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Research article

Genetic and physiological effects of the natural radioactive gas radon on the epiphytic plant *Tillandsia brachycaulos*

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ABSTRACT

Radon (^{222}Rn) is the most abundant natural radioactive gas in nature and triggers carcinogenesis. Few reports exist on whether radon can damage plants as it does animals. Therefore, we chose *Tillandsia brachycaulos,* a common indicator plant, as the material to detect the physiological and genetic changes caused by radon. With an increase in radon concentration, DNA indices (tail length, tail DNA, tail moment and Olive tail moment) from the comet assay and malondialdehyde (MDA) content increased significantly, suggesting that *T. brachycaulos* inevitably suffered from radiation damage. However, neither the leaf relative conductivity nor the soluble protein content changed significantly with radon fumigation, and no dose-dependent effect existed between the chlorophyll content and radon concentration, indicating that *T. brachycaulos* had resistance to radon stress. Foliar trichomes most likely excluded the pollutant from plants because DNA damage in *T. brachycaulos* with trichomes manually removed was considerably greater than that with trichomes. Moreover, the antioxidant enzyme system further reduced the damage of radon to plants because the activity of superoxide dismutase (SOD) increased significantly with the radon concentration.

1. Introduction

Radon (^{222}Rn) is widely present in the atmosphere and is the most abundant natural radioactive gas in nature (Little, 1997). After radon and its progenies are inhaled into the human body, the α particles generated during the decay irradiate in the body, which causes ionization of the damaged tissues or cells (primarily in the lung), damages the structure of DNA molecules, affects the cell regeneration process, and causes chromosomal aberrations, thereby triggering carcinogenesis (UNSCEAR, 2000). The International Agency for Research on Cancer (IARC) has categorized radon and its progenies as Group I carcinogenic factors, and the World Health Organization (WHO) has also listed radon as one of the 19 carcinogenic factors in humans (ICRP, 2008).

The efficient accumulation of toxic compounds by some plant species is well known, which therefore can be used for biomonitoring or phytoremediation (Pignata et al., 2002; Li et al., 2012; Kushwaha et al., 2016). However, few reports exist on the relationship between radon and plants. Vives i Batlle et al. (2011, 2017) speculated from modeling plants could adsorb radon and its progenies through the actions of gas diffusion, osmosis, and plant surface adsorption. Our recent experiment first demonstrated that the epiphytic indicator plant *Tillandsia brachycaulos* Schltdl. efficiently absorbs radon through its leaves (Li et al., 2018). A commonly used accumulation-type indicator plant must have one basic trait: strong resistance to pollutants, i.e., the plant does not die shortly after exposure to pollutants (Pignata et al., 2002; Li et al., 2012). However, no studies have been conducted on the effects of radon and its progenies on plants.

Tillandsia brachycaulos belongs to a special group of plants in the plant kingdom, known as “air plants,” that do not depend on soil and can live in the air. These plants are originally native to the southern U.S.A. and Central and South America and have been introduced worldwide for cultivation. Their leaf epidermis is covered with thick trichomes, which can absorb moisture and nutrients from the air. Hence, *Tillandsia* leaves have robust absorption capacities and can absorb and accumulate many pollutants in the atmosphere, thereby rendering themselves “indicator plants” capable of sensitively detecting environmental changes (Benzing, 2000). Many common heavy metal elements in the atmosphere such as Mn, Cu, Fe, Co, N, Pb, Zn, Sr and Cs and some common organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) can also be monitored using different *Tillandsia* species (Filhoa et al., 2002; Pignata et al., 2002; Pereira et al., 2007; Li et al., 2012).

As commonly used indicators of atmospheric pollution, *Tillandsia* plants have a closer relationship with atmospheric pollutants including radon than that of other plants and are more affected by atmospheric pollutants. Therefore, we chose *T. brachycaulos* as the experimental

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material to detect the physiological and genetic changes in plants treated by fumigation with different radon concentrations in a standard radon chamber, with the objective to clarify whether radon has a significant effect on *T. brachycaulos* plants and which indices reflect the extent of this effect. We also investigated the role of leaf epidermal trichomes in this process to provide a further basis for the study of the relationship between radon and living organisms.

2. Materials and methods

2.1. Radon chamber settings

Plant radon treatment was performed in a standard radon chamber at Peking University, China, following Li et al. (2018). This radon chamber consisted of the main chamber, a radon source (^{226}Ra), the measurement system (RAD7 radon detector; DURRIDGE, USA) and other ancillary equipment. The volume was approximately 1 m^3, and the chamber included a radon-filled gas circuit connected to the radon source (^{226}Ra) and a measurement gas circuit connected to the radon detector. The radon chamber was equipped with a small-scale fan for an even distribution of the radon concentration in the chamber.

2.2. Radon treatment for plants

Forty healthy *T. brachycaulos* individuals with similar growth size were selected and randomly divided into four groups. One group was used as the control group and was placed in a normal room environment with an average radon concentration of approximately 40 Bq·m^{-3}; one group was subjected to radon fumigation after manual removal of leaf epidermal trichomes according to the method used by Li et al. (2015), with the initial radon concentration of 2560 Bq·m^{-3}; and the other two groups were subjected to radon fumigation without removal of leaf epidermal trichomes, with their initial radon concentrations set as 2879 or 4525 Bq·m^{-3}. Radon was supplied from a^{226}Ra source, which could create the highest Rn concentration of approximately 5000 Bq·m^{-3} in the chamber. Considering that the error would be large if the initial radon concentration was too low, the low radon concentration exposed to the plants was approximately 2500 Bq·m^{-3}, and the high radon concentration exposed to the plants was approximately 4500 Bq·m^{-3}. Both levels were evidently higher than the radon concentration in the natural environment, but the total doses were equivalent to 6.25 and 11.25 months of exposure to a medium environmental radon concentration of 40 Bq·m^{-3} (Cheng et al., 2002).

To reduce the confounding effects of the individual plant growth state on the experimental results, the plants were immersed in deionized water for 20 min before the experiment to saturate them with water and then uniformly air-dried for 30 min under the same conditions. The dried plants were uniformly hung with thin wires in fixed positions in the radon chamber. The radon chamber was then closed, the initial radon concentration was adjusted, and the genetic and physiological indices were measured after the plants were fumigated for 72 h.

2.3. Determination of plant genetic indices

In this study, single cell gel electrophoresis (SCGE), also known as the comet assay, was used to detect the DNA damages in radon-fumigated plants. Because of the unique advantages of being sensitive, simple, rapid, low-cost, and reproducible, and because the DNA damage in a single cell can be directly observed, this method has become a popular method to detect DNA damage (Santos et al., 2015).

2.3.1. Extraction of plant cell nuclei

Plant samples were cut into pieces after rinsing with double-distilled water. A mixture of 2% cellulase and pectinase was added, and the samples were well mixed and allowed to stand at 4 °C for 2 h. The samples were harvested by gradient centrifugation, and the nuclei were observed under a microscope to ensure a complete morphology. The cells were washed with phosphate-buffered saline (PBS) two times and then suspended in PBS. After the cell concentration was adjusted to $2*10^4$ ml^{-1}, the suspension solution was placed in a 4 °C refrigerator for further use.

2.3.2. Alkaline single-cell gel electrophoresis assay

For gel preparation, 0.75% agarose gel with a normal melting point was boiled, and 100 μl was quickly and evenly spread in a homemade microelectrophoresis tank. The tank was then placed in a 4 °C refrigerator for 1 min to allow the gel to solidify; 25 μl of well-prepared cell suspension solution was evenly mixed with 75 μl of 0.75% low-melting-point agarose gel, which was evenly spread onto the first layer of the gel. The gel was placed in a 4 °C refrigerator for 1 min to allow the gel to solidify.

For lysis, the microelectrophoresis tank was placed in a freshly prepared alkaline lysis solution, which was then placed in a 4 °C refrigerator for lysis for 2 h.

After lysis, the microelectrophoresis tank was removed, and the excessive salt was rinsed off with double-distilled water. The tank was placed in a horizontal electrophoresis apparatus containing a 4 °C alkaline electrophoresis solution. After this stood for 20 min, electrophoresis was performed at 20 V and 200 mA for 20 min.

For staining and observation, after staining with 2 μg ml^{-1} ethidium bromide (EB), the gel was rinsed with double-distilled water to remove excess dye. Comets were first observed under a fluorescence microscope at a low magnification. Then, at a high magnification, comet images were randomly captured with an image acquisition system; 200 comet cells were imaged for each sample.

2.3.3. Image analysis for comet assay

Each comet image was analyzed in an automated manner using the CASP system provided by a university in Poland. Tail length, tail DNA, tail moment (TM), and Olive tail moment (OTM) were determined.

2.4. Determination of plant physiological indices

With reference to Li (2000), the relative conductivity was measured using a DDS-11 conductivity instrument; the chlorophyll content was determined using the improved acetone-ethanol mixture method; the soluble protein content was determined by UV spectrophotometry; the malondialdehyde (MDA) content was determined by the thiobarbituric acid (TBA) method; the superoxide dismutase (SOD) activity was determined by the nitro blue tetrazolium method; the catalase (CAT) activity was determined by the UV absorption method; and the peroxidase (POD) activity was determined by the guaiacol method.

2.5. Data analyses

Differences in the measured physiological and genetic indices between different radon concentration treatments were analyzed using the SPSS statistical software package 19.0. One-way analysis of variance was used to analyze the data, and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Radiation damage by radon to DNA of T. brachycaulos leaves

As shown in Fig. 1 (A, B, D), with the increase in radon concentration, the comet tail length gradually increased, indicating that DNA migration increased and that the DNA content in the tail increased. When the radon concentration was increased to 4525 Bq·m^{-3}, a clear head continued to be observed in the comet image, but the DNA migration increased significantly (Fig. 1D). As shown in Table 1, with