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ORIGINAL ARTICLE

Expression analysis and biochemical characterization of beans plants biofortificated with zinc

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Abstract The present work was carried out in greenhouse conditions at the Centro de Investigación en Alimentación y Desarrollo AC in Delicias, Chihuahua, México. Four different concentrations (0, 25, 50 and 100 μM L^{-1}) of Zn chelate and sulfate were used to study the antioxidant system of *Phaseolus vulgaris* L. Three genes related with antioxidant activity [superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT)] were selected for expression study. Results showed that when Zn chelate at 50 and 100 μM L^{-1} were applied SOD was repressed and GSH-Px expression was low at 0, 25 and 100 μM L^{-1} while with sulfate form SOD expression was low and GSH-Px expression was strong in all treatment. CAT was highly expressed in all form and treatments. For a biochemical study the same enzymes were spectrophotometrically measured. SOD activity shows differences in both forms of Zn, chelate form was different at 25, 50 and 100 μM L^{-1} with less activity at 100 μM L^{-1} and sulfate treatment shows differences in all concentrations used. GSH-Px activity shows significant differences with sulfate form at 25, 50 μM L^{-1} where at 50 μM the activity was higher and low at 100 μM L^{-1}, CAT does not exhibit significant differences but with chelate treatment at 50–100 μM L^{-1} the activity was higher compared to sulfate. Finally, to raise the Zn concentration in bean under biofortification program is a promising

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strategy in cropping systems in order to increase the ingestion of zinc and antioxidant capacity in the general population and provided the benefits that this element offered in human health.

1. Introduction

Zn is necessary for the activity of more than 100 enzymes involved in several metabolic routes and consequently participates in various biochemical and immunological functions. Zn deficiency affects physical development, immune system, reproductive function and neuro development. For children these deficiencies are manifested as little gain in weight and height, diarrhea, anorexia and neurological problems. Additionally, the deficiency of this element is associated with increases in mortality (Hotz and Brown, 2004).

Nowadays, an innovative approach to solve the problems of micronutrients malnutrition is called ''biofortification'' (Bouis, 2003). Biofortification has been defined as a process where the concentration of bioavailable essential elements in the edible parts of the crop plants increases through crop management (fertilization).

To ensure the effectiveness of Zn biofortification, the concentration should be established not only to prevent deficiencies of this element in the fruits, but also to maintain the GSH-Px, SOD and CAT enzymes at a high level, and something very important is to care that the concentrations used do not cause oxidative reactions (Hafeez et al., 2013).

Few studies have focused on gene expression of the enzymes mentioned in biofortification processes. Comprehension of the responses of gene expression of antioxidant enzymes is important for future understanding of the molecular factors that control the antioxidant defense in the bean plants, for this reason the objective of this research is to analyze the temporal expression and enzyme activity of SOD, GSH-Px and CAT in bean plants (*Phaseolus vulgaris* L.) biofortified with Zn-sulfate and Zn-chelate.

2. Materials and methods

The present work was carried out in greenhouse conditions at the Centro de Investigación en Alimentación y Desarrollo AC in Delicias, Chihuahua, México. Seeds of bean (*Phaseolus vulgaris* L. cv. Strike) were germinated in a chamber at 28 °C for 48 h. After this period, the plants were grown in a greenhouse. For 10 days after transplantation and prior to application of the experimental treatments, the plants received a complete Hoagland nutrient solution suited by Sanchez et al. (2004); the nutrient solution was renewed every three days. Subsequently, 10 days after germination the following zinc treatments were applied (for 50 days) in the forms of sulfate and chelate: T1 = 0 μM, T2 = 25 μM, T3 = 50 μM, T4 = 100 μM.

The intermediate leaves were collected after 60 days, after complete crop cycle, and were used to perform biochemical and molecular analyses. The fruits were also harvested for yield determination.

2.1. Molecular analyses

The total RNA was extracted using the RNA purification kit GenElute™ RNA/DNA/Protein Plus.

The first cDNA strand was synthesized using the SuperScript III System (Invitrogen Life Technologies, USA). A polymerase chain reaction (PCR) was carried out in three steps of one cycle each at 94 °C for 5 s, 55 °C for 30 s and 68–72 °C for 1 min.

Differential expression of genes encoding proteins widely related to oxidation mechanisms in plants (SOD, GSH-Px, and CAT) were analyzed using RT-PCR. The primers were designed using the Primer Express 2.00 (Applied Biosystems software), based on the sequences obtained from the database of the National Center for Biotechnology Information (NCBI) (Table 1). RT-PCR was performed in a BioRad standard cycle programmer system.

The amplification consisted of one cycle for denaturation at 94 °C for 2 min, 40 cycles of annealing at specific temperature for each gene (Table 1) for 30 s, 58 °C for 30 s and extension at 72 °C for 1 min followed by a cycle of final extension at 72 °C for 2 min. The results were visualized in agarose gel 1%.

2.2. Biochemical analyses

2.2.1. Biomass and bean yield (*P. vulgaris*)

Plants biomass was determined as the average dry weight of whole plants and expressed as $mg.DW^{-1}$. Yield was expressed as dry weight of fruits per plant (in grams).

2.2.2. GSH-Px assay

The activity of GSH-Px (EC 1.11.1.9) was determined spectrophotometrically as described by Rao et al. (1997). Activity was measured by following the decrease in absorbance at 340 nm and expressed as μmoles of NADPH oxidized $(mg\ prot)^{-1}\ (min)^{-1}$.

2.2.3. CAT assay

Total catalase (CAT: EC 1.11.1.6) activity was determined in a spectro-photocolorimeter as described by Sánchez et al. (2000). The activity was determined by monitoring the degradation of H_2O_2 at 240 nm during 1 min against a sample without plant extract. The enzyme activity was expressed in μmoles of H_2O_2 $(mg\ prot)^{-1}\ (min)^{-1}$.

2.2.4. SOD assay

Extraction and quantification of the SOD (EC 1.15.1.1) was performed as established by Sánchez et al. (2004). The activity of this enzyme was measured by optical density at 560 nm, based on the inhibition of the photochemical reduction of Nitro Blue Tetrazolium (NBT). The enzyme activity was expressed in units of SOD $(mg\ prot)^{-1}\ (min)^{-1}$.

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