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Enrichment of glucosinolate and carotenoid contents of mustard sprouts by using green elicitors during germination

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ABSTRACT

White mustard seeds is a rich source of the glucosinolate glucosinalbin, although these levels are reduced during seed germination. This study aimed to enrich glucosinolate contents of white mustard sprouts during 9 days of germination (22 ± 2ºC) using different elicitors. The effect of such elicitors on the carotenoid biosynthesis during germination was also studied. As chemical elicitors, methyljasmonate (MeJA-10–100 µM) and salicylic acid (SA-50–300 µM) were applied daily as a spray during germination (light/darkness photoperiod (16/8 h) or 24-h darkness). As an abiotic physical elicitor, UV-B radiation (52 kJ m^{-2}) was applied on 8-days-old sprouts (photoperiod or darkness) followed by 24-h acclimatization. The cotyledon area/stem length was not affected by elicitor treatments. The highest glucosinalbin retention was achieved with MeJA-25 or SA-300 after 9 days under the photoperiod, and even enhanced up to 280% with UV-B. The highest carotenoid contents were achieved in MeJA-50 or SA-50 samples after 9 days under photoperiod. UV-B applied to MeJA-50 and SA-50 samples enhanced β-carotene/lutein contents by 560/280 and 620/350%, respectively, under the photoperiod. Glucosinolate and carotenoid enhancements with elicitors were lower during germination under darkness. Conclusively, germination with MeJA or SA enriched glucosinolate and carotenoid contents of white mustard and even increased after UV-B treatment.

1. Introduction

White mustard (*Sinapis alba*) seeds are worldwide known for their culinary interest. Almost two centuries ago white mustard also attracted the attention of the scientific community since the first glucosinolate (glucosinalbin) was isolated in 1831 from white mustard seeds (Robiquet and Boutron-Charlard, 1831). Since then, around 200 distinct glucosinolates structures have been reported from different brassica plants (Clarke, 2010). Glucosinolates are characterized by a wide variety of chemical structures of their side chains and on the basis of their nature can be divided into three groups: aliphatic glucosinolates (derived from Ala, Leu, Ile, Val, and Met), aromatic glucosinolates (from Phe or Tyr) and indolic glucosinolates (from Trp) (Ciska et al., 2008; Sønderby et al., 2010). Intact glucosinolates of brassica vegetables are hydrolized (after plant cell disruption occurred during chewing, processing, cooking, etc.) to their respective breakdown products (mainly isothiocyanates and nitriles) upon enzymatic and nonenzymatic transformations depending of several factors (pH, presence of various cofactors like epithiospecifier protein, etc.) (Prieto et al., 2019; Rouzaud et al., 2004).

Glucosinolates have been widely studied and are well-known by the high anticarcinogenic properties of their breakdown products: isothiocyanates and nitriles. Generally, isothiocyanates showed higher anticarcinogenic activity than nitriles (Nastruzzi et al., 2000). In addition, other beneficial effects of glucosinolate products have been reported, including regulatory functions in inflammation and stress response, and antimicrobial properties, among others (Prieto et al., 2019). In particular, the breakdown products of glucosinalbin, a glucosinolate found in high quantities in white mustard (represents more than 95% of total glucosinolate content of white mustars), have shown higher anticarcinogenic activity than other breakdown products of glucosinolates like glucoraphanin, glucotropaeolin and epiprogoitrin (Ciska et al., 2008; Nastruzzi et al., 2000). Nevertheless, the high glucosinolate contents of brassica seeds, such as white mustard seeds, are decreased during seed germination due to the dilution effect since they

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are used like nutrient and defense reserve during tissue expansion (Baenas et al., 2012; Ciska et al., 2008). In addition, higher glucosinolate contents are reached during germination of white mustard seeds under dark conditions compared with light (Ciska et al., 2008). On the other side, other health-promoting compounds are synthesized during brassica seed germination under light conditions, like β-carotene, as observed in white mustar seedling (Schnarrenberger and Mohr, 1970).

Consumption of sprouts is a convenient way to increase the consumption (e.g. in salads) of some brassica species, like white mustard seeds. In particular, 10% of interviewed consumers affirmed that they purchased fresh sprouts in each of the past three years (Kresin, 2018). However, the expected glucosinolate levels of white mustard sprouts are lower than seeds and adult tissues (Ciska et al., 2008; Souci et al., 2016). In addition, the production of white mustard sprouts under darkness conditions, to meet the consumers' preferences according to white/-yellow colors, may lead to lower carotenoid biosynthesis due to the light absence (Frosch and Mohr, 1980). In that sense, enrichment with different elicitors may counteract the reduction of the glucosinolate contents of white mustard seed during germination under light conditions, while carotenoid biosynthesis is ensured under such light regime.

Enrichment with natural signaling molecules, such as methyl-jasmonate (MeJA) and salicylic acid (SA), or technologies with low environmental impact, such as UV-B illumination, may be considered as 'green elicitors' to enrich the contents of health-promoting compounds during germination of sprouts (Artés-Hernández et al., 2021). MeJA is approved as a flavoring in the European Union by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2004). On the other side, SA, widely used in the cosmetic and pharmacologic industry, is also usually present inside our body since it is formed when aspirin breaks down in the body (Nagelschmitz et al., 2014). MeJA and SA treatment during germination of broccoli sprouts was considered a useful tool for improving their bioactive content in glucosinolates, carotenoids, vitamin C, and phenolic compounds (Pérez-Balibrea et al., 2011). Furthermore, another study showed that different *Brassica* sprouts (turnip, rutabaga, radish, and broccoli) treated with MeJA (25 μM; daily spray) led to 50–220% induction of indole glucosinolate accumulation (Baenas et al., 2014).

UV-B radiation is considered safer compared to other non-ionizing radiations with more penetrating wavelengths like UV-C (Sliney and Stuck, 2021). UV-B illumination, in one treatment of periodically applied, has been reported to be a good abiotic 'green elicitor' to increase glucosinolate accumulation of several *Brassicaceae* sprouts (Castillejo et al., 2021a; Martínez-Zamora et al., 2021a; Mewis et al., 2012; Moreira-Rodríguez et al., 2017). Moreover, a UV-B treatment has been recently reported to stimulate carotenogenesis in bell peppers jointly with other illumination strategies (Martínez-Zamora et al., 2021b). On the other side, UV-B did not affect the hypocotyl and sprout length development of kale sprouts (Castillejo et al., 2021a). Nevertheless, the effects of the elicitors MeJA and SA on the glucosinolate and carotenoid contents of white mustard sprouts have not been previously reported to the best of our knowledge.

Therefore, the aim of this study was to enrich the glucosinolate contents of white mustard during germination using MeJA and SA treatments (daily spraying). The effect of such elicitors treatments was also studied on the carotenoid biosynthesis during germination. The additional effect of a UV-B treatment on 8-days-old sprouts, followed by 24 h acclimation prior to harvest, was likewise studied as a non-chemical 'green elicitor'. In addition, the effects of those elicitors treatments were studied under the following germination conditions: light/darkness (16/8 h) photoperiod or 24-h darkness conditions.

2. Materials and methods

2.1. Plant material and chemical elicitors

White mustard seeds (*Sinapis alba*) with the specification for the production of ecological sprouts were obtained from the Batlle company (Barcelona, Spain). According to the supplier, washing and soaking of seeds were not necessary due to the high sprouting percentage of the seeds (>90%) indicated by the company. Methyl jasmonate (MeJA) and salicylic acid (SA) (Merck KGaA, Darmstadt, Germany) were used as chemical elicitors.

2.2. Germination of seeds

Petri dishes (18 cm diameter) were lined with coconut fiber and mustard seeds were sown (approximately 80 seeds per tray). Germination of seeds was done in a controlled environment chamber (22 ± 2 ºC and relative humidity of 65%) under 2 illumination regimes: (1) light/darkness photoperiod of 16/8 h, as previously described (Pérez-Balibrea et al., 2011), and (2) 24-h darkness. The illumination system of the chamber consisted of fluorescent lights with white full spectrum (0.31 W m^{-2}; Philips 36 W/54–765) with a photon flux density of 9.6 ± 0.8 μmol m^{-2} s^{-1}. Petri dishes were placed at 60 cm from the light source.

Irrigation of seeds was daily made (until obtaining 9 days-old sprouts) with exogenous spraying with 20 mL (per Petri dish) of water containing the corresponding chemical elicitor treatment (described in the following section).

2.3. Chemical and UV-B elicitor treatments of sprouts

Elicitors were freshly prepared every day by dissolving MeJA or SA in distilled water (containing 0.2% ethanol; Merck KGaA, Darmstadt, Germany) at 10, 25, 50, and 100 μM (hereinafter referred to as MeJA-10, MeJA-25, MeJA-50 and MeJA-100), and 50, 100, 200, and 300 μM (hereinafter referred as SA-50, SA-100, SA-200 and SA-300), respectively. The elicitors and doses used were selected according to previous studies on sprouting of other brassica species to enhance their phytochemical contents (Pérez-Balibrea et al., 2011). MeJA and SA were applied daily as a spray (20 mL per petri dish). Control (CTRL) samples were sprayed with distilled water.

As an illumination elicitor, a single UV-B treatment was applied on 8 days-old sprouts, followed by a 24 h acclimatization prior to harvest time (day 9), as previously done in broccoli sprouts (Mewis et al., 2012; Moreira-Rodríguez et al., 2017). In particular, Mewis et al. (2012) found higher glucosinolate increments (comparing to untreated samples) in broccoli sprouts treated with UV-B (a similar UV-B treatment to our study) 24 h prior to harvest compared to broccoli sprouts UV-B-treated 2 h prior to harvest.

The used UV-B radiation chamber is fully described in (Martínez-Zamora et al., 2021b). Briefly, it consisted of a reflective stainless-steel chamber with two lamp banks (one bank suspended horizontally over the radiation vessel and the other one placed below it) being fitted to each bank 13 UV-B unfiltered emitting lamps (TL 40 W/01 RS; Philips, Eindhoven, The Netherlands). Petri dishes containing germinating sprouts (8-days-old sprouts) were placed between the two lines of lamps at 17.5 cm above and below over a polystyrene net. The applied UV-B intensity of 11.1 W m^{-2} was calculated as the mean of 18 readings using a radiometer (LP 471 UVB; Delta OHM, Selvazzano Dentro, Italy). The applied UV-B dose was 51.7 kJ m^{-2} (treatment time 1 h and 20 min), based on previous studies on sprouting of other brassica species to enhance their phytochemical contents (Moreira-Rodríguez et al., 2017). A UV-B illumination control treatment with 0 kJ m^{-2} (placing samples in the radiation chamber for 1 h and 20 min but with lamps switched off) was also done.

Sprouts (untreated and treated with elicitors) were harvested at germination days 3, 6, and 9 for analysis of glucosinolates and carotenoids. Furthermore, extra samples were harvested on day 8 to compare the UV-B effect on the harvested (9 days old) sprouts. Each harvesting day, samples were flash-frozen in liquid nitrogen and stored at − 80 °C until further analyses. Five replicates (consisting each replicate of a petri dish with approximately 80 sprouts) were taken every harvesting time

(germination time).

2.4. Sprout biometrics during germination

The biometric parameters (germination percentage, cotyledon surface area, and hypocotyl length) were determined during seed germination. Germination success was determined as the percentage of germinated seeds. For cotyledon surface area, pictures were taken with a smartphone (three integrated cameras of 12-megapixel (f/1.5) + 12-megapixel (f/2.4) + 16-megapixel (f/2.2)) and areas were determined with the software ImageJ (Laboratory for Optical and Computational Instrumentation; University of Wisconsin, Madison WI, USA). Hypocotyl length was measured with a ruler.

2.5. Glucosinolate extraction

Glucosinolates were analysed as previously described (Klug et al., 2018), but with slight modifications. Briefly, 0.2 g of freeze-dried samples were weighed into glass screw-cap tubes followed by the addition of 50 µL of a 3 mM solution of sinigrin (≥99.0% purity; Merck KGaA, Darmstadt, Germany) as internal standard, and immediately heated in a heating block at 75 °C for 2 min. Then, 10 mL of preheated (70 °C) methanol (Merck KGaA, Darmstadt, Germany)-water (70:30; *volume* (*v*):*v*) was added to each sample, and heating at 70 °C was continued in an agitated water bath for 20 min. Extracts were then allowed to cool down at room temperature. Subsequently, extracts were centrifuged (18,000g, 20 min, 4 °C) and the supernatant was loaded on prepared DEAE Sephadex A25 (GE Healthcare, Uppsala, Sweden) mini-columns as previously described. Briefly, the unbound material was removed using two washings with 0.5 mL of in-house ultrapure water followed by two washings with 0.02 mol L^{-1} (pH 5.0) sodium acetate (Merck KGaA, Darmstadt, Germany) per column. Then, purified sulfatase (from *Helix pomatia*; sulfatase activity: 10,000 units g^{-1}; Merck KGaA, Darmstadt, Germany) (75 µL) was loaded onto each column and desulfation was performed overnight (12 h) at room temperature. Desulfoglucosinolates (hereinafter "glucosinolates") were eluted with three ultrapure water washings (0.5 + 0.5 + 0.25 mL) and made up to a final volume of 1.5 mL with ultrapure water.

Glucosinolate extracts were analysed using an ultra-high-performance liquid chromatography (UHPLC) instrument (Shimadzu, Kyoto, Japan) equipped with a DGU-20A degasser, LC-30 CE quaternary pump, SIL-30 AC autosampler, CTO-10AS column heater, and SPDM-20A photodiode array detector. Chromatographic analyses were carried out onto a Gemini C18 column (250 mm × 4.6 mm, 2.6 µm particle size; Phenomenex, Macclesfield, UK). Mobile phases were water (A) and acetonitrile (B). The flow rate was 1.5 mL min^{-1} with a linear gradient eluent starting with 2% B to reach 20% B at 28 min and 2% B at 32 min. Chromatograms were registered at λ = 227 nm. Glucosinolate contents were calculated using sinigrin as an internal standard and the response factor of each compound relative to sinigrin (European Community, 1990). Results were expressed in µmol g^{-1} (dry weight). Each of the five replicates was analysed in duplicate.

Identification of glucosinolates was made using a Triple Quadrupole LC/MS System (Agilent 6420 Series) under the same chromatographic conditions for the above-described UHPLC analyses. Mass spectrometry was performed using an ion trap detector equipped with an electrospray ionization (ESI) system. Mass spectrometry parameters were set as previously described by Vallejo et al. (2003). Briefly, heated capillary and voltage were 350 °C and 4 kV, respectively, and nitrogen was used as the nebulizing gas. The full-scan mass spectra were measured in positive ionization mode from *m/z* 80–700.

2.6. Carotenoid extraction

Carotenoids were analysed as previously described (Gupta et al., 2015). Briefly, 0.2 g of freeze-dried samples were extracted with 1.5 mL of chloroform:dichloromethane (2:1 *v:v*) for 20 min under constant vortex at 4 °C. Subsequently, 0.5 mL of 1 M sodium chloride solution (≥99.0% purity; Merck KGaA, Darmstadt, Germany) was added for phase separation, followed by mixing inversion and centrifuged (15, 000× *g*, 4 °C, 10 min). The obtained organic phase was reserved, and the pellet was re-extracted similarly two more times. The three organic fractions were combined and dried under N_2. Finally, dry extracts were re-suspended in 1 mL of methanol:methyl tert-butyl ether (MTBE; Merck KGaA, Darmstadt, Germany) (60:40, *v:v*), filtered with a 0.22 µm polytetrafluoroethylene syringe filter and used as the carotenoid extract. Chromatographic separation of extracts was done in the UHPLC device using a C30 column (3 µm, 250 × 4.6 mm; YMC, Tokyo, Japan) and 3 mobile phases (methanol:water 98:2 (*v:v*); methanol:water 95:5 (*v:v*) and 100% MTBE). The chromatographic conditions are fully described by Gupta et al. (2015). The identification was done by comparing the retention times of authentic trans-isomer carotenoid standards (all of them of ≥95.0% purity; Carotenature; Münsingen, Switzerland) and UV–Vis spectral data reported (Gupta et al., 2015). In addition to the characteristic UV–Vis spectral data of cis-isomers (near 330 and 360 nm), the Q ratio (ratio of absorption peak heights from the trough between peak II and III) was used to identify cis-isomers and compared to literature (Gupta et al., 2015). Finally, carotenoids were quantified using the trans-isomer carotenoid standards (see Supplementary material) and expressed as µg g^{-1} (dry weight). Each of the five replicates was analysed in duplicate.

2.7. Statistical analysis

The experiment had a three-factor (darkness/light condition × MeJA/SA dose × germination time) design. For biometric data of 9-days-old sprouts, the experiment had a two-factor (darkness/light condition × MeJA/SA dose) design. Data were subjected to an analysis of normality and homoscedasticity and a variance (ANOVA) using SPSS software (International Business Machines Corporation-IBM; Armonk NY, USA). Statistical significance was assessed at p = 0.05, and Tukey's multiple range test was used to separate the means.

3. Results and discussion

3.1. Sprouts biometrics

Seed sprouting was very high reaching values of 92–97% as observed in Table 1, without remarkable differences among the light/darkness photoperiod or 24-h darkness regimes. Sprouting success is highly influenced by several factors, such as genetic aspects and seed pretreatments to increase the germination percentage of seeds (Baenas et al., 2014). SA-300 and MeJA-10 doses induced the highest reduction of germination percentage to levels of 93–94% in samples under the light/darkness photoperiod. The use of elicitors under the 24-h darkness regime generally (except for MeJA-10) affected in a higher degree to the germination percentage compared with the light/darkness photoperiod, with levels ranging from 92% to 95%. Interestingly, SA-50 induced the highest reduction of the germination percentage with a 92% germination percentage under the 24-h darkness regime. Even so, the germination success of sprouts percentages in all cases were very high (>92%) according to the indications of the seed supplier (as indicated in the Plant material Section 2.1). Nevertheless, other biometric parameters, such as cotyledon surface area and hypocotyl length, should be studied in detail since they define the sprout visual quality and consequently the consumer purchase decision.

Cotyledon surface area of sprouts germinated under the light/darkness photoperiod was higher (almost double) than under the 24-h darkness regime (Table 1, Fig. 1). Leaf and cotyledon expansion in dicotyledonous plants is a light-dependent developmental process (Neff and Van Volkenburgh, 1994). In particular, those authors reported that phytochrome B was involved in the enhancement of cotyledon cell

Table 1
Germination percentage, cotyledon surface area, and hypocotyl length of white mustard sprouts germinated for 9 d (22 ± 2 °C) with different methyljasmonate treatment under a light/darkness photoperiod (16/8 h) or 24-h darkness (mean ±SD).

	Seeds germination (%)	Cotyledon area (cm^2)	Hypocotyl length (cm)
Photoperiod[a]			
CTRL	97.3 ± 0.6 a	0.223 ± 0.034 a*	6.31 ± 0.07 a
MeJA-10	93.2 ± 1.8 c	0.178 ± 0.031 b*	4.06 ± 0.05 b
MeJA-25	95.4 ± 1.6 b	0.159 ± 0.026 b	4.22 ± 0.04 b
MeJA-50	96.4 ± 0.7 ab	0.161 ± 0.021 b*	3.31 ± 0.05 c
MeJA-100	96.0 ± 1.2 b	0.130 ± 0.021 c*	3.47 ± 0.21 c
Darkness[b]			
CTRL	97.3 ± 0.8 a	0.120 ± 0.035 ab	9.51 ± 0.52 a*
MeJA-10	97.3 ± 1.2 a	0.099 ± 0.036 b	8.30 ± 0.26 c*
MeJA-25	94.0 ± 0.9 bc	0.146 ± 0.032 a	6.41 ± 0.57 d*
MeJA-50	93.5 ± 0.8c	0.138 ± 0.038 a	6.87 ± 0.31 d*
MeJA-100	94.9 ± 0.7 b	0.131 ± 0.032 a	9.11 ± 0.19 b*
Photoperiod[a]			
CTRL	97.3 ± 0.6 a	0.223 ± 0.034 a*	6.31 ± 0.07 a
SA-50	96.5 ± 1.4 ab	0.235 ± 0.049 a*	3.40 ± 0.05c
SA-100	95.7 ± 1.2 b	0.234 ± 0.053 a*	4.12 ± 0.04 b
SA-200	96.1 ± 1.2 a	0.229 ± 0.035 a*	3.54 ± 0.04 c
SA-300	94.0 ± 1.3 c	0.178 ± 0.025 b*	2.11 ± 0.04 d
Darkness[b]			
CTRL	97.3 ± 0.8 a	0.120 ± 0.035 a	9.51 ± 0.52 a*
SA-50	91.6 ± 1.9 c	0.105 ± 0.036 a	3.88 ± 0.15 e*
SA-100	94.7 ± 1.2 b	0.083 ± 0.016 a	5.10 ± 0.37 c*
SA-200	94.5 ± 1.7 b	0.124 ± 0.016 a	4.71 ± 0.27 d*
SA-300	95.2 ± 1.0 b	0.134 ± 0.014 a	5.82 ± 0.19 b*

[a] light/darkness photoperiod of 16/8 h.
[b] 24-h darkness regime; MeJA, methyljasmonate elicitation treatment with different doses (10–100 μM); SA, salicylic acid elicitation treatment with different doses (50–300 μM); CTRL, control treatment with no elicitor. Different letters denote significant differences ($p < 0.05$) among different MeJA or SA doses for the same germination condition (photoperiod or darkness). *denote significant ($p < 0.05$) higher cotyledon area/hypocotyl length among photoperiod and darkness regimes for the same MeJA or SA dose.

expansion under light conditions leading to an increment of the cotyledon area of *Arabidopsis thaliana* seedlings (Neff and Van Volkenburgh, 1994). Attending to elicitors, the cotyledon area decreased as the MeJA dose increased showing MeJA-100 the lowest area with 0.13 cm^2 under the light/darkness photoperiod (Table 1). Similarly, *A. thaliana* seedlings germinated with jasmonic acid treatment reduced the cotyledon area under light conditions, which was accompanied by a declining level of free IAA (indole-3-acetic acid) and a considerable increase in the ABA (abscisic acid) level, both considered as the most important plant growth regulators (Karnachuk et al., 2008). Nevertheless, no high cotyledon area differences were found among all MeJA doses under the 24-h darkness regime (ranging from 0.10 to 0.15 cm^2) (Table 1). Similarly, no high influence of jasmonic acid treatment on the cotyledon area of *A. thaliana* seedlings was observed during germination under dark conditions (Karnachuk et al., 2008). For SA, no remarkable influence of the studied SA doses were observed on the cotyledon surface area of samples, regardless of light/darkness photoperiod or 24-h darkness regime (Table 1).

Hypocotyl length was higher under the 24-h darkness regime compared with the light/darkness photoperiod, contrary to the cotyledon surface area data, with values of 9.5 cm and 6.3 cm, respectively, for the 9-days-old sprouts (Table 1, Fig. 1). Germination can take place either through photomorphogenesis, which occurs under light conditions, or skotomorphogenesis, which occurs under dark conditions (Wei et al., 1994). In skotomorphogenesis, plants expend more energy on rapid elongation of the hypocotyls to search for light, rather than expansion of cotyledons (Chan et al., 2014; Josse and Halliday, 2008), which agrees with our data. In general, elicitor treatments reduced the hypocotyl length of sprouts. Thus, the hypocotyl length of treated sprouts under the light/darkness photoperiod ranged from 3.3 to 4.2 cm, although SA-300 showed the lowest length with 2.1 cm. Interestingly, for the 24-h darkness regime, the length reduction observed with the low-intermediate elicitor doses was counteracted with the highest MeJA and SA doses, which showed the longest sprout lengths compared to their respective lower doses. Hence, the combined stress of germination under the 24-h darkness regime with high elicitor doses led to an enhanced plant response observed in longer hypocotyls.

In conclusion, the germination percentage was not highly affected by either the 24-h darkness regime or the light/darkness photoperiod, and the elicitor treatments. The cotyledon surface area was not much influenced by the elicitor treatments under the 24-h darkness regime, while the cotyledon area was reduced under the light/darkness photoperiod as the MeJA concentration increased. Hypocotyl elongation was generally reduced when the elicitors were applied (with the highest reduction for SA-300), showing SA treatments the highest inhibition of

Fig. 1. White mustards sprouts after 9 days of germination at 22 ± 2 °C under 24-h darkness (A) or a light/dark (16/8 h) photoperiod (B).

sprout elongation under the darkness regime. Our data are in agreement with previous data found by Karnachuk et al. (2008), who suggested that regulation of *A. thaliana* seedling morphogenesis by jasmonic acid under light conditions is quite likely to be associated with the interaction of signal transduction systems triggered by these factors.

3.2. Glucosinolates

3.2.1. Influence of light/darkness on the glucosinolate content of white mustard seeds during germination

White mustard was characterized by the presence of one predominant glucosinolate: glucosinalbin (Fig. 2 [peak 2], Table 2/3). In particular, the glucosinalbin content of white mustard seeds (410.8 μmol g^{-1}; Table 2/3) represented 96% of the glucosinolate profile of this *Brassica* species, which is consistent with the literature (Baenas et al., 2012; Ciska et al., 2008). The minor glucosinolates identified in white mustard seeds were: gluconasturtiin [peak 5] (0.67 μmol g^{-1}), 4-hydroxyglucobrassicin [3] (0.96 μmol g^{-1}), glucobrassicin [4] (0.05 μmol g^{-1}), 4-methoxyglucobrassicin [6] (0.12 μmol g^{-1}), neoglucobrassicin [7] (0.11 μmol g^{-1}) and sinigrin [1] (14.1 μmol g^{-1}) (Table 2/3). The high predominancy of a single glucosinolate makes white mustard seeds unique compared with most *Brassica* seeds appreciated for sprouting (e.g. broccoli, kohlrabi, red cabbage, rutabaga, turnip greens, turnip, and radish) (Baenas et al., 2012; Ciska et al., 2008; Popova and Morra, 2014). The high predominancy of glucosinalbin may be explained since white mustard crop was bred for pungency as a condiment, and now it contains one of the highest glucosinalbin concentrations reported for *Brassica* seeds (Baenas et al., 2012).

During white mustard seed germination (without elicitors), glucosinalbin contents decreased, regardless of light or darkness conditions (Table 2/3). This finding is widely reported in the literature and is consistent with the defense and nutrient reserve functions of glucosinolates in plant seeds, whose contents are decreased during germination due to the dilution effect during tissue expansion (Baenas et al., 2012; Ciska et al., 2008). The observed consumption of seed reserves of major glucosinolate of several brassica species during germination has been widely observed in the literature (Castillejo et al., 2021a; Ciska et al., 2008; Martínez-Zamora et al., 2021a; McGregor, 1988).

In particular, we observed that the glucosinalbin content decreased more intensively during germination under the light/darkness photoperiod compared with the 24-h darkness regime (Tables 2 and 3). McGregor (1988) observed an increased gluconasturtiin content during the expansion of hypocotyl in rapeseed seedlings. It may link the higher hypocotyl length of mustard sprouts under the 24-h darkness regime (Table 1) with the better retention of glucosinalbin (another aromatic glucosinolate like gluconasturtiin) under darkness (Table 2 and 3). The highest glucosinalbin reductions were observed from day 6 to day 9 of germination. Hence, glucosinalbin decreased by 7/23% (24-h darkness/light-darkness photoperiod), 9/40% and 20/80% after 3, 6 and 9 d, respectively, compared with seed contents (Tables 2 Table 3). Similarly, Baenas et al. (2012) found 18% and 72% lower total glucosinolate contents of white mustard sprouts after 4 and 8 d of germination (compared with seed contents), respectively, under a similar light/darkness photoperiod (16/8 h). In addition, the higher intensity of the glucosinalbin content reduction as the germination advanced may be explained by its use as antioxidant/nutrient reserves during plant development, in special during cotyledon development that was higher under light conditions (Table 1). Similarly, Pérez-Balibrea et al. (2008) observed higher consumption of antioxidants (mainly, vitamin C) in cotyledons of broccoli sprouts during germination under a light/darkness regime compared with germination under complete darkness (24-h darkness regime).

Interestingly, a general increment was observed on day 6 for minor glucosinolates. Such increment was even more pronounced under the 24-h darkness regime. Particularly, glucobrassicin showed the highest increment from 0.05 (seeds at day 0) to ≈ 1 μmol g^{-1} after 6 days under the 24-h darkness regime (Tables 2 and 3). Ciska et al. (2008) also observed that glucobrassicin achieved the highest increment among minor glucosinolates after 7 d of germination of mustard seeds under complete darkness (24-h darkness regime). McGregor (1988) observed that glucobrassicin contents in the cotyledons of rapeseed decreased during seedling development. Hence, the higher hypocotyl:cotyledon mass ratio observed under the 24-h darkness regime (Table 1) could explain a lower proportion of glucobrassicin degradation related to that from the cotyledon part. The levels of the rest of minor glucosinolates were also increased (1.3–11.5-fold) although to a lesser degree compared with glucobrassicin (Tables 2 and 3). Similar findings of minor glucosinolate increments of several brassica seeds (broccoli, radish and kale) during germination have been previously found (Castillejo et al., 2021a; Martínez-Zamora et al., 2021a).

The complexity of glucosinolates changes during the germination of seeds may be explained by the complexity of the biochemical processes that occurred. Additionally, it may be justified by the nature of glucosinolates, which are secondary metabolites of plant metabolism whose levels are determined by their precursors: amino acids and glucose, which are also essential for the development of the young plant (Ciska et al., 2008). Furthermore, different glucosinolate accumulation has been observed depending on the sprout organ and the specific

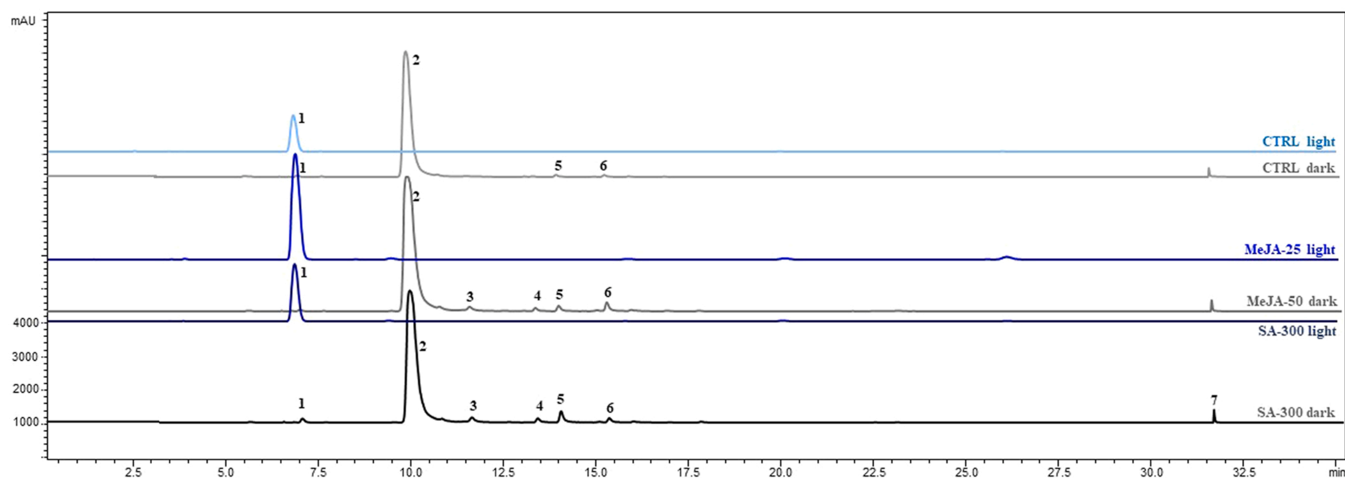

Fig. 2. Chromatogram of glucosinolates from white mustard sprouts after 9 days of germination at 22 ± 2 °C under 24-h darkness or a light/dark photoperiod (16/8 h) with different elicitor treatments (methyljasmonate, MeJA; or salicylic acid, SA). [1] sinigrin; [2] glucosinalbin; [3] 4-hydroxyglucobrassicin; [4] glucobrassicin; [5] gluconasturtiin; [6] 4-methoxyglucobrassicin; [7] neoglucobrassicin.

Table 2
Glucosinolate contents (μmol g^{-1}) of white mustard sprouts germinated for 9 d (22 ± 2 °C) with different methyljasmonate treatment under a light/darkness photoperiod (16/8 h) or 24-h darkness (mean±SD).

	Aromatic		Indole				Aliphatic
	SNB	GN	HGB	GB	MGB	NGB	SI
Seeds	410.8 ± 5.7	0.67 ± 0.07	0.96 ± 0.09	0.05 ± 0.01	0.12 ± 0.02	0.11 ± 0.03	14.1 ± 0.35
Photoperiod[a]							
Day 3							
CTRL	326.8 ± 16.5 Aa	0.19 ± 0.03 Cb	0.38 ± 0.03 Bb	0.03 ± 0.04 Ba	0.57 ± 0.07 Cb	0.05 ± 0.01 Cc	0.56 ± 0.06 Db
MeJA-10	308.4 ± 26.0 Ba	3.37 ± 0.20 Aa	0.71 ± 0.06 Ab	0.11 ± 0.01 Aa	0.89 ± 0.09 Aa	0.51 ± 0.19 Bc	4.50 ± 0.28 Ca
MeJA-25	281.7 ± 17.4 Cb	2.31 ± 0.18 Bb	0.74 ± 0.06 Ac	0.09 ± 0.03 ABa	0.61 ± 0.05 BCb	0.77 ± 0.09 Ac	4.70 ± 0.46 Ca
MeJA-50	309.5 ± 23.6 Ba	3.50 ± 0.14 Aa	0.72 ± 0.07 Ab	0.11 ± 0.01 Aa	0.89 ± 0.05 Aa	0.52 ± 0.09 Bc	6.65 ± 0.12 Ba
MeJA-100	317.7 ± 1.75 ABa	3.59 ± 0.27 Aa	0.79 ± 0.07 Aa	0.12 ± 0.01 Aa	0.67 ± 0.09 BCa	0.51 ± 0.15 Bb	7.61 ± 0.96 Aa
Day 6							
CTRL	251.5 ± 20.2 Bb	1.49 ± 0.34 Ca	0.86 ± 0.10 Ca	0.06 ± 0.02 Aa	0.93 ± 0.14 Aa	0.36 ± 0.03 Da	3.58 ± 0.44 Aa
MeJA-10	261.0 ± 48.1 Aab	2.59 ± 0.30 Bb	1.02 ± 0.15 Ba	0.08 ± 0.01 Aa	0.88 ± 0.18 Aa	1.17 ± 0.18 Aa	2.94 ± 0.58 Bb
MeJA-25	271.9 ± 4.0 Aa	3.10 ± 0.14 Aa	1.13 ± 0.15 Aa	0.09 ± 0.01 Aa	0.90 ± 0.04 Aa	1.21 ± 0.23 Ab	2.84 ± 0.43 Bb
MeJA-50	248.4 ± 6.6 Bb	2.51 ± 0.34 Bb	1.07 ± 0.03 ABa	0.06 ± 0.02 Aa	0.61 ± 0.18 Bb	1.09 ± 0.27 Ba	2.22 ± 0.12 Cb
MeJA-100	247.6 ± 17.0 Bb	1.54 ± 0.10 Cb	0.86 ± 0.10 Ca	0.04 ± 0.02 Aa	0.55 ± 0.29 Ba	0.81 ± 0.21Ca	2.28 ± 0.39 Cb
Day 9							
CTRL	79.4 ± 18.7 Cc	0.14 ± 0.03 Cb	0.28 ± 0.02 Cb	0.01 ± 0.01 Aa	0.24 ± 0.07 Cc	0.15 ± 0.03 Db	0.32 ± 0.05 Cb
MeJA-10	203.1 ± 13.5 Bb	1.62 ± 0.09 ABc	0.81 ± 0.16 Ab	0.03 ± 0.04 Aa	0.57 ± 0.13 Ab	0.91 ± 0.20 BCb	2.29 ± 0.27 Ab
MeJA-25	241.9 ± 37.3 Aa	1.90 ± 0.36 Ac	0.88 ± 0.09 Ab	0.05 ± 0.01 Aa	0.61 ± 0.21 Ab	1.34 ± 0.28 Aa	1.26 ± 0.40 Bc
MeJA-50	212.3 ± 30.2 Bb	1.66 ± 0.24 Ac	0.55 ± 0.13 Bc	0.03 ± 0.02 Aa	0.44 ± 0.08 Bc	0.93 ± 0.08 Bb	1.57 ± 0.34 Bb
MeJA-100	202.2 ± 16.6 Bb	1.33 ± 0.11 Bb	0.60 ± 0.12 Bb	0.04 ± 0.04 Aa	0.33 ± 0.32 BCc	0.86 ± 0.13 Ca	1.26 ± 0.20 Bc
Darkness[b]							
Day 3							
CTRL	385.8 ± 12.5 Aa	1.82 ± 0.47 Cb	0.65 ± 0.24 Bb	0.08 ± 0.01 Ac	0.25 ± 0.08 Cc	0.07 ± 0.05 Ab	10.66 ± 1.64 ABa
MeJA-10	358.3 ± 30.3 Bb	3.00 ± 0.13 Ab	0.84 ± 0.14 Ab	0.07 ± 0.05 Aa	0.59 ± 0.14 ABc	0.12 ± 0.04 Ab	13.07 ± 2.05 Aa
MeJA-25	357.8 ± 40.3 Bb	2.34 ± 0.61 Bc	0.55 ± 0.07 Bb	0.05 ± 0.04 Aa	0.68 ± 0.19 Ab	0.13 ± 0.04 Aa	10.39 ± 1.92 Ba
MeJA-50	314.9 ± 42.8 Dc	1.44 ± 0.57 Dc	0.38 ± 0.18 Cc	0.02 ± 0.02 Aa	0.48 ± 0.21 Bc	0.07 ± 0.04 Ab	8.26 ± 1.09 Da
MeJA-100	329.1 ± 22.4 Cc	3.27 ± 0.54 Ac	0.61 ± 0.08 Bc	0.04 ± 0.02 Aa	0.30 ± 0.04 Cc	0.08 ± 0.01 Ab	9.02 ± 0.63 Ca
Day 6							
CTRL	375.6 ± 32.8 Ea	3.32 ± 0.96 Ea	1.24 ± 0.27 Da	0.97 ± 0.78 Aa	1.36 ± 0.10 Ba	0.18 ± 0.03 Aa	5.24 ± 1.67 Cb
MeJA-10	535.7 ± 11.7 Aa	9,89 ± 0.52 Aa	2.12 ± 0.14 Aa	0.14 ± 0.04 Ba	2.04 ± 0.10 Aa	0.19 ± 0.04 Aab	7.52 ± 1.29 Ab
MeJA-25	393.5 ± 17.2 Da	8.44 ± 0.24 Ba	1.18 ± 0.09 Da	0.08 ± 0.02 Ba	1.35 ± 0.24 Ba	0.15 ± 0.02 ABa	5.14 ± 0.71Cb
MeJA-50	456.6 ± 23.2 Ca	7.93 ± 0.48 Ca	1.50 ± 0.26 Ca	0.09 ± 0.03 Ba	1.39 ± 0.07 Bb	0.11 ± 0.01 Bab	6.10 ± 0.79 Bb
MeJA-100	492.1 ± 18.6 Ba	6.69 ± 0.31 Da	1.89 ± 0.13 Ba	0.08 ± 0.02 Ba	1.31 ± 0.04 Bb	0.17 ± 0.03 ABa	4.47 ± 0.51 Db
Day 9							
CTRL	340.7 ± 14.4 Db	1.76 ± 0.16Cb	0.64 ± 0.12 Db	0.66 ± 0.12 Ab	0.93 ± 0.24 Cb	0.17 ± 0.04 Aa	3.04 ± 0.42 Ac
MeJA-10	310.8 ± 12.9 Ec	1.74 ± 0.48 Cc	0.83 ± 0.17 Cb	0.05 ± 0.01 BCa	0.93 ± 0.20 Cb	0.20 ± 0.06 Aa	1.37 ± 0.13 Cc
MeJA-25	355.7 ± 17.4 Cb	3.04 ± 0.19 Bb	1.08 ± 0.03 Ba	0.02 ± 0.04 Ca	1.38 ± 0.08 Ba	0.21 ± 0.02 Aa	0.60 ± 1.04 Dc
MeJA-50	433.5 ± 21.1 Ab	3.04 ± 0.48 Bb	1.13 ± 0.20 ABb	0.09 ± 0.03 Ba	1.75 ± 0.26 Aa	0.18 ± 0.03 Aa	1.40 ± 0.77 Cc
MeJA-100	394.7 ± 28.4 Bb	4.03 ± 0.69 Ab	1.18 ± 0.23 Ab	0.06 ± 0.02 BCa	1.81 ± 0.25 Aa	0.17 ± 0.01 Aa	1.90 ± 0.18 Bc

[a] light/darkness photoperiod of 16/8 h;
[b] 24-h darkness regime; SNB, glucosinalbin (4-hydroxybenzyl); GN, gluconasturtiin (2-phenylethyl); HGB, 4-hydroxyglucobrassicin (4-hydroxy-3-indolylmethyl); GB, glucobrassicin (3-indolylmethyl); MGB, 4-methoxyglucobrassicin (4-methoxy-3-indolylmethyl); NGB, neoglucobrassicin (1-methoxy-3-indolylmethyl); SI, sinigrin (2-propenyl). MeJA, methyljasmonate elicitation treatment with different doses (10–100 μM); CTRL control treatment with no elicitor. Different uppercase letters denote significant differences ($p < 0.05$) among different MeJA doses for the same germination time under the same germination condition (photoperiod or darkness). Different lowercase letters denote significant differences ($p < 0.05$) among different germination times for the same MeJA dose under the same germination condition (photoperiod or darkness).

glucosinolate; e.g. glucobrassicin was mainly linked to cotyledons while 4-methoxyglucobrassicin was predominant in the root of rapeseed sprouts (McClellan et al., 1993; McGregor, 1988).

Germination of white mustard seeds under a light/darkness photoperiod, and for most seed sprouts in general (except those appreciated by its whiteness), is preferred to germination under a 24-h darkness regime due to the light-dependent biosynthesis of other health-promoting compounds (e.g. carotenoids, chlorophylls, etc.). However, there is a need to find efficient and sustainable elicitors to counteract the observed glucosinalbin reduction during germination of white mustard under the 24-h darkness regime.

3.2.2. Influence of chemical elicitors on the glucosinolate content of white mustard seeds during germination

The application of the chemical elicitors (MeJA and SA) highly mitigated the high glucosinalbin reduction of seeds after 9 d of germination under the light/darkness photoperiod, while no remarkable glucosinalbin increments were observed in the previous germination days (Fig. 2, Tables 2 and 3). In particular, MeJA-treated samples showed 160–205% higher glucosinalbin contents, with the highest enhancement for MeJA-25 with a value of 241.9 μmol g^{-1}, compared with CTRL sprouts at day 9 of germination under the light/darkness photoperiod (Table 2). For SA, glucosinalbin contents of samples treated with SA-50–200 doses showed 140–155% higher levels than CTRL sprouts at day 9 under the light/darkness photoperiod, being such enhancements increased up to 190% in SA-300 sprouts under such conditions (Table 3). The observed most efficient doses (the low-intermediate MeJA dose (25 μM) and the highest SA dose (300 μM)) for glucosinolate elicitation under the light/darkness photoperiod in our study have also been found in previous studies with broccoli sprouts daily sprayed with similar MeJA and SA doses (Pérez-Balibrea et al., 2011). The better efficiency of the MeJA-25 dose to induce glucosinolate biosynthesis, contrary to higher MeJA doses, may be explained by a saturation of the elicitor molecule in the plant tissues after a certain dose (Baenas et al., 2016; Ku et al., 2014). In that sense, receptors of plant cells for SA elicitation seemed not to be saturated at 300 μM, although it need to be further verified at higher SA doses. It has been reported that SA is involved in the synthesis of compounds that lead to protection in plants against abiotic stresses (Khan et al., 2015; Liang et al., 2013). Hence, the threshold of the receptor saturation for SA elicitation could

Table 3
Glucosinolate contents (μmol g^{-1}) of white mustard sprouts germinated for 9 d (22 ± 2 °C) with different salicylic acid treatment under a light/darkness photoperiod (16/8 h) or 24-h darkness (mean±SD).

	Aromatic		Indole				Aliphatic
	SNB	GN	HGB	GB	MGB	NGB	SI
Seeds	410.8 ± 5.7	0.67 ± 0.07	0.96 ± 0.09	0.05 ± 0.01	0.12 ± 0.02	0.11 ± 0.03	14.1 ± 0.35
Photoperiod[a]							
Day 3							
CTRL	326.8 ± 16.5 Aa	0.19 ± 0.03 Db	0.38 ± 0.03 Bb	0.03 ± 0.04 Aa	0.57 ± 0.07 Bb	0.05 ± 0.01 Bc	0.56 ± 0.06 Db
SA-50	306.7 ± 32.7 Aa	2.72 ± 0.27 ABa	0.65 ± 0.15 Ab	0.09 ± 0.05 Aa	0.97 ± 0.28 Aa	0.11 ± 0.03 Ac	5.78 ± 0.97 Ca
SA-100	284.0 ± 34.9 Aa	2.49 ± 0.09 Ba	0.66 ± 0.07 Ab	0.09 ± 0.02 Aa	0.95 ± 0.14 Aa	0.12 ± 0.06 Ab	6.96 ± 0.98 Ba
SA-200	285.1 ± 31.1 Aa	2.08 ± 0.28Ca	0.60 ± 0.08 Aa	0.08 ± 0.04 Aa	0.90 ± 0.21 Aa	0.13 ± 0.01 Ab	7.50 ± 0.71 Ba
SA-300	288.9 ± 16.5 Aa	2.99 ± 0.25 Aa	0.65 ± 0.19 Ab	0.08 ± 0.02 Aa	0.97 ± 0.23 Aa	0.05 ± 0.01 Bc	8.43 ± 0.84 Aa
Day 6							
CTRL	251.5 ± 20.2 Ab	1.49 ± 0.34 Ca	0.86 ± 0.10 ABa	0.06 ± 0.02 Aa	0.93 ± 0.14 ABa	0.36 ± 0.03 Aa	3.58 ± 0.44 Aa
SA-50	212.0 ± 12.3 Ab	1.76 ± 0.19 BCb	0.83 ± 0.08 Ba	0.06 ± 0.01 Aa	0.94 ± 0.20 ABa	0.38 ± 0.17 Ab	2.92 ± 0.26 ABb
SA-100	220.4 ± 31.4 Ab	1.86 ± 0.40 Bb	0.82 ± 0.10 Ba	0.05 ± 0.02 Aa	1.05 ± 0.23 ABa	0.09 ± 0.02 Cb	3.60 ± 0.84 Ab
SA-200	207.1 ± 20.9 Ab	2.03 ± 0.15 ABa	0.68 ± 0.11 Ca	0.05 ± 0.01 Aa	0.85 ± 0.23 Ba	0.13 ± 0.03 BCb	2.53 ± 0.54 Bb
SA-300	233.1 ± 3.9 A	2.19 ± 0.20 Ab	0.92 ± 0.08 Aa	0.06 ± 0.01 Aa	1.04 ± 0.07 Aa	0.15 ± 0.05 Bb	3.22 ± 0.73 ABb
Day 9							
CTRL	79.4 ± 18.7 Bc	0.14 ± 0.03 Cb	0.28 ± 0.02 Db	0.01 ± 0.01 Aa	0.24 ± 0.07 Bb	0.15 ± 0.03 Db	0.32 ± 0.05 Cb
SA-50	198.9 ± 21.5 Ab	1.82 ± 0.28 Ab	0.65 ± 0.05 Ab	0.03 ± 0.03 Aa	0.79 ± 0.12 Ab	0.60 ± 0.06 Aa	3.41 ± 0.29 Ab
SA-100	190.3 ± 22.9 Ac	1.43 ± 0.10 Bc	0.69 ± 0.09 Ab	0.02 ± 0.01 Aa	0.97 ± 0.04 Aa	0.32 ± 0.03 Ba	1.52 ± 0.36 Bc
SA-200	202.7 ± 40.0 Ab	1.42 ± 0.44 Bb	0.55 ± 0.13 Ba	0.04 ± 0.02 Aa	0.86 ± 0.17 Aa	0.24 ± 0.06 Ca	1.87 ± 0.27 Bb
SA-300	231.1 ± 17.8 Ab	1.55 ± 0.14 ABc	0.40 ± 0.06 Cc	0.04 ± 0.02 Aa	0.90 ± 0.20 Aa	0.28 ± 0.11 Ca	3.00 ± 0.53 Ab
Darkness[b]							
Day 3							
CTRL	385.8 ± 12.5 ABa	1.82 ± 0.47 Ab	0.65 ± 0.24 ABb	0.08 ± 0.01 Ac	0.25 ± 0.08 Bc	0.07 ± 0.05 Ab	10.66 ± 1.64 Ca
SA-50	331.6 ± 21.5 Cc	1.44 ± 0.20 Bc	0.61 ± 0.20 Bb	0.02 ± 0.03 Aa	0.53 ± 0.14 Ab	0.04 ± 0.02 Ab	13.65 ± 1.05 Aa
SA-100	402.1 ± 6.80 Aa	1.82 ± 0.69 Ac	0.71 ± 0.27 Ab	0.14 ± 0.17 Aa	0.49 ± 0.06 Ab	0.06 ± 0.05 Ac	15.53 ± 2.04 ABa
SA-200	354.9 ± 34.6 BCb	0.62 ± 0.58 Cc	0.53 ± 0.22 Cc	0.14 ± 0.13 Aa	0.35 ± 0.17 Ab	0,07 ± 0.02 Ac	10.43 ± 0.82 Ca
SA-300	368.8 ± 30.8 ABCa	1.45 ± 0.40 Bc	0.57 ± 0.05 BCb	0.04 ± 0.03 Aa	0.45 ± 0.18 Ac	0.05 ± 0.04 Ab	12.96 ± 1.54 Ba
Day 6							
CTRL	375.6 ± 32.8 Aa	3.32 ± 0.96 Ca	1.24 ± 0.27 Aa	0.97 ± 0.78 Aa	1.36 ± 0.10 Ca	0.18 ± 0.03 Ba	5.24 ± 1.67 Bb
SA-50	399.1 ± 24.2 Aa	3.70 ± 0.46 Ba	1.09 ± 0.25 Ca	0.08 ± 0.02 Ba	1.74 ± 0.05 Ba	0.16 ± 0.04 Ba	9.69 ± 0.41 Ab
SA-100	404.4 ± 17.8 Aa	4.02 ± 0.03 Aa	1.18 ± 0.20 ABa	0.09 ± 0.02 Ba	2.17 ± 0.23 Aa	0.30 ± 0.05 Aa	8.01 ± 0.54 Ab
SA-200	399.0 ± 19.3 Aa	3.60 ± 0.09 BCa	1.12 ± 0.11 Ba	0.05 ± 0.01 Bb	2.10 ± 0.26 Aa	0.15 ± 0.02 Bb	5.67 ± 1.14 Bb
SA-300	326.7 ± 13.5 Bb	1.91 ± 0.65 Db	0.71 ± 0.25 Db	0.04 ± 0.01 Ba	1.75 ± 0.23 Bb	0.20 ± 0.01 Ba	2.69 ± 0.08 Cc
Day 9							
CTRL	340.7 ± 14.4 ABb	1.76 ± 0.16 Db	0.64 ± 0.12 Db	0.66 ± 0.12Ab	0.93 ± 0.24 Db	0.17 ± 0.04 Ba	3.04 ± 0.42 BCc
SA-50	377.6 ± 19.2 Ab	2.40 ± 0.15 Cb	1.07 ± 0.08 Ba	0.07 ± 0.01 Ba	1.81 ± 0.14 Ca	0.19 ± 0.01 ABa	3.57 ± 0.19 ABc
SA-100	305.7 ± 13.4 Cb	2.81 ± 0.03 Bb	0.86 ± 0.27 Cb	0.08 ± 0.01 Ba	2.11 ± 0.22 Ba	0.20 ± 0.02 ABb	2.67 ± 1.03 Cc
SA-200	332.4 ± 15.4 ABCc	2.90 ± 0.55 Bb	0.90 ± 0.10 Cb	0.06 ± 0.01 Bb	2.25 ± 0.12 Ba	0.21 ± 0.03 ABa	2.89 ± 0.50 BCc
SA-300	355.3 ± 14.1 Ab	4.60 ± 0.06 Aa	1.37 ± 0.03 Aa	0.02 ± 0.04 Ba	2.98 ± 0.17 Aa	0.24 ± 0.02 Aa	3.95 ± 0.14 Ab

[a] light/darkness photoperiod of 16/8 h;
[b] 24-h darkness regime; SNB, glucosinalbin (4-hydroxybenzyl); GN, gluconasturtiin (2-phenylethyl); HGB, 4-hydroxyglucobrassicin (4-hydroxy-3-indolylmethyl); GB, glucobrassicin (3-indolylmethyl); MGB, 4-methoxyglucobrassicin (4-methoxy-3-indolylmethyl); NGB, neoglucobrassicin (1-methoxy-3-indolylmethyl); SI, sinigrin (2-propenyl). SA, salicylic acid elicitation treatment with different doses (50–300 μM); CTRL control treatment with no elicitor. Different uppercase letters denote significant differences ($p < 0.05$) among different SA doses for the same germination time under the same germination condition (photoperiod or darkness). Different lowercase letters denote significant differences ($p < 0.05$) among different germination times for the same SA dose under the same germination condition (photoperiod or darkness).

be increased for a higher the synthesis of antioxidant compounds (e.g. glucosinolates). Attending to absolute values, the MeJA-50 led to the highest glucosinalbin content under the 24-h darkness regime at day 9 (16–27% higher contents than CTRL sprouts at day 9), while no glucosinalbin elicitation was observed ($p > 0.05$) for any of the studied SA doses during the whole germination period (Table 2). These data might show for the first time, that the possible saturation of MeJA receptors at 25 μM under light conditions does not occur under the 24-h darkness regime after 9 d of germination of white mustard seeds.

Attending to minor glucosinolate contents, a high elicitation was already observed after 3 d of germination under the light/darkness photoperiod (contrary to glucosinalbin data), especially for gluconasturtiin, sinigrin and neoglucobrassicin, whose levels were 11–19, 8–15, and 3–15-fold higher for MeJA and SA compared with CTRL samples (Tables 2 and 3). Such trends were maintained during the subsequent germination days under such light/darkness photoperiod leading MeJA-25 and SA-50 doses to the highest sinigrin, gluconasturtiin and neoglucobrassicin increments of 4/11-, 14/13- and 9/4-fold (MeJA-25/SA-50) compared with CTRL samples at day 9 under the light/darkness photoperiod. For the rest of the minor glucosinolates, elicitors showed 2–3-fold higher contents than CTRL under the light/darkness photoperiod at day 9 without high differences among elicitor doses. As observed, MeJA seemed to be more efficient to increase indole glucosinolates (attending to the major indole glucosinolates in white mustard: neoglucobrassicin and 4-hydroxyglucobrassicin), SA appeared to be more efficient for aliphatic glucosinolates (sinigrin), while MeJA seemed also to be more efficient for the aromatic glucosinolate gluconasturtiin (as observed for the aromatic glucosinolate glucosinalbin), which is explained by their different biosynthesis pathways. Hence, CYP81F4 genes (which have a significant role in the conversion of glucobrassicin to neoglucobrassicin), trans-activated by MYB34 genes (key regulators in jasmonic acid signaling as observed in other *Brassica* species), were expressed at remarkably high levels in broccoli, cabbage, and kale treated with MeJA, which also induced higher neoglucobrassicin (indole glucosinolate) accumulation in all three subspecies (Yi et al., 2016). However, not dramatically different gene expression levels (CYP83A) involved in aliphatic glucosinolate biosynthesis after application of elicitors have been studied (Ku et al., 2013). Interestingly, no high benefits (<20% increments) were observed applying MeJA and SA under the 24-h darkness regime (Tables 2 and 3). However,

gluconasturtiin, 4-methoxyglucobrassicin, and 4-hydroxyglucobrassicin responded only at high MeJA and SA doses with levels 130/160, 95/220, and 84/110% higher (MeJA-100/SA-300), respectively, compared with CTRL samples at day 9 under the 24-h darkness regime.

As observed, the studied MeJA and SA elicitors can greatly elicit glucosinolate biosynthesis. Then, they may be considered as key signal compounds leading to *de novo* transcription and translation and, ultimately, to the biosynthesis of secondary metabolites in plant cell cultures (Gundlach et al., 1992). Related to the effects of these elicitors on early plant development (9 days-old sprouts) of *Brassica* species, most studies refer to broccoli sprouts (Baenas et al., 2016; Hassini et al., 2017; Pérez-Balibrea et al., 2011). Nevertheless, the effect of chemical elicitors on *Brassica* sprouts with a predominant aromatic glucosinolate (e.g. in white mustard) was unknown until the present study. Even more interestingly, illumination elicitors (e.g. UV-B) combined with different chemical elicitor doses during germination have not been studied yet.

3.2.3. *Influence of UV-B elicitation on the glucosinolate content of white mustard during germination*

Fig. 3 represents increments/reductions of glucosinolates of white mustard seeds from germination day 8 (when the UV-B treatment was applied) to germination day 9 (when sprouts were harvested). UV-B treatment (without combination with chemical elicitors) did not show an enhancement effect, either under the light/darkness photoperiod (Fig. 3A/B) or the 24-h darkness regimes (Fig. 3C/D), on the glucosinolate contents of 9-days-old white mustard sprouts. Hence, 9-days-old UVB-treated sprouts showed 30–90% lower glucosinolate contents compared with non-UV samples at day 9. Contrary, other authors found that a similar UV-B treatment dose (51.5 kJ m^{-2}) applied to 8-days-old broccoli sprouts led to glucosinolate increments of 46% in the subsequent 24 h of germination under a comparable light/darkness (16/8 h) photoperiod (Moreira-Rodríguez et al., 2017). Castillejo et al. (2021a,b) also found that multiple UV-B illumination sessions (10–15 kJ m^{-2} distributed in 4 different times (days of germination) enhanced the

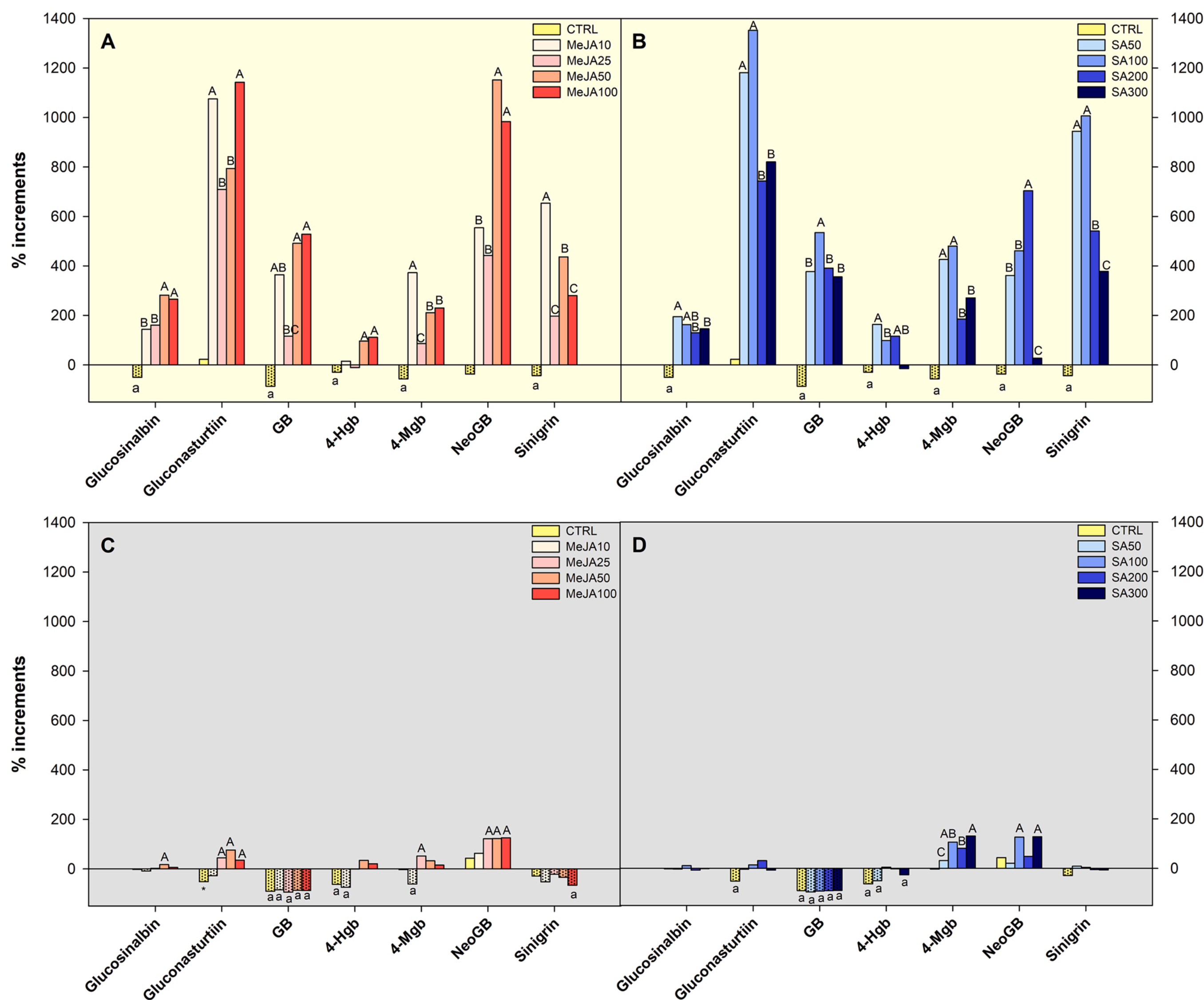

Fig. 3. Glucosinolate changes (%) after 24 h acclimatization following UV-B treatment (applied on 8-days-old sprouts) of white mustard sprouts previously germinated (at 22 ± 2 °C under either a light/darkness photoperiod (16/8 h) (A, B) or 24-h darkness regime (C, D)) with different daily doses of methyljasmonate (MeJA; A, C) or salicylic acid (SA; B, D) (mean). 4-Hgb (4-hydroxyglucobrassicin), 4-Mgb (4-methoxyglucobrassicin), NeoGB (neoglucobrassicin). The presence of uppercase or lowercase letters on bars indicates significant ($p < 0.05$) increments/reductions (from germination day 8 to germination day 9) of UV-B-treated samples compared with untreated samples. UV-B-induced increments (from germination day 8 to germination day 9) with different uppercase letters indicate significant differences ($p < 0.05$) among elicitor treatments. UV-B-induced reductions (from germination day 8 to germination day 9) with different lowercase letters indicate significant differences ($p < 0.05$) among elicitor treatments.

glucoraphanin and 4-hydroxyglucobrassicin contents of kale sprouts by 24–36% after 10 d of germination under a 24-h darkness regime. Thus, Mewis et al. (2012) found that gene families involved in the biosynthesis pathways of indolyl and aliphatic glucosinolates of broccoli sprouts were activated with UV-B treatments. Nevertheless, activation of glucosinolate biosynthesis in *Brassica* species with predominancy of one aromatic glucosinolate (i.e. glucosinalbin in white mustard) seems to be different, which deserves further investigation. Furthermore, Hernández-Cánovas et al. (2020) did not find significant differences in white mustard sprouts germinated under LED lighting (230 μmoL m^{-2} s^{-1} in the 400–700 nm spectrum being of 1 m length giving a wide sunlight spectrum with reduced ultraviolet radiation 0–1.5%; light/darkness photoperiod of 18/6 h) while they observed significant glucosinolates increments in broccoli, red cabbage, and red radish sprouts (all of them with predominancy of aliphatic and indolyl glucosinolates) under LED lighting. Additionally, single yellow (monochromatic yellow LED with a peak at 600 nm) or green (monochromatic yellow LED with a peak at 517 nm) illumination session after harvest of broccoli sprouts (germinated for 9 days under 24-h darkness regime) induced 77 or 35%, respectively, higher total glucosinolate contents at day 4 of storage of harvested sprouts (Castillejo et al., 2021b).

Nevertheless, the combination of MeJA (Fig. 3A) or SA elicitors (Fig. 3B) with the UV-B treatment enhanced the biosynthesis of all glucosinolates during germination under the light/darkness photoperiod. In particular, glucosinalbin contents increased by 140–280% and 130–200% (in the 24 h acclimation following the UV-B treatment) when UV-B was applied in MeJA- and SA-treated sprouts, respectively, with the highest enhancements for MeJA-50/100 and SA-50/100 (without high differences among both doses, with values of 290–300 and 210–250 μmol g^{-1} for MeJA and SA samples at day 9, respectively) under the light/darkness photoperiod. Gluconasturtiin (the other aromatic glucosinolate found in white mustard) showed the highest increments 700–1350% (reaching levels of 1.22–1.98 μmol g^{-1}) when UV-B was applied on MeJA- or SA-treated samples, showing MeJA-100 and SA-100 the highest gluconasturtiin increases (with values of 1.69 and 198 μmol g^{-1}, respectively) under the light/darkness photoperiod (Fig. 3 A/B). For indole glucosinolates, when UV-B was also applied on MeJA- or SA-treated samples the highest increments of neoglucobrassicin, glucobrassicin, 4-methoxyglucobrassicin, and 4-hydroxyglucobrassicin were observed of 1150/460 (MeJA-100/SA-100), 528/535, 210/480 and 110/98%, respectively, under the light/darkness photoperiod. Similarly, Moreira-Rodríguez et al. (2017) also found that neoglucobrassicin exerted the highest increment (≈380%) among indole glucosinolates of 8-days-old broccoli sprouts when UV-B (51.5 kJ m^{-2}; applied at day 7 of germination) and 25 μM MeJA (only one MeJA was studied by those authors) were combined during germination. Nevertheless, our study shows that 100 μM MeJA was more efficient (higher glucosinolate increments) than 25 μM MeJA when UV-B was applied.

Finally, the observed benefit from the application of UV-B combined with MeJA or SA during germination of white mustard sprouts under the light/darkness photoperiod was reduced when sprouts were germinated under the 24-h darkness regime (Fig. 3C/D). In particular, glucosinalbin contents remained almost unchanged (<17% changes) after the 24-h acclimation time under the 24-h darkness regime. Interestingly, 4-methoxyglucobrassicin and neoglucobrassicin reached the highest increments (60–130 and 20–13% for MeJA (Fig. 3C) and SA (Fig. 3D), respectively, showing MeJA and SA the highest increments), while glucobrassicin levels were highly decreased (80–90% reduction). It may be explained since neoglucobrassicin and 4-methoxyglucobrassicin (with 4-hydroxyglucobrassicin as intermediate) are synthesized from glucobrassicin (Liu et al., 2014). Then, glucobrassicin contents highly decreased as a consequence of neoglucobrassicin and 4-methoxyglucobrassicin synthesis after elicitation with the MeJA or SA combination with UV-B.

3.3. Carotenoids

3.3.1. Influence of light/darkness on the carotenoid content of white mustard seeds during germination

Lutein [Fig. 4, peak 3] and *β*-carotene [peak 7] were the predominant carotenoids of the total carotenoid content of mustard seeds (2.13 μg g^{-1}) (Fig. 4, Table 4/5). Among minor carotenoids, neoxanthin [1] represented 2.4% of the total carotenoid content of seeds. Among cis-isomers, 9-cis-lutein [4], 9'-cis-lutein [5], and 9-cis β-carotene [8] represented 2.7%, 1.6%, and 1.6% of the total carotenoid content of seeds. No lycopene was detected in the seeds (Tables 4 and 5). Similarly, Schnarrenberger and Mohr (1970) early observed that lutein was the major carotenoid of mustard seeds followed by *β*-carotene.

As expected, carotenoid biosynthesis occurred during seed germination, even under the 24-h darkness regime (Tables 4 and 5). As expected, chlorophyll synthesis occurred under the light/darkness photoperiod (Fig. 1/4). It is well known that some carotenoid biosynthesis takes place in an etiolated angiosperm seedling which grows in complete darkness while no chlorophyll is synthesized under these circumstances (Frosch and Mohr, 1980). Hence, only trace chromatographic signals of chlorophyll A were found in our study during germination under the 24-h darkness regime (Fig. 4, peak 6), which may be attributed to mild activation of photosynthetic receptors during door opening of the growing chamber for daily elicitor treatments.

Carotenoid accumulation was higher under the light/darkness photoperiod compared with the 24-h darkness regime (Tables 4 and 5). Frosch and Mohr (1980) reported that phytochrome-regulated carotenoid accumulation during white mustard germination takes place during etioplast to chloroplast conversion. Thus, carotenoids are essential in the protection of chlorophylls from pro-oxidation and are thus indispensable for chlorophyll accumulation (Lintig et al., 1997). In particular, the expression of phytoene synthase, the enzyme catalyzing the first step in carotenoid biosynthesis, during germination of white mustard seeds under the light/darkness photoperiod was induced during etioplast/chloroplast conversion (Lintig et al., 1997; Welsch et al., 2000). Furthermore, the last authors demonstrated that phytoene synthase expression is regulated by phytochrome in white mustard during germination under light conditions. In particular, white and red lights (applied for 24 h in 3-days old sprouts previously germinated under 24-h darkness regime) induced the highest phytoene synthase expression (6- and 7-fold, respectively; compared with germination under darkness conditions) (Lintig et al., 1997).

Focussing on individual carotenoids, the highest increments were observed for *β*-carotene and its major cis-isomer found (9-cis *β*-carotene), followed by lutein and their isomers (9- and 9'-cis lutein) with 38-, 16-, 13-, 5- and 7-fold higher contents, respectively, after 9 d of germination under the 24-h darkness regime compared with their respective initial levels in ungerminated seeds (Tables 4 and 5). This finding may be explained by the high antioxidant capacity of *β*-carotene (to protect chlorophylls from pro-oxidation), which is considered the major antioxidant compound in nature (Edge et al., 1997). In particular, for the light/darkness photoperiod, the highest lutein and *β*-carotene increments were observed after 6 d of germination reaching levels of 68.04 and 503.8 μg g^{-1}, respectively. Nevertheless, lutein and *β*-carotene levels decreased by 60% and 77%, respectively, from day 6 to day 9. The observed carotenoid reduction from day 6 to day 9 may be explained by the high carotenoid use as antioxidants for the protection of chlorophylls from pro-oxidation (greater than biosynthesis rates of these carotenoids), which is in accordance with the high chlorophyll B accumulation observed from day 6 to day 9 under light conditions in the chromatograms (data not shown).

Lycopene was neither detected in white mustard seeds nor after 3 d of germination under either light/darkness photoperiod or 24-h darkness regimes. Nevertheless, 0.08–0.12 ug g^{-1} and 1.5–1.6 ug g^{-1} lycopene levels were detected at days 6–9 of germination under the 24-h darkness regime and light/darkness photoperiod, respectively, without

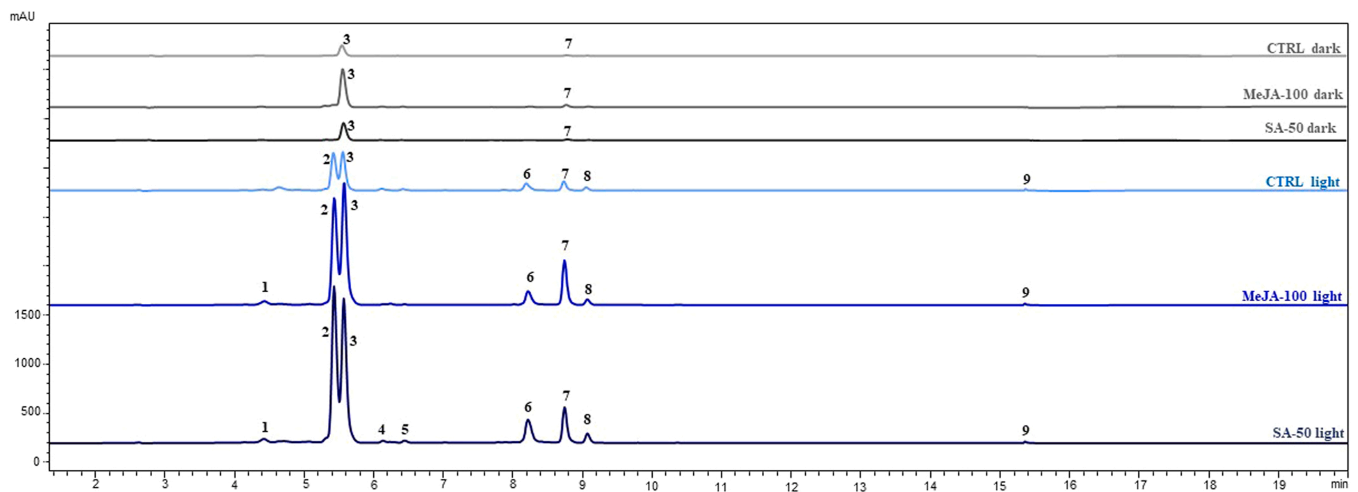

Fig. 4. Chromatograms of carotenoids of white mustard sprouts germinated for 9 d (22 ± 2 ºC) under a 16/8 h light/darkness photoperiod or 24-h darkness with different elicitor treatments (methyljasmonate, MeJA; or salicylic acid, SA). [1] neoxanthin; [2] chlorophyll B; [3] lutein; [4] 9-cis-lutein; [5] 9´-cis-lutein; [6] chlorophyll A; [7] β-carotene; [8] 9-cis-β-carotene; [9] lycopene.

Table 4
Carotenoid contents (μg g^{-1}) of white mustard sprouts germinated for 9 d (22 ± 2 ºC) with different methyljasmonate treatment under a light/darkness photoperiod (16/8 h) or 24-h darkness (mean±SD).

	All-trans isomers				Cis-isomers		
	Lutein	*β*-carotene	Neoxanthin	Lycopene	9 cis lutein	9' cis lutein	9-cis *β*-carotene*
Seeds	1.36 ± 0.08	0.59 ± 0.03	0.05 ± 0.01	nd	0.06 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
Photoperiod[a]							
Day 3							
CTRL	2.93 ± 1.30 Cc	6.6 ± 1.3 Bc	0.08 ± 0.04 Bc	0.06 ± 0.10 Cb	0.10 ± 0.07 Bc	0.04 ± 0.04 Bc	0.05 ± 0.08 Ab
MeJA-10	13.43 ± 6.0 Ab	52.1 ± 10.3 Ab	0.23 ± 0.10 Aa	0.40 ± 0.13 ABc	0.14 ± 0.08 Bb	0.09 ± 0.06 ABb	0.10 ± 0.02 Ac
MeJA-25	10.50 ± 4.81 Ac	53.1 ± 7.1 Ac	0.18 ± 0.04 Ac	0.44 ± 0.12 Ac	0.30 ± 0.24 Ac	0.13 ± 0.03 Ab	0.13 ± 0.03 Ac
MeJA-50	5.97 ± 3.07 Bc	28.8 ± 4.8 Bc	0.09 ± 0.07 Bc	0.17 ± 0.04 BCc	0.07 ± 0.03 Bc	0.04 ± 0.01 Bc	0.32 ± 0.38 Ab
MeJA-100	8.21 ± 3.30 Bb	56.9 ± 12.6 Ac	0.18 ± 0.12 Ac	0.44 ± 0.21 Ac	0.06 ± 0.03 Bc	0.03 ± 0.02 Bc	0.09 ± 0.03 Ac
Day 6							
CTRL	68.04 ± 6.43 Ba	503.8 ± 82.8 Aa	1.20 ± 0.19 Bb	1.46 ± 0.36 Ea	0.96 ± 0.37 Bb	0.64 ± 0.14 Db	0.98 ± 0.86 Bab
MeJA-10	65.55 ± 14.61 Ba	489.6 ± 94.5 ABa	1.09 ± 0.21 Bb	3.10 ± 0.43 Ca	1.01 ± 0.15 Ba	0.77 ± 0.11 Ca	1.58 ± 0.19 ABb
MeJA-25	73.54 ± 16.86 Aa	469.7 ± 98.1 Ba	1.60 ± 0.15 Ab	3.79 ± 0.65 Aa	1.33 ± 0.51 Ab	1.21 ± 0.14 Aa	1.78 ± 0.27 ABb
MeJA-50	67.41 ± 17.63 Ba	409.5 ± 74.4 Ca	1.71 ± 0.34 Ab	3.38 ± 0.52 Ba	0.96 ± 0.16 Bb	0.59 ± 0.11 Db	1.93 ± 0.49 ABa
MeJA-100	39.22 ± 4.05 Ca	213.6 ± 12.6 Db	1.66 ± 0.15 Aa	1.81 ± 0.13 Db	1.26 ± 0.18 Aa	1.01 ± 0.10 Ba	2.21 ± 0.72 Aa
Day 9							
CTRL	25.92 ± 2.96 Bb	114.4 ± 25.5Cb	1.77 ± 0.24 Ba	1.62 ± 0.23 Da	1.32 ± 0.04 Ba	1.03 ± 0.20 Ba	1.64 ± 1.68 Ca
MeJA-10	10.59 ± 2.54 Db	53.3 ± 17.0 Eb	1.07 ± 0.18 Cb	0.89 ± 0.50 Eb	0.88 ± 0.29 Ca	0.74 ± 0.20 Da	3.12 ± 4.16 Ba
MeJA-25	31.03 ± 5.89 Ab	244.8 ± 51.9 Bb	1.85 ± 0.17 ABa	2.08 ± 1.04Cb	1.53 ± 0.31 Aa	1.24 ± 0.11 Aa	8.57 ± 1.52 Aa
MeJA-50	18.83 ± 7.73 Cb	85.3 ± 18.9 Db	1.91 ± 0.16 Aa	2.50 ± 0.29 Bb	1.61 ± 0.26 Aa	1.05 ± 0.19 Ba	0.68 ± 0.56 Cb
MeJA-100	8.55 ± 1.26 Db	358.5 ± 13.0 Aa	1.16 ± 0.22 Cb	4.58 ± 0.48 Aa	0.92 ± 0.40 Cb	0.86 ± 0.11 Cb	1.68 ± 0.95 Cb
Darkness[b]							
Day 3							
CTRL	2.34 ± 0.42 Ac	1.8 ± 0.2 Aa	0.12 ± 0.03 Aa	nd	0.09 ± 0.01 Ac	0.04 ± 0.01 Ac	0.02 ± 0.01 Aa
MeJA-10	5.22 ± 1.35 Ab	9.0 ± 1.8 Aa	0.15 ± 0.05 Aa	nd	0.14 ± 0.03 Ab	0.07 ± 0.02 Ac	0.04 ± 0.01 Aa
MeJA-25	3.10 ± 0.81 Ac	3.6 ± 0.5 Ab	0.11 ± 0.04 Ab	nd	0.09 ± 0.03 Ac	0.05 ± 0.01 Ac	0.06 ± 0.04 Ab
MeJA-50	4.59 ± 1.44 Ac	7.0 ± 1.7 Aa	0.16 ± 0.04 Ab	nd	0.13 ± 0.05 Ab	0.07 ± 0.02 Ac	0.03 ± 0.01 Aa
MeJA-100	3.85 ± 0.69 Ac	4.1 ± 1.0 Ab	0.16 ± 0.04 Ab	nd	0.11 ± 0.03 Ab	0.05 ± 0.02 Ac	0.02 ± 0.01 Ab
Day 6							
CTRL	9.05 ± 1.50 Db	14.2 ± 3.1 Ba	0.07 ± 0.01 Ca	0.12 ± 0.01 Aa	0.44 ± 0.07 BCa	0.39 ± 0.06 Aa	0.44 ± 0.18 Aa
MeJA-10	8.92 ± 2.46 Db	10.8 ± 1.0 Ba	0.06 ± 0.03 Ca	0.03 ± 0.01 Aa	0.27 ± 0.08 Db	0.24 ± 0.05 Bb	0.62 ± 1.03 Aa
MeJA-25	26.5 ± 2.71 Ba	38.2 ± 5.2 Aa	0.36 ± 0.02 Ba	0.14 ± 0.02 Aa	0.62 ± 0.01 Aa	0.43 ± 0.01 Aa	0.07 ± 0.02 Ab
MeJA-50	21.5 ± 2.83Cb	27.6 ± 5.5 Aa	0.32 ± 0.06 Ba	0.12 ± 0.03 Aa	0.32 ± 0.06 CDa	0.25 ± 0.04 Bb	0.08 ± 0.02 Aa
MeJA-100	32.3 ± 2.5 Aa	34.4 ± 2.1 Aa	0.53 ± 0.05 Aa	0.15 ± 0.01 Aa	0.53 ± 0.01 ABa	0.42 ± 0.01 Aa	0.05 ± 0.01 Ab
Day 9							
CTRL	17.6 ± 0.54 Ba	22.5 ± 3.1 Aa	0.16 ± 0.02 ABa	0.08 ± 0.03 Aa	0.29 ± 0.06 Db	0.24 ± 0.08 Cb	0.57 ± 0.81 Aa
MeJA-10	19.2 ± 1.46 Ba	25.4 ± 2.9 Aa	0.14 ± 0.03 ABa	0.15 ± 0.03 Aa	0.54 ± 0.07 ABa	0.42 ± 0.07 ABa	0.53 ± 0.57 Aa
MeJA-25	20.7 ± 4.28 Bb	25.6 ± 5.8 Aab	0.05 ± 0.02 Bb	0.23 ± 0.04 Aa	0.42 ± 0.07 BCb	0.35 ± 0.05 Bb	1.12 ± 0.19 Aa
MeJA-50	28.3 ± 1.56 Aa	26.3 ± 3.6 Aa	0.22 ± 0.04 Aab	0.29 ± 0.05 Aa	0.40 ± 0.07 Ca	0.36 ± 0.02 Ba	0.40 ± 0.47 Aa
MeJA-100	29.8 ± 3.08 Ab	31.9 ± 2.6 Aa	0.08 ± 0.01 Bb	0.24 ± 0.03 Aa	0.57 ± 0.11 Aa	0.49 ± 0.09 Aa	1.27 ± 0.64 Aa

[a] light/darkness photoperiod of 16/8 h;
[b] 24-h darkness regime; MeJA, methyljasmonate elicitation treatment with different doses (10–100 μM); CTRL control treatment with no elicitor. Different uppercase letters denote significant differences ($p < 0.05$) among different MeJA doses for the same germination time under the same germination condition (photoperiod or darkness). Different lowercase letters denote significant differences ($p < 0.05$) among different germination times for the same MeJA dose under the same germination condition (photoperiod or darkness). nd, not detected; *9-cis *β*-carotene data expressed in ng g^{-1}.

Table 5
Carotenoid contents (μg g-1) of white mustard sprouts germinated for 9 d (22 ± 2 °C) with different salicylic acid treatment under a light/darkness photoperiod (16/8 h) or 24-h darkness (mean±SD).

	All-trans isomers				Cis-isomers		
	Lutein	β-carotene	Neoxanthin	Lycopene	9cis lutein	9' cis lutein	9-cis β-carotene[a]
Seeds	1.36 ± 0.08	0.59 ± 0.03	0.05 ± 0.01	nd	0.06 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
Photoperiod[a]							
Day 3							
CTRL	2.93 ± 1.30 Bc	6.6 ± 1.3 Cc	0.08 ± 0.04 Bc	0.06 ± 0.10 Ab	0.10 ± 0.07 Ac	0.04 ± 0.04 BCc	0.05 ± 0.08 Ac
SA-50	11.09 ± 4.71 ABc	50.0 ± 6.0 Ac	0.18 ± 0.11 ABc	0.37 ± 0.03 Ac	0.15 ± 0.03 Ab	0.09 ± 0.02 ABc	0.29 ± 0.20 Ac
SA-100	14.04 ± 3.87 Ab	50.5 ± 5.5 Ac	0.23 ± 0.05 Ac	0.41 ± 0.08 Ac	0.18 ± 0.06 Ab	0.11 ± 0.06 Ac	0.10 ± 0.02 Ac
SA-200	7.36 ± 1.24 ABc	18.9 ± 0.5 BCc	0.13 ± 0.03 ABc	0.14 ± 0.01 Ac	0.11 ± 0.03 Ac	0.05 ± 0.01 BCc	0.05 ± 0.01 Ab
SA-300	6.11 ± 2.33 ABc	26.2 ± 3.6 Bc	0.09 ± 0.01 Bc	0.23 ± 0.05 Ac	0.07 ± 0.01 Ac	0.03 ± 0.01 Cc	0.05 ± 0.01 Ac
Day 6							
CTRL	68.04 ± 6.43 Aa	503.8 ± 82.8 Aa	1.20 ± 0.19 Bb	1.46 ± 0.36 Ca	0.96 ± 0.37 Ab	0.64 ± 0.14 Cb	0.98 ± 0.86 Ab
SA-50	63.63 ± 20.32 Aa	194.4 ± 21.3 Eb	1.37 ± 0.23 Ab	4.36 ± 0.73 Ab	1.04 ± 0.09 Aa	0.78 ± 0.17 Bb	1.97 ± 0.34 Aa
SA-100	40.13 ± 7.72 Ba	298.0 ± 76.4 Cb	0.76 ± 0.15 Cb	2.37 ± 0.33 Bb	1.07 ± 0.57 Aa	0.92 ± 0.13 Aa	0.90 ± 0.33 Ab
SA-200	48.11 ± 14.57 Ba	236.3 ± 30.4 Db	0.74 ± 0.32 Cb	2.67 ± 0.57 Bb	0.93 ± 0.82 Ab	0.68 ± 0.13 Cb	1.01 ± 0.51 Aa
SA-300	48.20 ± 4.02 Ba	325.3 ± 66.6 Bb	0.55 ± 0.13 Db	2.44 ± 0.47 Bb	0.57 ± 0.09 Bb	0.36 ± 0.06 Db	0.61 ± 0.38 Ab
Day 9							
CTRL	25.92 ± 2.96 Bb	114.4 ± 25.5 Db	1.77 ± 0.24 Ca	1.62 ± 0.23 Da	1.32 ± 0.04 Aa	1.03 ± 0.20 Ca	1.64 ± 1.68 Aa
SA-50	31.07 ± 8.75 ABb	517.4 ± 68.6 Aa	2.09 ± 0.24 Aa	5.91 ± 0.83 Aa	1.10 ± 0.39 Aa	1.20 ± 0.13 Aa	1.17 ± 0.86 Ab
SA-100	38.08 ± 18.66 Aa	371.0 ± 21.2 Ca	1.65 ± 0.24 Da	3.09 ± 0.68 Ca	1.07 ± 0.39 Aa	1.06 ± 0.16 Ba	2.13 ± 1.07 Aa
SA-200	31.50 ± 8.82 ABb	367.1 ± 61.7Ca	1.89 ± 0.18 Ba	3.22 ± 0.71 Ca	1.18 ± 0.47 Aa	1.08 ± 0.24 Ba	1.27 ± 0.87 Aa
SA-300	32.19 ± 16.14 ABb	448.1 ± 50.0 Ba	1.72 ± 0.21 CDa	4.67 ± 0.88 Ba	1.13 ± 0.29 Aa	1.03 ± 0.15 Ba	3.11 ± 2.70 Aa
Darkness[b]							
Day 3							
CTRL	2.34 ± 0.42 Ac	1.8 ± 0.2 Aa	0.12 ± 0.03 Aa	nd	0.09 ± 0.01 Ab	0.04 ± 0.01 Ac	0.02 ± 0.01 Aa
SA-50	2.39 ± 0.38 Ab	2.6 ± 0.3 Aa	0.13 ± 0.01 Ab	nd	0.09 ± 0.01 Ab	0.04 ± 0.02 Ac	0.03 ± 0.01 Ab
SA-100	1.55 ± 0.29 Ab	2.2 ± 0.4 Aa	0.09 ± 0.02 Ab	nd	0.07 ± 0.01 Aa	0.02 ± 0.01 Ac	0.04 ± 0.02 Ab
SA-200	1.63 ± 0.16 Ac	1.9 ± 0.2 Aa	0.10 ± 0.01 Ab	nd	0.06 ± 0.02 Ab	0.03 ± 0.01 Ab	0.02 ± 0.01 Ab
SA-300	1.19 ± 0.12 Ab	1.4 ± 0.3 Aa	0.07 ± 0.01 Ab	nd	0.04 ± 0.1 Ac	0.02 ± 0.01 Ac	0.02 ± 0.01 Ab
Day 6							
CTRL	9.05 ± 1.50 Ab	14.2 ± 3.1 Aa	0.07 ± 0.01 Ca	0.12 ± 0.01 Aa	0.44 ± 0.07 Aa	0.39 ± 0.06 Aa	0.44 ± 0.18 Aa
SA-50	17.7 ± 3.30 Aa	26.6 ± 2.7 Aa	0.25 ± 0.04 Ba	0.10 ± 0.03 Aa	0.47 ± 0.03 Aa	0.37 ± 0.03 Aa	0.20 ± 0.06 Ab
SA-100	14.5 ± 3.41 Aa	26.3 ± 5.4 Aa	0.27 ± 0.07 ABa	0.09 ± 0.02 Aa	0.36 ± 0.04 Aa	0.27 ± 0.05 Ba	0.67 ± 0.98 Aa
SA-200	15.3 ± 2.19 Aa	24.4 ± 3.7 Aa	0.37 ± 0.04 Aa	0.12 ± 0.02 Aa	0.27 ± 0.05 Aa	0.18 ± 0.02 Ca	0.08 ± 0.01 Ab
SA-300	11.5 ± 1.24 Aa	21.0 ± 1.4 Aa	0.25 ± 0.01 Ba	0.06 ± 0.02Aa	0.28 ± 0.04 Ab	0.19 ± 0.03 Cb	0.06 ± 0.02 Ab
Day 9							
CTRL	17.6 ± 0.54 Aa	22.5 ± 3.1 Aa	0.16 ± 0.02 Aa	0.08 ± 0.03 Aa	0.29 ± 0.06 Ba	0.24 ± 0.08 BCb	0.57 ± 0.81 Aa
SA-50	17.2 ± 2.57 Aa	22.2 ± 4.9 Aa	0.06 ± 0.01 ABb	0.09 ± 0.02 Aa	0.38 ± 0.09 Ba	0.29 ± 0.03 Bb	0.81 ± 0.43 Aa
SA-100	12.3 ± 2.91 Aa	16.7 ± 4.2 Aa	0.07 ± 0.05 ABb	0.02 ± 0.02 Aa	0.22 ± 0.05 Ba	0.18 ± 0.03 Db	0.38 ± 0.31 Aab
SA-200	9.2 ± 1.95 Ab	16.8 ± 1.9 Aa	0.03 ± 0.01 Bb	0.10 ± 0.01 Aa	0.26 ± 0.01 Ba	0.21 ± 0.01 CDa	1.23 ± 0.63 Aa
SA-300	12.1 ± 0.93 Aa	19.4 ± 0.9 Aa	0.09 ± 0.04 ABb	0.07 ± 0.03 Aa	0.56 ± 0.04 Aa	0.56 ± 0.07 Aa	0.29 ± 0.18 Aa

[a] light/darkness photoperiod of 16/8 h;
[b] 24-h darkness regime; SA, salicylic acid elicitation treatment with different doses (50–300 μM); CTRL control treatment with no elicitor. Different uppercase letters denote significant differences ($p < 0.05$) among different SA doses for the same germination time under the same germination condition (photoperiod or darkness). Different lowercase letters denote significant differences ($p < 0.05$) among different germination times for the same SA dose under the same germination condition (photoperiod or darkness). nd, not detected; *9-cis β-carotene data expressed in ng g-1.

significant ($p > 0.05$) differences among these germination times (Tables 4 and 5).

3.3.2. *Influence of chemical elicitors on the carotenoid content of white mustard seeds during germination*

Different responses for individual carotenoids were observed depending on the chemical elicitor used (MeJA or SA), as well as the elicitor dose, during germination of white mustard (Fig. 4, Tables 4 and 5). In general, all carotenoid contents were enhanced at day 3 of germination when MeJA or SA was applied. In particular, the lowest doses of both elicitors, i.e. MeJA-10/MeJA-25 (Table 4) and SA-50/SA-100 (Table 5), induced the highest accumulation of the major carotenoids (lutein and β-carotene) under the light/darkness photoperiod, without high differences among MeJA or SA, with values 260–380% and 580–650% higher for lutein and β-carotene, respectively, compared to untreated samples at day 3 under the light/darkness photoperiod. This behavior was also observed on day 3 of germination under the 24-h darkness regime for MeJA treatments, although in a lower degree than light/darkness photoperiod (Table 4). Meanwhile, no remarkable differences (<20% changes) were observed with SA treatments under the 24-h darkness regime at day 3 (Table 5). Focussing on cis-isomers, a higher isomerization was also observed for the treatments MeJA-25 and SA-100 under the light/darkness photoperiod, and MeJA10–25 under the 24-h darkness regime. The observed initial accumulation of major carotenoids at day 3 after MeJA and SA treatments, shifted to reductions of those levels (or low accumulation (<20%)) at days 6 and 9 when MeJA was applied under the light/darkness photoperiod. This observation is consistent with previous publications where 9-day broccoli sprouts treated with 25 μM MeJA (daily spraying) had lower lutein content than untreated sprouts (Moreira-Rodríguez et al., 2017). It might be caused by a MeJA-induced stimulation of both ethylene formation and a senescence-like symptom leading to the high carotenoid consumption observed (Moreira-Rodríguez et al., 2017; Wierstra and Kloppstech, 2000). However, β-carotene contents were enhanced by 213% when a higher MeJA dose (MeJA-100) was used compared with untreated samples at day 9 under the light/darkness photoperiod (Table 4).

Contrary to light conditions, accumulation of major carotenoids was still observed under the 24-h darkness regime at days 6 and 9 (although in a lower degree than day 3) when MeJA was used with the highest increments observed for MeJA-100 (Table 4). Interestingly for major carotenoids, a high β-carotene accumulation (220–350%) at day 9 under the light/darkness photoperiod was observed when SA treatments were applied (Table 5), contrary to the commented reduction of major

carotenoid contents at day 9 under the light/darkness photoperiod in MeJA-treated samples (Table 4). Nevertheless, the benefit of using SA under the light/darkness photoperiod was not observed under the 24-h darkness regime at day 9 with a general reduction behavior (or unchanged levels; $p > 0.05$) for all-trans carotenoid forms.

Attending to minor carotenoids, no high neoxanthin changes were observed when MeJA or SA treatments were applied at days 3–9 of germination (Tables 4 and 5). Nevertheless, very high neoxanthin enhancements (280–715%) were observed when all MeJA and SA treatments (except MeJA-10) were applied on day 6 under darkness conditions, with the highest increment for MeJA-100 (Table 4). This finding was not observed under the light/darkness photoperiod since plants activate a mechanism to regulate photosynthesis under excessive illumination and radiation in which the carotenoid violaxanthin (the precursor of neoxanthin) is converted into zeaxanthin, which modulates the photosynthetic apparatus and increases photoprotection at various levels. After such light stress, zeaxanthin is converted back to violaxanthin, closing the so-called xanthophyll cycle (Niyogi et al., 2015).

Focussing on lycopene, MeJA and SA treatments led to lycopene accumulation under light/darkness photoperiod with the highest increments at day 3 (up to 580–660% and 528–610% for MeJA10–25 (Table 4) and SA50–100 (Table 5), respectively, compared to control samples). Such high lycopene accumulation observed under the light/darkness photoperiod at day 3 with MeJA and SA treatments was reduced in the following days 6 and 9 with increments ranging from 60% to 160% among all MeJA and SA treatments. In particular, MeJA-100 and SA-50 induced the highest lycopene accumulation at day 9 with values of 4.58 and 5.91 $\mu g\ g^{-1}$, respectively, under light/darkness photoperiod. Under the 24-h darkness regime, lycopene was not detected at day 3 for any of the treatments being only detected low lycopene contents (0.03–0.15 $\mu g\ g^{-1}$) at days 6 and 9. Nevertheless, MeJA-25/50/100 treatments led to lycopene accumulation of 190–270% at day 9 under darkness compared to control samples at day 9 (Table 4).

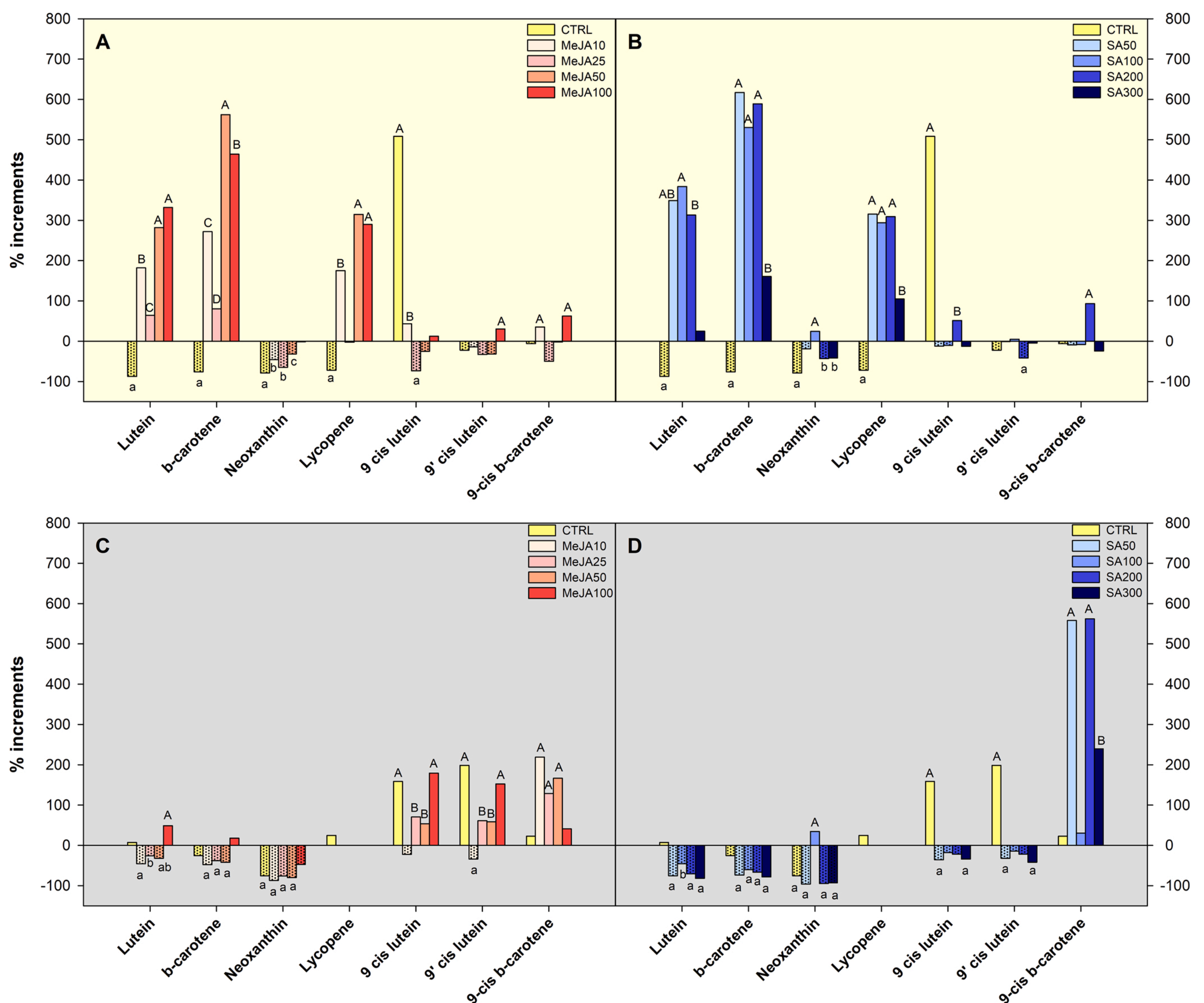

Fig. 5. Carotenoid changes (%) after 24 h acclimatization following UV-B treatment (applied on 8-days-old sprouts) of white mustard sprouts previously germinated (at 22 ± 2 °C under either a light/darkness photoperiod (16/8 h) (A, B) or 24-h darkness regime (C, D)) with different daily doses of methyljasmonate (MeJA; A, C) or salicylic acid (SA; B, D) (mean). The presence of uppercase or lowercase letters on bars indicates significant ($p < 0.05$) increments/reductions (from germination day 8 to germination day 9) of UV-B-treated samples compared with untreated samples. UV-B-induced increments (from germination day 8 to germination day 9) with different uppercase letters indicate significant differences ($p < 0.05$) among elicitor treatments. UV-B-induced reductions (from germination day 8 to germination day 9) with different lowercase letters indicate significant differences ($p < 0.05$) among elicitor treatments.

3.3.3. Influence of UV-B elicitation on the carotenoid content of white mustard during germination

Fig. 5 represents increments/reductions of carotenoids of white mustard seeds from germination day 8 (when the UV-B treatment was applied) to germination day 9 (when sprouts were harvested). UV-B treatment induced 80–90% lower *β*-carotene and lutein contents in CTRL sprouts under the light/darkness photoperiod (Fig. 5A/B) while no remarkable effect of the UV-B treatment was observed on the contents of those major carotenoids under the 24-h darkness regime (Fig. 5C/D). Similarly, neoxanthin and lycopene levels of 9-days-old sprouts (light conditions) were reduced ($p < 0.05$) by 70–80% in UVB-treated samples. Isomerization of lutein was observed under the 24-h darkness regime with 160–200% higher 9- and 9'-cis lycopene contents in UVB-treated samples. Mewis et al. did not observe significant differences in *β*-carotene content of broccoli sprouts after a lower UV-B dose (0.1 kJ m^{-2}) applied to 12-days-old sprouts with subsequent 24-h adaptation time (Mewis et al., 2012). In that sense, high UV-B doses would lead to the observed *β*-carotene reductions during germination under the light/darkness photoperiod in other *Brassica* sprouts as hereby observed for white mustard. A strong *β*-carotene isomerization was not apparently observed after UV-B treatment under either 24-h darkness regime or light/darkness photoperiod, with slightly or unchanged contents ($p > 0.05$), respectively, of the identified 9-cis *β*-carotene. That finding may be explained by a probable high *β*-carotene isomerization, but also high 9-cis *β*-carotene use due to its high antioxidant potential (as previously reported by Levin and Mokady (1994)) counteract the UV-B stress.

The application of UV-B in MeJA or SA-treated samples highly enhanced the all-trans *β*-carotene, lutein, and lycopene biosynthesis under the light/darkness photoperiod (Fig. 5A/B). In particular, MeJA-50/100 induced the highest enhancements with 460–560, 280–330, and 290–315% higher all-trans *β*-carotene, lutein, and lycopene contents, respectively, for UV-B-treated samples (Fig. 5A). Contrary, the highest SA dose (combined with UV-B) led to the lowest all-trans *β*-carotene, lutein, and lycopene enhancements (25–160%), while 530–620, 310–380, and 290–315% enhancements were observed for SA-50/100/200 for UV-B-treated samples under the light/darkness photoperiod (Fig. 5B). Interestingly, UV-B treatments of SA-200 samples led to lutein and *β*-carotene isomerization (50–90% higher levels of those cis-isomers) of samples under the light/darkness photoperiod, which was not observed (unchanged levels; $p > 0.05$) for the other SA doses (Fig. 5A/B).

Attending to the 24-h darkness regime, the application of UV-B in MeJA-100 samples was the only combined treatment that did not affect the *β*-carotene content (Fig. 5C), while the rest of MeJA and SA doses reduced the levels of this all-trans carotene (Fig. 5C and D). Similar findings were observed for all-trans lutein, which levels were even enhanced with 50% higher contents for UVB-treated MeJA-100 samples (Fig. 5C). Interestingly, a general higher *β*-carotene isomerization was observed when UV-B was combined with MeJA or SA compared with *β*-carotene isomerization of CTRL samples under the 24-h darkness regime. This finding highlights the high antioxidant potential of cis *β*-carotene, which levels were enhanced as a response to the combined stress MeJA/SA+UVB. It might also lead to high benefits on the health-promoting properties of white mustard sprouts when they are germinated under darkness conditions due to the well-known higher plasma levels of cis-isomers compared with all-trans carotene isomers (Boileau et al., 2016).

In Table 6 are summarized the doses for MeJA and SA treatments that induced the highest contents of major glucosinolates and carotenoids in white mustard sprouts after 9 days of germination under light/darkness photoperiod (16/8-h) or 24-h darkness. In addition, the MeJA and SA doses that induced the highest glucosinolates and carotenoids increments when the UV-B (52 kJ m^{-2}) treatment was applied are included between parentheses.

Table 6
Methyljasmonate and salicylic acid doses that reached the maximum elicitation of glucosinolate and carotenoid contents of white mustard sprouts germinated for 9 d (22 ± 2 ºC) under a light/darkness photoperiod (16/8 h) or 24-h darkness. Best elicitor treatments, implemented with UV-B (52 kJ m^{-2}), are also included between parentheses.

	Photoperiod[a]	Darkness[b]
Glucosinolates (96% glucosinalbin)	MeJA-25 (↘MeJA-50)	MeJA-50 (↘MeJA-50)
	SA-300 (↘SA-50)	SA-50 (↘SA-100)
Lutein	MeJA-25 (↘MeJA-100)	MeJA-100 (↘MeJA-100)
	SA-100 (↘SA-100)	SA-50 (↘SA-50)
β-carotene	MeJA-100 (↘MeJA-100)	MeJA-100 (↘MeJA-100)
	SA-50 (↘SA-50)	SA-50 (↘SA-50)

[a] light/darkness photoperiod of 16/8 h;
[b] 24-h darkness regime; MeJA, methyljasmonate elicitation treatment with different doses (10–100 μM); SA, salicylic acid elicitation treatment with different doses (50–300 μM); ↘ refers to the best elicitor treatment implemented with UV-B (52 kJ m^{-2}) treatment (applied on 8-days-old sprouts).

4. Conclusions

The glucosinolate contents of brassica sprouts are very low compared with adult tissues, since the high contents of seeds are used as antioxidant/nutrient reserve during germination, which is even aggravated under light conditions compared to darkness. Hence, this study aimed to enrich the glucosinolate contents (mainly glucosinalbin) of white mustard seeds during germination using the elicitors methyljasmonate and salicylic acid. In addition, the effects of those elicitor treatments on the carotenoid profile biosynthesis of white mustard during germination was studied. Daily spraying with methyljasmonate or salicylic acid aimed to mitigate glucosinalbin reduction during germination under photoperiod (16 h light+8 h darkness), inducing 25 μM methyljasmonate the highest glucosinolate contents in 9-days-old sprouts under photoperiod. On the other side, SA-50 induced the highest carotenoid contents of sprouts after 9 days under photoperiod. In addition, a UV-B (52 kJ m^{-2}) treatment applied to 8-days-old sprouts (followed by an acclimation period of 1 day), which were daily sprayed with methyljasmonate or salicylic acid, led to even higher elicitation of glucosinolate and carotenoid levels after 9 d under photoperiod. Such treatments did not affect the normal sprout morphological development. Photoperiod was preferred for germination compared with a 24-h darkness regime due to the higher carotenoid biosynthesis. In future studies, the observed elicitation of these and other health-promoting compounds may be linked with the activity of related key enzymes and transcription factors of their respective biosynthesis pathways.

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CRediT authorship contribution statement

Francisco Artés-Hernández: Resources; Writing – review & editing; Visualization; Supervision; Project administration; Funding acquisition. **Francisco Daniel Miranda-Molina**: Investigation; Data curation; **Tâmmila Venzke Klug**: Investigation; Data curation; **Ginés Benito Martínez–Hernández**: Conceptualization; Methodology; Formal analysis; Investigation; Data curation; Writing – original draft; Writing – review & editing; Visualization; Supervision.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2022.104546.

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