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LIGHT MODULATED PHENOLIC SYNTHESIS IN *CHENOPODIUM QUINOA* MICROGREENS AS A POTENTIAL BIOTECHNOLOGICAL TOOL

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Abstract: *Chenopodium quinoa* microgreens are a culinary product that, as well as other species, is rich in bioactive, health promoting compounds. The present paper shows that light spectrum alone can be used as a convenient tool for enhancing phenolic synthesis in quinoa plantlets.
The methods used involved pot growth of quinoa plantlets and irradiation with a 16 : 8 photoperiod of either full spectrum (white) or a combination of blue:red:UV wavelengths and morphometric and biochemical assessments.
Results shows that, while full spectrum light is more suitable for biomass accumulation, modulated light spectrum can be used for increases in the amount of phenolic compounds. Further optimization of light spectrum modulation can lead to economically higher value microgreens, with positive outcomes for both horticultural biotechnology but also for the consumer.

Keywords: *biomass, metabolism, phenylpropanoid, photosystem, UV*

INTRODUCTION

Due to the increasing awareness regarding the importance of a healthy lifestyle, functional foods emerge as a valuable dietary means of preventing or alleviating numerous conditions. In the last 20 years, this concept has been established with the development of a market segment that grows up with approximately 10 % per year, because nutraceutical foods provide bioactive compounds with high bioavailability and clinical effects [1, 2]. At the present time different natural, sustainable and ecological sources are being actively sought to develop such products. Microgreens are successfully included in the category of functional foods because they contain a series of bioactive substances that can be used as prevention or even adjuncts in certain pathologies. Moreover, the content of these chemicals is higher than in the mature counterpart, making them concentrated sources of micronutrients [3]. In addition to such specific substances, microgreens are considered rich sources of vitamins, minerals, enzymes, pigments, etc. [4], that provide numerous health benefits. Their content is associated with growing conditions and the level of micronutrients is correlated to the environmental factors, being often applied to controlled conditions, such as natural or artificial light, soil or growing media and others [3].
Quinoa sprouts represent a very important category of microgreens, rich in functional ingredients such as polyphenols, peptides, carbohydrates and saponins, which are of great significance for improving human health. Besides these, quinoa sprouts are rich in essential amino acids (histidine, leucine, isoleucine, valine, lysine, phenylalanine etc.) with values of content between 0.7 and 2.0 g/100 g dry weight and minerals (calcium, cooper, iron, magnesium, phosphorus, potassium, sodium, zinc) ranging from 0.2 to 525.2 mg/100 g dry weight [5]. Due to these bioactive compounds, the consumption of quinoa ensures protection against diseases such as diabetes, allergies, acute inflammation and cardiovascular diseases [6]. The high content of polyphenols is associated with antioxidant properties, due to the high number of hydroxyl groups. Polyphenols are present in different quinoa species in free or glycosylated form. Their concentration is dependent on the genotype of *Chenopodium quinoa* and may reach up to 30.69 mg GAE/g dry matter in black quinoa [7]. The main phenolic acids detected in different quinoa species are rutin, vanillic acid, ferulic acid, kaempferol, quercetin derivatives, p-coumaric acid, caffeic acid, pinocembrin and apigenin [8]. Among these, vanillic and ferulic acids were identified in the high concentration in free form [9].
Vanillic acid has numerous health benefits beyond their very well-known flavour, and their dosage is dependent on the disease. In low concentrations, 250 - 500 $\mu g \cdot mL^{-1}$, vanillic acid reduces the intestinal colonization and inhibits biofilm formation in bacterial infection [10]. In higher content, 30 - 50 $mg \cdot kg^{-1}$, vanillic acid has therapeutic effect in some diseases such as hypertension by regulating the expression of eNOS and ET1 protein [11], osteoporosis by reducing cartilage destruction caused by IL-1β induced inflammation responses [12] and diabetes by reducing HbA1c and glucose levels [13]. Moreover, vanillic acid improves spatial learning and memory retention and inhibits neuroinflammatory processes in cognitive impairments at a dosage of 100 $mg \cdot kg^{-1}$. Besides these effects, vanillic acid has a protective role in cancer chemotherapy, reducing cisplatin nephrotoxicity, by inhibiting NF-κB activation, stimulating renal antioxidant defence system and restoring the levels of serum urea, uric acid and creatinine [14]. Ferulic acid has, as the other phenolic compounds, antioxidant

capacity and reduces the number of the free radicals. This effect leads to health benefits such as anti-diabetic effect by helping the pancreatic β cell to proliferate and radiate more insulin, and anti-ageing effect, being a photo protector against UV-induced skin damage. Beside these, ferulic acid stimulates detoxification enzymes, inhibits the growth of colon cancer cells and decreases the side effects of chemotherapy, having anticancer properties [15].

Due to the biological activity of chemical compounds, especially phenolic compounds, quinoa have different effects on human health. Phenolic content of quinoa has been reported to inhibit, dependent on the dosage, the release of pro-inflammatory factor IL-8 and also downregulate the expression of IL-6, IL-8, TNF-α, IL-1β and COX-2, having anti-inflammatory effect [16]. Polyphenols from quinoa were reported to have anti-obesity and anti-diabetic activities through the inhibition of α-glucosidase and pancreatic lipase enzymes, that catalyse the digestion of carbohydrates and absorption of triglyceride lipids, respectively [17]. Some reports show that quinoa ethanol extracts had beneficial effects *in vivo*, such as hypolipidemic effect, decreasing lipid peroxidation and regulating the level of leptin and adiponectin hormone [18], or neuroprotective effect by reducing the activity of scopolamine, that induces memory deficits [19].

The biosynthesis of polyphenols can be influenced by different growth factors, such as illumination, to improve food quality. For example, blue illumination increases the content of rosmarinic and gallic acid [20], two phenolic acids with numerous biological applications.

MATERIAL AND METHODS

Plant material and growth conditions

Microgreens production was obtained from quinoa Vikinga seeds from the research and educational seed stock of the Life Sciences University "Ion Ionescu de la Brad" Iași, Romania. For growth, two treatments were applied provided by a Phytofy RL LED unit (OSRAM, Golden Dragon, Munich, Germany). Approximately 150 seeds were sowed in a mixture of general-purpose soil and peat moss 2:1 for each light treatment from 8 plastic boxes (4 for biochemical analyses and 4 for phenotypic measurements), 10 × 10 × 12 cm. After seeding, the boxes were kept in the dark for 3 days and then were placed at 30 cm from the light sources. Each light treatment was provided by a Phytofy RL LED unit (OSRAM, Golden Dragon, Munich, Germany), from a distance of 30 cm from the top of the boxes. The two light treatments applied, with a 16 : 8 hours photoperiod, were a control variant, using a full spectrum (white) LED program (0:0:0:0:0:1, UV:blue:green:red:far-red:white, in µmoles) and a blue-red-UV (BRUV) program (1:9:0:9:3:0, UV:blue:green:red:far-red:white), respectively. After seeding, the boxes were kept in the dark for 3 days and, afterwards, total PPFD (Photonic Flux Density) for the two treatments were 160 and 161 $\mu moles \cdot m^{-2} \cdot s^{-1}$, respectively. The emission spectra of LED lights (according to OSRAM software) used are given in Figure 1. Plants were recorded for morphometry and collected for biochemical analyses 10 days after germination.

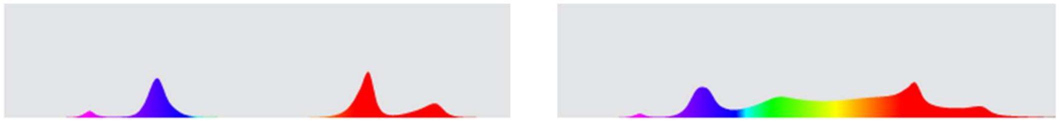

Figure 1. *LED emission spectra (left) BRUV Treatment; (right) full spectrum treatment (X scale 300-800 nm)*

Analyses

Biomass related measurements were performed using calibrated scales and calipers. Phenotypic measurements were performed non-destructively, using a MC-100 Chlorophyll Concentration Meter (Apogee Instruments) for Chlorophyll determination and a FMS2 fluorometer (HansaTech, Norfolk, UK) for Chlorophyll fluorescence. The analyses were performed before harvest by measuring 30 leaves or cotyledons/treatment for each determination.

For biochemical analyses, ethanolic extracts were prepared from dry plant and 70 % (w/w) ethanol in a ratio of 1 : 9, by maceration for 60 minutes at 50 °C. Total phenolic content and antioxidant activity were determined in microtiter plates according to the methods described by Herald [21]. Briefly, total phenolic contents were assayed using Folin-Ciocalteu reagent, expressing results as gallic acid equivalents (GAE)/mg, while antioxidant activity was measured as percent of inhibition of DPPH free radical in ethanolic extracts. The reads were performed with Biotek Epoch 2 microplate spectrophotometer (Agilent, United States).

RESULTS AND DISCUSSION

Under modulated LEDs illumination, quinoa plantlets recorded quantitative differences regarding various morphometrical and biochemical parameters. As such, regarding growth, plantlets under full spectrum (white) light had significantly higher mass/100 plantlets, with up to 50 % more than plantlets grown under blue-red-UV (BRUV) light and also marginally higher leaf-area index and plant height compared to BRUV plantlets (Figure 2).

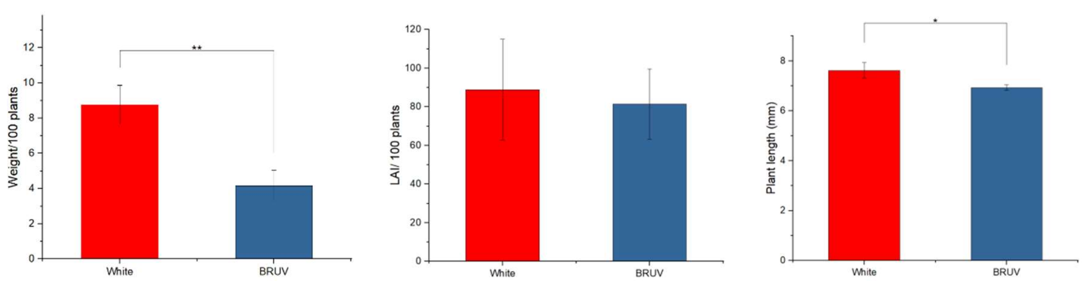

Figure 2. *Morphometric parameters of quinoa grown under modulated and full spectrum light*

Regarding photosystem related parameters, BRUV light spectrum induced significantly higher accumulation of chlorophyll, with up to 50 % more than full spectrum light. In the meantime, BRUV light altered the behavior of photosystem II (PSII), by raising the steady-state fluorescence levels (Fs) and maximal fluorescence (Fm') of the photosystem and by lowering with only 0.03 the overall efficiency of the PSII (Table 1).

Table 1. *Photosystems related parameters of quinoa grown under modulated and full spectrum light*

Properties	Treatment	
	BRUV	**White**
Chlorophyll (AU)	3.889±0.163^a	2.692±0.077^b
Fs	395.97±15.4^a	266.82±7.16^b
Fm'	1622.63±55.85^a	1249.23±29.84^b
ΦPSII	0.76±0.01^a	0.79±0.01^b

In the meantime, total phenolic contents significantly increased in BRUV treated plantlets, with approximately 15 % compared to full spectrum plantlets. A similar increase was noted in the free radical scavenging activity of ethanolic extracts (Figure 3).

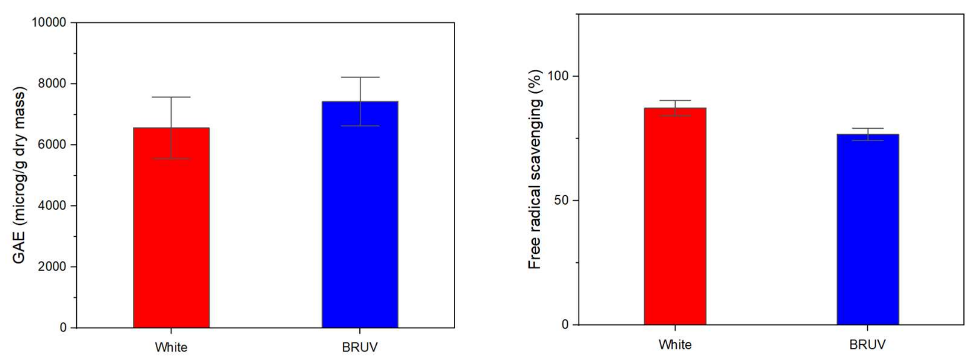

Figure 3. *Total phenolic contents (left) and free radical scavenging activity (right) of quinoa plantlets extracts*

Phenolic compounds, characterized by their extensive structural heterogeneity, are acknowledged for their health-related properties. These compounds may be integrated into customary dietary practices or exploited as precursors for the sophisticated augmentation of their molecular architecture and biological activity [22]. As a distinct class of secondary metabolites, phenolic substances are critical to plant physiological processes and environmental adaptability. Their functions are multifaceted, encompassing the defense against pathogenic entities, amelioration of ultraviolet radiation damage, mediation of pollinator attraction, and regulation of symbiotic plant-microbe interactions [23].

The composition of light in terms of its spectrum substantially influences photosynthesis and the operation of photosystems in plants. This impact is primarily due to factors such as the triggering of different photoreceptors, varying efficiencies of spectral wavelengths in photosynthesis, and the differing depths to which these wavelengths penetrate the leaf tissue [24]. Plant photoreceptors, like phytochromes for red light and cryptochromes for blue light, are activated by specific light wavelengths, affecting plant growth and metabolic processes. While the effects of blue light (with a peak at approximately 450 nm) and red light (peaking around 660 nm) on plant growth and metabolism are acknowledged, their full impact remains unclear. However, light spectral composition alters the expression of light-responsive genes, influencing growth, photosynthesis, and physiological reactions in plants, as seen in seedlings [25].

Different wavelengths within the light spectrum can trigger various photoreceptors, affect photosynthesis efficiency, and penetrate leaf tissue to varying depths. The use of white LEDs, which incorporate blue light at varying percentages, has shown species-dependent growth and developmental responses in plants like radishes, soybeans, and wheat [24]. The percentage of blue light in these LEDs can significantly alter plant morphology, affecting stem elongation and leaf area expansion, with higher blue light levels generally leading to more compact plant growth [26]. Also, red and blue light is more strongly absorbed by photosynthetic pigments and is predominantly absorbed by the upper cell layers of leaves, while green light penetrates deeper into the leaf tissue. This more uniform distribution of green light can be beneficial for leaf photosynthesis, potentially leading to higher efficiency under certain conditions [27]. Furthermore, the interaction between light quality (wavelength) and light intensity (PPFD) significantly affects photosynthetic efficiency. For example, red light has been shown to produce higher quantum yields and electron transport rates at low PPFD compared to blue and green light. Significant influences of blue and red light on both Photosystem II (PSII) and Photosystem I (PSI) have been documented. Blue light, especially within the 400 - 500 nm range, is noted for increasing photosynthesis rates and stomatal opening, thereby enhancing CO_2 uptake [28]. Additionally, the employment of red and blue LED light spectra has been observed to heighten the accumulation of polyphenols, flavonoids, and other phytochemicals in plants, although this does not consistently boost antioxidant activities, possibly due to the premature redirection of metabolites to other pathways like curcumin synthesis. These effects appear to stem from the stress induced by intense light or specific spectral compositions, triggering plant responses that include the synthesis of phenolic compounds via hormonal pathways [29].

Regarding the effects of ultraviolet (UV) radiation on plants, exposure to it, especially UV-B (280 - 320 nm), plays a regulatory role in plant development but also acts as a stressor, leading to deleterious effects [30]. UV-B exposure can promote the accumulation of phenolic compounds, such as anthocyanins, which serve protective functions against UV damage [31]. Plants can increase the accumulation of flavonoids and phenylpropanoids in response to oxidative damage caused by UV light, which also helps protect the photosynthetic cell layers [32]. UV-B radiation has also been employed to enhance the content of bioactive compounds in controlled environments, although changes in plant structure throughout growth can affect the distribution of UV-B radiation interception and, consequently, the evaluation of its effects on bioactive compound biosynthesis [33]. The C to N ratio in plants is influenced by the interactive effects of nitrogen, UV, and photosynthetically active radiation (PAR), highlighting the complex interplay between these factors on plant morphology and biochemistry [34].

CONCLUSION

Our results showed that the different spectrums we used led to a modified C:N ratio usage in quinoa plantlets, partly shifting the metabolism of the plantlets, from biomass accumulation, to secondary, phenylpropanoid metabolites production. However, no significant stress was recorded at photosystem level, creating the premises of biotechnologically modulating quinoa microgreens using only light as an environmental cue. The effects of light spectrum on plant growth and development are complex and highly species-specific. While white light is broadly effective for photosynthesis, the role of specific wavelengths like blue, red, and green light can vary depending on the plant species, developmental stage, and environmental conditions. Understanding these interactions can be essential for optimizing growth lighting in horticultural practices.

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