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Organogenesis and Antioxidant Capacity of *Streptocarpus* ×*hybridus* In Vitro under Different LED Light Spectrum Composition

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Abstract: Plants, whilst also having an ornamental role, are also a source of beneficial, bioactive compounds, and in vitro cultures are helpful in finding and obtaining them. *Streptocarpus* ×*hybridus* can be a source of antioxidants. The effect of different LED light qualities on the growth, development and metabolite composition of *Streptocarpus* multiplied in vitro on Murashige and Skoog medium was investigated. The used spectra were: 100% blue (B), 100% red (R), red and blue in 7:3 proportion (RB), 50% RB + 50% ultra violet (RBUV), 50% RB + 50% green (RBG), 50% RB + 50% yellow (RBY), 50% RB + 50% far red (RBfR), and white (WLed). A fluorescent lamp served as the control (Fl). For the best morphological quality of multiplied plants, the use of RBY light is recommended. Fl light reduced the antioxidant properties of extracts compared to LEDs. The most recommended spectrum in this aspect is RBfR, WLed, RBG and R light. These lights (except for R) stimulated the polyphenol content. RBY and R light influenced the highest content of free amino acids and reducing sugars. Studies showed that a selected LED light spectrum can influence the production of plant biomolecules with antioxidant properties and, compared to Fl light, improves the growth and development of multiplied plants.

Keywords: Cape Primrose; antioxidants; secondary metabolites; light quality; polyphenols; micropropagation

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1. Introduction

Plants are a source of valuable compounds that exhibit active, often positive and health-promoting effects on the human body when used appropriately. Scientists are constantly searching for new sources of such compounds, and have also studied the composition of metabolites produced by plants. Initially, the secondary metabolites were considered anti-nutritional, but over time their protective potential and health-promoting benefits have been discovered, which range from stimulating antioxidant mechanisms to reducing the risk of incidence of, for instance, cancer or cardiovascular diseases [1–3]. Phenolic compounds, for instance, exhibit potent biological activities. They can help prevent chronic and oxidative stress-related disorders, including cancer, cardiovascular diseases, and neurodegenerative diseases, by scavenging free radicals [4]. In vitro culture technology is helpful in finding and obtaining sources of active plant compounds. Using the technology, under controlled conditions, it becomes possible to multiply large amounts of plant material in a short period of time and of a uniform quality. In specific and specialized tissues and structures, plants are capable of accumulating metabolites of high value, and sometimes only at a

certain stage of development. The microenvironment of in vitro cultures allows for the manipulation of growth and production [5,6]. Currently, plant-derived metabolites are the basis of most pharmaceuticals, and new products are constantly being discovered. In vitro culture conditions, as a source of their acquisition, provide a geographically independent source. They eliminate the need to rely on plants growing in natural sites. Maintaining plants using this method also allows the further use of the plants for producing engineered compounds, such as vaccines [7]. Nowadays, the search is not only taking place within vegetable plants, fruiting plants or herbs—species known as ornamental plants can also be a source of active compounds [8,9]. At present, they top the list of micropropagated plant species on the largest commercial scale [10].

Streptocarpus ×*hybridus*, also known as a Cape Primrose, is a popular ornamental potted plant. It is naturally native to southern and southeastern Africa. It is also found in Madagascar and some parts of Asia. Its natural habitat is tropical moist forests. It is a perennial plant with oval, elongated and almost stalkless leaves and large funnel-shaped flowers with usually ruffled edges, growing in groups of two to three. It is acaulescent, producing only a single leafy organ attached to the petioles—a rosulate. It is a plant that can be easily propagated generatively by sowing seeds or vegetatively by cuttings, but also by micropropagation techniques [11,12]. In fact, in vitro shoot proliferation is usually used for mass propagation and for year-round production [13]. Due to its beautiful flowers, the plant is important to the horticultural sector and is extensively cultivated worldwide [14]. The potential value of *Streptocarpus* may be much higher than its exclusive use as an ornamental plant. As reported by Hârţa et al. [13], a variety of chemical compounds can be identified in its tissues, which can be applied in the food and pharmaceutical industries. For example, *Streptocarpus* flowers can be an important source of antioxidants. However, to date, the growth patterns of *Streptocarpus* with the function of any secondary metabolites have only been rarely considered [15].

The production of secondary metabolites in plants is influenced by environmental factors. They are produced in response to various forms of stress associated with growth conditions in order to perform specific physiological tasks [16]. One type of such interaction under in vitro culture conditions may be the quality of light [17]. Studies in recent years have conclusively shown that radiation is an important factor affecting plant secondary metabolites [1,18]. In practice, light stress is used to increase the production of secondary metabolites in the production of in vitro cultures of plants that are valuable from a medical point of view [19]. With light-emitting diode (LED) technology, it is possible to use a spectrum of light with a specific composition, which results in a diverse developmental response of plants multiplied under conditions of different light qualities [20–22].

The goal of the study was to investigate the effect of different qualities of light emitted by LEDs on the growth and development of *Streptocarpus* within in vitro cultures, as well as to identify the composition and content of biomolecules in the multiplied plants as their potential source. Determination of their antioxidant potential through free radical scavenging tests was also conducted. The effect of a wide range of wavelengths of ultraviolet, blue, green, yellow, red, far red and the effect of white LED light was investigated.

2. Materials and Methods

2.1. Plant Material

The plant material consisted of plants of *Streptocarpus* ×*hybridus* cultivar 'Paula' introduced into the in vitro culture collection of the Department of Ornamental Plants at the University of Agriculture in Kraków. The variety was bred by Rafał Firszt and is registered with The Gesneriad Society under the number IR191437. Leaf blade fragments, cut transversely with a fragment of the main nerve, were taken as the explant for the experiment.

2.2. Experiment Design and Culture Conditions

The experiment in adventitious buds in in vitro cultures of *Streptocarpus* was conducted in two stages: a pre-experiment and a main experiment were performed. During the pre-

experiment, the morphological response of *Streptocarpus* to 3 variants of Murashige and Skoog [23] (MS) medium of basic composition, enriched with 30 g/L sucrose, solidified with 0.5% BioAgar (Biocorp, Warsaw, Poland) was examined. The pH was adjusted to 5.7. The media differed in the levels of the growth regulators cytokinin 6-benzyladenine (BA) and auxin 1-naphthaleneacetic acid (NAA) (Duchefa Biochemie, Haarlem, the Netherlands). The combinations used were: 5 µM BA + 0.5 µM NAA, 10 µM BA + 1 µM NAA and medium without growth regulators as control (MS0). Cultures were maintained in 100 mL Erlenmeyer flasks for 6 weeks. The phytotron used a 16-h photoperiod, a temperature of 25/23 °C (day/night) and a relative humidity of 70%. The second factor studied, in addition to the different composition of the medium, was the different quality of light emitted by the LED. Biometric observations of the multiplied plant material were analyzed and the following were measured: the shoot regeneration rate, the average number of regenerating shoots, leaves and flower buds per explant, as well as the average height of the obtained plants.

Based on the pre-experiment, a growth medium was selected for the main experiment. The process of multiplication of *Streptocarpus* by the method of regeneration of adventitious buds was repeated on MS basal medium with the same composition as during the pre-experiment, but already with only one variant, enriched with 5 µM BA and 0.5 µM NAA. The conditions in the phytotron were the same as in the pre-experiment, and culture was conducted for 8 weeks. The aim of the main experiment was to test the content of polyphenols, flavonoids, free amino acids and reducing sugars in the multiplied plant tissues of *Streptocarpus*. Determination of antioxidant potential through free radical scavenging tests by DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) as well as FRAP (the ferric reducing antioxidant power) and reducing power. FTIR (Fourier-transform infrared spectroscopy) spectroscopic analyses were also carried out to examine the chemical composition of biological macromolecules. Photosynthetic pigments in the tissues in response to different qualities of LED light were also measured. The spectral qualities used were the same as in the pre-experiment.

2.3. Light Conditions

In this study, specially designed LED panels were used as a light source for the multiplied plants [24]. Light parameters were set using an LI-250A light meter with a Q 50,604 sensor (Li-COR) and BTS256 spectrometer (Gigahertz-Optik, Türkenfeld, Germany). The experiment involved 8 different LED spectra of various wavelengths: 100% blue of 430 nm (B); 100% red of 670 nm (R); mix of 70% red and 30% blue (RB); 50% RB and 50% ultraviolet of 400 nm (RBUV); 50% RB and 50% green of 528 nm (RBG); 50% RB and 50% yellow of 600 nm (RBY); 50% RB and 50% far red of 730 nm (RBfR) and white LED light composed of 33.3% warm white (2700 K), 33.3% neutral white (4500 K), and 33.3% cool white (5700 K) (WLed). The control was white light emitted by fluorescent lamps (Fl) (OSRAM LUMILUX Cool White L 36W/840). In each variant of different quality of light, the photosynthetic photon flux density (PPFD) was set at the same level of 40 µmol m^{-2} s^{-1}.

2.4. Plant Tissue Analyses

2.4.1. Extracts Preparation

Extracts were obtained by solvent extraction according to the method described by Grzeszczuk et al. [25]. A mixture of methanol (Merck, Darmstadt, Germany) and distilled water (7:3, *v*/*v*) was used as the solvent. Then, 0.5 g of lyophilizate was transferred to a 50 mL Falcon tube (Bionovo, Legnica, Poland) and mixed with 40 mL of solvent mixture. The solutions were then extracted for 30 min in an ultrasonic bath (Elmasonic S30H, Elma Schmidbauer GmbH, Singen, Germany) and centrifuged for 5 min at 5000× *g* (Centrifuge 5418 Eppendorf, Warsaw, Poland). The resulting extracts were filtered using 0.22 µm nylon membrane filters (Merck, Darmstadt, Germany). The plant extracts were made in triplicate and stored in a freezer at −20 °C.

2.4.2. Determination of Total Polyphenolic Content (TPC) and Total Flavonoid Content (TFC)

The TPC of the extracts was determined spectrophotometrically with the microplate reader (Synergy LX, Bio Tek, Winooski, VT, USA) using the Folin–Ciocalteu method as described elsewhere [26]. This method involves mixing 20 µL of extract with 150 µL of distilled water and 100 µL of Folin–Ciocalteu reagent. The solution was supplemented with 80 µL of saturated Na_2CO_3 (after 5 min). Finally, the mixture was incubated in the dark at 40 °C (30 min). Absorbance was measured at 765 nm, and TPC was expressed as milligrams of GAE (gallic acid equivalent) per g dry weight (DW). The TFC was determined by mixing 25 µL of extracts with 100 µL of H_2O and 7.5 µL of $NaNO_2$. After 5 min, the mixture was supplemented with 7.5 µL of 10% $AlCl_3$ solution. The mixture was kept for 6 min at room temperature before adding 25 µL of 1 M NaOH. Then, the mixture was diluted with 135 µL of distilled water. The final absorbance was read at 510 nm and expressed as milligrams of QE (quercetin equivalent) per g dry weight (DW).

2.4.3. Determination of Reducing Sugars Content (RSC)

The determination of RSC by DNS reagent was carried out according to the method described by Łopusiewicz et al. [27]. To prepare DNS reagent, a total of 10 g of DNS (3,5-dinitrosalicylic acid) was dissolved in 200 mL of distilled water by continuous stirring, followed by slowly adding 16 g of NaOH (dissolved first in 150 mL of distilled H_2O). The mixture was incubated at 50 °C with stirring to obtain a clear solution. Then, 403 g of potassium sodium tartrate tetrahydrate was added in small portions. The mixture was filtered using a paper filter and the volume was made up to 1000 mL with distilled water. One milliliter of extract was combined with 1 mL of 0.05 M acetic buffer (pH 4.8) and 3 mL of DNS reagent. The entire mixture was shaken vigorously and then incubated for 5 min in hot water (~96 °C). The tubes were cooled at room temperature. The calibration curve was prepared using known concentrations of glucose (0.1–100 mg/mL). Absorbance measurement was performed at 540 nm.

2.4.4. Determination of Total Content of Free Amino Acids

The total content of free amino acids was analyzed spectrophotometrically using Cd-ninhydrin reagent according to Łopusiewicz et al. [28]. To prepare the reagent, 0.8 g ninhydrin was dissolved in a mixture of 80 mL ethanol and 10 mL glacial acetic acid, followed by the addition of 1 g $CdCl_2$ dissolved in 1 mL of distilled water. To this end, 1 mL of the extract was combined with 2 mL of ninhydrin-Cd reagent in test tubes. The samples were vortexed, heated at 84 °C (5 min), and then cooled on ice. The mixture was transferred onto a plate, and the absorbance was measured at 507 nm. Results were calculated as mg of glycine (Gly) equivalent per 1 g sample (DW).

2.4.5. Fourier-Transform Infrared Spectroscopy (FTIR) Studies

FTIR analyses were performed using a FTIR spectrometer (Perkin Elmer 100 Spectrometer, Waltham, MA, USA). The lyophilized samples were directly placed on spectrometer crystal and scanned in the wavenumber range 650–4000 cm^{-1} (100 scans, resolution 1 cm^{-1}). The resulting FTIR spectra were normalized, corrected against the baseline and analyzed using SPECTRUM software (v10, Perkin Elmer, Waltham, MA, USA).

2.4.6. Determination of DPPH and ABTS Scavenging Activity

The ability test of DPPH and ABTS radical scavenging was measured according to the method described by Łopusiewicz et al. [27]. A total of 0.5 mL of methanolic DPPH solution (0.01 mM) was mixed with 0.5 mL of extract in a ratio of 1:1. Samples were incubated at 22 ± 2 °C for 30 min, in the absence of light. Finally, absorbance was measured at 517 nm. To determine ABTS radical scavenging activity 1.5 mL of ABTS solution was mixed with 25 µL of extract and incubated at room temperature for 10 min in the dark. The final absorbance was measured at 734 nm and expressed as µmol TE/g DW (TE—Trolox equivalent).

2.4.7. The Ferric Reducing Antioxidant Power (FRAP) Assay

The determination of the reduction of iron ions to ions of a lower oxidation state (FRAP) by the antioxidant was measured based on the methodology described previously [29]. FRAP reagent was prepared by mixing 2.5 mL of TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) in 40 mM HCl, 2.5 mL $FeCl_3$, and 25 mL of acetate buffer pH = 3.6 (300 mM). Then, 20 µL of the extract in triplicate was transferred to a 96-well TC plate by adding 280 µL of FRAP reagent, and the plate was gently shaken for 10 s. The absorbance was read at 595 nm. Results are expressed as mg of ascorbic acid equivalent per g dry sample weight (mg AAE/g DW). Each analysis was performed three times in triplicate of each sample, and the obtained results are the mean value.

2.4.8. Reducing Power (RP) Determination

The reducing power of the methanolic extracts was measured according to the method of Salachna et al. [30]. A total of 100 µL of extract was introduced into 1.5 mL Eppendorf tubes, 250 of phosphate buffer (0.2 M, pH 6.6) and 250 µL of 1% potassium hexacyanoferrate. Samples were incubated at 50 °C for 20 min before 250 µL of trichloroacetic acid was added. Samples were centrifuged at 3000× *g* for 10 min (Centrifuge 5418 Eppendorf, Warsaw, Poland). Then, 500 µL of the solution was taken into Eppendorf tubes (1.5 mL), and 0.5 mL of H_2O and 0.1 mL of $FeCl_3$ (1%) were added. Finally, the absorbance was measured at 700 nm.

2.5. Statistical Analysis

The results collected during the experiment underwent statistical analysis using a two-factor ANOVA analysis of variance for the pre-experiment stage of the experiment to test the effects of light spectrum conditions and medium composition on biometric parameters of *Streptocarpus* development. The effects of the treatments and the interactions between them were estimated at two levels of significance: $p \leq 0.05$ and $p \leq 0.01$.

For the main experiment, a one-way ANOVA analysis of variance was used to determine the effect of different light quality on the studied parameters. In the conducted laboratory analysis, Duncan's post hoc multiple range test was used in both stages of the experiment to separate significantly different means and provide homogeneous groups for the measures (at $p \leq 0.05$). Also, a correlation analysis was calculated to determine the strength of relationships between the polyphenols and flavonoids and their antioxidant properties regarding the different methods used; and the correlations between the identified compounds' content. A map of the correlations was created. Statistica 13.3 software was used to carry out the aforementioned analyses (StatSoft, TIBCO Software Inc., Palo Alto, CA, USA).

3. Results and Discussion

3.1. Pre-Experiment

The goal of the first stage of the research conducted was to select the optimal composition of the nutrient solution for further stages of the experiment. During the arrival organogenesis of the *Streptocarpus* plant, the development of adventitious buds was observed, which then developed into adventitious shoots (Figure 1B). Pre-experiment analyses showed that regardless of the light quality used, the best medium for obtaining good quality multiplied plants was Murashige and Skoog (MS) medium supplemented with 5 µM of 6-benzyladenine (BA) and 0.5 µM of 1-naphthaleneacetic acid (NAA) (Table 1). The use of a supplement of plant growth regulators (PGRs) to the medium was necessary because those plants multiplied on the medium without them (MS0) had statistically significantly reduced all biometric parameters compared to the media containing BA and NAA in the composition. In addition, on the media with PGRs, flower bud formation was observed in the multiplied plants, especially under the influence of light with far-red in the spectrum (Figure 1D).

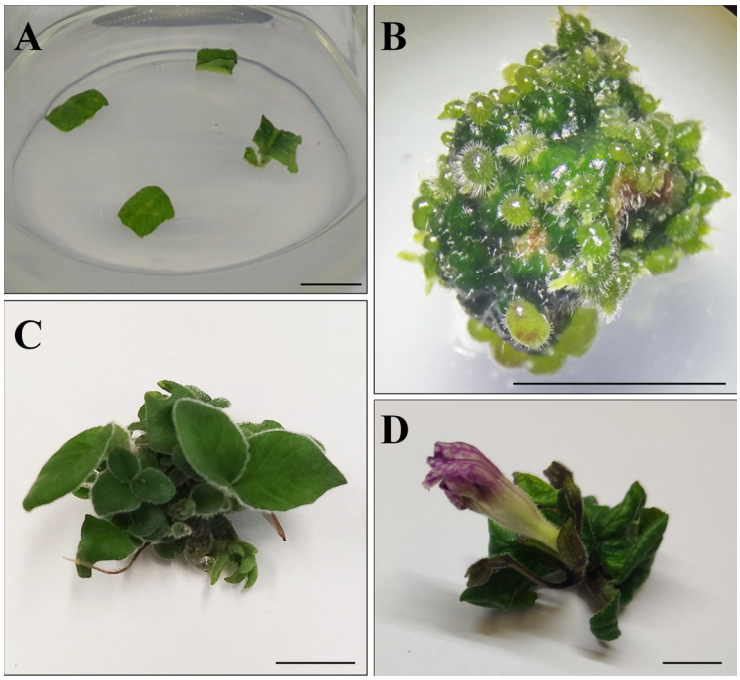

Figure 1. *Streptocarpus* ×*hybridus* organogenesis: (**A**) initial explants, (**B**) adventitious bud formation, (**C**) plants multiplied on medium containing 5 µM BA and 0.5 µM NAA, (**D**) flower buds obtained from plants grown under the far red LED light influence. Bar—1 cm.

Table 1. Biometrical observations of *Streptocarpus* ×*hybridus* multiplicated in vitro on MS. medium with different BA and NAA content (µM), regardless of the used light quality.

PGR		Multiplication Rate	Shoots No.	Plant Height (mm)	Leaves No.	Flower Buds No.
BA	NAA					
0	0	0.38 ± 0.30 a	3.61 ± 4.28 a	1.66 ± 1.74 a	0.72 ± 0.62 a	0.00 ± 0.00 a
5	0.5	0.92 ± 0.18 b	17.33 ± 4.10 b	4.96 ± 1.72 c	1.98 ± 0.61 c	0.26 ± 0.53 b
10	1	0.86 ± 0.20 b	17.75 ± 4.23 b	4.31 ± 1.34 b	1.71 ± 0.52 b	0.63 ± 0.84 c

Means ± standard deviations within a column followed by the same letter are not significantly different according to Duncan's multiple range test at $p \leq 0.05$ (different letters indicate significantly different means).

The process of organogenesis is dependent on the use of plant growth regulators, as well as on the individual ability of tissues to respond to introduced hormonal changes during culture [31]. The use of properly tailored growth regulators in in vitro culture is crucial for the efficiency of the process and the proper development of plants, as well as enabling it to be targeted [32,33]. In our experiment, *Streptocarpus* growing on the medium with lower PGR content was statistically higher (by an average of 3.3 mm than on MS0), and had more leaves (by an average of 1.26 leaves more than on MS0) also compared to *Streptocarpus* growing on the medium with higher PGR content (10 µM BA and 1 µM NAA). Parameters such as the shoot regeneration rate and the average number of shoots were at the same statistical level in both variants of the medium with PGRs. Therefore, it is economically reasonable to choose a medium with a lower and sufficient content of PGRs, and a main experiment was carried out on such a medium composition, in which a number of laboratory analyses were performed and focused on the effect of different light quality on the development and growth of *Streptocarpus*. Indeed, the light quality factor is crucial in the life of the plant by regulating the biochemical and morphological and even molecular processes that are at the basis of its growth and development [34].

3.2. Main Experiment

3.2.1. Biometrical Observations

The different quality of light received by *Streptocarpus* plants during in vitro growth, on growth medium enriched with 5 µM BA and 0.5 µM NAA, elicited differential responses at the biometric level (Figure 2). The shoot regeneration coefficient was at a high level and ranged on average from 0.83 (B and RBfR LED lights) to 1.00 (R, RB, RBG, RBY and WLed LED lights and Fl light); however, UV light had a significant limiting effect on the growth of the plants tested (regeneration coefficient at an average level of 0.58). This light also had a limiting effect on the mean number of shoots (average 13.97) and leaves (average 1.07) obtained, as well as plant height (2.33 mm). The highest number of shoots was produced by the *Streptocarpus* explants grown under the influence of mixed LED light: red and blue in the ratio of 7:3 (RB—average 21.00) and white light (WLed—average 23.77)—almost 1.5-fold more compared to the white light of the control fluorescent lamp (Fl—average 15.77). The highest plants were obtained under the addition of yellow LED light in the spectrum (RBY—average 6.90 mm), which also had a stimulating effect on the number of forming leaves (average 3.00). A shoot height at a statistically high level was also obtained under the influence of red light (R—5.83), red with blue (RB—5.33), the addition of green (RBG—5.47) LED and under fluorescence (Fl—6.53).

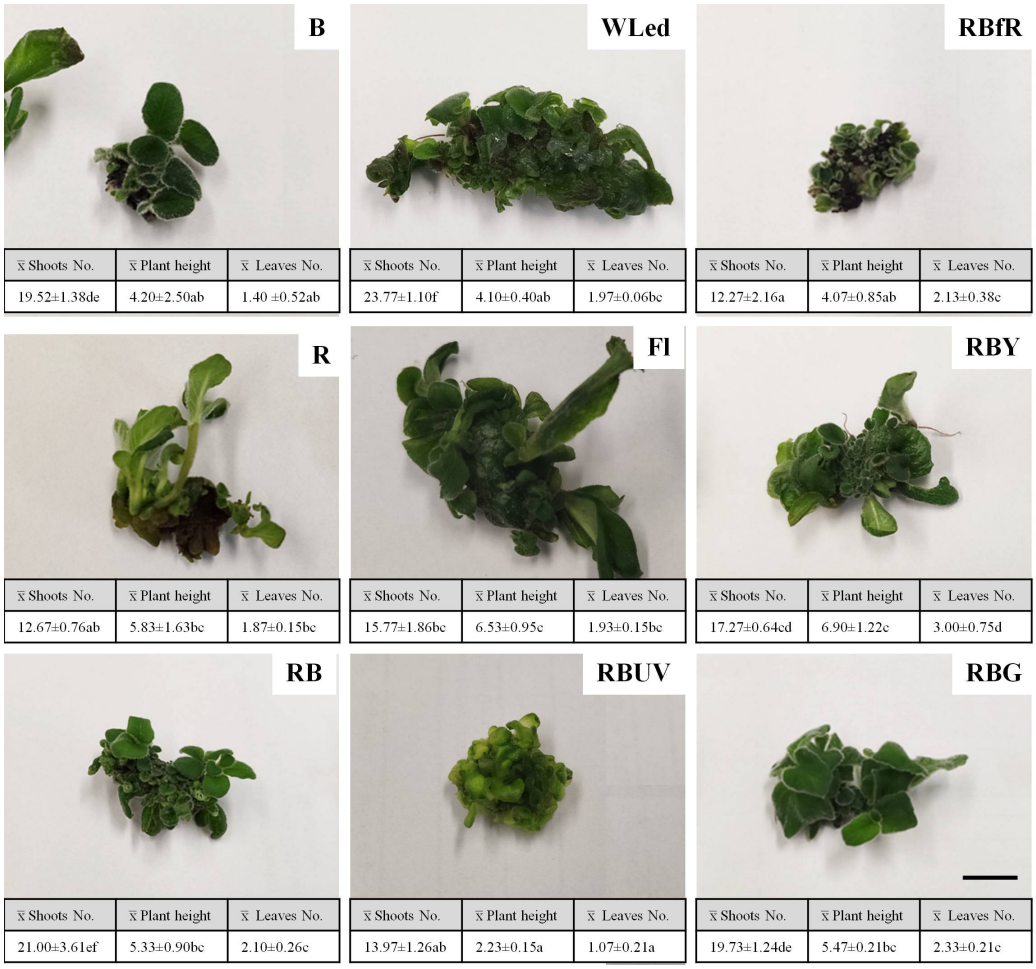

Figure 2. *Streptocarpus* ×*hybridus* multiplicated in vitro on MS medium with 5 µM BA and 0.5 µM NAA (x̄ Plant height in mm) under different LED light conditions: B–100% blue, R–100% red, RB–red and blue in 7:3 proportion, RBUV–50% RB + 50% ultra violet, RBG–50% RB + 50% green, RBY–50% RB + 50% yellow, RBfR–50% RB + 50% far red, WLed–white and Fl–control; bar–1 cm. Means ± standard deviations within a column followed by the same letter are not significantly different according to Duncan's multiple range test at $p \leq 0.05$ (different letters indicate significantly different means).

In the studies carried out to date on plant in vitro cultures of *Streptocarpus* by various authors, there have been no reported studies that have examined the effect of different quality of LED light on its developmental parameters [13,35–37]. Moreover, as for the influence of light alone on the biometrics of proliferated plants of different species, the majority of analyses carried out by various authors concern the effect of red and blue LED light and their combinations in various proportions ([21,38–42] and many others). The response of plants to different qualities of light is highly specific to both species and variety, and even the type of explant. The influence of the other wavelength ranges of the spectrum is already beginning to be studied by scientists. The growth-stimulating effect of an addition in the yellow light wavelength spectrum observed in our experiment (increased number of leaves and height of multiplied shoots) is also confirmed by the work of other authors. Pałka et al. [43] obtained the formation of more *Lilium candidum* L. bulbs in vitro on the same light quality. The addition of yellow wavelength to the red and blue spectrum also appeared to promote the vegetative growth of *Solanum tuberosum* seedlings in vitro [44], although in a study by Yang et al. [45] on *Nicotiana tabacum* L. grown in climate chambers, yellow light showed an inhibitory effect on plant growth. In our experiment, the addition of green light in the spectrum also influenced the production of tall *Streptocarpus* shoots, and additionally in large numbers. Dougher and Bugbee [46] highlighted the similarities in the effects of green light and yellow light on some plant mechanisms of physiological responses. However, this may be due to the fact that researchers usually classify wavelengths of 500–600 nm as green light, which further limits observations of the characteristics of yellow light (580–600 nm) and makes them not yet well understood [44]. In a study by Li et al. [44], green light enhanced root regeneration and elongation in *L. candidum*. In their experiments, Miler et al. [47] examined the effects of different LED wavelength ranges on the growth and development of various ornamental species, demonstrating a positive effect of green/yellow wavelengths (500–600 nm) on the propagation coefficient of *Gerbera jamesonii* and *Lamprocapnos spectabilis* in vitro. They also confirmed that the effect of light quality, for instance, on shoot length, number of leaves, callus regeneration, and chlorophyll biosynthesis is species-dependent. In our research on *Streptocarpus*, the use of wavelengths from the far-red or ultraviolet range (RBfR, RBUV) in the RB LED mixed spectrum had an inhibitory effect on the growth and development of the plant—fewer shoots were obtained, which were additionally low, and in the case of light with UV addition, there were also fewer leaves. There have also been reported cases of degrading effects of UV light, especially when applied in excessive amounts, on plant growth and development, e.g., the restriction of growth of gametophytes of *Cyathea delgadii* in vitro [48], decrease in cell proliferation and even death of callus of *Fagonia indica* [49], or tissue damage and reduction in the number of shoots of *Phylanthus tenellus* [50]. In general, UV light is considered harmful by plants and they seek to protect themselves from its effects. Thus, applying a small amount of it during micropropagation can cause some morphogenetic effects such as thickening of leaves, or elongation of the stem and leaves. Moreover, it strongly affects the growth aspects of generative organs in plants which can include an increased number of flowers, or their diameter [51]. The presence of far-red wavelengths in the RB mixed light spectrum also has a stimulating effect on flowering [52]. The addition of far red can also increase the number of stomata (*Chrysanthemum*—Kim et al. [41]), elongate stems (*Ficus benjamina*—Werbrouck et al. [53]), or increase propagation coefficient [47]. Studies are also increasingly comparing the effect of white light emitted by LED compared to fluorescent lamps due to the additional economic aspect. These studies often confirm the effectiveness of white LED lights on micropropagation, such as the bulb formation of *L. candidum* [43] or shoot induction of *Vanilla planifolia* [21]. In our study, *Streptocarpus* formed a statistically high number of shoots under this light (WLed), but they were low.

3.2.2. The Changes in Biomolecule Level Content and Antioxidant Activity

Modern advances in science and technology enable increasingly accurate measurements of the content of specific compounds in various plant species. However, despite the

existence of a number of commonly used methods for determining antioxidant activity (e.g., the DPPH, ABTS or FRAP tests), none alone provides a sufficiently effective assessment of the activity of all compounds present in a given plant extract. Therefore, it is important to select a method appropriately, taking into account its advantages and disadvantages, and to use more than one of them, in order to assess the real potential of plant samples [30,54]. In the experiment conducted, analyses were performed on the antioxidant activity of the obtained plant extracts of *Streptocarpus* multiplied under different qualities of light under in vitro culture conditions. Four different methods were used: the DPPH and ABTS radical scavenging tests, the ferric reducing antioxidant power (FRAP) and reducing power determination (Table 2). They provided different results, so the antioxidant potential of the plant samples tested could depend both on the light spectrum, but also on the analytical method used, the extraction time and the type of solvent and its concentration. The highest level of antioxidant activity of *Streptocarpus* plant samples was shown using the reducing power determination method (activity at an average level of 65 µmol TE/gDW). Statistical differences due to the use of different light quality were not high, but the highest activity was obtained in plant samples taken from growing material grown in the RBY LED light spectrum (72.2 µmol TE/gDW). This method involves determining the antioxidant potential in the reaction mixture based on the reduction of ferrous ions (Fe^{3+} to Fe^{2+}). On the other hand, determination of DPPH and ABTS methods are based on the scavenging of free, stable radicals. However, the ABTS assay is routinely used to determine the total antioxidant activity of both hydrophobic and hydrophilic antioxidant samples [30]. In this case, the results of our analysis indicate the highest antioxidant properties in samples from plants grown under white LED light (WLed) (average 11.55 µmol TE/gDW), but this activity was also statistically high when yellow light and UV light were added to the RB LED spectrum (RBY—8.27 µmol TE/gDW and RBUV—8.70 µmol TE/gDW). LED light narrowed to only the blue or red spectrum significantly reduced these properties (B—1.75 µmol TE/gDW, R—2.61 µmol TE/gDW). The DPPH method also showed a stimulating effect of antioxidant activity in plant samples grown under RBUV and RBY light, but the best result in this method was provided by RBfR light. Also, this spectrum resulted in the highest rating in the FRAP assay. The DPPH method mainly measures the antioxidant potential of lyophilic compounds due to the solubility of the compound only in organic solvents, while the FRAP assay is based on determining the reducing power of iron ions [55–57], similar to the RP determination method where the reduction of a ferric-tripyridyltriazine complex to its ferrous form is measured. A FRAP assay is more complex and time-consuming but also more sensitive than the RP method. It can be used to determine the total antioxidant capacity of a sample, and the RP method does not provide such information [58,59]. Both methods also showed a positive effect of red LED light (R) and, in the case of the FRAP assay, also white light (WLed and Fl) on the increase in antioxidant activity. Very importantly, both our study and the literature note that regardless of the type of antioxidant activity assay performed, it is statistically significantly reduced when treated with white light from fluorescent lamps (Fl) compared to LED light [60]. LED light increases the content of biomolecules which leads to a significant improvement in antioxidant properties [61] and free radical scavenging potential [62].

From the prepared *Streptocarpus* extracts, some of the primary and secondary metabolites which may have antioxidant properties were determined and extracted. They belonging to four different groups: reducing sugars (reducing sugar content—RSC) and free amino acids—primary metabolites; as well as flavonoids (total flavonoid content—TFC was determined) and polyphenols (total polyphenolic content—TPC)—secondary metabolites. The content level of the identified substances is shown in Figure 3 and Table S1, and the FTIR spectra of *Streptocarpus* grown under different qualities of light are presented in Figure 4. The data in Figure 3 and Table S1 demonstrate that treatment with different light quality significantly influenced the content of biomolecules. The highest level of polyphenols was noticed for samples RBG (41.87 ± 0.63 mg GAE/g), WLed (41.71 ± 4.05 mg GAE/g), and RBfR (41.13 ± 4.44 mg GAE/g), whereas the lowest was

found in samples RBY and R (29.71 ± 3.24 mg GAE/g and 30.85 ± 0.42 mg GAE/g, respectively). On the other hand, the highest content of flavonoids was found in *Streptocarpus* grown in blue light (B) and fluorescent light (Fl) (649.78 ± 48.02 mg QE/g and 610.02 ± 35.36 mg QE/g, respectively), whereas the lowest content was noticed for sample RBUV and RBY (493.45 ± 43.81 mg QE/g and 485.56 ± 16.96 mg QE/g, respectively). The lowest free amino acids as well as reducing sugar contents were found in sample RBfR (3.35 ± 0.15 mg GLY/g and 253.86 ± 12.48 mg/g, respectively. Phenolic compounds are secondary metabolites involved in plant adaptation processes under stress conditions, such as injury, infection or UV exposure [63], so the quality of light applied during plant growth in vitro can be linked to their content in plant tissue sample. Moreover, it can be assumed that the light quality can upregulate the expression level of genes involved in metabolite biosynthesis and induce the activity of plant defense enzymes, resulting in increased accumulation of secondary metabolites.

Table 2. Antioxidant activity analyses of extracts obtained from *Streptocarpus* plants multiplied in vitro under the influence of different light quality.

Light Quality	DPPH (µmol TE/gDW)	ABTS (µmol TE/gDW)	FRAP (mg AAC/gDW)	Fe^{3+} Reducing Power (µmol TE/gDW)
B	2.24 ± 0.06 a	1.75 ± 0.48 a	8.15 ± 1.11 b	65.82 ± 1.63 ab
R	3.04 ± 0.04 d	2.61 ± 0.97 a	9.19 ± 0.41 bc	63.41 ± 1.97 ab
RB	2.41 ± 0.01 b	6.73 ± 0.81 c	8.27 ± 0.49 b	60.80 ± 6.44 a
RBUV	2.73 ± 0.10 c	8.70 ± 0.11 d	8.75 ± 1.80 b	65.59 ± 5.32 ab
RBG	2.40 ± 0.05 b	4.08 ± 0.54 b	8.40 ± 0.89 b	60.81 ± 4.90 a
RBY	2.82 ± 0.04 c	8.27 ± 0.54 d	3.41 ± 0.93 a	72.20 ± 6.35 b
RBfR	3.06 ± 0.10 d	4.80 ± 0.27 b	10.66 ± 0.51 c	63.59 ± 4.43 ab
WLed	2.34 ± 0.05 ab	11.55 ± 0.70 e	9.43 ± 0.82 bc	62.49 ± 5.87 a
Fl	2.28 ± 0.02 a	4.30 ± 0.19 b	9.01 ± 0.53 bc	66.56 ± 2.77 ab

B–100% blue LED of 430 nm; R–100% red LED of 670 nm; RB–combination of red (70%) and blue (30%) LED; RBUV–50% RB and 50% ultraviolet LED of 400 nm; RBG–50% RB and 50% green LED of 528 nm; RBY—50% RB and 50% yellow of 600 nm; RBfR—50% RB and 50% far red of 730 nm; WLed—white LED light composed of 33.3% warm white (2700 K), 33.3% neutral white (4500 K), and 33.3% cool white (5700 K); Fl—control, fluorescence lamps. Means ± standard deviations (n = 3) within a column followed by the same letter are not significantly different according to Duncan's multiple range test at $p \leq 0.05$ (different letters indicate significantly different means).

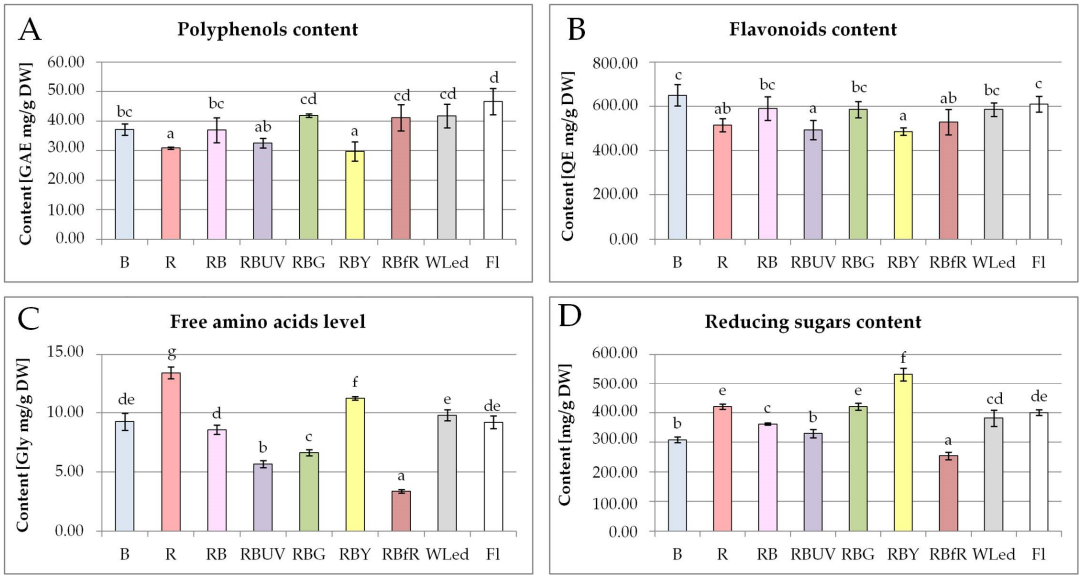

Figure 3. The level content of identified substances: (**A**) polyphenols, (**B**) flavonoids, (**C**) free amino acids and (**D**) reducing sugars. Used light spectra during multiplication: B—100% blue LED of 430 nm;

R—100% red LED of 670 nm; RB—combination of red (70%) and blue (30%) LED; RBUV—50% RB and 50% ultraviolet LED of 400 nm; RBG—50% RB and 50% green LED of 528 nm; RBY—50% RB and 50% yellow of 600 nm; RBfR—50% RB and 50% far red of 730 nm; WLed—white LED light composed of 33.3% warm white (2700 K), 33.3% neutral white (4500 K), and 33.3% cool white (5700 K); Fl—control, fluorescence lamps. Means ± standard deviations (n = 3) within a chart column followed by the same letter are not significantly different according to Duncan's multiple range test at $p \leq 0.05$ (different letters indicate significantly different means).

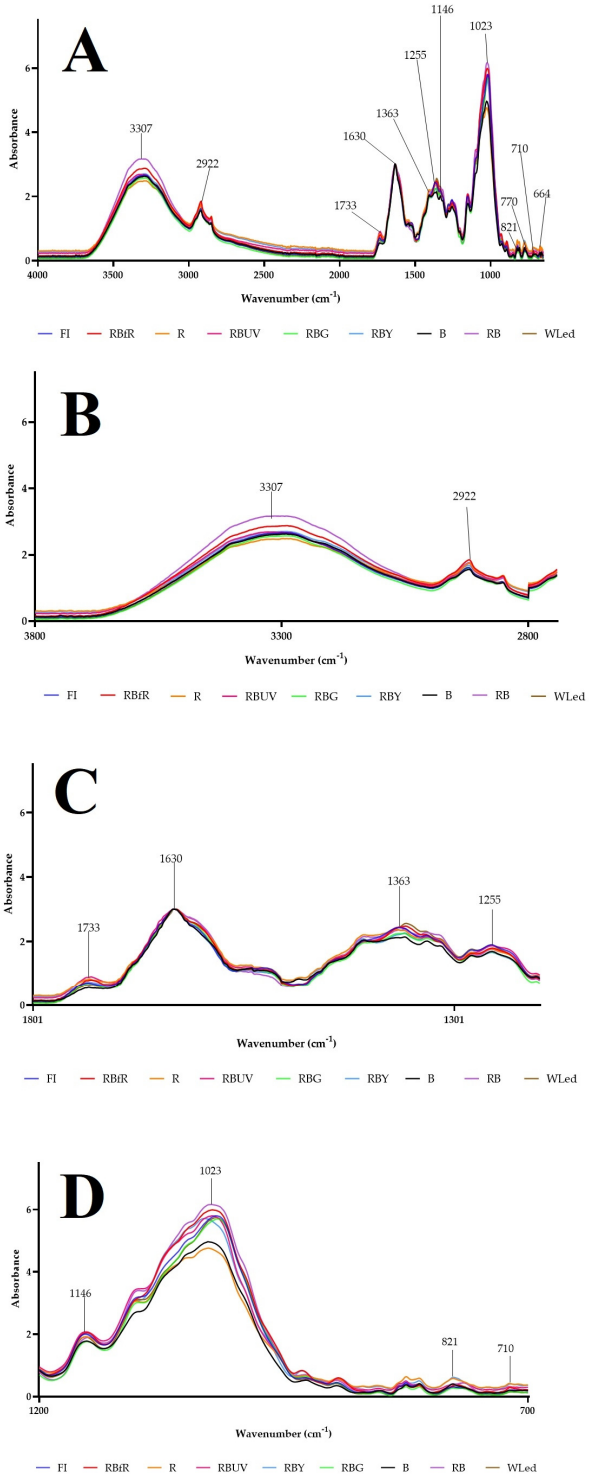

Figure 4. Fourier-transform infra-red spectroscopy (FTIR) spectra of *Streptocarpus* under different light quality (**A**) whole FTIR spectrum (4000–700 cm^{-1}); (**B**) in the range of 3600–2400 cm^{-1} (–OH, –NH, $–CH_2$

and –CH_3 groups); (**C**) in the range of 1800–1200 cm^{-1} (proteins); (**D**) in the range of 1250–700 cm^{-1} (polysaccharides). LED light conditions: B—100% blue, R—100% red, RB—red and blue in 7:3 proportion, RBUV—50% RB + 50% ultra violet, RBG—50% RB + 50% green, RBY—50% RB + 50% yellow, RBfR—50% RB + 50% far red, WLed—white and Fl—control.

FTIR spectroscopy can provide comprehensive data on macromolecule composition in plants [26]. This technique enables the detection of specific functional groups of macromolecules in the sample and the monitoring of alterations in different chemical bonds. The FTIR spectra of plants exposed to different light quality are presented in Figure 4. Characteristic vibrations at 3600–2400 cm^{-1} (attributed to -OH, -NH_2, -CH_2, -CH_3 groups), 1800–1200 cm^{-1} (proteins) and 1250–700 cm^{-1} (polysaccharides) were observed [26,64]. As can be seen, the quality of light influenced the macromolecule content in *Streptocarpus* samples. Differences in intensity were detected particularly for bands denoting -OH groups (approximately at 3298 cm^{-1}), that in a previous study were linked with a higher quantity of polyphenols and greater antioxidant activity [26]. Interestingly, the highest band intensity was observed for sample RB, that did not show the highest antioxidant activity and polyphenols, flavonoids, free amino acids and reducing sugar content. However, hydroxyl groups can also be present in other macromolecules such as proteins or complex sugar (e.g., lignin, cellulose), which are part of plant cell walls. In fact, some differences and shifts were observed in the amide region (1800–1200 cm^{-1}), suggesting different plant protein composition, as also indicated by the varying content of free amino acids (Figure 3, Table S1). Similarly, the highest band intensity at 1023 cm^{-1} was noticed for sample RB. This region can be assigned to a symmetric stretching mode of C-O-C in the glycosidic bond in cellulose as well as C-C ring modes in carbohydrates [64]. Some shifts to 1015 cm^{-1} were observed for the samples RBG, WLed, RBY, that suggest some alternations in the composition of cell wall carbohydrates. Thus, to comprehend better the effects of light quality on plant composition and structure, it is necessary to support the results of macromolecule FTIR analyses obtained by the spectroscopic methods by further in-depth biochemical and molecular studies such as HPLC (high-performance liquid chromatography) and SDS-PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) [26].

Many studies have proved that the ability to scavenge free radicals is strongly correlated with the total flavonoid and polyphenol content [65–67]. The research of Salachna et al. [30] confirmed this conclusion, as the authors found a very strong correlation between the total polyphenol content and the antioxidant activity. Our research has confirmed this relationship in part (Table 3). We observed a statistically significant positive correlation between FRAP assay, and total polyphenolic content (TPC) (r = 0.47). Such a correlation was also observed for RP and the content of reducing sugar (r = 0.41). RBfR light stimulates an increase in TPC content and thus an increase in antioxidant activity according to the FRAP method. In a study of Manivannan et al. [60], the highest total antioxidant capacity of *Rehmannia glutinosa* extracts was observed for blue LED, followed by red LED, which was correlated precisely with higher total polyphenolic content in plants propagated under these lights. The literature indicates a link between the enhancement of antioxidant properties and increased phenolic content, as they have structural properties to scavenge free radicals resulting from the presence of hydroxyl groups, and thus to prevent oxidative damage [68]. However, the data obtained as a result of our study are not so clear. The analyses conducted showed a negative correlation between DPPH scavenging activity analysis and identified polyphenolic content (TPC and TFC) (average r = −0.62), as well as between FRAP assay and reducing sugar content (RSC) (r = −0.69). Antioxidant activity as measured by DPPH increased under LED light: R, RBUV and RBY, which simultaneously reduced the content of TPC and TFC, and in the case of TFC, also the light of RBfR. However, the differences in the antioxidant capacity and radical scavenging potential of plant extracts after treatment with light of different qualities can also be attributed to the nature of individual tests. The authors also suggest a relationship in which an increase in reactive

oxygen species (ROS) production triggers antioxidant enzyme activity to prevent cellular damage from light-induced oxidative stress [60,69,70].

Table 3. Correlation between the identified groups of substances and their antioxidant activity depending on the method used and connections with the Streptocarpus morphogenetic response.

Identified Groups of Substances:	r>=	−1	−0.8	−0.6	−0.4	−0.2	0	0.2	0.4	0.6	0.8	1
	Substances				Antioxidant Activity Analysis				Morphogenetic Response			
	Poly-phenols	Flavo-noids	Free Amino Acids	Reducing Sugars	DPPH	FRAP	Fe3+ RP	ABTS	Multip-lication rate	Shoots No.	Plant Height	Leaves No.
Polyphenols	1.000000	**0.547244**	−0.362720	−0.293390	**−0.494739**	**0.466165**	−0.093278	−0.064599	0.297957	0.287706	0.035849	0.039762
Flavonoids	**0.547244**	1.000000	0.040343	−0.211401	**−0.742946**	0.262876	−0.029212	−0.295366	0.295423	**0.419967**	0.181231	−0.148513
Free amino acids	−0.362720	0.040343	1.000000	**0.669383**	−0.049907	−0.372783	0.219875	−0.078758	**0.460682**	0.154611	**0.504087**	0.217701
Reducing sugar	−0.293390	−0.211401	**0.669383**	1.000000	0.008416	**−0.690694**	**0.405933**	0.190531	**0.469714**	0.174554	**0.591941**	**0.608063**

Colors show the strength of correlation. Red font indicates the presence of correlation; $p \leq 0.05$.

Antioxidant activity can also be related to the content of reducing sugar. Sugars are essential in the plant nutritional, but also play a signaling and regulatory role by influencing the expression of genes related to growth and development, and the stress response or disease resistance, among others [71]. Reducing sugars play an important role in central metabolic pathways and help produce secondary metabolites that enhance the healing potential of plants [72]. They may also act as ROS scavengers [73,74]. Moreover, similar mechanisms can counteract ROS-related diseases in the human body. Natural antioxidants extracted from plants are becoming increasingly popular as functional food ingredients [75]. In our study, the content of reducing sugar was particularly stimulated by RBY light. In the case of FRAP analysis, the correlation was negative: the highest antioxidant activity was observed on RBfR light and occurred at a reduced content of reducing sugar. On the other hand, measuring with the RP method, the correlation was positive. Increased antioxidant activity occurs with the increased accumulation of reducing sugar, so in plants multiplied under RBY light. Jeong et al. [76] also noted that LED light affects the accumulation of reducing sugar in grape (*V. berlandieri* × *V. riparia*) in vitro cultures. RB light in a 1:1 proportion increased the accumulation compared to B LED and Fl lights. The light also had a positive effect in a study of Gangadhar et al. [77] investigating bell pepper (*Capsicum annuum*). In our study, RB light increased the accumulation of reducing sugar (average 362 mg/gDW), but to a lesser extent than the control Fl light (average 400 mg/gDW). RB light enriched with the yellow spectrum (600 nm) stimulated this accumulation more than 30% more (average 533 mg/gDW) compared to RB LED light.

Analyzing the relationships between the identified substances, it was also noted that as the content of reducing sugar in the tissues increases, the content of free amino acids also increases (the content of both compounds stimulated by RBY) (Figure 3, Tables S1 and 3). Amino acids and peptides are compounds that can act as antioxidants by breaking down peroxides, scavenging singlet oxygen or chelating metals [78,79]. Studies indicate that their content correlates with total phenolic and flavonoid amounts and radical scavenging capacity. Thus, they can be used as an indicator of antioxidant properties [30]. A study conducted by Gao et al. [80] using different LED light showed that the level of free amino acids, as well as the antioxidant capacity of *Allium fistulosum* L., was significantly higher under blue-white light, followed by a combination of white and red-white LED light, while a combination of green-white and yellow-white light significantly reduced these values. In ex vitro studies conducted on *Lactuca sativa* L. cultivars on the content of primary metabolites (including free amino acids), a positive effect of additional spectrum wavelengths was noted compared to the applied LED basic lighting [81]. In particular, the addition of UV-A and green light influenced the increased accumulation of identified amino acids.

Biometric parameters of multiplied *Streptocarpus* plants are also correlated with the content of identified compounds in their tissues (Table 3). A positive statistically significant correlation was detected for the content of free amino acids and reducing sugar, and the regeneration rate (mean r = 0.47) and the height of the obtained adventitious shoots (mean

r = 0.55)—all these traits were stimulated by RBY LED light and R. The number of shoots was positively correlated with the content of TFCs (r = 0.42) (white LED light and RB light), while the number of leaves was correlated with the content of reducing sugars (r = 0.61) (RBY LED light).

4. Conclusions

The scientific literature has extensively studied and proved that light quality affects the biometric growth parameters of plants multiplied in in vitro cultures. This was also confirmed by our experiment carried out using the adventitious shoots of *Streptocarpus*. For the best morphological quality of multiplied plants, with high shoots and a large number of leaves, the use of RBY light is recommended. The application of white LED light also provided favorable results, which had a stimulating effect on the highest number of shoots obtained. These data may be of interest for producers of this ornamental plant in selecting the best light conditions to obtain plants with desired morphometric characteristics. The light spectrum also influences the content of polyphenols, flavonoids, free amino acids and reducing sugars produced by these plant. Moreover, it also affects the resulting antioxidant properties. In our experiment, white light from a fluorescent lamp (Fl) reduced antioxidant properties compared to different qualities of LED light, regardless of the method used. The most recommended spectrum in this aspect was RBfR, WLed, RBG and R light. These lights (except for R) stimulated the polyphenol content of the tested *Streptocarpus* extracts. RBY and R light, on the other hand, influenced the highest content of free amino acids and reducing sugars. RBY, at a medium statistical level, also positively affected the antioxidant properties of the plant samples according to all the methods tested for their determination, except for FRAP analysis. In this aspect, it is suggested to conduct further research on this ornamental plant, which may be a potential source of metabolites with health-promoting properties. It will be necessary to investigate the actual bioactivities and activity of identified groups of biomolecules.

The plant response to different quality light is highly specific depending on the examined aspect. Therefore it is not possible to recommend one ideal light composition for a specific species growing requirements. However, it is possible to influence and targeted process of plant development on many levels.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13123009/s1, Table S1: the content of identified substances: polyphenols, flavonoids, free amino acids and reducing sugars (mg/gDW).

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Abbreviations and Acronyms

ABTS	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
AAE	Ascorbic Acid Equivalent
B	100% blue light
BA	6-benzyladenine
DNS	3,5-dinitrosalicylic acid
DPPH2,2-diphenyl-1-picrylhydrazyl	
DW	Dry Weight
Fl	Fluorescent Lamp Light
FRAP	Ferric Reducing Antioxidant Power
FTIR	Fourier-Transform Infrared Spectroscopy
GAE	Gallic Acid Equivalent
Gly	Glycine
HPLC	High-Performance Liquid Chromatography
LED	Light-emitting diode
MS	Murashige and Skoog medium
NAA	1-naphthaleneacetic acid
PGR	Plant growth regulator
R	100% red light
RB	Red and blue light in 7:3 proportion
RBfR	50% of RB light + 50% of far red light
RBG	50% of RB light + 50% of green light
RBUV	50% of RB light + 50% of ultra violet light
RBY	50% of RB light + 50% of yellow light
RP	Reducing Power
RSC	Reducing Sugars Content
TE	Trolox Equivalent
TFC	Total Flavonoid Content
TPC	Total Polyphenolics Content
TPTZ	2,4,6-tris(2-pyridyl)-s-triazine
WLed	White LED light

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