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Enhancement of Glucosinolate Content in *Camelina sativa* (L.) Crantz Sprouts

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Abstract

Camelina sativa sprouts were investigated as a source of glucosinolates (GLSs), and their modulation in response to germination time and elicitor treatments was evaluated. Total GLS content significantly decreased during sprouting, from 21.18 µmol/g DW in seeds to 0.75 µmol/g DW at 24 days, with 5-day-old sprouts selected as the optimal harvest stage, with 8.82 ± 0.08 µmol g^{-1} DW. HPLC analysis identified three major aliphatic GLSs (GLS9, GLS10, GLS11), with GLS10 being the most abundant. Prior to treatment, tolerance assays showed that glucose (75 mM) and DL-methionine (2.5 mM) significantly increased total GLS content, whereas sulfur supplementation had no effect. Genotype-dependent responses were observed among Calena, Alan, and Pearl sprouts. Two-way ANOVA revealed a significant interaction between genotype and elicitor for total GLS content. Glucose and DL-methionine enhanced GLS accumulation in a cultivar-specific manner, with DL-methionine being more effective in Pearl, while Calena and Alan were more responsive to glucose. Sulfur treatments did not induce GLS accumulation in any genotype or condition tested. At the individual compound level, GLS9 was consistently increased by both elicitors, whereas GLS10 and GLS11 showed genotype and treatment-specific responses. Overall, these findings highlight the potential to increase the total GLS content in camelina sprouts through targeted elicitation and cultivar selection. By optimizing elicitor type, concentration, and timing, camelina sprouts could become a richer source of bioactive compounds.

Keywords: false flax; DL-methionine; glucose; sulfur; GLSs; genotype-dependent responses; elicitation; two-way ANOVA

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1. Introduction

The family of *Brassicaceae* is widely recognized as an excellent source of nutrients and bioactive compounds [1]. Among the species belonging to this family, *Camelina sativa* (L.) Crantz has attracted considerable interest due to its valuable nutritional profile, which is largely attributable to the high content of phytochemicals [2–4]. *Camelina sativa*, commonly referred to as false flax, is an emerging crop valued for its high omega-3 fatty acid content in the seeds and adaptability to diverse growing conditions. Beyond its oil-rich seeds, there is a growing interest in camelina sprouts, which share similarities with the *Brassicaceae* family in terms of nutrient and phytochemical content [5–7]. *Brassicaceae* sprouts are recognized for their rich composition of bioactive compounds, including glucosinolates

(GLSs), vitamins, and phenolic compounds, which in the early stages of development can reach concentrations significantly higher than those found in mature plants, although this trend varies depending on the species and growing conditions [8–11]. Glucosinolates, secondary metabolites predominantly found in *Brassicaceae* species, play a crucial role in human health as precursors of bioactive isothiocyanates (ITCs). These compounds are released upon enzymatic hydrolysis and have been widely studied for their protective effects against chronic diseases, including certain types of cancer [12–15]. As demonstrated by Lin et al. [16], glucosinolate (GLS) profiles in broccoli show significant variability across cultivars and developmental stages, particularly during germination and early seedling growth, suggesting that optimizing germination conditions may enhance their accumulation. The metabolic pathways involved in GLS biosynthesis are influenced by external factors, including elicitors such as sugars, sulfur compounds, and amino acids [17,18]. Although the regulation of glucosinolate metabolism has been extensively studied in model species, information on non-model *Brassicaceae*, including camelina, remains limited. Preliminary studies indicate that elicitation strategies and environmental factors can influence GLS accumulation, but a comprehensive understanding is still lacking, particularly during germination. Exogenous application of signaling molecules, including glucose and methionine, has been shown to stimulate the production of GLSs in sprouts of several *Brassica* species [18,19]. Glucose, beyond its role as an energy source, acts as a signaling molecule, regulating key metabolic and stress-response pathways [20]. Similarly, sulfur is a critical element of GLSs, and its supplementation has been demonstrated to boost their biosynthesis [17,21]. Methionine, an essential amino acid, serves as a direct precursor for the synthesis of specific GLS types, enhancing their levels when applied exogenously [22,23].

Despite the extensive body of research on sprouts from other Brassicaceae species [18–23], studies focusing on camelina sprouts remain limited [5–7]. The present study aims to evaluate the effect of the exogenous application of glucose, sulfur, and DL-methionine on the biosynthesis and accumulation of GLSs in camelina sprouts. In particular, the objective is to optimize germination conditions in order to enhance the content of these bioactive compounds, which are known for their potential health-promoting properties. Therefore, this work aims to address this research gap by providing new insights into the GLS modulation of camelina sprouts.

2. Materials and Methods

2.1. Plant Material and Germination Conditions

Camelina seeds of the commercial cultivar Calena, and two improved camelina lines, Alan (developed at the Institute of Agricultural Biology and Biotechnology—IBBA of the National Research Council—CNR, Milan, Italy) and Pearl (supplied by Smart Earth Camelina, Saskatoon, SK, Canada), were soaked for 3 h in water. Then the soaked seeds were left to germinate and further grow in custom-made containers comprising a glass jar (10 cm diameter) with a fine-mesh net (Figure S1). Approximately 350 mg of seeds (about 300 seeds) were used per container. The soaked seeds were spread on the top of the net inserted in the jar that contained approximately 350 mL of water to maintain a moist environment. During the first days, the jars were covered with light impermeable paperboard cups to avoid light exposure. The paperboard covers were removed after 66 h from the sowing to allow light exposure of the sprouts. Throughout the experiment, the sprouting jars were kept in an environment-controlled chamber with a cycle of 16 h light with an air temperature of 22 °C and 8 h dark at 18 °C with a photosynthetically active radiation (PAR) level of 220 μmol m^{-2} s^{-1}.

2.2. Time Course of Sprout Development

In order to find the best size of seedlings and the highest glucosinolate content during germination of seedlings, 21 glass jars were prepared using Calena seeds. One set of three glass jars (3 biological replicates) of the Calena cultivar was removed every two days during the sprouting period. The remaining jars were filled (if necessary) with additional water every 24 h. Harvesting took place on the 3rd, 5th, 7th, 9th, 11th, 13th and 24th days after sowing. The sprouts were harvested separately from each jar (three independent jars for each time point) and lyophilized for 4 days. Lyophilized samples were ground to a fine powder with a mortar and pestle, and if not used the same day, stored at −20 °C to avoid myrosinase activity.

2.3. Treatments with Elicitors

Glucose (1.0–200 mM), amino acid DL-methionine (1.0–20 mM), and sulfur (S) supplied as K_2SO_4 (10–80 mg/0.35 L) were selected as elicitors according to Baenas et al. [17] and Pérez-Balibrea et al. [21]. All elicitors were supplied by Merck Life Science S.r.l., Milan, (IT) and were dissolved in distilled water. For each control (Ctr) and for each concentration of each treatment, three biological replicates (i.e., three independent jars) were prepared. Glucose, sulfur, and DL-methionine solutions were applied as soaking (350 mL per jar) from day 3 to day 5 of sprouting (48 h of treatment) using water as a control. Simultaneously, the solution in all glass jars was removed and replaced. Control jars were replenished with fresh water, whereas treatment jars received the corresponding glucose, sulfur, or DL-methionine solution. The five-day-old sprouts (120 h) were harvested, weighed, and then lyophilized as reported above. Regarding the sulfur (K_2SO_4), in addition to the 48 h treatment (described above), it was also supplied for only 3, 6, 9, and 24 h at a dose of 10 mg/0.35 L (S_{10}), after which the five-day-old sprouts were processed as above. Each experiment was performed independently under the same growth conditions, and the corresponding control was included in each experimental run.

2.4. Extraction and Determination of Glucosinolates

Total GLS content was determined as reported in Pozzo et al. [24] with some modifications. Briefly, GLSs were extracted from approximately 20 mg of fine lyophilized powder using 600 µL of 70% hot methanol at 75 °C (w:v 1:30). The samples were incubated for 10 min in hot methanol at 75 °C with vortexing every 2 min. After cooling down for 2 min, the extract was centrifuged at 15,000 *g* for 15 min. The resulting supernatant was transferred to a new tube, and the residue was re-extracted again with 400 µL of 70% hot methanol. The combined extracts (about 1 mL) were added on top of a DEAE Sephadex A-25 column (Merck Life Science S.r.l., Milan, Italy). The substance not retained by the resin was washed twice with 1 mL of 20 mM Na acetate at pH 4.0. Bound GLSs were desulfated by the addition of 30 µL (10 U) of sulfatase Type H1 at 37 °C overnight. Desulfoglucosinolates were eluted from the DEAE column with 1 mL of 100% ethanol, and the samples were dried at 55 °C. The pellets were resuspended in 20% ethanol and filtered with a 0.22 µm Costar Spin-X Centrifuge Tube Filter (Corning Incorporated, Corning, NY, USA) before conducting HPLC analysis. Desulfoglucosinolates were separated by HPLC according to Russo et al. [25]. Three GLS extractions were performed for each jar or biological replicate (experimental unit), N = 3. A calibration curve with desulfosinigrin was generated. Sinigrin hydrate from horseradish was purchased from Merk Life Science S.r.l. (Milan, Italy). Glucosinolate quantification was performed using sinigrin as an external standard, applying a response factor of 1 for all compounds, as described by Grosser and van Dam [26]. Concentrations were expressed as µmol/g of dry weight (DW). Peak identification was done using the three standard GLSs: Glucoarabin potassium salt, Glucocamelinin potassium salt,

and Homoglucocamelinin potassium salt. The standards were purchased from Lab Service Analytical S.r.l. (Anzola dell'Emila, Bologna, Italy).

2.5. Statistical Analysis

All statistical analyses were performed using Jamovi, an open statistical software (The Jamovi project, 2025, https://www.jamovi.org), in the Desktop-version (v. 2.3.28.0). One-way ANOVA and Tukey's post hoc test (95% Confidence) were used to analyze the significance of differences in the time course and elicitor dose experiments (N = 3). The effect of different elicitors on GLS content in sprouts of different genotypes of camelina was evaluated by 3 (no elicitor, glucose, and DL-methionine) × 3 (Calena, Pearl, and Alan) ANOVA (N = 3). In the case of significant interaction ($p \leq 0.05$), main effects of factors were not discussed, and grouping of means was performed using Tukey's test (95%).

3. Results and Discussion

Total GLS content in camelina sprouts was strongly influenced by germination time, since germination brought a significant decrease in glucosinolate concentration during the sprouting period (Figure 1A). Our data are in agreement with the results of Berhow et al. [27], who observed a GLS amount reduction during the course of camelina sprouting. A similar behavior has also been reported in other species belonging to the *Brassicaceae* family [16]. In our experiment, the total aliphatic GLS content in camelina seeds was 21.18 µmol/g dry weight (DW), which decreased to 11.81 µmol/g DW at day 3, and dropped to 7.13 µmol/g DW at day 7 and finally to 0.75 µmol/g DW at day 24 (Figure 1A). This trend is consistent with observations in *Brassica* species, where GLSs have been proposed to play a role during germination and early seedling development [28,29]. Based on the size of the seedlings and GLS content, the best time for sprout harvesting was fixed at 5 days after sowing (DAS) with a GLS content of 8.82 ± 0.08 µmol g^{-1} DW. At this stage, the two cotyledons were fully expanded, and the seedling length (shoot + root) was 8.5 ± 0.63 cm; thus, it was considered the ready-to-eat stage. HPLC analysis demonstrated that, as observed in the seeds [24,30,31], also in camelina sprouts, three main aliphatic GLSs were detected (Figure 1B).

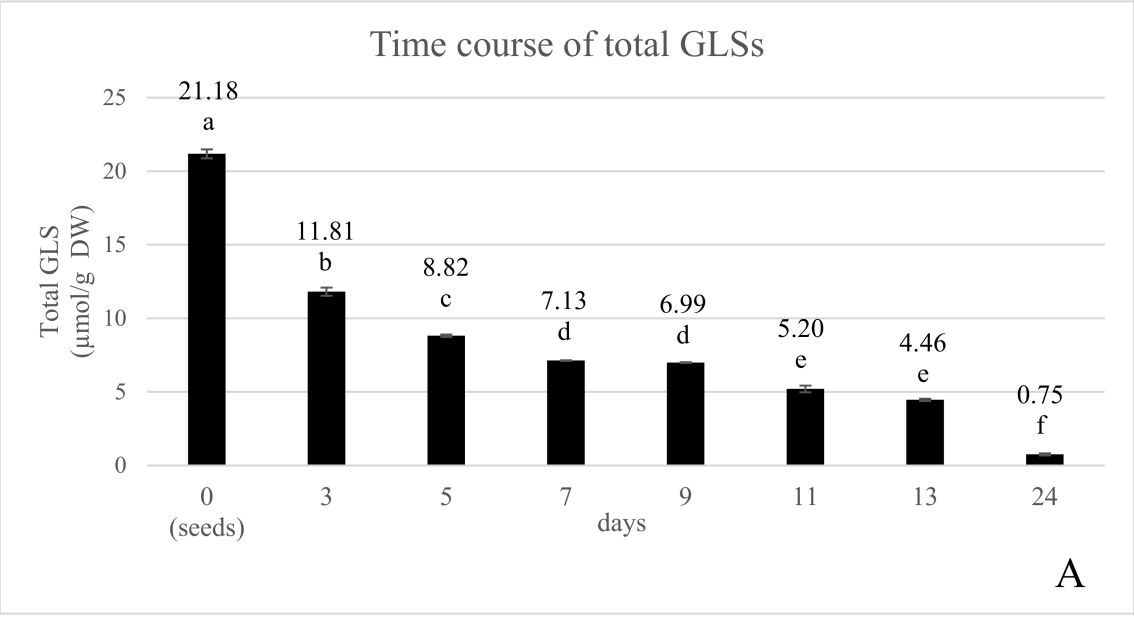

Figure 1. *Cont.*

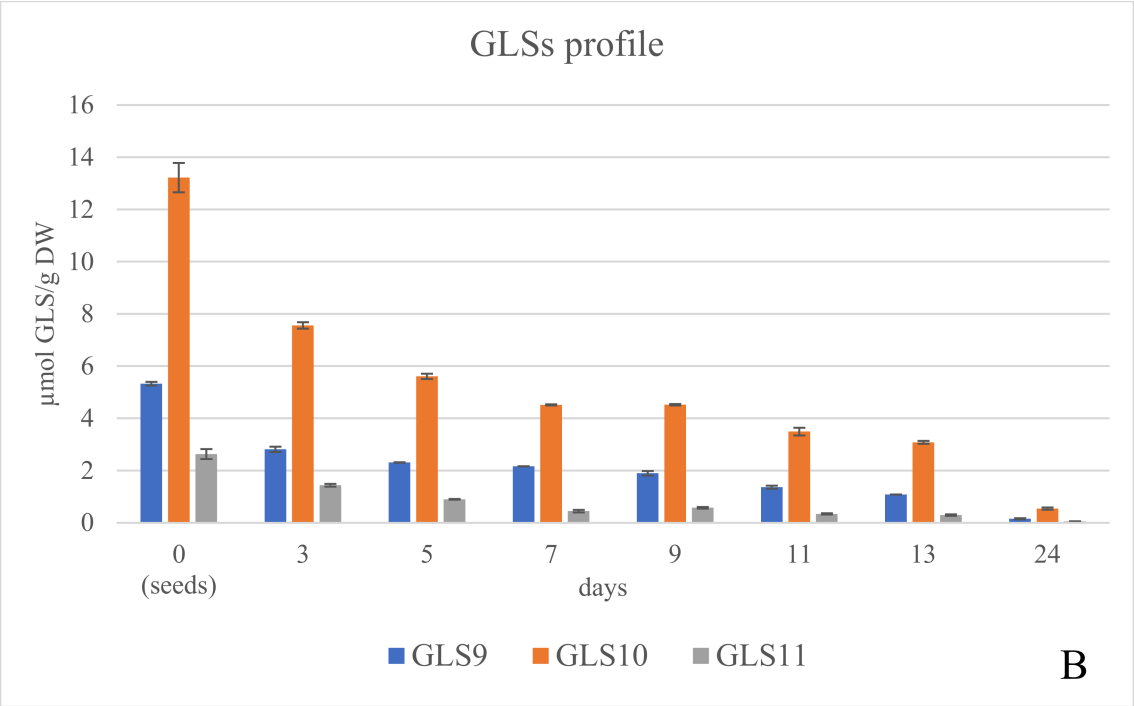

Figure 1. (**A**) Content of total glucosinolates (GLSs) during the course of 24-day sprouting studies expressed in µmol/g dry weight (DW). Data analyzed by one-way ANOVA. Different lowercase letters are significantly different at $p \leq 0.05$. (**B**) Content of the three types of GLSs (Glucoarabin = GLS9; Glucocamelinin = GLS10; Homoglucocamelinin = GLS11) during the course of 24 days of sprouting. Error bars indicate the standard deviation of the triplicates for each data point.

Each peak was recognized by the HPLC profile of the commercial standards and assigned as GLS9 for Glucoarabin, GLS10 for Glucocamelinin, and GLS11 for Homoglucocamelinin (Figure 2A,B). Therefore, in camelina sprouts, three major glucosinolates (Figure 2B) were present, and the relative quantity of each GLS, contrary to the profile observed by Bravi et al. [5], was similar to that detected in the seeds, with GLS10 proving to be the most abundant among the three GLSs (Figures 1B and 2B) [24,30,31].

Prior to supplementing the sprouts with glucose, DL-methionine, and sulfur salt (K_2SO_4), tolerance studies were carried out to ensure that substances would not have a serious inhibitory effect on sprout growth. Calena cultivar was used for this test. Glucose was initially tested in six concentrations (1, 10, 50, 75, 100, 200 mM). The total contents of GLSs tended to increase when the sprouts were exposed to 1 mM to 75 mM and decreased with higher concentrations (Table 1). At 75 mM of glucose, the GLS content value was approximately 25% higher than in the control. The sprouts treated with 200 mM of glucose showed a reduced GLS content and marked inhibition of root growth since they were noticeably shorter in length than the control. This dual effect is consistent with the role of glucose as both a metabolic substrate and a signaling molecule [20,32]. Moderate levels of glucose may activate transcriptional pathways involved in secondary metabolism; instead, excessive glucose can impose osmotic stress and inhibit normal development. The observed increase in GLSs following glucose treatment aligns with previous studies in *Brassicaceae* sprouts, where sugars acted as effective elicitors of GLS biosynthesis [17]. Similar increases in glucosinolate accumulation have been reported in broccoli sprouts treated with either sucrose or mannitol, suggesting that the increase in glucosinolate contents in broccoli sprouts treated with sucrose was due to the osmotic stress caused by sucrose, rather than to gene expression regulation [33]. Moreover, Gigolashvili et al. [34] demonstrated that glucose significantly induces the expression of HAG1/MYB28, a key R2R3-MYB transcription factor

controlling the biosynthesis of methionine-derived aliphatic glucosinolates in *Arabidopsis thaliana*. Therefore, the enhanced glucosinolate accumulation observed in our glucose-treated sprouts may reflect the combined action of osmotic stress and glucose-mediated regulation of glucosinolate biosynthetic pathways.

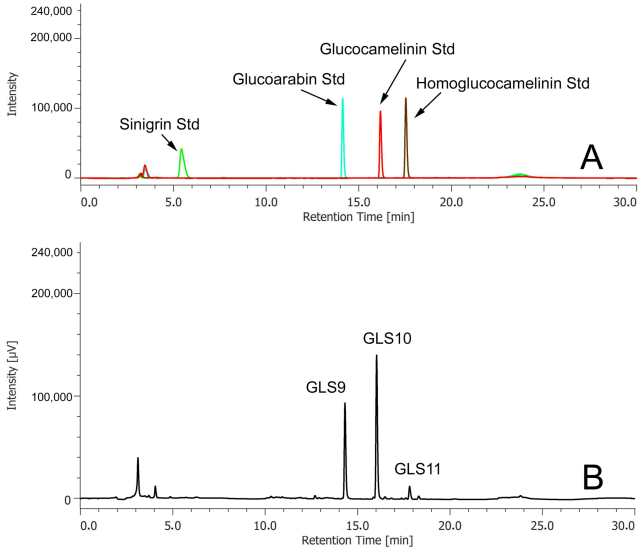

Figure 2. (**A**) Chromatogram of reference standard mixture—Glucoarabin (light blue peak), Glucocamelinin (red peak), Homoglucocamelinin (brown peak), and Sinigrin standard (green peak)—used to identify the peaks in the sample. (**B**) Chromatogram of glucosinolates from camelina sprouts: GLS9 = Glucoarabin; GLS10 = Glucocamelinin; GLS11 = Homoglucocamelinin. Std = standard.

Table 1. Effect of glucose, DL-methionine, and sulfur treatment (K_2SO_4) on total glucosinolate (GLStot) content in Calena sprouts treated for 48 h with different elicitor concentrations expressed in milliMolar (mM) or milligrams (mg). Data analysis by one-way ANOVA. For each treatment, values with different letters are significantly different ($p \leq 0.05$) according to Tukey's post hoc test. ± represent the standard deviation (N = 3).

Calena	Dose	GLStot µmol/g
Glucose	0	8.69 ± 0.36 c, d
	1 mM	9.00 ± 0.16 b, c, d
	10 mM	9.30 ± 0.05 b, c
	50 mM	9.84 ± 0.02 a, b, c
	75 mM	10.89 ± 0.06 a
	100 mM	10.15 ± 0.71 a, b
	200 mM	7.78 ± 0.09 d
DL-Methionine	0	8.14 ± 0.02 e
	1 mM	9.39 ± 0.05 b
	2.5 mM	10.17 ± 0.12 a
	5 mM	9.08 ± 0.78 c
	10 mM	8.68 ± 0.11 d
	20 mM	7.42 ± 0.20 f
K_2SO_4	0	8.58 ± 0.27 a
	$S_{10\ mg}$	7.58 ± 0.51 b
	$S_{20\ mg}$	7.39 ± 0.50 b, c
	$S_{40\ mg}$	6.83 ± 0.39 b, c
	$S_{60\ mg}$	6.81 ± 0.19 b, c
	$S_{80\ mg}$	6.61 ± 0.55 c

DL-methionine was tested at five concentrations (1, 2.5, 5, 10, 20 mM) (Table 1). A significant increase in GLS content was observed at 2.5 mM of DL-methionine (approximately 25%, with respect to the control). At higher doses, the GLS content decreased. Methionine is a known precursor for aliphatic GLS biosynthesis, and its exogenous supply likely increased substrate availability for chain-elongation and core GLS pathways. However, the decrease observed at higher DL-methionine concentrations may reflect the presence of regulatory or metabolic constraints affecting glucosinolate biosynthesis.

In contrast to glucose and DL-methionine, sulfur supplementation for 48 h did not stimulate GLS accumulation in camelina sprouts at any tested concentration (Table 1). Since any increase in GLS content was observed at all tested sulfur doses, an additional experiment was made using the lowest sulfur concentration (S_{10}) applied for different exposure times (3, 6, 9, and 24 h). No increase in GLSs was detected with S_{10} at any time point (Table 2). This finding contrasts with results obtained in other *Brassicaceae*, where sulfur fertilization significantly increased GLS accumulation [21,35]. The lack of response observed may indicate that camelina possesses a distinct regulatory mechanism for sulfur assimilation. An identical result was obtained when a different concentration of inorganic sulfur salt, such as Na_2SO_4, was tested. The consistency of results obtained using different sulfur salts further supports this hypothesis of a species-specific response. Moreover, the absence of a positive effect of sulfur treatments on GLS content observed here is consistent with recent field studies reporting no significant increase in GLS content in camelina seeds under several concentrations of sulfur-based fertilizers [36].

Table 2. Effect of sulfur treatment on total glucosinolate (GLStot) content in Calena sprouts treated for 3 h, 6 h, 9 h, and 24 h with 10 mg of sulfur (S_{10}). Data expressed as mean ± standard deviation (N = 3).

Calena	Dose	Time of Treatment	GLStot µmol/g
K_2SO_4	0	0 h	8.90 ± 0.30
	$S_{10\ mg}$	3 h	8.79 ± 0.36
	$S_{10\ mg}$	6 h	8.39 ± 0.30
	$S_{10\ mg}$	9 h	8.19 ± 0.69
	$S_{10\ mg}$	24 h	8.05 ± 0.42

In order to verify whether different genotypes would have a similar response to glucose and DL-methionine elicitors, Pearl and Alan genotypes were tested together with Calena. The results of the two-factor (3 × 3) ANOVA are depicted in Figure 3, showing the effect on the total GLS content after 48 h of treatment with 75 mM glucose or 2.5 mM DL-methionine across the three genotypes. A significant interaction was detected between genotype (G) and elicitor (E) ($p_{G\times E}$ = 0.001). Control samples differed significantly in total GLS content, with Pearl sprouts showing the highest levels (14.02 ± 0.47 µmol/g DW) among the three genotypes. Although Alan and Calena sprouts (controls and treated samples) exhibited a lower content of total GLSs compared to Pearl, they were more responsive to glucose elicitor than Pearl. No significant difference in total GLS content was observed between control and glucose-treated Pearl sprouts (p = 1), whereas Calena and Alan sprouts both showed a significant increase in GLSs ($p < 0.001$). Compared to the control, the greatest increase in total GLS content following glucose treatment was observed in Calena (11.00 ± 0.15 vs. 8.56 ± 0.64) sprouts. Calena and Alan genotypes exhibited a similar response to glucose and DL-methionine treatments, with a significant increase in total GLS content of 2.40 ± 0.66 and 1.56 ± 0.93 µmol/g DW, respectively, following glucose treatment, and 1.90 ± 0.82 and 2.05 ± 1.18 µmol/g DW, respectively, following DL-methionine treatment. In contrast to glucose, DL-methionine treatment significantly

increased total GLS content in Pearl sprouts, reaching 16.41 µmol/g DW. The increase in GLS content following DL-methionine treatment in camelina is similar to that reported in other *Brassicaceae* by Pérez-Balibrea et al. [21].

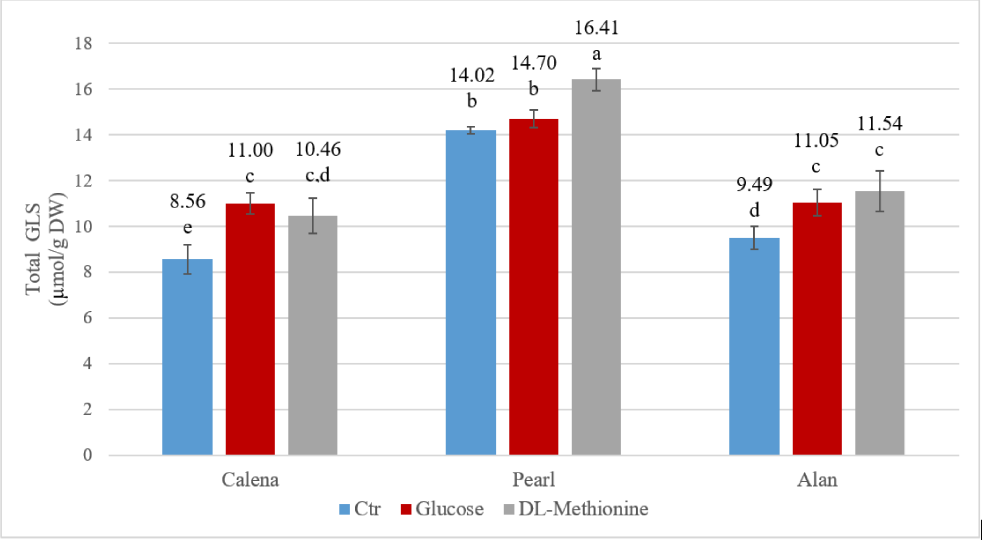

Figure 3. Total glucosinolates (µmol/g dry weight) detected in the sprouts of different camelina genotypes (Calena, Pearl, Alan) treated for 48 h with glucose and DL-methionine vs. control samples. Data analyzed by two-way (genotype × elicitor) ANOVA and Tukey's post hoc test (95% Confidence); statistically significant differences ($p \leq 0.05$) are indicated by different letters; N = 3.

The differential glucosinolate responses among genotypes may reflect differences in carbon sensing and metabolic regulation. In particular, sugar-signaling pathways and methionine-associated metabolism could contribute to the observed variability, although dedicated molecular analyses are required to verify these hypotheses. However, these results highlight that elicitor efficiency depends not only on the applied compound but also on the genetic background. Such variability is well documented in *Brassicaceae* and emphasizes the importance of genotype selection when designing.

Consequently, given that plant responses often vary among species and varieties [19], an experiment was also conducted using the lowest sulfur concentration (S_{10}) on Pearl and Alan genotypes, despite the Calena cultivar showing no response to sulfur treatment. None of the three genotypes tested exhibited an increase in total GLS content after 6 h of S_{10} treatment (Table S1).

Regarding the three types of GLSs, the distinct effect of 48 h of treatment with glucose or DL-methionine elicitors on the content of GLS9, GLS10, and GLS11 was analyzed by two-way (3 × 3) ANOVA, and the results are summarized in Table 3. In the case of GLS9, no significant interaction ($p_{G\times E} = 0.523$) between the two factors was observed, whereas significant main effects of both factors ($p_G < 0.001$, $p_E < 0.001$) were detected. In the case of GLS10 and GLS11, however, a significant interaction between factors was observed ($p_{G\times E} < 0.001$ for GLS10 and $p_{G\times E} = 0.017$ for GLS11), and in these cases, main effects were not further discussed. Both elicitors significantly enhanced GLS9 accumulation across all three genotypes. DL-methionine, however, determined a more incisive increase in GLS9 than glucose. In contrast, DL-methionine and glucose showed different effects on GLS10 and GLS11 content compared to control samples. Pearl was the only genotype to respond to DL-methionine, showing a significant increase restricted to GLS10 content (with no significant change in GLS11 content), whereas Calena and Alan did not show any response to DL-methionine treatment. The effect of glucose treatment, in contrast, significantly affected Calena and Alan genotypes limited to GLS10 content. Although no

differences in GLS11 content were detected between treatments and control samples in any of the three genotypes, a significant difference was observed in the Calena genotype between glucose and DL-methionine-treated samples, suggesting that Calena may be slightly more responsive to glucose than to DL-methionine in GLS11 synthesis. The preferential accumulation of GLS9, which requires fewer elongation cycles than GLS10 and GLS11, may suggest a tighter regulation of downstream chain-elongation steps. The comparatively limited response of GLS11 further supports the possibility of progressive constraints along successive elongation reactions.

Table 3. Effect of 48 h of treatment with glucose or DL-methionine elicitors on different glucosinolate content (GLS9, GLS10, GLS11) in sprouts of different camelina genotypes (Calena, Pearl, Alan); Ctr = control sprouts without treatment. Data analyzed by two-way (genotype × elicitor) ANOVA and Tukey's post hoc test (95% Confidence); data expressed as µmol/g dry weight; N = 3.

Factors		**Response Variables**		
Genotype (G)	**Elicitor (E)**	**GLS9 µmol/g DW**	**GLS10 µmol/g DW**	**GLS11 µmol/g DW**
Calena		3.45 b	5.61	0.58
Pearl		4.64 a	8.95	1.23
Alan		2.94 c	6.43	1.02
	Ctrl	3.06 c	6.69	0.96
	glucose	3.70 b	7.62	1.01
	DL-methionine	4.85 a	7.18	0.91
Calena	Ctrl	2.82 e	5.16 e	0.58 d, e
	glucose	3.44 d	6.85 c	0.71 d
	DL-methionine	4.73 b	5.28 e	0.45 e
Pearl	Ctrl	4.07 c	8.72 b	1.24 a
	glucose	4.58 b	8.91 a, b	1.21 a, b
	DL-methionine	5.76 a	9.41 a	1.24 a
Alan	Ctrl	2.34 f	6.12 d	1.03 c
	glucose	3.03 d, e	6.96 c	1.06 b, c
	DL-methionine	4.05 c	6.52 c, d	0.97 c
ANOVA			*p*	
	G	<0.001	<0.001	<0.001
	E	<0.001	<0.001	0.006
	G × E	0.523	<0.001	0.017

a–f Statistically significant differences ($p \leq 0.05$) in columns obtained by Tukey's post hoc test (95% Confidence) are indicated by different superscripts.

Overall, these results demonstrate that glucose and DL-methionine can enhance glucosinolate accumulation in camelina sprouts and provide useful insights into genotype-dependent elicitation responses. However, further transcriptomic, enzymatic, and physiological analyses would be valuable to better clarify the regulatory mechanisms underlying these responses. In addition, the mechanisms associated with the lack of response to sulfur supplementation deserve further investigation. Future studies should therefore focus on these aspects, as well as on assessing the impact of elicitor-induced GLS enrichment on sensory quality, bioavailability, and biological activity.

4. Conclusions

Our results demonstrate that elicitation with moderate concentrations of glucose (75 mM) and DL-methionine (2.5 mM) significantly increased total GLS content in 5-day-old camelina sprouts, whereas higher doses were ineffective or inhibitory. Both elicitors preferentially enhanced GLS9 accumulation, suggesting a selective modulation of GLS

biosynthesis. In contrast, sulfur supplementation did not affect GLS accumulation, indicating that sulfur uptake and allocation in this species require further investigation. These findings highlight the potential of targeted elicitation and cultivar selection as effective strategies to enhance the glucosinolate content in *Camelina sativa* sprouts. However, this study should be considered primarily phenotypic and exploratory, as it was limited to increasing glucosinolate content and did not include mechanistic analyses. Nevertheless, the results provide a useful basis for future targeted molecular and functional investigations aimed at elucidating the mechanisms underlying glucosinolate modulation and its potential relevance for sprout quality and bioactivity.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/crops6040062/s1, Figure S1. Sprouting jars (**A**) disassembled (left: glass jar, elastic and fine-mesh net) and assembled jars (right). (**B**) approximately 300 soaked camelina seeds. (**C**) camelina sprouts after 3 days. (**D**) camelina sprouts after 5 days. Table S1. Effect of sulfur treatment on total glucosinolate (GLS) content in Calena, Pearl and Alan sprouts treated for 6 h using the same concentration of sulphur express in milligrams (mg) (S_{10}). Data expressed as mean ± standard deviation (N = 3).

Author Contributions: I.G.: conceptualization, writing—review and editing original draft; S.P.: writing—review, editing original draft, and data analysis; E.P.: writing—review and sample preparation; I.M.B.: sample preparation and laboratory analyses. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data are included in the article or in the Supplementary Materials.

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