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Irradiation with low-dose gamma ray enhances tolerance to heat stress in *Arabidopsis* seedlings

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ABSTRACT

Gamma irradiation at low doses can stimulate the tolerance to environmental stress in plants. However, the knowledge regarding the mechanisms underlying the enhanced tolerance induced by low-dose gamma irradiation is far from fully understood. In this study, to investigate the physiological and molecular mechanisms of heat stress alleviated by low-dose gamma irradiation, the *Arabidopsis* seeds were exposed to a range of doses before subjected to heat treatment. Our results showed that 50-Gy gamma irradiation maximally promoted seedling growth in response to heat stress. The production rate of superoxide radical and contents of hydrogen peroxide and malondialdehyde in the seedlings irradiated with 50-Gy dose under heat stress were significantly lower than those of controls. The activities of antioxidant enzymes, glutathione (GSH) content and proline level in the gamma-irradiated seedlings were significantly increased compared with the controls. Furthermore, transcriptional expression analysis of selected genes revealed that some components related to heat tolerance were stimulated by low-dose gamma irradiation under heat shock. Our results suggest that low-dose gamma irradiation can modulate the physiological responses as well as gene expression related to heat tolerance, thus alleviating the stress damage in *Arabidopsis* seedlings.

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1. Introduction

High temperature is one of the major limiting factors that affect the life of all living organisms. At the cellular level, heat stress differentially affects the stability of various proteins, fluidity of membrane lipids, RNA synthesis and organization of cytoskeleton, and inhibits activities of metabolic enzymes (Kotak et al., 2007; McClung and Davis, 2010). These injuries lead to reduced ion flux, metabolic imbalance, accumulation of toxic by-products, such as reactive oxygen species (ROS), and eventually starvation and inhibition of growth (Schöffl et al., 2005; Ruelland and Zachowski, 2010).

Heat stress can have an adverse impact on almost all aspects of plant growth and development. High temperatures can cause declines in seed germination and photosynthetic efficiency, leaf senescence, shoot and root growth inhibition, changes of anatomical structures, impairment of pollen and anther development, thereby reducing grain yields (Ismail and Hall, 1999; Karim et al., 1999; Zhang et al., 2005; Sato et al., 2006). Nevertheless, plants have evolved various physiological and molecular mechanisms to against heat stress, including maintenance of membrane stability, production of antioxidant enzymes and antioxidants, accumulation of compatible osmolytes, regulation of hormone signaling, induction of mitogen-activated protein kinase (MAPK), and, most importantly, activation of heat stress transcription factors (Hsfs) that mediate the expression of heat shock proteins (Hsps) (Sangwan and Dhindsa, 2002; Baniwal et al., 2004; Wahid and Shabbir, 2005; Gill and Tuteja, 2010; Sakata et al., 2010). All these mechanisms, which are regulated at the molecular level, enable plants to survive under heat stress.

Gamma irradiation has been widely applied for improvement techniques in various plant species because of their easy availability and power of penetration (Rodrigues et al., 2012). It has been proved that various growth parameters, such as seed germination, root and shoot length and leaf area, were improved by the exposure of dry seeds to low doses of gamma irradiation in

Abbreviations: ABA, abscisic acid; ANOVA, one-way analysis of variance; CAT, catalase; DTNB, 5,5-dithiobis (2-nitrobenzoic); FW, fresh weight; GI, germination index; GSH, glutathione; H_2O_2, hydrogen peroxide; HSF, heat stress transcription factor; HSP, heat shock protein; MAPK, mitogen-activated protein kinase; MAPK, mitogen-activated protein kinase; MDA, malondialdehyde; NBT, nitrobluetetrazolium; $O_2^{\cdot-}$, superoxide radical; $\cdot OH$, hydroxyl radical; POD, peroxidase; ROS, reactive oxygen species; SOD, superoxide dismutase; TCA, trichloroacetic acid

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plants (Kim et al., 2005; Marcu et al., 2013). Irradiation with low gamma rays also improved growth traits related to production in crop (Maity et al., 2005). Furthermore, the enhanced tolerance induced by low-dose gamma irradiation were observed in plants in response to abiotic stress (Moussa, 2011; Mohammed et al., 2012; Abo-Hamad et al., 2013). Recently, increasing the tolerance of plants to abiotic stress through low-dose gamma irradiation has received considerable attention.

The present study was carried out in an attempt to evaluate the physiological and molecular responses to heat stress with gamma-irradiated *Arabidopsis* seeds and explore the potential mechanisms underlying the heat tolerance induced by low-dose gamma irradiation in *Arabidopsis* seedlings. This work extends our knowledge on the roles of low-dose gamma irradiation in modifying responses of *Arabidopsis* seedlings to environmental stress.

2. Materials and methods

2.1. Plant materials and growth conditions

The Columbia-0 ecotype *Arabidopsis thaliana* was used for gamma irradiation. For growing seedlings on agar-containing plates, *Arabidopsis* seeds were pretreated with 70% ethanol for 1 min, surface-sterilized in 2.5% bleach for 10 min, and washed with distilled water. The seeds were planted on 1/2 MS medium (Sigma) supplemented with 1% (w/v) sucrose, 1% (w/v) agar (pH 5.8), and placed at 4 °C in the dark for 48 h. Then, the plates were placed under a light bar at 23 °C and providing 140 ± 20 μmol m^{-2} s^{-1} from cool white fluorescent lamps with a 16-h-light/8-h-dark cycle.

2.2. Gamma irradiation

Gamma irradiation was performed as described previously (Qi et al., 2014). Uniform seeds were randomly divided into two groups: (1) non-irradiated seeds for the controls and (2) seeds exposed to gamma irradiation. Gamma irradiation was conducted using a ^{60}Co [Cobalt-60] gamma source at a dose rate of 8.5 Gy/min. The doses of exposure used in this study were 25, 50, 75, 100, and 150 Gy.

2.3. Heat stress

The heat treatment was performed based on the methods described previously with some modifications (Suzuki et al., 2005). The seeds pretreated with gamma irradiation were planted onto solid 1/2 MS medium. After vernalization at 4 °C in the dark for 48 h, the seeds were germinated and vertically grown for 3 days at normal condition. Then, parafilm-sealed plates containing seedlings were subjected to heat stress (40 °C; 4 and 6 h) with a temperature-controlled air oven, and recovered at 23 °C in the growth chamber for another 4 days. Then, seedlings were sampled for the analysis of growth parameters including germination index, root length, fresh weight and survival rate, physiologic changes and gene expression in response to heat stress.

2.4. Morphological observations

For seed germination test, 100 seeds irradiated with gamma rays at different doses were planted on solid 1/2 MS medium and placed at 4 °C in the dark for 48 h. Then, the seeds were subjected to heat stress (40 °C; 4 and 6 h) before germination under normal growth condition. The seeds were considered germinated when they exhibited a radical extension of 0.2 cm. Counts of germinated seeds were made daily for 5 days to determine the germination index (GI) calculated using the following formula:

$$GI = \frac{N_1}{1} + \frac{N_2}{2} + \frac{N_3}{3} + \ldots \frac{N_n}{n}$$

where N_1, N_2, N_3,..., N_n represents the number of seeds that germinated on day 1, 2, 3..., n. Each result represents the mean of three biologic replicates.

The root growth of control and irradiated samples in response to heat stress was determined by subtracting the root length of seedlings normally grown before heat stress from the root length of seedlings recovered after heat stress. 30 individual seedlings were measured for each treatment. After heat treatments, the number of viable seedlings was quantified to determine the survival rate. Seedlings that were still green and generated new leaves were scored as survivors. 60 individual seedlings were measured for each treatment. Fresh weight was measured from 300 seedlings exposed to heat stress.

2.5. Measurement of superoxide radical ($O_2^{\bullet-}$) production rate, hydrogen peroxide(H_2O_2) and malondialdehyde (MDA) content

The production rate of superoxide radical ($O_2^{\bullet-}$) was determined according to the method of Elstner and Heupel (1976). The whole seedlings (0.2 g fresh weight, FW) were homogenized with 1 mL of 50 mM cold phosphate buffer (pH 7.8) and then centrifuged with 10,000*g* for 15 min at 4 °C. The supernatants (0.5 mL) were mixed with 0.5 mL of 65 mM phosphate buffer (pH 7.8) and 0.1 mL of 10 mM hydroxylamine chlorhydrate and then incubated at 25 °C for 1 h. After incubation, the mixture was combined with 1 mL of 17 mM sulfanilamide and 1 mL of 7 mM α-naphthylamine and incubated for 20 min at 25 °C. The absorbance was recorded at 530 nm and the production rate of $O_2^{\bullet-}$ was calculated based on a standard curve of $NaNO_2$.

The hydrogen peroxide (H_2O_2) content in the whole seedlings in response to heat stress was measured according to Patterson et al. (1984). Samples (0.50 g of FW) were homogenized in 5 mL cold acetone, and the homogenate was centrifuged with 10,000*g* at 4 °C for 15 min. The supernatant was collected and added into concentrated hydrochloric acid solution of 0.1 mL 20% $TiCl_4$ and 0.2 mL concentrated ammonia. After a 5-min reaction at 25 °C, the reaction mixture was centrifuged with 8000*g* at 4 °C for 10 min. The pellets were washed with cold acetone twice and added into 3 mL 1 M H_2SO_4. The absorption was measured at 410 nm, and the concentration of H_2O_2 was determined using a standard curve plotted with known concentrations of H_2O_2.

Malondialdehyde (MDA) concentration in the whole seedlings exposed to heat stress was determined by the trichloroacetic acid (TCA) reaction (Shalata and Neumann, 2001). Samples (0.30 g of FW) were collected and ground sufficiently with 5 mL 10% TCA and a little of SiO_2. After centrifugation with 5000*g* for 10 min, the supernatant (2 mL) was combined with 0.6% (w/v) thiobarbituric acid (2 mL) and incubated in boiling water for 15 min. The reaction was stopped by placing the tubes in an ice bath. The mixture was centrifuged at 5000*g* for 15 min and the supernatant was assayed at 532 and 450 nm.

2.6. Determination of antioxidant enzyme activity

The whole seedlings (0.20 g of FW) germinated from control or 50-Gy-gamma-irradiated seeds under heat stress were homogenized in a mortar and pestle with 2 mL of 50 mM cold phosphate buffer (pH 7.8) containing 4% PVP and 1 mM EDTA. The homogenate was centrifuged with 15,000*g* for 15 min at 4 °C. The supernatant was used for assaying the activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT). All of the procedures were conducted at 4 °C.

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