertilizers for years.[2,3] Cerium (Ce), an important REE as well as the cheapest, has regularly been used in various industries including pharmacology, electronics, and agriculture.[4] Its effect on development, differentiation, and lesions in plants and animals has attracted a lot of attention since 1989.[5] Moreover, cerium oxide in its engineered nanoparticle form now plays a more important role than the compound itself as the former is used to induce catalysis and is also employed in solar cells, solid fuel cells, ultraviolent absorbents for chemical mechanical planarization, gas sensors, oxygen pumps, and glass applications.[6–8] Due to its widespread application, the level of environmental Ce has increased in recent years and is expected to continue to increase in the near future. This situation requires us to take note of its biological effects on humans and their environments.[9]

Ce^{3+} has been identified as a "super calcium" since it shares several chemical properties with Ca^{2+} and exhibits higher binding ability with Ca^{2+}-binding sites than Ca^{2+} itself. Thus Ce^{3+} may competitively displace and replace Ca^{2+} in biological systems, causing metabolic disorders and toxic effects. As we know, Ca^{2+} is a

☆ **Foundation item**: Project supported by the National Natural Science Foundation of China (31271698, 31771367), The Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD), China Agriculture Research System (CARS-10).
* Corresponding author.
** Corresponding author.
E-mail addresses: hanyonghua@jsnu.edu.cn (Y.H. Han), zongyun_li@yeah.net (Z.Y. Li).
[d] These authors contributed equally to the work.

http://dx.doi.org/10.1016/j.jre.2017.06.006
1002-0721/

crucial intracellular second messenger with crucial roles in many physiological processes, including responses to biotic and abiotic stresses as well as pathogens.[10–13] Disruption of Ca^{2+} activity under conditions of abiotic or biotic stress induces cell death in animals and plants.[12,14,15] One researcher has revealed that high doses of REEs, such as Ce, can interact with Ca^{2+}-release channels, resulting in ER stress; this indicates that REEs could negatively affect various ionic channels.[16] In addition, Ce^{3+}, like Ca^{2+}, plays a role in increasing the mitochondrial permeability of rice mitochondria, resulting in mitochondrial swelling and mitochondrial potential change, and is more strongly induced than Ca^{2+} is under the same circumstances.[16]

Elevated cytosolic Ca^{2+} causes a significant change in the production of reactive oxygen species (ROS) which can damage basic metabolism in aerobic organisms.[17,18] Normally, intracellular ROS are mainly produced at low levels in organelles; under stress conditions, however, ROS increase rapidly. Previous studies have demonstrated that different Ce concentrations can exert contrary effects: one report has shown that a high concentration of Ce significantly increased H_2O_2 in the roots and leaves of rice, causing electrolyte leakage followed by lipid peroxidation, which is represented by increased malondialdehyde (MDA) production, whereas a low concentration of Ce exerted a scavenging effect, resulting in removing H_2O_2 and reducing ROS accumulation and thereby playing a protective role in plant resistance to oxidative stress.[19] A similar study by Liang et al showed that a low concentration of Ce decreased MDA content, while a high concentration actually increased MDA and H_2O_2 content in a dose-dependent manner.[20] To avoid ROS overproduction, plants have evolved an enzymic system, such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX). Interestingly, Ce also influences the activities of these enzymes as reported by Ma et al, a low concentration of $nCeO_2$ exposure did not effectively change SOD activity, whereas a high concentration increased SOD activity evidently[21] during vegetative development of *Brassica rapa*. In CAT and APX, similar phenomena have been seen in several plant species: a high concentration of $nCeO_2$ causes obvious increases in CAT and APX activity, while a low concentration results in no change.[22–24] These results suggest that Ce at low concentrations could inhibit ROS production without activating antioxidant enzymes, while Ce at high concentrations could effectively increase ROS production while activating antioxidant enzymes. Moreover, the dosage threshold separating these contrary effects is unique for and needs to be investigated in each plant species. In sweetpotato, the oxidative effect of Ce has not been thoroughly observed.

ROS overproduction in plants leads to programmed cell death (PCD), a remarkable process that serves as a major controller for various biological processes and plays key roles in the development and survival of organisms.[25,26] In plants, it not only functions in support of various developmental processes, including reproduction, differentiation, and senescence[27,28] but is also involved in adaption to abiotic and biotic stresses including drought, heavy metal, salt, and extreme temperature.[29,30] Although PCD by that name only occurs in plants, it has some remarkable features in common with apoptosis in animals and microorganisms, including DNA fragmentation, activation of caspase-like protease, and DNA cleavage (also called DNA laddering).[31,32] One of the key organs in the execution of PCD is the vacuole, a component unique to plant cells, which produces a crucial protease called vacuolar processing enzyme (VPE), which is known to be a hallmark of PCD induction.[33–35] VPE, as a cysteine protease, shares enzymatic functions with caspase, which is a key factor in the execution of apoptosis in animals.[34] It has long been documented that VPE displays caspase-1-like activity. It has also been reported that OsVPE2 and OsVPE3 (two types of VPE) can be mediated by hydrogen peroxide-induced programmed cell death in rice and that NtVPEs are transcriptionally up-regulated in aluminum-induced PCD in tobacco.[33,36] These studies suggest that VPE could be a key controller of the PCD process. Yet even though numerous biotic and abiotic factors have been shown to induce VPE followed by PCD in various plants, the questions of whether the VPE gene exists in sweetpotato and whether the process of VPE-induced PCD caused by Ce occurs in sweetpotato remain unresolved.

To explore the potential effects of Ce on sweetpotato, we investigated root elongation, cell viability, and chlorophyll content to assess the impact of its different concentrations on development. Then, we assessed the oxidative effect of Ce in an oxidative stress assay measuring ROS generation, MDA content, and antioxidant enzyme activity (SOD, peroxidase (POD), CAT) after Ce treatment. Finally, we tested for caspase-1-like activity to identify the PCD pathway in sweetpotato. Together with the previous findings discussed above, our results shed light on the effects that may be induced by Ce in sweetpotato.

2. Materials and methods

2.1. Cerium treatment

Branches of sweetpotato plants [*Ipomoea batatas* (L.) Lam. cv Xuzi 3; all samples collected from Institute of Agricultural Science, Xuzhou, Jiangsu], each with six to eight fully developed leaves, were used in this study. The branches were set in distilled water and incubated in a growth chamber (27/25 °C day/night temperature, 16:8 h light/dark cycle) to allow rooting for three days after cutting from the mother plants. At this point, the branches that were to be used in the root elongation assay were placed directly into Ce-exposure solution ($CeCl_3 \cdot 7H_2O$). All other branches were placed in a 1/4 Hoagland nutrient solution to allow root development for 14 days in the same growth chamber and then placed into exposure solution containing Ce^{3+} at concentrations of 10, 20, 40 and 80 mg/L for one, three or seven days, respectively. Whole sweetpotato seedlings were collected for fresh weight assay. Root tips were collected for assay of root Ca^{2+} level and caspase-1-like activity and for cell viability detection. The third or fourth leaf (counting from the primary leaf) on each sweetpotato branch was collected for chlorophyll content assay. In addition, the branches were placed into exposure solution (containing different concentrations of Ce^{3+}) with caspase-1 inhibitor (Z-YVAD-FMK, biovision, Milpitas, CA, USA) added to a final concentration of 0.5 mmol/L for assay of caspase-1-like activity and cell viability.

2.2. Fresh weight assay

Before Ce^{3+} treatment, the fresh weight of each sweetpotato branch was measured and recorded as W_{T0}. After treatment with exposure solution (0, 10, 20, 40, or 80 mg/L) for one, three, or seven days, the primary fresh weight of each sweetpotato branch was measured again and recorded as W_{T1}, W_{T3}, or W_{T7}, respectively. Changes in fresh weight were calculated according to the given equation:

$$\Delta W = W_{\text{T treatment days}} - W_{\text{T0}}$$

2.3. Root elongation assay

Before treatment, the primary root lengths of the 3-day-old seedlings were measured and recorded as L_{C0} and L_{T0}. After treatment with exposed solution (0, 10, 20, 40 and 80 mg/L) for 3 days, the primary root length was recorded as L_{C3} and L_{T3}. Root relative