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Effect of chemical stress on germination of cv Dalia bean (*Phaseolus vularis* L.) as an alternative to increase antioxidant and nutraceutical compounds in sprouts

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ABSTRACT

The aim of this study was to determine the effect of chitosan (CH), salicylic acid (SA) and hydrogen peroxide (H_2O_2) at different concentrations on the antinutritional and nutraceutical content, as well as the antioxidant capacity of bean sprouts (cv Dalia). All elicitors at medium and high concentrations reduced the antinutritional content of lectins (48%), trypsin inhibitor (57%), amylase inhibitor (49%) and phytic acid (56%). Sprouts treated with CH, SA and H_2O_2 (7 μM; 1 and 2 mM, and 30 mM respectively) increased the content of phenolic compounds (1.8-fold), total flavonoids (3-fold), saponins (1.8-fold) and antioxidant capacity (37%). Furthermore, the UPLC-ESI-MS/MS analysis showed an increase of several nutraceutical compounds in bean sprouts treated with SA such as coumaric (8.5-fold), salicylic (115-fold), gallic (25-fold) and caffeic (1.7-fold) acids, as well as epigallocatechin (63-fold), rutin (41-fold) and quercetin (16.6-fold) flavonoids. The application of elicitors in bean seed during sprouting enhances their nutraceutical properties.

1. Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important legume worldwide, and is an excellent source of high quality proteins, as well as starch, dietary fiber, minerals and vitamins. Furthermore, beans are a rich source of bioactive compounds with several health benefits such as phenolic acids, flavonoids, non-digestible polysaccharides, saponins and phytosterols (Ramírez-Jiménez, Reynoso-Camacho, Tejero, León-Galván, & Loarca-Piña, 2015).

In addition, common bean has antinutritional compounds such as phytic acid, protease inhibitors (trypsin and chymotrypsin), α-amylase inhibitors and lectins, which decrease the bioavailability of trace elements, carbohydrates and proteins (Doria, Campion, Sparvoli, Tava, & Nielsen, 2012). Therefore, the inactivation or removal of these undesirable components is essential to improve the nutritional quality of common bean (Shimelis & Rakshit, 2007).

In recent years, the consumption of low-processed food has increased, and sprouting represents an effective process to improve the nutritional quality of legumes (Tang, Dong, Ren, Li, & He, 2014). Several studies have reported that common bean sprouts have higher levels of nutrients and a lower content of antinutrients as compared to dry seeds (López et al., 2013). Furthermore, phenolic composition and dietary fiber levels are enhanced during bean germination (Dueñas et al., 2016).

On the other hand, the application of exogenous elicitors, such as salicylic acid, during germination of common bean seeds enhances seedling growth and increases their content of total soluble phenolic compounds (Limón, Peñas, Martínez-Villaluenga, & Frias, 2014; Rivas-San Vicente & Plasencia, 2011). Similarly, it has

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been reported that soybean and broccoli seeds soaked in salicylic acid and chitosan solutions presented an increased sprout growth and nutritional improvement (Anaya, Fghire, Wahbi, & Loutfi, 2015; Pérez-Balibrea, Moreno, & García-Viguera, 2011).

Therefore, germination and elicitation could be considered alternative technologies for the production of low-cost functional foods, diversifying bean market. Nevertheless, it is important to identify elicitors that stimulate sprouts growth and yield, improving their nutritional properties. The aim of this study was to evaluate the effect of chitosan (CH) (0.7, 3.3 and 7 μM), salicylic acid (SA) (0.1, 1 and 2 mM) and hydrogen peroxide (H_2O_2) (10, 20 and 30 mM) during germination of common beans (*P. vulgaris* L. cv Dalia) on sprout growth, antinutritional and nutraceutical compounds, as well as antioxidant capacities.

2. Materials and methods

2.1. Materials and reagents

Campo Experiemental Bajío (CEBAJ-INIFAP), Celaya, Guanajuato, Mexico provided the seeds of dry-bean cultivar Dalia (Flor de Junio commercial seed). Dalia is a cultivar with high adaptation and yield stability; it is disease resistant and tolerant to acid soils (Acosta-Gallegos, Montero-Tavera, Jiménez-Hernández, Anaya-López, & Gonzalez-Chavira, 2014). In addition, in previous studies in our laboratory, Dalia, without elicitation, showed great germination percentage, radicle size and water absorption.

2.2. Elicitors and chemical elicitation treatments

Elicitors were dissolved in distilled water at the following concentrations: 0.7 μM, 3.3 μM and 7 μM chitosan (CH); 0.1 mM, 1 mM and 2 mM salycilic acid (SA); 10 mM, 20 mM and 30 mM hydrogen peroxide (H_2O_2). All solutions were freshly prepared the day of their application.

2.3. Germination process

Seeds (100 g) were soaked in 1% sodium hypochlorite (1:6 w/v) for 30 min at room temperature. Then, seeds were drained and washed with distilled water until they reached a neutral pH. Afterwards, in order to soften seed coat and promote uniform water uptake, seeds were soaked in distilled water (1:6 w/v) for 6 h and shaken every 30 min. Finally, hydrated seeds were placed in trays where a wet filter paper was extended, and then were covered. The trays were introduced into a germination chamber and filter paper was watered daily with each elicitor solution. Germination was performed in darkness for 3 days at 25 °C. Germinating seeds in distilled water performed the control of the experiment. Each germination experiment included three replicates.

2.4. Vigor seedling: germination percentage and radicle size

The germination percentage was determined based on the total number of seedlings fully emerged. The radicle length (mm) was measured with a Vernier Caliper. Both parameters were obtained daily. The results of germination percentage and radicle length were analyzed using mathematical exponential models.

2.5. Flour preparation

At the end of the process, germinated seeds were collected and immediately plunged into liquid nitrogen, ground in a mill, passed through a mesh with a particle size of 1 mm, and then the flours were stored at −70 °C until further analysis. This storage temperature ensures stability of phytochemical compounds during storage.

2.6. Quantitation of antinutritional compounds

2.6.1. Haemagglutinating activity

Haemagglutination assays were carried out using trypsin-treated human erythrocytes by the serial dilution method as described by Grant, More, McKenzie, Stewart, and Pusztai (1983). Blood samples were collected into pre-heparinised tubes and immediately after collection were diluted 1:19 with a solution of sodium chloride saline (1%). Then, human erythrocytes were pretreated with pronase (20 mg/ml of diluted blood for 30 min at 25 °C). Dried flours (1 g) were extracted with 0.04 M glycine-HCl buffer, pH 2.2, for 16 h at 1 °C in a ratio of 1:20 (w/v) flour/buffer. Finally the mix was centrifuged. The haemagglutinating activity of the sample was evaluated against human erythrocytes by a serial dilution method (Grant et al., 1983). One unit of haemagglutinating activity (HU) was defined as that contained in the amount of sample in the last dilution which caused 50% agglutination of the blood cells.

2.6.2. Trypsin inhibitor activity

The trypsin inhibitor activity (TIA) was determined following the method of Kakade, Rackis, McGhee, and Puski (1974) with some modifications. Dried flours (1 g) were incubated with 50 mL of 0.01 N NaOH (pH 8.2) for 3 h. The mix was filtered, and then the extract (200 μL) was incubated with 250 μL of trypsin solution (Sigma Chemical Company, St. Louis, MO, USA) (0.004% trypsin in 0.025 M glycine-HCl buffer) and diluted to 1 mL with TRIS buffer (pH 8.2). Then, 2.5 mL of 0.001 M Nα-Benzoyl-L-arginine 4-nitroanilide hydrochloride (BAPNA) solution in TRIS buffer (pH 8.2) previously warmed to 37 °C, was added. Finally, absorbance was measured at 410 nm, and TIA was calculated as trypsin inhibitor units per milligram of sample (TIU), and one trypsin unit was defined as the increase by 0.01-absorbance unit at 410 nm of reaction mixture.

2.6.3. Alpha-amylase inhibitor

The α-Amylase inhibitor activity (AIA) was evaluated according to Deshpande, Sathe, Salunkhe, and Cornforth (1982). Dried flours (1 g) were extracted with 10 mL of a saline solution (NaCL 0.1 M) for 12 h at 5 °C. The mix was filtered, and then the extract (0.25 mL) was incubated with 0.25 mL of α-amylase (from *Bacillus subtilis*, Sigma Chemical Company, St. Louis, MO, USA) solution (0.003% in 0.2 M sodium phosphate buffer (pH 7) and 0.006 M NaCl) for 15 min at 37 °C. Then, 0.5 mL of 1% starch solution preincubated at 37 °C was added. After 10 min, the reaction was stopped with 2 mL of dinitrosalicylic acid, and then the mixture was warmed in a water bath for 10 min. Finally, the absorbance was read at 540 nm. One unit of amylolytic activity was defined as the milligrams of protein required for hydrolyzing 0.06 mg starch per minute. One unit of amylase inhibition (AIU) was defined as the milligrams of protein required to inhibit one unit of amylase activity under the specified conditions. The Bradford method was used to determine proteins of the extracts.

2.6.4. Phytic acid

Phytic acid (PA) content was determined following the method of Frühbeck, Alonso, Marzo, and Santidrián (1995). Dried flours (1 g) were extracted with 20 mL of 0.66 N HCl, and was incubated with agitation for 2 h. Then, the sample was centrifuged at 17,300 *g* for 30 min at room temperature and the supernatant was recovered. PA was purified using a glass column (0.7 × 15 cm) packed with 0.5 g of anion exchange resin (AG 1-X4, 100-200 dry mesh Bio-Rad). 10 mL of 1:25 extract dilution (pH 6) were applied to