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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 the use of LEDs has been implemented in food production in order to reduce the energy costs and to facilitate the 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 versus 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 spe 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 the public health systems worldwide including obesity, diabetes, chronic pain and inflammation, as well as different types of cancer [1,2]. The cruciferous sprouts 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 with better nutritional value than the seeds and suitable for human consumption with higher contents of phytochemicals than the mature tissues [3,4]. Healthy living needs of 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 the daily diet, 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 production practices (e.g., greenhouses or growth chambers with fluorescent lamps, etc.) and having 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 irrigation water and 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 bioactive compounds have been very effective and positive in terms of glucosinolates and phenolic compounds [5,7]. Taking into consideration all these premises we 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 versus experimental) designed for indoor food production systems, with the aim to gain knowledge on the response by means of performance (germination rate, biomass yield) and phytochemical composition of fresh edible 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 olreacea 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, England, UK) and were kept for 48 h in the dark and 80% Relative Humidity. The 3-day old sprouts were then placed in the growth chamber with controlled growth conditions (Photoperiod 18 h/6 h; temperature 24 °C/18 °C; and relative humidity 60%/80%), irrigated every other day to keep enough humidity in substrate using 1% bleach in distilled water and collected on day 7th. 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 analysis.

2.2. Ilumination System and Elicitation

The growth chamber used LED lamps with average intensity of 230 μmol/m^2/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® equiped 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 disolved in 0.1% EtOH (96% r.a.) the trays of germinating seeds were evenly sprayed daily with 10 mL of solution during 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 shoeing the clear green color of the mustard in contrast to dark green color of broccoli and the reddish tone of the red cabbage with the deep brown-red color of the radish (Figure 1).

(**A**) (**B**)

Figure 1. White Mustard, Broccoli, Red Cabbage and Red Radish, under the two different LED lighting comparing Commercial (**A**) *versus* Experimental LEDs (**B**).

The analysis of fresh weight as a measure of production of biomass from 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).

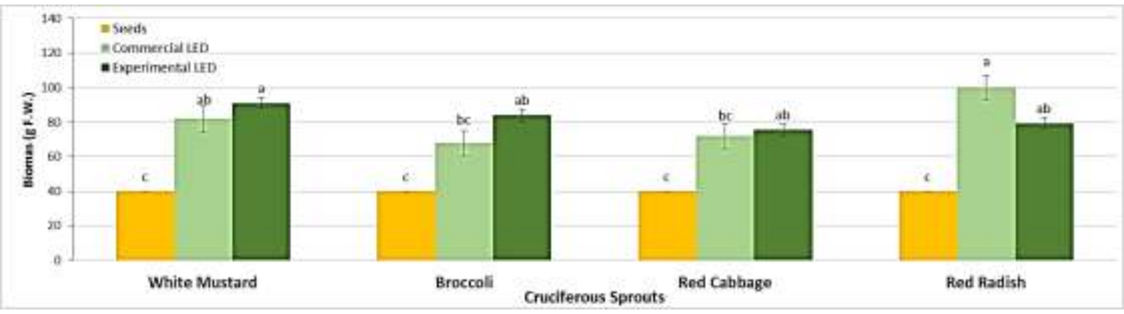

Figure 2. Biomass production (g fresh weight) of seeds and edible (7-day old) sprouts of White Mustard, Broccoli, Red Cabbage and Red Radish, under the two different LED lighting comparing Commercial *versus* Experimental LEDs. The numbers show the average values per treatment (n = 3) ± standard deviation.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

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 the 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.

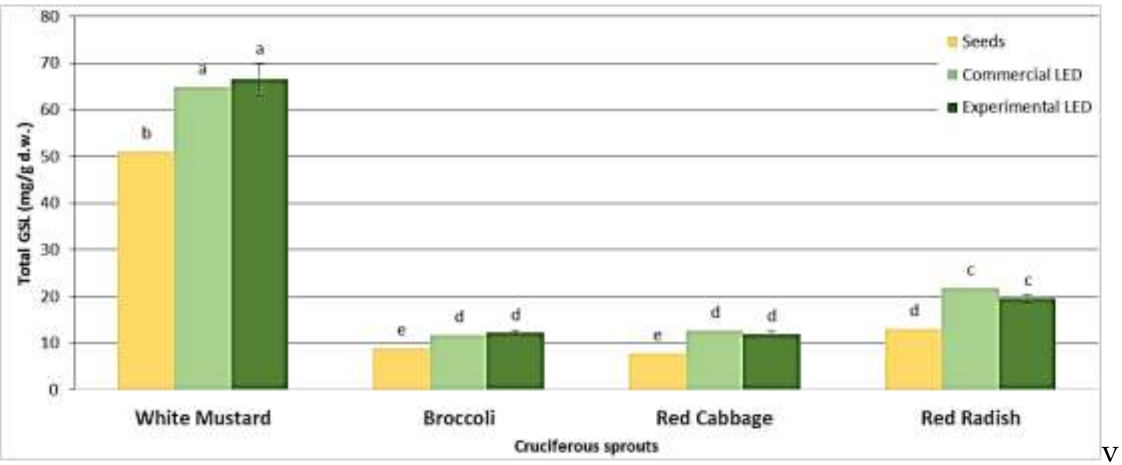

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Figure 3. Total Glucosinolates (mg/100 g fresh weight) of seeds and harvested sprouts of White Mustard, Broccoli, Red Cabbage and Red Radish, under the two different LED lighting comparing Commercial *versus* Experimental LEDs. The numbers show the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

The commercial *versus* 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 glucoerucin in red cabbage could be selected as the major GSL in these sprouts (data not shown). From a practical point of view, 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 the recommendation to the growers would be to use prefereably 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 the 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 *versus* Experimental LEDs and elicited with MeJA (250 μM). The numbers show the average values per treatment (n = 3).

Sprout Variety	Experimental LEDs		Commercial LEDs	
	Control	MeJA	Control	MeJA
White Mustard	179.2	220.5	177.4	231.3
Broccoli	45.8	63.1	42.8	59.4
Red Cabbage	112.6	275.2	124.1	272.9
Red Radish	55.4	62.9	55.1	62.8

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% om red radish. The influence of the MeJA is clear but the effect of the LEDs treatments is limited, eventhough of 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 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 versus experimental lights are not especially different for the purposes of growing cruciferous sprouts (broccoli, red cabbage, red radish and white mustard), therefore, it would recommendable in future experiments and for the growers, the use of the experimental LEDs.

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 validate statistically 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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Conflicts of Interest: The authors declare no conflict of interest.

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