The Quality and Glucosinolate Composition of Cruciferous Sprouts under Elicitor Treatments Using MeJA and LED Lights †

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Abstract: Background: Cruciferous sprouts (Brassicaceae) are rich in glucosinolates (GSL) as health-promoters involved in the prevention and modulation of different pathological conditions. Only recently has the use of LEDs been implemented in food production in order to reduce energy costs and to facilitate soil-less systems for producing edible sprouts. The aim of this research was to obtain cruciferous sprouts enriched in bioactive compounds (GSL) by means of soil-less production using LEDs and Methyl-Jasmonate as elicitors. Methods: Seeds of broccoli, red radish, red cabbage, and white mustard varieties for sprouting, were sanitized (2 h) and water imbibed (22 h) before sowing and growing in the dark (2 d). The 3-day old sprouts were transferred to growth chamber under 18/6 h photoperiod, with controlled relative humidity (60/80%) and LED lights (Experimental vs. Commercial) with spraying Methyl-Jasmonate (250 µM) as elicitor. The germination efficiency, biomass production, and GSL contents were analysed. Results: The LED treatments affected the fresh biomass production. The GSL analysis revealed qualitative differences and suggested the potential of using specific GSL as markers of every variety: glucoraphanin in broccoli; Dehydro-Erucin in radish; hydroxybenzyl-GLS in mustard, and glucoerucin in red cabbage. The combination of LED lighting and MeJA is a promising tool for increasing GSL contents in sprouts, rendering healthier fresh foods or ingredients for functional products.

Keywords: brassica oleracea; raphanus sativus; sinapis alba; elicitor; LED light

1. Introduction

Nowadays, there are numerous non-communicable diseases challenging public health systems worldwide including obesity, diabetes, chronic pain and inflammation, as well as different types of cancer [1,2]. Cruciferous sprouts are obtained by germination of seeds and developed in hydroponics or any other substrate. Collecting these fresh foods before the development of true leaves are immature vegetables with two cotyledons, hypocotyl and radicle, have better nutritional value than the seeds and are suitable for human consumption with higher contents of phytochemicals than the mature tissues [3,4]. Healthy living requires balanced diets in order to prevent non-communicable diseases, and fresh edible sprouts are a good option for incorporating foods naturally-rich in bioactive compounds in a daily diet; they are ready to eat, fresh, nutritionally rich and safe [5].

The use of LED (light-emitting diode) for the development of cruciferous sprouts and microgreens has been incorporated to food production systems in the last few years in order to facilitate development in a more sustainable way than common food produc-
tion practices (e.g., greenhouses or growth chambers with fluorescent lamps, etc.) and has in mind the needs for the future of food security of being able to produce fresh foods in urban environments and in agri-food areas without increasing the use of soils or irri-
gation water, and also improving the nutritive and phytochemical content of the sprouts and microgreens produced under LED lighting conditions [6]. On the other hand, previous experiences on the use of elicitors to obtain cruciferous sprouts enriched in bioa-
tive compounds have been very effective and positive in terms of glucosinolates and
phenolic compounds [5,7]. Taking into consideration all these premises we have planned
the development of cruciferous sprouts in hydroponics elicited with LED lighting and
Methyl-Jasmonate (MeJA) to bio-stimulate the production of glucosinolates comparing
the effects of two types of LEDs (commercial vs. experimental) designed for indoor food
production systems, with the aim to gain knowledge on the response by means of per-
formance (germination rate, biomass yield) and phytochemical composition of fresh ed-
ible sprouts of cruciferous varieties (broccoli, radish, cabbage and mustard) under these
conditions for future food production recommendation.

2. Materials and Methods

2.1. Cruciferous Sprouts Seeds and Growth Conditions

Seeds of different species of Brassicaceae ready for sprouting (Intersemillas S.A.,
Loriguilla, Valencia) including broccoli (Brassica oleracea var. italica L. cv. Calabrese),
white mustard (Sinapis alba L.), red radish (Raphanus sativus var. sativus L. cv. Sango)
and red cabbage (B. oleracea var. capitata f. rubra L.). The sanitation, imbibition and
germination conditions were the same as described previously [4,5]. The imbibed seeds
were sown on GrowFelt White media (80% viscose, 20% polyester) to promote rapid
germination (Anglo Recycling Tech. Ltd., Lancashire, UK) and were kept for 48 h in the
dark and 80% Relative Humidity. The 3-day old sprouts were then placed in a growth
chamber with controlled growth conditions (Photoperiod 18/6 h; temperature 24/18 °C;
and relative humidity 60/80%), irrigated every other day to maintain enough humidity in
substrate using 1% bleach in distilled water and collected on day 7. The measure of fresh
weight per tray was registered upon collection of the plant material from every tray and
saved for comparison with dry weight after freeze-drying prior to phytochemical analy-

2.2. Illumination System and Elicitation

The growth chamber used LED lamps with an average intensity of 230 µmol/m²/s in
the 400–700 nm spectrum being of 1 m length giving a wide sunlight spectrum (with
reduced ultraviolet radiation 0–1.5%) and the possibility of regulation of the intensity of
light. Two systems were compared: Commercial LEDs. LEDs available in the market for
industry and research model Phillips Xitanium® equipped with 60 W-LED, 400–700 nm
spectrum (Koninklijke Philips N.V., Amsterdam, The Netherlands). Experimental LEDs.
LEDs customized to reduce energy consumption, equipped with 20 W-LED, spectrum
400–700 nm, model Protect BioLED 100W (SysLed Spain, S.L.). For the treatment of
sprouts with MeJA 250 µM dissolved in 0.1% EtOH (96% r.a.) the trays of germinating
seeds were evenly sprayed daily with 10 mL of solution for 4 days. The control treatment
was the 0.1% EtOH solution only.

2.3. Phytochemical Analysis (Glucosinolates)

The analysis of the freeze-dried plant material (100 mg) was carried out to study the
glucosinolate composition of the sprouts, by means of hydromethanolic extraction an
HPLC-DAD analysis of intact glucosinolates following already established protocol in
the research group [4,5].
2.4. Statistical Analysis

For the experiments, two-way ANOVA analysis was performed, using an HSD Tukey as a post hoc. All de analyses were executed in RStudio (version 3.5.1). A value of $p < 0.05$ was considered significant.

3. Results and Discussion

3.1. Performance and Biomass

The expression of phenotype of the cruciferous varieties was the characteristic of each variety, the pigment composition of the different varieties was expressed in the differential aspect of the different sprouts showing a clear green color of the mustard in contrast to the dark green color of broccoli and the reddish tone of the red cabbage with the deep brown-red color of the radish (Figure 1).

![Figure 1. White Mustard, Broccoli, Red Cabbage and Red Radish, under the two different LED lighting comparing Commercial (A) vs. Experimental LEDs (B).](image)

The analysis of fresh weight as a measure of production of biomass from the seed was significant and we could observe statistically significant differences between the LED treatments for the different types of sprouts, mustard, broccoli, red radish and red cabbage (Figure 2).

![Figure 2. Biomass production (g fresh weight) of seeds and edible (7-day old) sprouts of White Mustard, Broccoli, Red Cabbage and Red Radish, under the two different LED lighting comparing Commercial vs. Experimental LEDs. The numbers show the average values per treatment ($n = 3$) ± standard deviation. Different letters indicate statistically significant differences ($p < 0.05$).](image)

The production of biomass from the seeds was significantly higher in all the varieties and in the two LED treatments, with better similar results between the light treatments and with a ×2 factor in terms of fresh weight increase from seed (d0) to harvest (d7) in all the varieties.
3.2. Glucosinolate (GSL) Contents

In Figure 3, we included the results of the total glucosinolates (GSL) in the seeds, as reservoir of GSL, and the sprouts at harvest. The regular trend from seed to sprout is to dilute the content of glucosinolates with growth, but in this case, and for all the studied varieties, the total GSL contents were significantly higher in the sprouts than in the seed; there was not a dramatic increase, but there was no reduction, that is already positive, for the delivery of enough mg of GSL per edible portion to the consumer.

![Figure 3. Total Glucosinolates (mg/100 g fresh weight) of seeds and harvested sprouts of White Mustard, Broccoli, Red Cabbage and Red Radish, under the two different LED lighting comparing Commercial vs. Experimental LEDs. The numbers show the average values per treatment (n = 3) ± standard deviation. Different letters indicate statistically significant differences (p < 0.05).](image)

The commercial vs. the experimental LEDs slightly but significantly affected the content of GSL in the varieties under study. In order to select a characteristic GSL for each variety as marker, glucoraphanin for broccoli, glucoraphenin and dehydro-erucin for radish, glucosinalbin for mustard and glucocruerin in red cabbage could be selected as the major GSL in these sprouts (data not shown). From a practical point of view, and taking into consideration the little effect on the GSL composition, and the fact that the experimental LEDs are much less expensive than the current commercial LEDs, future studies, as well as a recommendation to the growers, would be to preferably use the experimental LEDs. Nonetheless, we aimed to know the effects of elicitation with MeJA (250 µM) together with the LED treatments as a potential strategy to enrich the cruciferous sprouts in GSL, and tested this effect in a separate experiment, as shown in Table 1.

![Table 1. Total Glucosinolates (mg/100 g fresh weight) of sprouts of White Mustard, Broccoli, Red Cabbage and Red Radish, under the two different LED lighting comparing Commercial vs. Experimental LEDs and elicited with MeJA (250 µM). The numbers show the average values per treatment (n = 3).](table)

* Indicates statistically significant differences between the Control and the MeJA treatments at \( p < 0.05 \).

The preliminary evaluation of the effects of combining MeJA spraying with a different LED lightening treatment showed clear increases in total GSL contents for all the
studied sprouts when sprayed for 4 days with the MeJA 250 µM. The intensity of the increase was different depending on the variety, ranging by 23–30% for white mustard, by 37% for broccoli, by a dramatic 220–240% in red cabbage, and by 15% on red radish. The influence of the MeJA is clear but the effect of the LEDs treatments is limited, even though there was a similar intensity in both systems of LEDs. The relevance at the statistical level of these results needs to be evaluated (ongoing work).

4. Conclusions

The use of LED lights for the growing of edible cruciferous sprouts is positive in terms of biomass production and phytochemical content (glucosinolates) without any negative effects of significance. The use of LED lights is of great economical interest in the production of foods because of the reduced energy consumption, and in this case, the commercial vs. experimental lights are not especially different for the purposes of growing cruciferous sprouts (broccoli, red cabbage, red radish and white mustard); therefore, it is recommendable in future experiments and for the growers, that experimental LEDs be used.

From the point of view of using elicitation to enrich the bioactive compounds in cruciferous sprouts, the use of MeJA is positive, confirming previous results. The intensity of the response for the different species is clearly useful to focus the production of sprouts for specific purposes (e.g., red cabbage is rich in glucosinolates—richer than broccoli or radish—and increased dramatically the total GSL contents under MeJA elicitation). More work is undergoing to statistically validate the results and to incorporate strategies of priming and elicitation to the production of edible sprouts enriched in bioactive compounds from cruciferous species.

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References

1. World Health Organization. Noncommunicable Diseases. 2018. Available online: https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases (accessed on 23 October 2020).
2. Prüss-Ustün, A.; van Deventer, E.; Mudu, P.; Campbell-Lendrum, D.; Vickers, C.; Ivanov, I.; Forastiere, F.; Gümü, S.; Dora, C.; Adair-Rohani, H. Environmental risks and non-communicable diseases. BMJ 2019, 364, l265, doi:10.1136/bmj.l265.
3. Samuoliene, G.; Brazaityte, A.; Viršile, A.; Miliauskienė, J.; Vaštakaitė-Kairienė, V.; Duchovskis, P. Nutrient levels in Brassicaeae microgreens increase under tailored light-emitting diode spectra. Front. Plant Sci. 2019, 10, 1–9, doi:10.3389/fpls.2019.01475.
4. Baenas, N.; Moreno, D.A.; García-Viguera, C. Selecting sprouts of brassicaceae for optimum phytochemical composition. J. Agric. Food Chem. 2012, 60, 11409–11420, doi:10.1021/jf302863c.
5. Baenas, N.; Gómez-Jodar, I.; Moreno, D.A.; García-Viguera, C.; Periago, P. Broccoli and radish sprouts are safe and rich in bioactive phytochemicals. Postharvest Biol. Technol. 2017, 127, 60–67, doi:10.1016/j.postharvbio.2017.01.010.
6. Zhang, X.; Bian, Z.; Yuan, X.; Chen, X.; Lu, C. A review on the effects of light-emitting diode (LED) light on the nutrients of sprouts and microgreens. Trends Food Sci. Technol. 2020, 99, 203–2016, doi:10.1016/j.tifs.2020.02.031.
7. Baenas, N.; García-Viguera, C.; Moreno, D.A. Elicitation: A tool for enriching the bioactive composition of foods. Molecules 2014, 19, 13541–13563, doi:10.3390/molecules190913541.