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

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Running title: Elicitation and seed priming to enhance glucosinolates in sprouts

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), methyl jasmonate (250 μM), jasmonic acid (250 μM) and DL-methionine (10mM) 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.

Keywords: elicitation, edible sprouts, natural functional foods, glucosinolates.

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 last bioactive phytochemicals have been widely investigated because their hydrolysis compounds, the isothiocyanates (ITC) and indoles (biologically active molecules which may impact in diseases prevention). 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-thioglucosidase activity of the gut microflora is largely responsible for converting ingested glucosinolates to their cognate isothiocyanates and indoles (Dinkova-Kostova & Kostov, 2012). The ITC sulforaphane, hydrolysis compound of the GLS glucoraphanin predominant in broccoli sprouts, has demonstrated to have neuroprotective effects (Tarozzi, Angeloni, Malaguti, Morroni, Hrelia & Hrelia, 2013) and anti-inflammatory and chemoprotective activity (Surh & Na, 2008) through induction of the Nrf2 factor and consequently the activation of phase II and endogenous antioxidant cell systems, as well as by the inhibition of NFkB factor, activated after proinflammatory stimuli. 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 glucoraphanin 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 3,3'-diindolymethane, which have potentially biological effects, including activity on carcinogen metabolizing enzyme system (Aggarwal & Ichikawa, 2005) and affect adiposity decreasing body weight and modulating lipid metabolism in mice (Chang, Wang, Chan, Chiu, & Chen, 2011).

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 clorhydre (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 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 $g \cdot L^{-1}$ 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 a 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 ºC. Photosynthetically active radiation (PAR) of 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was supported by a combination of fluorescent tubes (Philips TLD 36 W/83, Hamburg, Germany; Sylvania F36W/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 ºC 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) (1-10mM) were selected as elicitors according to literature review (Baenas, García-Viguera & Moreno, 2014a). 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 hours, with three different treatments: MeJA and JA in a concentration of 250 µM and MET in 10nM. 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 were extracted according to Baenas, García-Viguera, & Moreno, (2014b) with modifications. Briefly, 50 mg of powder were extracted with 1 mL of methanol 70% V/V, then heated at 70 ºC for 30 min in a heating bath, with shaking every 5 min and centrifuged (17 500 × *g*, 15 min, 4 ºC). 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*, et al*., (2014b) protocol. Briefly, the intact GLSs 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 analysed and quantified in a Waters HPLC-DAD system (Waters Cromatografia S.A., Barcelona, Spain) as described by Pérez-Balibrea, Moreno & García-Viguera (2011). The intact GLSs 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 (*Brassica oleracea* var *italica*) and radish (*Raphanus sativus* cv. Rambo) 8-day-old sprouts show different glucosinolates profiles (Figure 1). These species are interesting due to their high content in total GLSs, being 302.84 and 379.71 $mg \cdot g^{-1}$ F.W., in broccoli and radish sprouts, respectively (Table 1 and 2), if compared with other 7 and 8-days-old sprouts (100-250 $mg \cdot g^{-1}$ F.W.; Zhou, Zhu, & Luo, 2013; Pereira, Rosa, Fahey, Stephenson, Carvalho, & Aires, 2002), and adult plants (30-100 $mg \cdot g^{-1}$ F.W.; Verkerk, *et al.*, 2009). These results are fairly consistent with previous studies from our group, as the growing

conditions, a factor which affect the bioactive compounds content in the plant, are completely controlled (Baenas, *et al*., 2014b). The predominant individual glucosinolates have been widely studied because of their hydrolysis products, the isothiocyanates 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), which belongs to the aliphatic group (mainly derived from methionine, but also from alanine, leucine, isoleucine, and valine), accounting for almost the 50% of the total, 144 $mg \cdot g^{-1}$ F.W. (Table 1). This compound is hydrolyzed by the enzyme myrosinase to sulforaphane (being 10% the overall yield from glucoraphanin). Also glucoiberin (3-methylsulphinylpropyl), precursor to the isothiocyanate iberin, and glucoerucin (4-methylthiobutyl), precursor to the isothiocyanate erucin, are aliphatic GLSs which account for the 15% of the total GLSs in broccoli sprouts (48.06 and 45.21 $mg \cdot g^{-1}$ F.W, respectively). The glucosinolates 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 $mg \cdot g^{-1}$ F.W, respectively). Both, broccoli and radish sprouts, contain indole glucosinolates, accounting for the 20% and 5% of the total GLSs, respectively. Glucobrassicin (3-indolylmethyl) (GB), 4-hydroxiglucobrassicin (4-hydroxy-3-indolylmethyl) (HGB) and 4-methoxyglucobrassicin (4-methoxy-3-indolylmethyl) (MGB) are common to both species, but broccoli sprouts also present the glucosinolate neoglucobrassicin (*N*-methoxy-3-indolylmethyl) (NGB).

3.2. Effect of the application of elicitors: priming and exogenous spray.

192). In this work, MeJA (250µM), JA (250µM) and MET (10mM) were applied as priming
193 treatment during 24 hours, founding only slight enhanced GLSs content, but when
194 priming was applied in combination with exogenous spray from day 4 to 7 of germination,
195 the effect was improved in terms of GLSs content. Conrath *et al*., (2015) defined defense
196 priming as an induced physiological state in which cells respond to very low levels of a
197 stimulus in a more rapid and robust manner than unprimed cells. The higher increase of
198 total GLS content after combination of priming seeds and exogenous application of
199 elicitors, may be related to an activation of the seeds resistance, which produce enhance
200 molecular mechanism of defense in sprouts. This idea could be substantiated by the fact
201 that plants treated with elicitors such as UV radiation, salt or temperature, caused an
202 increased resistance for subsequents generations, and also treatment of seeds with JA and
203 MeJA primes plants enhance herbivore resistance weeks later and in next generetions
204 (Rasmann, *et al*., 2012). Estos elicitadores activan mecanismos de defensa de la planta,
205 en concreto el sistema de resistencia sistémica inducida (ISR), en los que se produce la
206 producción de enzimas implicadas en la biosíntesis de compuestos naturales como los
207 GLSs, ampliamente estudiados por su efecto en la protección de cultivos y su beneficioso
208 para la salud.
209 No existe un protocolo establecido para utilizar los elicitadores con el objetivo de
210 incrementar los compuestos bioactivos en crucíferas, sin embargo, trabajos como este nos
211 proporcionan información sobre la dosis más eficaz de aplicación de estos compuestos.
212 Under MeJA treatment, there was a statistically significant increase in the total GLSs in
213 both species, not only after exogenous application of the elicitor (50-250 µM) but also
214 after priming treatment (250 µM) together with spray treatment on the cotyledons. Este
215 incremento no es proporcional al aumento de la dosis del elicitador, de acuerdo con lo
216 publicado por Ku, Jeffery & Juvika, (2014), ya que puede existir una saturación del

compuesto en los diferentes tejidos de la planta. Por ello, es interesante seleccionar para cada especie una dosis del elicitador eficaz, en este trabajo, tanto para los brotes de brócoli como los de rábano, podríamos seleccionar estos tratamientos: Prim + 50 µM MeJA, Prim + 125 µM JA and Prim + 5 mM MET (Table 1 and 2). Aunque algunos autores mostraron que cada especie de planta adulta responde de forma diferente al tratamiento con elicitador, como fue el caso de la aplicación de MeJA en broccoli florets y kale leafs (Ku & Juvik, 2013), y la aplicación de DL-methionine in broccoli heads or radish hypocotyls (Scheuner, Schmidt, Krumbein, Schonhof & Schreiner, 2005), en el presente estudio los resultados obtenidos son similares en ambas especies, esto podría deberse a que los brotes, por su estado de desarrollo joven, poseen un metabolismo similar, al contrario que lo ocurrido al elicitar diferentes tejidos en la planta adulta, como en el caso de la aplicación de MeJA en las raíces, hojas o cabezas florales de brócoli (Ku et al., 2014)., donde se obtuvieron diferencias en el incremento de GLSs según el tratamiento. The elicitors treatments in these sprouts were effective, as well as other results found in bibliography which show an increase in GLS after applying exogenous MeJA 250 µM on broccoli florets (60% increase) (Kim & Juvik, 2011) or MeJA 10 µM on broccoli sprouts (22% of increase) (Pérez-Balibrea *et al.*, 2011), these variations in the % of increases of GLS could be produced by different environmental factors, physiological states of the plant or doses of treatment.

Los mecanismos fisiológicos y celulares por los cuales los jasmonatos controlan dichos procesos aún no se han determinado, ya que la ruta metabólica que se ve afectada por ellos no es lineal, forman parte de una red amplia de respuestas celulares, including induction of pathogenesis-related proteins and enzymes of oxidative stress protection, the activation of defense-related genes, changes in the potential of plasma membrane cell and enhanced ion fluxes, rapid changes in protein phosphorylation, lipid oxidation, structural

242 defensive barriers and the activation and the *de novo* biosynthesis of transcription factors,
243 which directly regulate the expression of genes involved in secondary metabolites
244 production, such as glucosinolates (García-Brugger, *et al*., 2006). The glucosinolate
245 levels were elevated to a maximum of 2-fold in case of priming + 250µM MeJA in
246 broccoli (Table 1) and priming + 50µM of MeJA in radish sprouts (Table 2). The
247 combination of these treatments in sprouts were more effective that which were found in
248 other studies with exogenous phytohormones, such as treatment with MeJA 250 µM on
249 broccoli florets (60% of increase) (Kim & Juvik, 2011) and MeJA 10 µM on broccoli
250 sprouts (22% of increase) (Pérez-Balibrea *et al.*, 2011). Concerning individual GLSs of
251 broccoli sprouts (Table 1), it is noted that not all compounds were increased equally:
252 glucoiberin was enhanced to a maximum around 75% after some treatments, such as
253 priming + 50 and 250µM MeJA, and priming + 5mM MET. Glucoraphanin (recently
254 accepted as safe, GRAS), increased by 40% and 70% after some MeJA (250µM
255 exogenous spray with or without priming) and MET (priming + 1-10mM exogenous
256 application) treatments, respectively, achieving significant differences compared to the
257 control (Table 1). However, JA treatments had a very limited effect on this GLS. The 4-
258 HGB and GER GLSs in broccoli sprouts remained unchanged. The aliphatic GLS of
259 radish sprouts, such as GRE and DER also were enhanced after MeJA, JA and MET
260 treatments (Table 2). GRE achieved the maximum concentration of 374 $mg \cdot g^{-1}$ F.W. after
261 priming + 125µM MeJA, 345 $mg \cdot g^{-1}$ F.W. after 250µM JA and 277 $mg \cdot g^{-1}$ F.W. after
262 priming + 5mM MET. Regarding DER, the elicitor JA did not show any effect, however,
263 this compound increased to a maximum of 80% in case of MeJA priming with or without
264 exogenous treatment, and a maximum of 67% after MET priming + exogenous
265 application of this elicitor (Table 2).

Studying indole GLSs, GB from broccoli sprouts was enhanced almost by all treatments under study, achieving a 2-fold increase with MeJA priming (53 $mg \cdot g^{-1}$ F.W.) and MET priming + 1mM of exogenous spray (60 $mg \cdot g^{-1}$ F.W.) (Table 1). In case of radish sprouts, GB was increased 13-fold and 16-fold by priming + the higher concentrations of MeJA and JA exogenous treatments, respectively. The GLS 4-HGB only was increased in radish sprouts after MeJA and MET treatments (to a maximum of 2-fold in MeJA priming + 50µM) and in broccoli sprouts after MET treatments. We also found an increase of 2-fold and 3-fold in MGB in both sprouts species, and 10-fold increase of NGB concentration in broccoli sprouts, after application of the higher concentrations of MeJA and JA priming + exogenous treatments. We could highlight that phytohormones were more effective in increasing indole GLS than MET, according to different authors (Ku, Jeffery, & Juvik, 2013; Brader, Tas, & Palva, 2001) as MET only enhanced GB and MGB contents after application of priming + exogenous spray (Table 1).

Various studies in the model plant *Arabidopsis thaliana* have led to the identification of most glucosinolates biosynthetic pathway genes and their key transcriptional regulators (Sønderby, Geu-Flores, & Halkier, 2010). In recent years, MYB transcription factors were shown to be the most important components of the regulatory network controlling glucosinolates biosynthesis, MYB28, MYB29 and MYB76 are involved in the regulation of aliphatic glucosinolates biosynthesis, whereas MYB34, MYB51 and MYB122 are regulators of indole glucosinolates biosynthesis in *Arabidopsis* (Gigolashvili, Berger, & Flügge, 2009). Achieving upon 2-fold increase of health glucosinolates concentration, such as after MeJA priming + 250 µM in broccoli sprouts (626 $mg \cdot g^{-1}$ F.W. of total GLS), involve a high increase of GLS in the daily diet. Besides, sprouts could be consumed uncooked, allowing the activity of the enzyme myrosinase and, therefore, the isothiocyanates and indoles production and absorption is more extensive than when

crucifers are subjected to cooking (Cramer & Jeffery, 2011). Priming and exogenous elicitation with plant hormones (generally accepted as safe, GRAS) are a sustainable tool to improve the content of health-promoting compounds, enhancing the concentrations of potentially anti-inflamatory, anticarcinogenic and antioxidant bioactives in *Brassica* foods.

4. Conclusions

Combining priming of seeds and spray treatments with natural elicitors, mainly with very low dosages of phytohormones (MeJA 50-250 μM), offer effective and environmentally friendly strategies to trigger the synthesis of target natural products in cruciferous foods without using transgenic technology. These enriched ready-to-eat sprouts can be used in preclinical and clinical trials with potential for protective effects in cells against oxidative and inflammatory processes, and therefore to study and research on the prevention of the development of neurodegenerative, cardiovascular diseases and certain types of cancer, through dietary interventions with naturally healthy foods.

Acknowledgements

This work was supported by the Spanish Ministry of Economy and Competitiveness through Research Project AGL2013-46247-P. N. Baenas was funded by a FPU (Formación Profesorado Universitario) grant of the Fellowships Programme from the Spanish Ministry of Education.

References

Aggarwal, B.B. & Ichikawa, H. (2005). Molecular targets and anticancer potential of indole-3-carbinol and its derivatives. *Cell cycle*, 4, (9), 1201-1215.

317 Baenas, N., García-Viguera, C., & Moreno, D.A. (2014a). Elicitation: a tool for enriching
318 the bioactive composition of foods. *Molecules*, 19, 13541-13563
319 Baenas, N., García-Viguera, C., & Moreno, D.A. (2014b). Biotic elicitors effectively
320 increase the glucosinolates content in Brassicaceae sprouts. *Journal of*
321 *Agricultural and Food Chemistry,* 62, 1881-1889.
322 Barillari, J., Iori, R., Broccoli, M., Pozzetti, L., Canistro, D., Sapone, A., Bonamassa, B.,
323 Biagi, G.L., & Paolini, M. (2007). Glucoraphasatin and glucoraphenin, a redox
324 pair of glucosinolates of *Brassicaceae*, differently affect metabolizing enzymes in
325 rats. *Journal of Agricultural and Food Chemistry*, 55, 5505-5511.Brader, G., Tas,
326 E., Palva, E.T. (2001). Jasmonate-dependent induction of indole glucosinolates in
327 *Arabidopsis* by culture filtrates of the nonspecific pathogen *Erwinia carotovora*.
328 Plant Physiology, 126(2),849-60.
329 Cartea, E., & Velasco, P. (2008). Glucosinolates in Brassica foods: bioavailability in food
330 and significance for human health. *Phytochemistry Reviews*, 7, 213–229.
331 Chang, H.P., Wang, M.L., Chan, M.H., Chiu, Y.S., & Chen, Y.H. (2011). Antiobesity
332 activities of indole-3-carbinol in high-fat-diet-induced obese mice. *Nutrition*, 27
333 (4), 467-470.
334 Conrath, U., Beckers, G.J.M., Langenbach, C.J.G. & Jaskiewicz, M.R. (2015). Priming
335 for enhanced defense. Annual Review of Phytopathology, 53,97–119.
336 Cramer, J.M., & Jeffery, E.H. (2011). Sulforaphane absorption and excretion following
337 ingestion of semi-purified broccoli powder rich in glucoraphanin and broccoli
338 sprouts in healthy men. *Nutrition and Cancer*, 63 (2), 196-201.
339 Dinkova-Kostova, A.T., & Kostov, R.V. (2012). Glucosinolates and isothiocyanates in
340 health and disease. *Trends of Molecular Medicine*, 18, 337-347.

341 Fahey, J.W., Zhang, Y., & Talalay, P. (1997). Broccoli sprouts: An exceptionally rich
342 source of inducers of enzymes that protect against chemical carcinogens.
343 *Proceedings of the National Academy of Sciences, USA,* 94(19), 10367-10372.
344 García-Brugger, A., Lamotte, O., Vandelle, E., Bourque, S., Lecourieux, D., Poinssot, B.,
345 Wendehenne, D., Pugin, A. 2006. Early signaling events induced by elicitors of
346 plant defenses. *Molecular Plant Microbe Interactions*, 19, 711–724.
347 Gigolashvili, T., Berger, B., & Flügge, U.I. (2009). Specific and coordinated control of
348 indolic and aliphatic glucosinolate biosynthesis by R2R3-MYB transcription
349 factors in *Arabidopsis thaliana*. Phytochemistry Reviews, 8, 3–13
350 Guo, R., Yuan, G., Wang, Q. (2011). Sucrose enhances the accumulation of anthocyanins
351 and glucosinolates in broccoli sprouts. Food Chemistry. 129, 1080-1087.
352 Jahangir, M., Abdel-Farid, I.B., Kim, H.K., Choi, Y.H., & Verpoorte, R. (2009). Healthy
353 and unhealthy plants: The effecr of stress on the metabolism of *Brassicacea*e.
354 *Environmental and Experimental Botany*, 67 (1), 23-33.
355 Kim, H.S. & Juvik, J.A. (2011). Effect of selenium fertilization and methyl jasmonate
356 treatment on glucosinolate accumulation in broccoli florets. Journal of the
357 American Society for Horticultural Science, 136, 239–246.
358 Ku,K-M., Jeffery, E.H., & Juvik, J.A. (2014). Exogenous methyl jasmonate treatment
359 increases glucosinolate biosynthesis and quinone reductase activity in kale leaf
360 tissue. *PLoS One*, 9(8), e103407.
361 Pereira, F.M., Rosa E., Fahey, J.W., Stephenson, K.K., Carvalho, R., Aires, A. (2002).
362 Influence of temperature and ontogeny on the levels of glucosinolates in broccoli
363 (*Brassica oleracea* var. *italica*) sprouts and their effect on the induction of
364 mammalian phase 2 enzymes. *Journal of Agricultural and Food Chemistry*,
365 50(21), 6239-6244.

366 Pérez-Balibrea, S.; Moreno, D.A.; García-Viguera, C. (2011). Improving the
367 phytochemical composition of broccoli sprouts by elicitation. Food Chem. 2011,
368 129, 35–44.
369 Rasmann, S., De Vos, M., Casteel, C.L., Tian, D., Halitschke, R., Sun, J.Y., Agrawal,
370 A.A., Felton, G.W., Jander, G. 2012. Herbivory in the previous generation primes
371 plants for enhanced insect resistance. Plant Physiology, 158(2), 854–863.
372 Roberto, O. & Solano, R. (2005). Molecular players regulating the jasmonate signalling
373 network. Current Opinion in Plant Biology, 8:532–540
374 Sønderby, I.E., Geu-Flores, F., & Halkier, B.A. (2010). Biosynthesis of glucosinolates-
375 gene discovery and beyond. Trends in Plant Science, 15(5), 283-290.
376 Surh, Y-J., & Na, H-K. (2009). NF-κB and Nrf2 as prime molecular targets for
377 chemoprevention and cytoprotection with anti-inflammatory and antioxidant
378 phytochemicals. *Genes & Nutrition*, 2(4), 313–317.
379 Tarozzi, A., Angeloni, C., Malaguti, M., Morroni, F., Hrelia S., & Hrelia, P. (2013).
380 Sulforaphane as a potential protective phytochemical against neurodegenerative
381 diseases. *Oxidative Medicine and Cellular Longevity*, 415078.
382 Verkerk, R., Schreiner, M., Krumbein, A., Ciska, E., Holst, B., Rowland, I., De Schrijver,
383 R., Hansen, M., Gerh, C., Mithen, R., & Dekker, M. (2009). Glucosinolates in
384 Brassica vegetables: The influence of the food supply chain on intake,
385 bioavailability and human health. Molecular Nutrition & Food Research, 53, S219
386 – S265.
387 Wagner, A.E., Terschluesen, A.M., & Rimbach, G. (2013). Health promoting effects of
388 Brassica-derived phytochemicals: from chemoprotective and anti-inflammatory
389 activities to epigenetic regulation. *Oxidative Medicine and Cellular Longevity*,
390 2013, ID 964539, 1-12.

391 Wang, W., Wang, S., Howie, A.F., Beckett, G.J., Mithen, R., & Bao, Y. (2005).
392 Sulforaphane, erucin, and iberin up-regulate thioredoxin reductase 1 expression
393 in human MCF-7 Cells. *Journal and Agricultural and Food Chemistry*, 53 (5),
394 1417–1421.
395 Wei, J., Miao, H., Wang, Q. (2011). Effect of glucose on glucosinolates, antioxidants and
396 metabolic enzymes in *Brassica* sprouts. Science Horticulturae, 129, 535-540.
397 Worrall D, Holroyd GH, Moore JP, Glowacz M, Croft P, et al. (2011). Treating seeds
398 with activators of plant defense generates long-lasting priming of resistance to
399 pests and pathogens. New Phytology, 193:770–78
400 Yuan, G., Wang, X., Guo, R., Wang, Q. (2010). Effect of salt stress on phenolic
401 compounds, glucosinolates, myrosinase and antioxidant activity in radish sprouts.
402 Food Chemistry, 121, 1014-1019.
403 Zhou, C., Zhu, Y., & Luo, Y. (2013). Effects of sulfur fertilization on the accumulation
404 of health promoting phytochemicals in radish sprouts. *Journal of Agricultural and*
405 *Food Chemistry*, 61, 7552−7559.
406

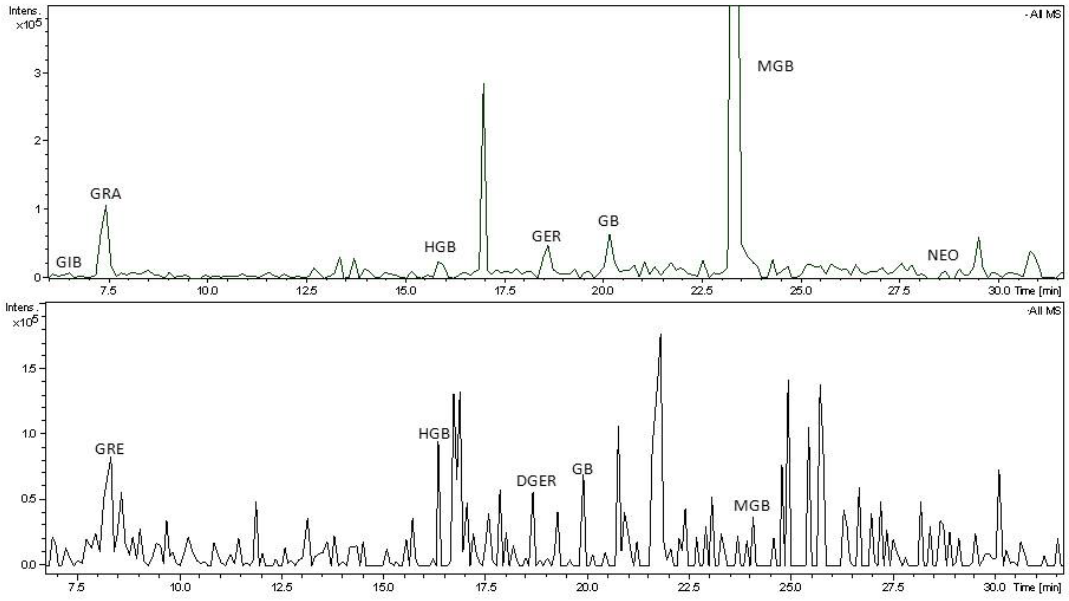
Figure 1. A) Identification of individual GLS present in broccoli and radish sprouts. B) Full MS data of broccoli and radish sprouts. C) MS2 and MS3 spectra of the fragmentation of MGB glucosinolate, consisting of the aglycone m/z 259 and the MS3 of this ion, a fragment of m/z 97, corresponding to the sulfate molecule.

A.

Code	Glucosinolate (GLS)	Semisystematic name	R_t (min)	$[M-H]^-$ (m/z)	MS2 and MS3	Broccoli	Radish
GIB	Glucoiberin	3-methylsulfinylpropyl-gls	6.5	422	259 and 97	+	
GRE	Glucoraphenin	4-methylsulfinyl-3-butenyl-gls	7.1	434			+
GRA	Glucoraphanin	4-methylsulfinylbutyl-gls	7.4	436		+	
4-HGB	4-Hydroxyglucobrassicin	4-hydrony-3-indolylmethyl-gls	16.9	463		+	+
GER	Glucoerucin	4-methylthiobutyl-gls	18.6	420		+	
DER	Dehydroerucin	4-methyltio-3-butenyl-gls	19.9	418			+
GB	Glucobrassicin	3-indolylmethyl-gls	20.1	447		+	+
MGB	4-Methoxyglucobrassicin	4-methoxy-3-indolylmethyl-gls	23.5	477		+	+
NGB	Neoglucobrassicin	N-methoxy-3-indolylmethyl-gls	28.4	477		+	

R_t , retention time; +, compound presence

B.



C.

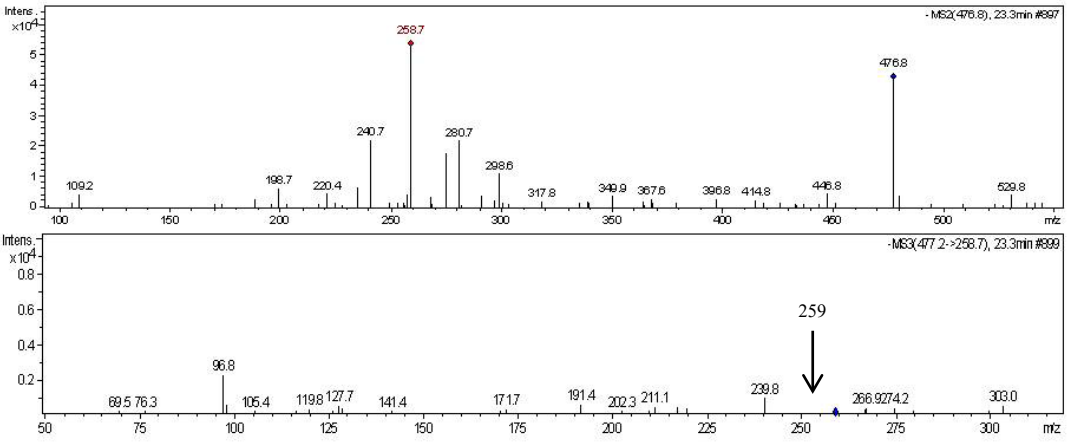


Table 2. Individual and total glucosinolates (mg $100g^{-1}$ F.W.) in broccoli sprouts under priming and elicitation treatments.

Treatment	Glucosinolates													
	GIB		GRA		4-HGB		GER		GB		MGB		NGB		TOTAL	
Control	48.06	j	144.36	i	19.07	b-e	45.21	a-c	17.27	l	11.93	g	16.95	n	302.84	l
Methyl jasmonate																
25 µM	69.75	b-g	185.48	c-f	19.37	b-e	48.48	a-c	31.08	h-j	15.86	e-g	53.81	jk	426.45	e-i
50 µM	61.59	e-j	173.36	e-h	19.11	b-e	44.00	a-c	31.17	h-j	14.88	fg	67.11	ij	415.95	f-j
125µM	58.98	e-j	165.09	f-i	15.75	d-f	36.78	a-c	32.81	g-i	14.12	fg	78.09	hi	405.87	f-j
250µM	**78.42**	**a-d**	**187.71**	**c-f**	21.15	a-e	40.98	a-c	40.79	d-g	20.30	c-f	**135.59**	**de**	**537.60**	**bc**
Prim$^{\Delta}$ (250 µM)	67.02	d-h	177.27	d-g	23.64	a-c	41.96	a-c	**53.09**	**ab**	16.98	e-g	48.86	j-l	427.59	e-i
Prim + 25 µM	71.75	a-f	176.30	e-g	17.75	b-f	36.59	bc	45.05	b-e	17.48	d-g	115.74	fg	490.04	c-e
Prim + 50 µM	**82.85**	**a-c**	**199.77**	**b-e**	23.54	b-f	40.77	a-c	**49.49**	**bc**	21.24	c-f	**133.44**	**d-f**	**564.45**	**ab**
Prim + 125 µM	68.43	c-g	151.37	g-i	15.80	d-f	32.10	c	37.31	e-h	16.13	e-g	107.24	g	437.55	d-h
Prim + 250 µM	**83.34**	**ab**	**205.49**	**b-d**	17.82	b-f	34.40	c	**42.54**	**c-f**	**22.80**	**b-e**	**189.21**	**a**	**626.46**	**a**
Jasmonic acid																
25 µM	55.89	g-j	144.56	i	17.16	c-f	38.12	a-c	36.33	f-h	17.42	d-g	54.86		369.93	i-k
50 µM	64.89	d-i	174.74	e-g	18.12	b-e	37.53	a-c	44.69	b-f	19.50	d-g	86.91	h	456.27	d-f
125µM	49.64	j	151.79	g-i	14.64	ef	39.12	a-c	41.33	c-g	18.54	d-g	151.86	cd	467.93	d-f
250µM	55.68	g-j	115.38	j	10.77	f	40.62	a-c	29.85	h-j	16.28	e-g	117.93	e-g	385.59	g-j
Prim (250 µM)	57.25	e-j	162.17	f-i	21.67	a-e	43.97	a-c	25.20	i-l	16.40	e-g	25.67	mn	355.07	j-l
Prim + 25 µM	59.88	e-j	166.49	f-i	18.72	b-e	36.92	a-c	41.84	c-f	18.27	d-g	56.36	jk	406.70	f-j
Prim + 50 µM	59.55	e-j	169.10	f-i	16.83	c-f	36.05	bc	43.31	c-f	18.84	d-g	77.55	hi	428.82	e-i
Prim + 125 µM	69.24	b-g	163.64	f-i	18.50	b-e	50.18	a-c	**49.31**	**b-d**	**24.72**	**a-d**	**165.54**	**bc**	**544.38**	**bc**
Prim + 250 µM	21.51	h-j	145.38	hi	16.00	d-f	39.35	a-c	44.12	c-f	**24.93**	**a-d**	**178.50**	**ab**	**495.84**	**cd**
DL-Methionine																
1 mM	58.20	e-j	186.69	c-f	24.69	ab	35.37	c	43.59	c-f	16.32	e-g	31.38	l-n	385.94	g-j
2.5 mM	68.15	d-g	178.34	d-g	17.88	b-f	42.35	a-c	23.30	j-l	15.09	fg	21.72	n	363.41	i-l
5 mM	60.93	e-j	180.42	d-f	21.17	a-e	54.75	ab	27.30	i-k	14.78	fg	22.58	n	379.40	h-j
10 mM	51.27	ij	144.12	i	17.00	c-f	46.43	a-c	20.63	kl	12.65	g	16.35	n	307.08	kl
Prim (10 mM)	51.68	ij	145.26	hi	16.17	d-f	41.61	a-c	19.07	kl	20.88	c-f	16.10	k-m	299.55	l
Prim + 1 mM	72.48	a-e	**221.60**	**b**	**26.78**	**a**	55.76	a-c	**60.26**	**a**	**32.27**	**a**	43.49	n	**496.76**	**cd**
Prim + 2.5 mM	61.40	e-j	170.81	f-i	19.70	a-e	45.78	a-c	37.37	e-h	27.42	a-c	25.97	mn	379.76	h-j
Prim + 5 mM	**85.77**	**a**	**254.45**	**a**	21.17	a-e	54.75	a-c	27.30	i-k	14.78	fg	22.58	n	**555.98**	**bc**
Prim + 10 mM	77.81	a-d	**209.25**	**bc**	22.68	a-d	55.11	a-c	42.95	c-f	30.3	ab	20.60	n	450.14	d-g
$LSD_{0.05}$‡	3.77		7.29		1.86		4.91		2.175		1.95		4.83		16.88	

†Mean values (n=3). a-d, Different lowercase-letters mean statistically significant differences between treatments (for each glucosinolate).
‡, Least Dignificant Difference (LSD) for separating means in the respective row. The LSD was computed only after analysis of variance indicated a significant ($p<0.05$) entry effect.. ANOVA p value, $p<0.001$.
$^{\Delta}$Prim (Priming)
The most effective treatments are indicated in bold, as well as, the results of intact and total GLS.

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Table 3. Individual and total glucosinolates (mg $100g^{-1}$ F.W.) in radish sprouts under priming and elicitation treatments.

Treatment	Glucosinolates					
	GRE	4-HGB	DER	GB	MGB	TOTAL
Control	162.20 k	9.49 k	195.22 g	3.42 l	7.05 g	379.71 i
Methyl jasmonate						
25 µM	215.46 g-k	14.58 e-k	240.84 b-g	18.10 g-k	11.74 d-g	507.23 e-i
50 µM	300.24 b-f	18.05 d-h	295.91 a-f	20.21 f-j	16.33 b-f	663.75 b-e
125µM	253.59 d-i	17.01 d-i	239.05 c-g	22.03 e-i	12.81 d-g	557.00 d-h
250µM	**304.82 b-e**	20.20 b-f	280.31 a-g	35.11 c-e	16.81 a-e	**678.33 a-d**
Prim$^{\Delta}$ (250 µM)	**312.49 a-d**	**24.73 a-c**	**356.74 a**	31.87 d-f	7.56 g	**721.20 a-c**
Prim + 25 µM	238.90 f-i	20.77 b-e	247.93 b-g	33.45 c-e	12.45 d-g	563.81 c-h
Prim + 50 µM	**374.23 a**	**28.75 a**	**339.07 ab**	**48.78 ab**	17.88 a-d	**829.88 a**
Prim + 125 µM	**331.14 a-c**	**25.09 ab**	308.88 a-e	43.34 b-d	12.87 d-g	**738.86 ab**
Prim + 250 µM	294.39 b-f	21.75 b-d	212.73 e-g	**45.63 a-c**	12.99 c-g	610.63 b-g
Jasmonic acid						
25 µM	190.94 i-k	10.95 i-k	196.06 fg	14.20 h-l	9.51 e-g	419.14 hi
50 µM	204.47 h-k	12.10 h-k	194.68 g	20.10 f-j	8.38 g	437.59 hi
125µM	261.65 d-i	14.03 f-k	193.88 g	28.42 e-g	12.50 d-g	501.96 f-i
250µM	**345.16 ab**	19.74 b-g	219.55 d-g	**53.98 ab**	**23.99 a**	653.34 b-f
Prim (250 µM)	228.30 g-j	13.07 h-k	239.68 b-g	8.98 i-l	13.63 c-g	512.10 e-i
Prim + 25 µM	223.69 g-k	13.47 g-k	215.63 e-g	22.33 e-h	12.38 d-g	483.65 g-i
Prim + 50 µM	246.06 e-i	10.10 jk	216.41 e-g	29.52 e-g	12.85 d-g	508.90 e-i
Prim + 125 µM	**312.96 a-d**	20.31 b-f	267.48 a-g	**45.34 a-c**	**21.39 ab**	**660.44 b-f**
Prim + 250 µM	**328.62 a-c**	17.28 d-i	216.92 e-g	**58.20 a**	**20.38 a-c**	**628.91 b-g**
DL-Methionine						
1 mM	189.17 i-k	13.95 f-k	261.94 a-g	7.07 j-l	13.53 c-g	485.27 g-i
2.5 mM	214.79 g-k	13.22 g-k	271.58 a-g	5.31 kl	13.39 c-g	517.52 e-i
5 mM	276.81 d-h	17.15 d-i	309.16 a-e	7.49 j-l	14.43 b-g	624.92 b-g
10 mM	172.39 jk	9.97 jk	226.81 c-g	3.74 l	9.35 fg	422.30 hi
Prim (10 mM)	206.58 h-k	10.88 i-k	196.75 fg	4.52 l	12.26 d-g	431.01 hi
Prim + 1 mM	191.57 i-k	15.84 d-k	260.42 a-g	7.20 j-l	14.18 b-g	488.69 g-i
Prim + 2.5 mM	248.54 d-i	16.26 d-j	319.13 a-d	6.42 kl	13.94 c-g	604.16 b-g
Prim + 5 mM	**277.26 c-g**	18.37 c-h	**326.64 a-c**	6.66 kl	12.70 d-g	**641.53 b-g**
Prim + 10 mM	190.53 i-k	13.21 g-k	278.57 a-g	12.86 h-l	10.71 d-g	505.40 e-i
$LSD_{0.05}$‡						

†Mean values (n=3). a-d, Different lowercase-letters mean statistically significant differences between treatments (for each glucosinolate).
‡, Least Dignificant Difference (LSD) for separating means in the respective row. The LSD was computed only after analysis of variance indicated a significant ($p<0.05$) entry effect.. ANOVA p value, $p<0.001$.
$^{\Delta}$Prim (Priming)
The most effective treatments are indicated in bold, as well as, the results of intact and total GLS.

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