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Optimizing elicitation and seed priming to enrich broccoli and radish sprouts in glucosinolates

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ABSTRACT

Elicitation is a cheaper and socially acceptable tool for improving plant food functionality. Our objective was to optimize the treatment doses of the elicitors: methyl jasmonate (MeJA), jasmonic acid (JA) and DL-methionine (MET), in order to find a successful and feasible treatment to produce broccoli and radish sprouts with enhanced levels of health-promoting glucosinolates. Also a priming of seeds as a novel strategy to trigger the glucosinolates content was carried out with water (control), MeJA (250 μM), JA (250 μM) and MET (10 mM) before the elicitor exogenous treatment. The results showed that almost all treatments could enhance effectively the total glucosinolates content in the sprouts, achieving the most significant increases from 34% to 100% of increase in broccoli and from 45% to 118% of increase in radish sprouts after MeJA priming and treatments. Consequently, our work demonstrates the feasibility of using elicitors, such as plant stress hormones, by priming and exogenously, as a way of increase the phytochemical profile of these sprouts to enhance their consumption in the diet.

1. Introduction

Consuming cruciferous vegetables is associated with many health benefits due to their composition in antioxidant compounds (mainly phenolic compounds and vitamin C) and glucosinolates (GLS – sulfur and nitrogen compounds with a glucose and a variable side chain derived from amino acids) (Dinkova-Kostova & Kostov, 2012; Jahangir, Abdel-Farid, Kim, Choi, & Verpoorte, 2009). Particularly, *Brassicaceae* sprouts content higher amount of GLS (20 times more), compared to the mature plants because their young physiological state (Fahey, Zhang, & Talalay, 1997). These bioactive phytochemicals have been widely investigated because their hydrolysis compounds, the isothiocyanates (ITC) and indoles. In plants, GLS are accompanied, but physically separated, by myrosinases (EC 3.2.1.147). These enzymes are responsible of their hydrolysis when there is a tissue disruption, mastication of fresh plants, and also upon ingestion by humans, because β-D-thioglucosidase activity of the gut microflora is largely responsible for converting ingested GLS to their cognate ITC and indoles, biologically active molecules which may impact in diseases prevention (Dinkova-Kostova & Kostov, 2012). The ITC sulforaphane, produced by hydrolysis of the predominant GLS of broccoli glucoraphanin, has demonstrated to have neuroprotective effects (Tarozzi, Angeloni, Malaguti, Morroni, Hrelia, & Hrelia, 2013) and anti-inflammatory and chemoprotective activity (Surh and Na, 2009). Other broccoli ITC, such as iberin and erucin, have shown similar antiproliferative activity in cancer cell lines, even though these compounds have not been widely studied (Wang, Wang, Howie, Beckett, Mithen, & Bao, 2005). The hydrolysis compounds of the GLS glucoraphenin and dehydroerucin, from radish sprouts, also showed inhibition of phase I or induction of phase II xenobiotic metabolizing enzymes (Barillari et al., 2007). Indole GLS, such as glucobrassicin, are hydrolyzed to indole-3-carbinol and its derived compound 3,3′-diindolymethane, which have potentially biological effects, including activity on carcinogen metabolizing enzyme system (Aggarwal & Ichikawa, 2005).

The glucosinolates content of broccoli and radish sprouts can be manipulated through treatments with elicitors, such as plant hormones (methyl jasmonate (MeJA), jasmonic acid (JA), salicylic acid (SA), ethylene (ET) or abscisic acid (ABA), among others) (Roberto & Solano, 2005), sucrose (Guo, Yuan, & Wang, 2011), sodium chloride (Yuan, Wang, Guo, & Wang, 2010), or the amino acid DL-methionine (MET) (Scheuner, Schmidt, Krumbein, Schonhof, & Schreiner, 2005), which act as stressors in the plants, activating an array of mechanisms similar to the defense responses to pathogen infections or environmental stimuli, affecting the plant metabolism and enhancing the synthesis of phytochemicals. Elicitors are usually applied daily by spraying over the cotyledons, not as irrigation procedure. In this work, using elicitors as a priming

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treatment is a novel tool to increase bioactive compounds, as this method has been widely used only to reduce the time from sowing to radicle emergence. Therefore, this work reports the effect of combination of priming and elicitation with MeJA, JA and MET, in order to maximize the total GLS contents in broccoli and radish sprouts, to include the naturally healthy and functional food in future human clinical trials and to enhance the bioactive compounds intake through dietary interventions, in view of increased interests in healthy foods from natural origin.

2. Material and methods

2.1. Plant material

Seeds for sprouts production were provided by Intersemillas S.A (Valencia, Spain). Two varieties were used: broccoli (*Brassica oleracea* L. var *italica*) and red radish (*Raphanus sativus* cv. Rambo). Seeds were equally hydrated by immersion in 5 g L^{-1} sodium hypochlorite under aeration during 2 h, then, were immersed with aeration in distilled water (control samples), and MeJA, JA and MET (treated samples), involving the priming treatment, during 24 h until radicle protrusion, in order to reduce the time from sowing to emergence. After pouring off the soaking water, the seeds were weighed (day 0) and spreaded on trays (5 g per tray) lined with cellulose (CN Seeds, UK) and irrigated everyday with Milli-Q water. Three replicates (trays) per sample were transferred to an environment controlled chamber with a cycle of 16 h light with 60% relative humidity and air temperature of 25 °C and 8 h dark with 80% relative humidity and 20 °C. Photosynthetically active radiation (PAR) of 400 μmol m^{-2} s^{-1} was supported by a combination of fluorescent tubes (Philips TLD 36 W/83, Hamburg, Germany; Sylvania F36 W/GRO, Danvers, Massachusetts, USA) and metal halide lamps (Osram HQI.T 400 W, Munich, Germany). During the first 3 days all trays were kept in controlled dark for increasing the stem elongation of sprouts. Then, three replicates per treatment of broccoli and radish sprouts were rapidly collected at day 8 after germination, in the middle of the light period, for analysis. All samples were weighed (fresh mass), flash frozen in liquid nitrogen and stored at −80 °C prior to analyses.

2.2. Treatments with elicitors: priming and exogenous spraying

The phytohormones jasmonic acid (JA) and methyl jasmonate (MeJA) (25–250 μM), and the amino acid DL-methionine (MET) (1–10 mM) were selected as elicitors according to literature review. JA (SIGMA–ALDRICH, Co., 3050 Spruce Street, St. Louis, MO. 63103, USA) and MeJA (SAFC, 3050 Spruce Street, St. Louis, MO. 63103, USA) were dissolved in 0.2% ethanol in Milli-Q water. DL-methionine (Alfa Aesar GmbH & Co KG, Karlsruhe, Germany) was dissolved in 0.04% ethanol in Milli-Q water.

Priming was performed with 100% imbibition and aeration of the seeds for 24 h, with three different treatments: MeJA and JA in a concentration of 250 μM and MET in 10 mM. Elicitors during germination of sprouts were applied as exogenous spraying on the cotyledons (not as soaking or irrigation solution) with 30 mL of test solution per sample (10 mL per tray) from day 4 to day 7 of sprouting (4 days of treatment), using Milli-Q water as control.

2.3. Extraction and determination of glucosinolates

2.3.1. Sample extraction

Freeze-dried samples powder (50 mg) were extracted with 1 mL of methanol 70% V/V, then heated at 70 °C for 30 min in a heating bath, with shaking every 5 min and centrifuged (17,500×*g*, 5 min). The supernatants were collected and methanol was removed using a rotary evaporator. The dry material obtained was re-dissolved in Milli-Q water and filtered (0.45 μm Millex-HV13 filter, Millipore, Billerica, MA, USA).

2.3.2. HPLC-PAD-ESI-MS^n analysis of glucosinolates

The qualitative and quantitative analysis of glucosinolates was performed according to Baenas, García-Viguera, and Moreno (2014) protocol. Briefly, the intact GLS were identified following their MS^2 $[M-H]^-$ fragmentations patterns in an HPLC-PAD-ESI-MSn (Agilent Technologies HPLC 1200, Waldbronn, Germany; coupled to a mass detector Bruker in series, model UltraHCT, Bremen, Germany). Chromatograms were recorded at 227 nm. Mass spectrometry data were acquired in the negative ionization mode for glucosinolates. Then, the extracted samples were analyzed and quantified in a Waters HPLC-DAD system (Waters Cromatografia S.A., Barcelona, Spain) as described by Pérez-Balibrea, Moreno, and García-Viguera (2011). The intact GLS were identified following their UV spectra and order of elution already described for similar acquisition conditions. Glucosinolates were quantified using sinigrin and glucobrassicin as standard of aliphatic and indole GLS, respectively (Phytoplan, Germany).

2.4. Statistical methods

The data were processed using the SPSS 15.0 software package (LEAD Technologies, Inc., Chicago, USA). The assays were conducted by triplicate. We carried out an ANOVA and the Tukey's Multiple Range Test to conclude significant differences at P values < 0.05.

3. Results and discussion

3.1. Glucosinolates profiles of broccoli and radish sprouts

The glucosinolates content in *Brassicaceae* vegetables varies with genotype, and environmental and growth conditions (Cartea & Velasco, 2008). Broccoli (*B. oleracea* var *italica*) and radish (*R. sativus* cv. Rambo) 8-day-old sprouts show different glucosinolates profiles (Fig. 1). These species are interesting due to their high content in total GLS, being 302.84 and 379.71 mg g^{-1} F.W., in broccoli and radish sprouts, respectively (Tables 1 and 2), if compared with other 7 and 8-days-old sprouts (100–250 mg g^{-1} F.W.; Pereira, Rosa, Fahey, Stephenson, Carvalho, & Aires, 2002; Zhou, Zhu, & Luo, 2013), and adult plants (30–100 mg g^{-1} F.W.; Verkerk et al., 2009). These results are fairly consistent with previous studies from our group, using controlled growth conditions to reduce the influence of external factors to the minimum (Baenas et al., 2014). The predominant individual GLS have been widely studied because of their hydrolysis products, the ITC and indoles (derived from tryptophan), which might play a role in diseases prevention through the anti-inflammatory and chemopreventive pathways (Wagner, Terschluesen, & Rimbach, 2013). In broccoli sprouts, the predominant glucosinolate is glucoraphanin (4-methylsulphinylbutyl), accounting for almost the 50% of the total, 144 mg g^{-1} F.W. (Table 1), which belongs to the aliphatic group (mainly derived from methionine, but also from alanine, leucine, isoleucine, and valine) and is hydrolyzed to the ITC sulforaphane. Also glucoiberin (3-methylsulphinylpropyl), precursor to the ITC iberin, and glucoerucin (4-methylthiobutyl), precursor to the ITC erucin, are aliphatic GLS which account for the 15% of the total GLS in broccoli sprouts (48.06 and 45.21 mg g^{-1} F.W, respectively). The GLS glucoraphenin (4-methylsulphinyl-3-butenyl) and dehydroerucin (4-methylthio-3-butenyl, also known as glucoraphasatin), are the predominant in radish sprouts (162.20 and 195.22 mg g^{-1} F.W, respectively). Both, broccoli and radish sprouts, contain indole