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Modification of catalase and MAPK in *Vicia faba* cultivated in soil with high natural radioactivity and treated with a static magnetic field

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ABSTRACT

The effects of a static magnetic field (SMF) and high natural radioactivity (HR) on catalase and MAPK genes in *Vicia faba* were investigated. Soil samples with high natural radioactivity were collected from Ramsar in north Iran where the annual radiation absorbed dose from background radiation is higher than 20 mSv/year. The specific activity of the radionuclides of ^{232}Th, ^{236}Ra, and ^{40}K was measured using gamma spectrometry. The seeds were planted either in the soil with high natural radioactivity or in the control soils and were then exposed to a SMF of 30 mT for 8 days; 8 h/day. Levels of expression of catalase and MAPK genes, catalase activity and H_2O_2 content were evaluated. The results demonstrated significant differences in the expression of catalase and MAPK genes in SMF- and HR-treated plants compared to the controls. An increase in catalase activity was accompanied by increased expression of its gene and accumulation of H_2O_2. Relative expression of the MAPK gene in treated plants, however, was lower than those of the controls. The results suggest that the response of *V. faba* plants to SMF and HR may be mediated by modification of catalase and MAPK.

Introduction

According to the latest report of the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR), the greatest contribution to mankind's exposure comes from natural background radiation, and the worldwide average annual effective dose per capita is 2.4 mSv (UNSCEAR 2000). Ramsar is a coastal city in the north of Iran where the annual radiation absorbed dose from background radiation is substantially higher than 20 mSv/year, 8 times higher than the maximum threshold determined by UNSCEAR (Ghiassi Nejad et al., 2000). Ionizing radiation is known to have general effects on plant growth and development, ranging from stimulatory effects at very low doses, to pronounced decreases in reproductive fitness and yield at high radiation levels (Kovalchuk et al., 2004). Previous studies have shown that relatively low-doses of ionizing radiation on plants were manifested as accelerated cell proliferation, enzyme activity, and stress resistance (Chakravarty and Sen, 2001). Absorption of ionizing radiation by plants cells can indirectly influence cell physiology by radiolysis of cellular water and stimulation of oxidases and nitric oxide synthases that lead to the generation of reactive oxygen species (ROS). Ionizing radiation has been shown to induce oxidative stress with overproduction of reactive oxygen species. Generation of ROS, particularly H_2O_2, has been proposed to be part of the signaling cascades that lead to protection from stresses (Hossam et al., 2011).

The magnetic field is another factor that can induce the production of ROS, whose distribution in the environment is burgeoning with the development of technology. It has been shown that static magnetic fields (SMF) can cause an inconsistency in the function of antioxidant enzymes in plant cells and thereby lead to oxidative stress (Sahebjamei et al., 2007). SMF in conjunction with other physical disturbing factors e.g., radioactive radiations or chemical signaling agents e.g., salicylic acid, show considerable effects on the balance between ROS and the antioxidant system (Javani et al., 2011; Rezaei et al., 2010). Scavenging and detoxification of H_2O_2, can be achieved by either non-enzymatic antioxidants or scavenging enzymes e.g., CAT and peroxidases. Interestingly, it has been documented that H_2O_2 stressors of varying strengths can induce the expression of catalase genes in the yeast (Quinn et al., 2002). A rapid increase of CAT activity was induced by H_2O_2 in suspension cultures of *Xanthophyllomyces dendrorhous* (Liu and Wu, 2006). A similar increase was observed in the activity of CAT in calli of the halophyte plant *Nitraria tangutorum* treated with exogenous H_2O_2 (Yang et al., 2012). Repetitive H_2O_2 stress caused an elevation both

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in the levels of catalase protein and its activity in fibroblasts (Sen et al., 2003).

The mitogen-activated protein kinase (MAPK) cascade is one of the major pathways by which extracellular stimuli are transduced into intracellular responses in all eukaryotic cells (Wrzaczek and Hirt, 2001). Most of the biotic and abiotic stresses, including oxidative stresses, can induce defense responses in plants through the MAPK pathway (Mishra et al., 2006). Activated MAPK can facilitate its translocation to the nucleus, where it can phosphorylate and activate transcription factors, thereby modulating gene expression (Melanie et al., 1995).

MAPKs have been suggested to control the expression of antioxidant enzymes and inhibit cell cycle progression and cell proliferation (Hu and Kong, 2004). Applying artificial magnetic fields on cultured zebra fish cells, Uchida et al. (2012) showed that an extracellular signal-regulated kinase (ERK)/MAPK was activated. To the best of our knowledge, however, such studies on the plant cells have not yet been conducted. The present study was undertaken to evaluate the effects of SMF and high natural radiations on the H_2O_2 level, activity and expression level of catalase as the major H_2O_2 scavenging enzyme, and expression of MAPK, which is suggested to control the expression of CAT, in broad bean. This evaluation can highlight the potential hazard risk of SMF in biological systems.

Materials and methods

Six soil samples with high natural radioactivity were collected from Ramsar, located in north Iran. The specific activity of the radionuclides ^{232}Th, ^{226}Ra and ^{40}K in the soil samples was measured by gamma spectrometer equipped with a high purity germanium semiconductor (Canberra GC4020) and the soil sample with the highest natural radioactivity was selected. The soil was silt-loam, with EC 3.5, pH 7.2, and the specific activity of ^{226}Ra, ^{232}Th, and ^{40}K of it was 9830 ± 124, 22.8 ± 4.1, and 654 ± 49.1 Bq/kg, respectively. Control soil samples similar to Ramsar soils just in terms of minerals, texture, EC, and pH were collected from an experimental field of Tarbiat Modars University in Tehran.

Magnetic field exposure

Seeds of broad bean (*Vicia faba* L.) were prepared from The Seed and Plant Improvement Institute, Golestan, Iran. The seeds were surface sterilized by subsequent washes with detergent, sodium hypochlorite (containing 5% active chlorine), and 70% EtOH. The seeds were then rinsed three times with distilled water and were sown in 500 g plastic pots in four groups. The first group of the seeds was sown in the control soil (without radiation) and was also kept far enough from the MF-producing apparatus to avoid any potential exposure to the MF. In addition, other electric appliances and laboratory facilities were turned off so that the control samples were only exposed to the local GeoMF (47 ± 5 μT, according to The Geophysics Institute of Tehran University). The second group of seeds was sown in the soils with HR, but was again kept far from MF. The third group was cultivated in the control soil but was exposed to SMF. The fourth group was cultivated in HR and also exposed to SMF. The pots were irrigated every day with either tap water or ½ Hoagland nutrient solutions. Treatment with SMF (30 mT) was started from the day of sowing until day 8, 8 h each day. The description of the SMF-generating apparatus, and the tests which were conducted for accuracy and homogeneity of the field have been described previously (Payez et al., 2013). At the end of treatment period, the seedlings were harvested. The plants were washed, and their roots and shoots were separated, wiped, and frozen in liquid N_2 and kept at −80 °C until used for biochemical and molecular analyses.

Biochemical procedure

Frozen samples (200 mg fresh weight) were homogenized in 3 mL Na-phosphate buffer, followed by centrifugation at $12{,}000 \times g$ for 10 min. All operations were performed at 4 °C. Catalase (CAT) activity was measured in a reaction mixture that consisted of 25 mM Na-phosphate buffer (pH 6.8), 10 mM H_2O_2 and diluted enzyme extract (supernatant) in a total volume of 1 mL. The decomposition of H_2O_2 was followed by the decline in absorbance at 240 nm by spectrophotometer (GBC, Cintra 6, and Australia), and the CAT activity was expressed based on the change in absorbance against protein content (Hajnorouzi et al., 2011). Protein contents were determined by the method of Bradford using bovine serum albumin (BSA) as a standard (Bradford, 1976).

Hydrogen peroxide content was assayed according to the following method. The samples (0.2 g) were homogenized with 5 mL of 0.1% (w/v) trichloracetic acid in an ice bath, followed by centrifugation at $12{,}000 \times g$ for 10 min. 0.5 mL of supernatant was then mixed with 0.5 mL of 10 mM potassium phosphate buffer (pH 7) and 1 mL of 1 M KI. Absorbance of the mixture solution was measured at 390 nm and the content of H_2O_2 was determined using a standard curve of 0–30 μM H_2O_2.

Molecular procedure

Semi-quantitative RT-PCR was used for detection of the relative expression levels of *CAT* and MAPK using Actin as an internal control. Total RNA was isolated from samples by RNX^{TM+} (CinnaGen Inc.) reagent according to the manufacturer's instructions, in the presence of chloroform, isopropanol and ethanol 70%. The concentration and purity of RNA were determined by Agarose gel electrophoresis. First strand cDNA was synthesized from 3 μg of total RNA using 1 μL oligo-(dT)18 primer (MWG-Biotech AG) and 0.5 μL RNase inhibitor (Fermentas), at 70 °C for 10 min. The mixture was then quickly cooled on ice to allow annealing of the primer and RNA. In a separate microtube, other components of reverse transcription (RT), i.e., 1 μL dNTPs, 0.5 μL RNase inhibitor, 1 μL reverse transcriptase (Revert Aid^{TM} M-MuLV, Fermentas), and 4 μL 109 RT buffer were mixed together and then were added to the former mixture. The RT reaction was performed at 42 °C for 2 h.

Forward and reverse specific primers of *Linum usitatissimum* were used to amplify the ACT (NCBI accession No. DQ846903) gene.

Catalase and MAPK gene Primers were designed from a highly conserved region of *Pisum sativum* (NCBI accession No. X60169 and X70703), All primers used in this study with PCR conditions are listed in Table 1. PCR products were separated using 1.2% agarose gel electrophoresis. The band intensity on the gel stained with ethidium bromide was measured by UV Documentation Luminescent Image Analysis software (England), and then quantified by Image Gauge software. The nucleotide sequences of amplified fragments were confirmed by DNA bidirectional sequencing using specific primers (Macrogen, Korea).

Statistical analysis

The experiment was structured following a completely randomized design arranged in a 2×2 factorial with three replications. For all variables, analysis of variance (ANOVA) was performed to test the differences between HR and SMF treatments and their interactions (SPSS version 16, Chicago, IL, USA). Each treatment was analyzed in three replications. When analysis of variance (ANOVA) showed significant treatment effects, Duncan's multiple range test was applied to compare the means at $P < 0.05$.