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Interaction of Culture Medium and Artificial Light Type on Pigmentation of Micro-Propagated *Opuntia* Plants

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Abstract: *Opuntia* is recognized economically as a significant crop for its nutritional, functional, and therapeutic properties and its potential in the pharmaceutical, cosmetic, and bioenergy industries. *Opuntia* is conventionally propagated by seed or vegetative propagation via rooted scions or grafting. However, multiplication procedures are insufficient for extensive spreading. One of the alternative techniques is in vitro. For this reason, the objective was to evaluate the growth of different components combined with light conditions to induce callus, embryogenesis, and the possible synthesis of a component in the genus *Opuntia*. The existence of genes involved in pigment synthesis in genotypes of different fruit colors was complementarily analyzed. In this study, we used different combinations of fructose (2 g/L) with prickly pear juice (2 mL/L), mannitol (4 g/L), silver nitrate ($AgNO_3$: 1 mg/L), 2,4-dichlorophenoxyacetic acid (2,4-D: 2 mg/L), and indole-3-acetic acid (IAA: 1 g/L), with white, blue, and red LEDs (light-emitting diodes) and laser beams. These explants yielded sufficient contents of simple phenols, gallic acid equivalents (GAE: 2283.30 ± 6.29 mg/100 g), and coumaric (2155.0 ± 35.0 mg/100 g) and ferulic (2176 ± 27.9 mg/100 g) acids for the genotype Tapón aguanoso, and chlorogenic acid (CGA: 380.22 ± 22.05 mg/100 g) for the Copena V1 genotype. Upon analyzing the genotypes of different fruit pigmentations, we also observed the following: enzyme 4,5 estradiol dioxygenases can be present in all genotypes (regardless of pigmentation); enzyme 5,3 glucosyltransferases (GTs) could be induced in pigment synthesis in the genotypes of orange to purple fruits. Sequencing primer-amplified fragments for GT showed high similarity to uridine diphospho (UDP)-glucose from other species. This allows us to infer that it is possible to obtain products of high therapeutic value in the near future under controlled conditions.

Keywords: opuntia species; LED and laser light; pigmentation; components in culture medium

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1. Introduction

Opuntia spp., the most important genus of Cactaceae concerning food, comprises many species and varieties [1,2]. *Opuntia* species have been the subject of several studies due to their chemical, therapeutic, and nutritional benefits. Butera et al. [3] estimated the antioxidant activity of fruit extracts from various colored prickly pear genotypes. Chávez-Santoscoy et al. [4] determined the in vitro cytotoxic capacity against human cancer cells of fruit juices (*Opuntia* spp.), and Ramírez-Ramos et al. [5] analyzed the behavior of the nutritional and therapeutic value components during the fruit ripening process to find a

commercialization and industrialization option. However, the cultivation methods, the harvest, and the industrial process can all have high costs. An alternative is using plant cultures to direct the synthesis of a specific compound. Several authors attempted to grow *Opuntia* species for in vitro cultures. Murashige and Skoog medium [6] is commonly used for the micropropagation of the *Opuntia* genus [7,8]. García-Saucedo et al. [9] demonstrated that the addition of 6-benzyl aminopurine (BAP) at 1.12 mg/L induces sprouting and indole-3-butyric acid (IBA) rooting in *Opuntia* genotypes such as Naranjona, Cristalina, and Reyna. Gomes et al. [10] worked with the genotype *O. ficus indica* and found that the combination of 4-amino-3,4,5-trichloropicolinic acid (picloram) at 4 mg/L induces somatic embryogenesis, depending on the explant age, light condition, cutting, and sucrose concentration. In a recent review of *Opuntia* species for mass propagation by axillary budding, Bouzroud et al. [11] found limited information. In this sense, new technologies, such as artificial light in Opuntia tissues, could contribute to the efficiency of plant production systems under in vitro conditions, as demonstrated in other species [12–15]. According to light quality, Miler et al. [16] showed that red light generally benefits the propagation of ornamental species (*Chrysanthemum* sp., *Gerbera* jamesonii, *Ficus* Benjamina, and *Lamprocapnos spectabilis*), depending on the species. Lofi et al. [17] found that red LEDs induce larger shoot sizes in the genus *Pyrus comunis*. Meanwhile, blue LEDs increase callus weight, far-red LEDs increase shoot number, and the addition of cinnamic acid increases rooting. Additionally, laser light has been tested to produce favorable bioeffects since it can increase product yield, stem, and seed production, in addition to reducing the vegetative period, improving crop quality, and stimulating germination [16]. Accordingly, this study aims to analyze the effect of different components in the culture medium and light conditions (LED and laser light) on the morphology and pigment content of prickly pear genotypes in the presence of two enzymes.

2. Materials and Methods

The experiment generally consisted of two stages using different *Opuntia* genotypes with different pigmentation in their fruits. In the first stage, the *Opuntia* genotype was grown on diverse culture media to induce any morphological response. At the same time, different artificial (white, red, and blue) LED and laser lights were configured. The second stage consisted of amplifying enzyme genes linked to fruit pigmentation.

2.1. Lighting System Characteristics

2.1.1. Artificial LED System

The design and manufacture of the lighting system were developed by the Artificial Lighting Laboratory (LIA) at the Instituto Tecnológico de Pabellón de Arteaga in Aguascalientes, México. The illumination system was characterized by obtaining the maximum values of PPFD for each light channel (color). The maximum intensity of white, blue, green, and red light was 150, 90, 55, and 150 μmol m^{-2} s^{-1}, respectively.

2.1.2. Laser Light Characteristics

In this experiment, we used a HeNe Laser Head 1100 Series (Photonic Solutions Ltd., Edinburg, UK) that produces a continuous-wave helium-neon laser at a wavelength of 632.8 nm and power amid 0.5–22.5 mW provided by Optics Research Center A.C-CONAHCyT, Campus Aguascalientes, México.

2.2. Red Fruit Cactus Explant Preparation

Different LEDs (white, red, blue, and red-blue) and one laser light were applied to prickly pear explants in a growing room. The PPDF for each light treatment was 105 ± 5 μmol m^{-2} s^{-1}. The laser treatment was established for 30 s at a wavelength of 632.8 nm. The photoperiod established for the experiment was 16/8 h of light. Explants were first adapted to 100 μmol m^{-2} s^{-1} for a period of 3 days to achieve the same

illumination conditions for all explants; then, different LED and laser light treatments were applied.

Culture Media to Evaluate the Growth Effect Regulators with the LED and Laser Light Influence

In vitro grown explants of two species that produce red fruits (Copena V1 and Tapon Aguanoso) were used on the Murashige and Skoog (MS 1962) basic medium [6], supplemented with different concentrations and combinations of growth regulators, including 2,4-D, kinetin, BAP, and IAA, in addition to compounds (carbohydrate sources, antioxidants, etc.) and induced responses, such as different lights, to form the callus. The media was prepared with 7 g/L agar and a pH of 5.7 and simultaneously sterilized at 121 °C for 15 min (15 lbs.). Table 1 shows the different combinations and preparations used to elicit the morphological response. The first column (cactus explant) contains the preparation name, which is given in alphabetical order. The second column (MS components) contains the proportions used to prepare the medium. It should be noted that, depending on the ingredients, one or more preparations may be produced.

2.3. Total Phenol and Flavonoid Determination

Total phenols and flavonoids were quantified using plant material (the callus of the Tapón aguanoso genotype and the whole plant of Copena V1, the species most commonly used by the food industry in Mexico). The components dextrose (10 g/L), prickly pear juice (7.5 mL/L), and maguey honey (7.5 mL/L) were used and analyzed by the Folin–Ciocalteu method proposed by Singleton et al. [18].

2.4. Genes Involved in Pigment Synthesis Identification

2.4.1. Genomic Deoxyribonucleic Acid (DNA) Extractions from Opuntia Spp.

According to the procedure reported by Doyle and Doyle [19] with some modifications, genomic DNA was extracted from 15 prickly pear genotypes grown under in vitro conditions, each one selected under the following fruit colors: light green (Villanueva, Cristalina, Xoconostle Blanco, Blanca Pepina, and Blanca San José), yellow (Atlixco, Rubí Reina, Amarilla Zacatecas, Amarilla Montesa, and Naranjón Legítimo), and red (Rojo vigor, Tapón Aguanoso, Cardona, Copena V-1, and Pico Chulo).

2.4.2. DNA Fragment Amplification and Sequencing

The pigmentation-related gene amplification oligonucleotides in *Opuntia* spp. are described as follows: the enzyme 5–0 glucosyltransferase is responsible for the red-purple pigments, and the oligonucleotides 5′-GCT GAT ATG TTC TTC AC-3′ forward (1678) and 5′-CGAGTTCCACCCACCCACAATG-3′ reverse (1679) are sequencing DNA fragments, or the enzyme 4,5 estradiol dioxygenases is responsible for yellow-red pigments, and the oligonucleotides 5′-TGTCTTCATATATCTCATGGRACTCCA-3′ forward (1680) and 5′-CCAGARCCAATGATGAGRACACACCTTC-3 reverse (1681), reported in the research by Felker et al. [20] described above are a multigene family of glycosyltransferases that generate glycosylation to orchestrate the bioactivity, metabolism, and localization of small molecules in plant cells [18]. The reaction mixture for gene amplification was 12.5 µL for each PCR tube containing the following: Gotaq 2X Buffer (6.25 µL), oligonucleotide 1, 10 µM (0.625 µL), oligonucleotide 2, 10 µM (0.625 µL), DNA (1 µL), and eater (4 µL). The reaction conditions were as follows: denaturation, 94 °C (1 min); alignment, 57 °C (2 min); and extension, 72 °C (2.5 min). The selected bands were sent to LANBAMA of IPICyT. The samples were sequenced with the dideoxynucleotide-labeled method in the 3130 Genetic Analyzer sequencer. The equipment used was an automated sequencer, the 3500 Series Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), a Verity thermal cycler for endpoint PCR (Applied Biosystems, Foster City, CA, USA), a NANODRP 1000 spectrophotometer (Thermo Scientific, Wilmington, NC, USA), and a centrifugal concentrator, Eppendorf (Merck KGaA, Darmstadt, Germany).

Table 1. Culture medium elements and conditions to induce a morphological response in prickly pear cactus explants.

Cactus Explants	MS Components	Additional Components		
		I	II	III
A	50% and activated carbon (1 g/L)	sucrose (at 10, 20, 30, 50, and 60 g/L), maguey honey (10 g/L)	BAP (1 mg/L)	white LED and red LED light
B	50% (liquid state) with activated carbon (1 g/L) 50% (liquid state) without activated carbon (1 g/L)	sucrose (at 25, 30 y 50 g/L), maguey honey (at 15, 25, and 50 g/L)	BAP (1 mg/L)	white LED light
C	50% and 100% with activated carbon (1 g/L) and 50% and 100% without activated carbon (1 g/L)	sucrose (50 g/L), maguey honey (25 g/L)	2,4-D (1, 2 y; 3 mg/L)	white LED and red LED light
D	50% and activated carbon (1 g/L)	sucrose (30, 50, and 75 g/L)	2,4-D (1, 2, 3 y; 4 mg/L)	white LED and red LED light
E	50% and $AgNO_3$ (0, 1, 2, and 4 y; 8 mg/L)	sucrose (30 g/L). mannitol (3% and 4%)	IAA (1 g/L) and BAP (3 mL/L)	white LED and red LED light
F	50% and activated carbon (1 g/L)	fructose (at 10, 15, 20, and 30 g/L)	2,4-D (1, 2 y; 3 mg/L) and kinetin (0.5, 1, and 2 mg/L)	white LED and red LED light
G	50% and $AgNO_3$ (0,0.75, 1, and 2 mg/L)	fructose (2 g/L), prickly pear juice (0, 2, 4, and 6 mL/L), mannitol (3, 4, and 5 g/L)	2,4-D (2 mg/L) and IAA (1 g/L)	white LED light
H	50% and activated carbon (1 g/L)	sucrose (30 g/L), fructose (20 g/L), prickly pear juice (16 mL/L), and mannitol (0.375 mL/L)	BAP (3 mg/L), 2,4-D (2 y; 3 mg/L), IAA (0.5 mg/L), kinetin (1 mg/L), and IAA (1 mg/L)	white, red, and blue LED light and laser beam (during 30 s)
I	50%, pH of 5.5, 5.7, and 6, citric acid (50 mg/L), and activated carbon (1 g/L)	sucrose (5, 10 y 20 g/L), prickly pear juice (10, 20, 30, and 40 mL/L)	2,4-D (0.1, 0.5, 0.75 and 1 g/L) and kinetin (0.1, 0.2, 0.5, and 1 mg/L), putrescine (10 mg/L)	white LED light
J	50% and $AgNO_3$ (0, 2, and 4 y; 8 mg/L) and activated carbon (1 g/L)	sucrose (3 and 100 g/L), mannitol (30 and 40 g/L)	2,4-D (3 mg/L)	white LED light
K	50% and activated carbon (1, 2 g/L)	sucrose (30, 40, and 60 g/L), maguey honey (10 g/L)	BAP (1 mg/L)	white LED light
L	MS (0%, 10%, and 50%), pH 5.7, and activated carbon (1 g/L)	maguey honey (15, 20, 25, and 30 g/L)	none	white LED light
M	50% and activated carbon (1 g/L)	dextrose (10 g/L), prickly pear juice (7.5 mL/L), and maguey honey (7.5 mL/L)	picloram (1 and 2 mg/L), 2,4-D (0.7 mg/L)	white LED light

I: carbohydrate source; II: growth regulator; III: lighting condition.

2.5. Statistical Data Analyses

The experiments were carried out under a completely randomized experimental design. Treatment consisted of at least 10 replicates (flasks with 1–2 explants). The mean and standard deviation (SD) were determined for each variable. Data acquisition was obtained when the plants reached thirty and/or sixty days of growth (depending on the evidence

for each experiment). Two tests were performed to statistically validate the artificial LED light effect on explant growth. The first confidence intervals were established for each sample based on a significance level of 95% and using a T-Student distribution, according to the quantity of data and the known standard deviation of the sample. According to the obtained data, the lower limit was taken as the basis for the hypothesis, since the increase from this limit is considered a change that forms the second statistical test. The hypotheses to be tested are listed below in Equations (1) and (2):

$$H_0: \mu = \text{lower limit} \quad (1)$$

$$H_A: \mu \neq \text{lower limit} \quad (2)$$

3. Results and Discussion

3.1. Prickly Pear Explant Development Effects of Components in the Culture Medium and Lighting Treatments

Figure 1 displays the growth and development of each prickly pear explant under different conditions of the in vitro culture medium and LED and laser light.

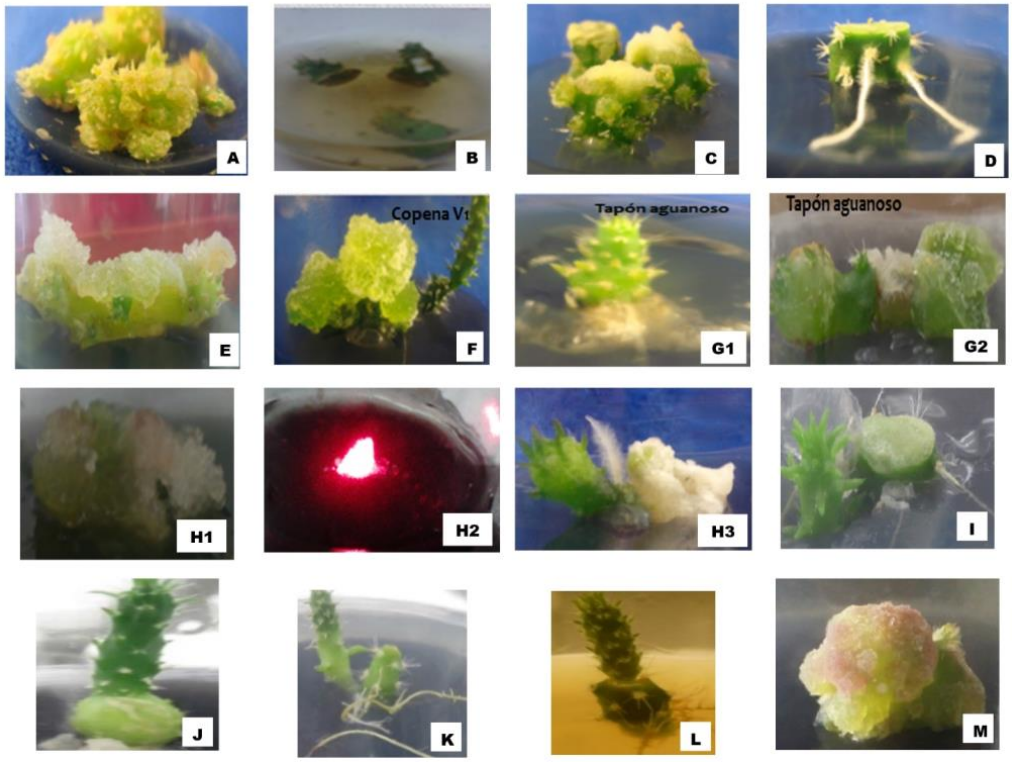

Figure 1. Effect sequence of the components in the culture medium and light treatments on the explants of two prickly pear genotypes (Tapón Aguanoso and Copena V-1). These are presented in alphabetical order, following the information in Table 1. (**A**,**C**–**F**) were exposed to white and red LED lights. (**B**,**G1**,**G2**,**I**–**M**) show explants exposed to white LED. (**H1**–**H3**) shows explants exposed to white, red, and blue LED light and laser beams.

Table 2 shows the results obtained regarding the added components' effects on the culture medium and the lighting condition to find the best combination for callus formation and pigmentation in prickly pear explants. All explants were first adapted to white light at 100 µmol m^{-2} s^{-1} for 20 days to establish the same illumination conditions. Then, prickly pear explants were introduced in the corresponding light treatments. Sucrose (50 g/L) plus BAP (1 mg/L) caused unsuccessfully developed explants, i.e., the generation of few shoots, and resulted in deformed explants when changed by maguey honey (10 g/L), as shown in Figure 1A. According to Figure 1B, the same combination from explant A changed

the liquid medium and caused no effect on *Opuntia* spp. propagation. Sucrose (50 g/L) and maguey honey (25 g/L) combined with 2,4-D (2 mg/L) stimulated shoots and roots, varying the response among genotypes (Figure 1C). Root formation is preferably the result obtained in explant D using the equivalent combination (2,4-D), except for increasing the sucrose concentration from 50 to 75 g/L, as shown in Figure 1D. BAP, in addition to IAA, combined with sucrose (30 g/L), mannitol (3%), and $AgNO_3$ (4 mg/L) stimulated callus formation (see Figure 1C) and obtained an outstanding result. Moreover, the mixture of fructose with 2,4-D (2 mg/L), and kinetin (1 mg/L) generated calluses and shoots, as shown in Figure 1F.

Table 2. Best effect on explants of pricky pear genotypes (Tapón Aguanoso and Copena V1) at 60 days of growth in culture medium and artificial light.

Explant Cactus	Genotype		Morphological Response						Major Response
			Callus		Shoot		Roots		
	Type	Amount Per Treatment	(%)	(cm)	(%)	(cm)	(%)	(cm)	
A	Tapón	12	33.3	1.4 ± 0.2	41.6	1.7 ± 0.8	58.3	8.5 ± 6.6	Red and white LED lights have the same effect on explant growth.
	Copena VI	8	25.0	1.2 ± 0.2	62.5	2.4 ± 1.4	100.0	7.6 ± 5.1	
B	Tapón	12	There was no influence on explant growth in callus, shoots, or roots.						The explants were necrotic after 15 days.
	Copena VI	10	The explant did not develop callus, shoots, or roots.						
C	Tapón	6	14.2	0.5 ± 0.3	100.0	1.7 ± 0.6	33.3	4.6 ± 2.7	Light treatments had poor response in callus formation.
	Copena VI	7	14.2	0.7 ± 0.2	21.8	0.7 ± 0.4		7.3 ± 5.0	
D	Tapón	3	No callus formation		66.0	0.6 ± 0.3	66.6	8.3 ± 0.5	Root development.
	Copena VI	3	Absent		33.3	0.2 ± 0.1	100.0	1.6 ± 1.1	
E	Tapón	3	100.0	4.0 ± 3.0	66.3	0.8 ± 0.35	No root formation.		Outstanding callus development.
	Copena VI	4	25.0	0.3 ± 0.0	33.3	0.7 ± 0.0	Absent		
F	Tapón	7	42.0	4.0 ± 3.0	71.0	0.6 ± 0.3	No root formation.		White LED light affected shoot generation. Red LED light effect on sprout development.
	Copena VI	7	Absent		100.0	0.7 ± 0.0	Absent		
G	Tapón	5	Present		No effect in shoots and roots.				Embryogenesis generation in the genotype Tapón Aguanoso with small callus.
	Copena VI	5	Active		Not present in shoots and roots.				
H	Tapón	5	Active		No effect shoots and roots.				The callus produced exhibited red pigmentation, and the laser light induced embryogenesis.
	Copena VI	5	Present		Not present in shoots and roots.				
I	Tapón	5	Light		80.0	2.12 ± 0.8	80.0	4.7 ± 1.8	Very few but vigorous shoots were produced, and roots were formed. No red pigmentation.
	Copena VI	4	Light		83.6	2.0 ± 1.06	83.0	5.6 ± 6.2	
J	Tapón	3	Light		66.6	0.8 ± 0.28	Absent		Few shoots, no roots, and a few whitish calluses. No red pigmentation.
	Copena VI	4	Light		66.6	0.4 ± 0.14	No present.		
K	Tapón	4	No effect		50.0	1.5 ± 0.70	50.0	17.8 ± 6.5	Limited sprouting and thin roots. No red pigmentation.
	Copena VI	4	No present		75.0	1.8 ± 0.76	75.0	13.4 ± 9.1	
L	Tapón	2	No present		100.0	Very small	100.0	1.7 ± 0.0	Very small shoots and roots.
	Copena VI	2	No effect		100.0	Too small	100.0	2.7 ± 1.7	
M	Tapón	12	Occurrence		Not present		No effect		Callus with red pigmentation.
	Copena VI	10	Presence		No effect		Absent		

Fructose (20 g/L) combined with prickly pear juice (2 mL/L), mannitol (4 g/L), $AgNO_3$ (1 mg/L), 2,4-D (2 mg/L), and IAA (1 g/L) induced callus and embryogenesis (Figure 1G1,G2) by white LED light treatment. Figure 1H1–H3 clearly show that the components in the culture medium were effective in callus formation and embryogenesis. When the medium slightly raised the pH (to 6), citric acid (50 mg/L), 2,4-D, kinetin, and putrescine (10 mg/L) were added with white LED light radiation, it resulted in the production of vigorous shoots and roots (Figure 1I). Few sprouting, callus, and color deficiencies were

caused by the medium and the mixture of 2,4-D with BAP, mannitol, $AgNO_3$, and sucrose 10% (Figure 1J). At a low concentration of maguey honey (10%), without mineral salts, activated carbon, and growth regulators, very thin roots and at least one shoot were generated (Figure 1K). The mixture of dextrose, prickly pear juice, maguey honey, Picloram, and 2,4-D produced callus in some explants and showed pigmentation (Figure 1L). Prickly pear juice (7.5 mL/L), maguey honey (7.5 mL/L), and dextrose (10 g/L) were used in combination with Picloram (1 and 2 mg/L), and 2,4-D (0.7 mg/L). These treatments, when coupled with white LED light, caused the formation of calluses with red pigmentation (Figure 1M). For explants pigmented and caused in the same way, embryogenesis occurred when the culture medium contained prickly pear juice, 2,4-D, and red LED or laser light. Garcia-Saucedo et al. [9] indicated that the genus *Opuntia* produced in vitro can generate shoots and roots, but the generation of callus and embryos in *Opuntia* species presents limitations, despite Gomes et al. [10] inducing somatic embryogenesis using picloram (4.0 mg/L) and sucrose as growth regulators applied to *O. ficus indica* species. The results of this study revealed the formation of completed plants (generated with embryogenesis), preferentially produced with red laser light, as shown in Figure 1H1–H3, for both genotypes.

Phenolic and Flavonoid Content

Table 3 presents the results on the content of phenols and flavonoids in the cactus explants propagated in vitro. It showed a high content of simple phenols in each genotype analyzed, demonstrating that combining the different elements outstandingly added to the culture medium. This is even higher than the data reported in fruits recognized for their therapeutic application [21], such as Noni (*Morinda citrifolia*) and Camu (*Myrciaria dubia*). In previous studies of the genus *Opuntia*, Chávez-Santoscoy et al. [4] found a total amount of phenolics of 226.3 ± 26.4 µg GAE in the Red Peach variety and a flavonoid content of 338.1 ± 21.5 (µg quercetin eq./g in the purple fruit genotype Tapón (*O. robusta*). Additionally, Ramirez-Ramos et al. [5] reported a phenolic compound concentration of 165.5 mg GAE/100 g in the Alteña Blanca genotype and a flavonoid content of 11.21 mg EC/100 g in Tapón (*O. robusta*) purple fruit. The components we found in the explants cultivated under in vitro conditions showed that it could be an ideal technique to produce these in an innocuous, standardized method without using open-air crops exposed to variant environmental conditions that would limit their production. It is worth mentioning that recent revisions of the genus *Opuntia* highlight its traditional use in therapeutic treatments by reducing lipids and alterations in blood sugar in humans [22]. Then, what we found in callus and in vitro cultivated plants represents a viable option for phenol synthesis under controlled conditions.

Table 3. Phenolic and flavonoid content of *Opuntia* genotype explants under in vitro conditions.

Genotype	Simple Phenolic Acids in Prickly Pears (mg/100 g)				Total Phenolics mg GAE/100 g	Flavonoids mg EC/100 g
	Gallic	Coumaric Acid	Ferulic	Chlorogenic		
Tapón	1887.2 ± 1.7	1121.0 ± 49.5	863.32 ± 111.8	380.22 ± 22.05	391.39 ± 2.1	67.06 ± 0.015
Copena V1	2283.30 ± 6.2	2155.0 ± 35.0	2176 ± 27.9	314.14 ± 26.47	374 ± 5.9	68.5 ± 0.03

3.2. Data Analysis

According to the statistical results obtained (Table S1) in the experiment, the explants significantly different from the lower limit are in the A explants for both Tapón aguanoso and Copena VI genotypes, which belong to the white and red LED lights. This means that there was a significant change in the growth of callus, roots, and shoots in the explants. The difference in the value reported in both tests can be observed. In the C explants, a significant difference was observed only in Copena VI, as poor callus growth was observed in them. In the F explants, only the Tapón aguanoso explant is considered significant since the white light contributed to the formation of shoots and calluses but did not form roots. A clear trend can be observed: white and red LED lights affect the formation of shoots but

do not contribute to callus formation (the information on the test is presented in Table S1 of Supplemental Materials).

3.3. Genes Involved in Pigment Synthesis

This experiment stage consisted of identifying the genes involved and promoting pigment synthesis (Table 4). We found that according to the primers of enzyme 4,5 estradiol dioxygenases, only three fragments were amplified in all the nopal genotypes (see Figure 2). We ascertained that all prickly pear species contain these genes; therefore, sequencing was discarded. The enzyme 5,3 glucosyltransferases were amplified in various fragments of each pricky pear genotype (4–10) within a range of 300–2000 bp. According to the analysis, most genotypes were identical in size; on the other hand, those that showed differences in their presence were selected for sequencing inspection. The genotypes were Genotype Atlixco (orange fruit), Tapón Aguanoso (purple fruit), and Cristalina (light green fruit). Table 4 shows the results of the sequences identified for each genotype; at the same time, each record was compared within the National Center for Biotechnology Information (NCBI) with the UDP-galactose transport gene of *Terenaya hassleriana*, obtaining similarities of 100%. Similarly, Sequence 3 reached 100% compatibility with *Solanum pennelli* (ch05) and *Hordeum vulgare* (clone BAC 455J22). On the other hand, species such as soybean, peach, alfalfa, oat, tomato, melon, and grapevine showed lower compatibility. The best results were obtained in Sequence 2 since it has the closest relationship to pigment synthesis, given that it coincides with a gene related to galactose transport; in addition, the *Terenaya* plant has flowers with a high betalain content. The genotypes were then compared with glucosyl transferase enzymes, determining a similarity between 71, 72, and 92% for *Phytolaca americana*, *Cicer aietinum*, *Rivina humillis*, and *Rosa hybrida*, respectively. Each sequence is compared with UPD-galactose since it is a substrate for the synthesis of noncellulosic polysaccharides and glycoproteins, as reported by Norabuena et al. [23]. Furthermore, Hou et al. [24] indicated that polysaccharides perform an irreparable function throughout the life history of plants, becoming one of the main components of the cell wall. The evidence found in this research specifies that the identified fragments of different sizes are highly related to pigmentation and carbohydrate synthesis in the different prickly pear genotypes, leaving an open niche for research at the in vitro level to demonstrate the degree of influence on callus pigmentation or whole plant generation (embryogenesis).

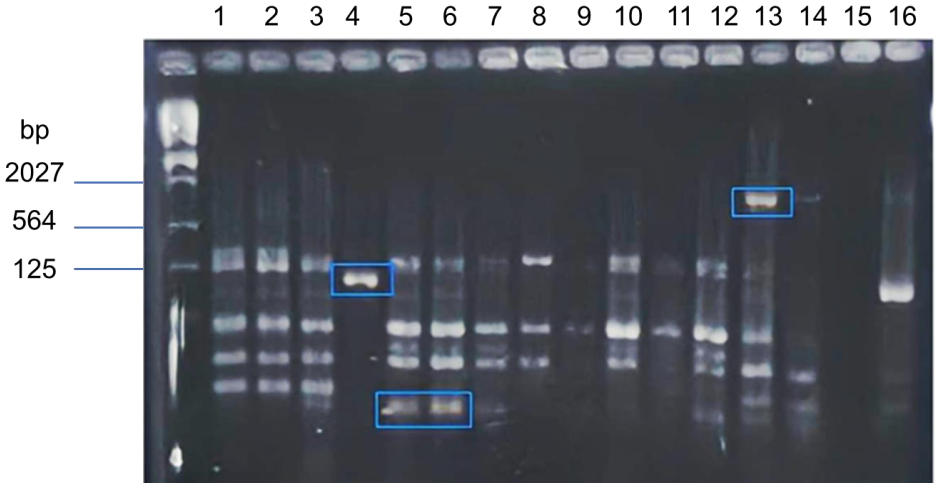

Figure 2. Fragments obtained with the indicators for the enzyme 5,3 glucosyltransferase for all the genotypes used in this study. Lanes 1 and 2 correspond to the Cardona, lane 3 to Xoconostle Blanco, lane 4 to Atlixco, and lanes 5 and 6 to Tapón Aguanoso and Tapón Aguanoso, respectively. Callus DNA was obtained in columns 7 and 8, Copena V1 in column 9, Cristalina in columns 12 and 13, and the Villanueva genotype from columns 14 to 16. The fragments used in the sequencing are denoted by blue boxes.

Table 4. Sequences obtained from fragments amplified with the 5,3-glucosyltransferase enzyme primers in different prickly pear genotypes.

Genotype	DNA Sequence in the 5′-3′ Enzyme for Each Cactus Genotype.
Atlixco	CTCACTCATCTCTTCACCTGTTCTTGGATGCAAACAGTCAGTACTA-ACACTA-GAATGCCTGTTTGGATGTCCTGATTGAACAACATGCTCAAGTGAAGA AATTATCAATTAGGTTTGTTATCATCAAAACACTACATCCTAAAGAG-GATGAATTCAACAAGGTGGCAGTGTCAAGGGCATTGAACGGCATTGG ACTAGCCATTGTAATACCCGCCATTCAGTCCCTCGTGG-CAGACTCAACTGAGGATCACAAC-CGTGGCACAGCCTTTGGGTGGCTACAGCTCACAGGAAACATGGGCTC AATCCTTGGCGGCCTTTGTTCTGTCTTGTT-GGCTTCCAGGTCCTTCATGGGAGTCCCTGGTT-GGAGGATAGCCTTCCATTTAGTTGGCCTAGTTAGTGTCATTGTTGGAG TTCTGGTTTGCCTCTTTGCCAATGATCCCCGCTATGCAG-GAAGTGATCACAAGGCTAGAGAG-GAGAAACGTCTGTCCTTTTGGTTAGAATTGAAAGGCGGTGTTGCAGGA AGCAAAATCAGTTCTCCGAATTCCATCATTTCAGATATTGTT-GCCCAAGGTGTGTCTGGGTCTTTTCCGTGTCAGCATTGTCATTTAACAT CATTGTGGGTGGGAACTCCGAA.
Cristalina	GTAGGTGTAGATGGCAAGTACTTCTT-GGCTTTTCATTAATTCTGTTCTGCTATCAACTGCC-TATGTTTACTCCATTTTAGCAACTTAGTGATGTGAACCAGATTCTTTGT TTGAACAAAGGCAAACTCTTCATCCAA-TATCAAAGTCCCCAAAATGGTTATTTACAAACTTATTAATAACTTAAG AAATTGGCCACCTTCTAGGAAGGAGTTCCCCTCTCCTCAAC-CTCTAAACAA-TACTGTTTCCAATACCTATATTTCCCGTTATGCAAGGTCAAGAACTTA GCTTTCTTTATTTGGAATCTAAGGGTTTTTCTGGGTGTT-GCAAGTGAGGCCG-CAGAAACTTTCTTTGATTTGACCGAATTTGGCAGGGTAAAATTTACTG CCTTTTTCGAAAGTTTTACTTCATGGTTTGCCAATCCTGGGTTAGA-GAAATCAGGGG-TAAATCCCTTAAAACCCAAAGCAACCCCCCTGGAATTGCCCCAAGGC GGTTTCGGGGGGGCGCCCCTGGCCCAAGTCCCAACCCGATTGGTCG-GACCCCTCCCATAAC-GGTTTTCCTAGGGCGGGGGGTGTTTAAACCCGGGGGTTTGGGGAGGG GGGGGAATTTTAAGAACCAAACACCCTCGA-GAGGGAAATTTTTTAAAAATGGGGGGTTCCAG-GATATTTTTGTTTTTGGCGTTATAAAAAGAATGTTGATTAAAAAAAAG CAGTCCCAGGATG.
Tapón aguanoso	CGCAATCGCTTATTAGGGACATATACGCAGCTTGGTCCCGC-TACCCAAACAAGAAAAGAAA-TAGATTTTTGGGGGTTTTTTTTTCACAAAATGGGCTTCGGTAAAAAGTT CGCCTTATTTTTGAAGAATTGGAACAAGTTTT-GAAAGGACAAATCCTCAAGGAAACTGTCTTAAATTCAAAACAATTAC GGCCTCGATTTAATTTCGGAATTGTCACTGATCC-TATAATCCATACATAATGTCTAG-TGTGGTGGAGACGGGTTTGTAACCAGTACCCTCTGGCTGGACCAAAT ACGAAAAAGGCCGATTCCCCTGGATTCCTCGAACAAAATCCTGGGG-GAATACCGGCCTTGTGGAATCGAG.

4. Conclusions

We determined that the culture medium combined with cactus fruit juice and red-light treatment (LED and laser) was outstanding for callus formation, embryogenesis, and pigment synthesis. At the same time, the quantification of simple phenols (ferulic, caffeic, and chlorogenic) obtained the best results. This research found an alternative to synthesizing *Opuntia* spp. components for therapeutic interest. We found that, when analyzing the geno-

types of different fruit pigmentation, the enzyme 4,5 estradiol dioxygenase is present in all genotypes (without considering pigmentation), and the enzyme 5,3 glucosyltransferase helps to differentiate the synthesis of pigments in the genotypes that have orange to purple fruits. Finally, the amplified fragments showed a high percentage of similarity with UDP galactose from other species, presenting themselves as an alternative to continue studying its function under in vitro conditions and to direct the synthesis of interested compounds in *Opuntia* species.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9121348/s1. Table S1: Hypothesis testing performed with a 95% confidence level.

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