agronomy

MDPI

Article

In Vitro Propagation of Easter Island *Curcuma longa* from Rhizome Explants Using Temporary Immersion System

María José Marchant [1], Paula Molina [2], Miriam Montecinos [2], Leda Guzmán [1], Cristobal Balada [1], Claudia Fassio [2] and Mónica Castro [2,*]

[1] Laboratorio de Biomedicina y Biocatálisis, Instituto de Química, Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso, Avenida Universidad 330, Valparaíso 2340000, Chile; marchant.mariajose@gmail.com (M.J.M.); leda.guzman@pucv.cl (L.G.); criss.tbc@gmail.com (C.B.)

[2] Laboratorio de Propagación, Escuela de Agronomía, Facultad de Ciencias Agronómicas y de los Alimentos, Pontificia Universidad Católica de Valparaíso, La Palma S/N, Quillota 2260000, Chile; paula.javiera@hotmail.cl (P.M.); miriam.montecinos@pucv.cl (M.M.); claudia.fassio@pucv.cl (C.F.)

* Correspondence: monica.castro@pucv.cl

Abstract: *Curcuma longa* (*C. longa*) is widely known for its medicinal properties. However, the potential overexploitation of this plant raises doubts about its long-term survival on Rapa Nui. Micropropagation using a temporary immersion system (TIS) could be the basis for developing a cost-effective and highly productive method of large-scale cultivation of this plant. Our objective was to develop and refine the in vitro multiplication system for mass propagation of *C. longa*, and thus help restore the fragile ecosystem of Rapa Nui. Three parameters were evaluated: number of explants per flask, flask capacity, and LEDs spectrum. For each parameter evaluated, four aspects were analyzed: fresh weight per plant, number of shoots, percentage of non-sprouting explants, and the proliferation rate. The use of 30 explants per two-liter flask results in more plants with high fresh biomass than other configurations. In addition, LEDs with a red:blue ratio of 2:1 provided the best lighting conditions for in vitro propagation and positively affected *C. longa* proliferation and rooting. Therefore, our results show that 30 explants per two-liter flask and an LED source with a red:blue ratio of 2:1 allow a higher number of *C. longa* plants to be obtained using TIS.

Keywords: turmeric; micropropagation; LED light; conservation; Rapa Nui; automatization

Citation: Marchant, M.J.; Molina, P.; Montecinos, M.; Guzmán, L.; Balada, C.; Fassio, C.; Castro, M. In Vitro Propagation of Easter Island *Curcuma longa* from Rhizome Explants Using Temporary Immersion System. *Agronomy* **2021**, *11*, 2121. https://doi.org/10.3390/agronomy11112121

Academic Editors: Valeria Cavallaro and Maurizio Lambardi

Received: 28 September 2021
Accepted: 20 October 2021
Published: 22 October 2021

1. Introduction

Easter Island or Rapa Nui is one of the most isolated inhabited places in the world. Ancestral medicine is very important to the people of Rapa Nui, who rely on several plants to make preparations widely used for medical purposes [1]. However, due to excessive harvesting, several plant species have disappeared and others, among them, *Curcuma longa* L., (*Pua* in the Rapa Nui language), are overexploited. The natives consider it a magical plant and use it to treat diseases such as cancer, diabetes, muscle pain, and others [2]. The widespread use of this species has put it in danger of extinction, and it is now under state protection [3]. A study of the biological properties of the Rapa Nui ecotype of *C. longa* revealed high relative concentrations of polyphenols and curcumin, highlighting its antioxidant and anti-inflammatory properties [3]. The results demonstrate the importance of conservation, reproduction, protection, and proper management of this species. Particular attention should be given to efforts aimed at optimizing its propagation on Rapa Nui through in vitro multiplication.

The rhizome of *C. longa* is one of the plant materials from which adult plants can be produced by in vitro micropropagation, preserving the characteristics of the mother plant [4]. The micropropagation method that we used in this study is known as the Temporary Immersion System (TIS) and involves intermittent contact between liquid culture medium and explants [5,6]. In addition, this system improves the proliferation

of shoots in a liquid medium with a temporary immersion of the explant in a liquid medium for a specific interval, thus providing the medium at one interval and oxygenation during the other [7]. However, a few studies about the development of a method of immersion using fed-batch liquid culture as bioreactors to micropropagation of *C. longa* were analyzed the response to minerals in the culture medium [8]. In TIS, the nutrient medium is pneumatically propelled to reach and immerse the explants while air is pumped to completely refresh the explants headspace with each immersion, renewing oxygen and avoiding CO_2 and ethylene accumulation, as occurs in semi-solid and solid media, where it can negatively affect plant morphogenesis [9]. Short immersion times allow good control of water content to reduce the possibility of plant vitrification [10]. In addition, this method allows for adequate nutrient mixing, relatively low frequency of subculturing, easy medium replacement, and minimal damage to explants. For these reasons, several authors have described TIS as the most appropriate approach to tissue culture [11,12].

TIS is a complex system influenced by several factors such as light, temperature, and medium composition [12]. Explant density is also important for the multiplication and growth of plants within the flask. The higher the density of explants per flask, the more nutrients and space needed for plant movement and proper development. An excessive number of explants in a limited space can reduce the proliferation rate and the quality of the plants produced [13,14]. Another parameter that affects plant growth is the light source. A common light source used for in vitro propagation is fluorescent lamps. However, they have the problem of high power consumption and produce a wide range of wavelengths (350–750 nm) that are not necessary for plant development [15]. Light-emitting diodes (LEDs) have recently become a viable alternative for commercial micropropagation. LEDs have several advantages: low thermal radiation, monochromatic spectrum, high durability, and low power consumption. LED lighting systems for in vitro culture provide light in the spectral range involved in photosynthesis and photomorphogenic responses in plants [16]. In the case of *C. longa*, LED light has been reported to affect the production of metabolites such as curcumin and its derivatives, thus increasing the antioxidant capacity of the plant [17]. However, the effect of this type of light on the proliferation and rooting of *C. longa* in vitro has not been reported so far.

TIS micropropagation of various plants species is an alternative method that offers advantages over mini-cutting, such as: higher multiplication rate, higher rooting rate; plant health, effective control of pests and diseases, environmental control, and reduced need for physical space [18]. Several TIS setups have been successfully used for micropropagation of various plant species of agricultural, medicinal and conservational value in recent years. In this study, we used the Twin-Flask system (BIT®) described by Escalona [19]. This system consists of a container for growing plants and a reservoir for the liquid medium. When the solenoid valve is opened, and the air compressor is turned on, the airflow pushes the medium into the flask with the plants and the plants are immersed. This process is reversed when another solenoid valve is opened, and the medium is returned to the original reservoir by air pressure. The system is connected to an automated irrigation mechanism, which controls the frequency and duration of each immersion [19,20]. The operating parameters of the TIS are adjusted for each species to be propagated in vitro in order to achieve optimal results.

In this study, we successfully propagated *C. longa* in vitro by TIS and evaluated the effects of three factors: number of explants per flask, flask capacity (volume), and red:blue ratio of LEDs. The ultimate goal is to establish and refine an in vitro propagation system with reasonable production costs that can be used for the mass propagation of *C. longa* to restore the compromised Rapa Nui ecosystem.

2. Materials and Methods

2.1. Explant Collection, Disinfection and Establishment of Rhizome Cultures

Rhizomes of *C. longa* L were harvested from the Mataveri Otai nursery on Easter Island (geographical coordinates: 27°09′50, 17′ S–109°26′24, 63 O). The excised long creeping

rhizomes were thoroughly washed with water and Tween-20 for 10 min. The rhizomes were then cut into 2–3 cm pieces and placed under water flow for 30 min. Subsequently, the rhizomes were immersed in the Phyton solution (3 mL/L) under gentle agitation for 15 min. Next, the rhizomes were cut into 1 cm pieces and active buds were separated and washed with 1.5% sodium hypochlorite solution, 0.5 g/L citric acid, and 0.5 g/L ascorbic acid for 15 min under constant agitation. The pieces were transferred to a laminar flow hood and washed four times with a sterile solution of 400 mg/L citric acid and 400 mg/L ascorbic acid. Finally, the pieces were placed in a Murashige and Skoog [21] (MS) culture medium supplemented with 0.1 mg/L thiamine, 200 mg/L glycine, 170 mg/L NaH_2PO_4, 2.5 mL/L plant preservative mixture (PPM), agar 6.5 g/L, sucrose 30 g/L, at pH 5.8. The flasks were incubated in vitro in the dark at 25 °C for 10 days and then placed under 1R:1B LEDs for one month.

2.2. In Vitro Growth of Explants

The shoot rhizomes were transferred to MS growth medium, supplemented with 0.1 mg/L thiamine, 200 mg/L glycine, 170 mg/L NaH_2PO_4, 1 mg/L 1-Naphthaleneacetic acid (NAA), 2 mg/L N^6-Benzylaminopurine (BAP), 6.5 g/L agar, 30 g/L sucrose, at pH 5.8. The plant material was subcultured three times (30 days each time) to obtain more explants for TIS. The initial explants for TIS were 1–2 cm long, rootless and showed no symptoms of vitrification (hyperhydricity), or oxidation (Figure 1).

Figure 1. Initial explants of *C. longa* used in the assay.

2.3. In Vitro Proliferation of C. Longa by TIS

TIS was used for the proliferation and rooting of *C. longa* shoots. Ten flasks for each set of conditions were prepared and three independent assays were performed. Flasks were maintained under the following conditions during proliferation and rooting (unless otherwise noted): 15 mL of culture medium (proliferation or rooting) per explant, immersion of 4 min each time in a 4 h cycle, the temperature of 23 ± 1 °C under for 16 h/day, and white LED illumination. For proliferation, MS culture medium was used supplemented with 200 mg/L glycine, 0.1 mg/L thiamine, 0.5 mg/L nicotinic acid, 0.5 mg/L pyridoxine, 100 mg/L myoinositol, 170 mg/L NaH_2PO_4, BAP 3 mg/L, 30 g/L sucrose, at pH 5.8. For the root stage, the same medium was used and BAP was replaced with 1 mg/L NAA. After the proliferation stage, the flask of proliferation medium was replaced with the flask of rooting medium under a laminar flow hood, while the explants were not disturbed by handling. After both stages, all shoots were placed in a greenhouse to acclimate.

2.3.1. Evaluation of the Number of Explants per Flask

We evaluated 20, 30, and 40 explants of *C. longa* per culture flask with a capacity of 1 L. The cultures were grown for 90 days (60 days for proliferation and 30 days for rooting), at a temperature of 23 °C, under 16 h of light per day, and white LED illumination. Subsequently, the resulting plants were acclimated in a greenhouse. Four parameters were measured prior to acclimation: fresh plant weight (total weight of shoots/number of shoots), number of shoots, percentage of non-sprouting explants (explants that did not germinate), and proliferation rate (number of shoots/number of initial explants).

2.3.2. Evaluation of Flask Capacity

Two different volumes of culture flasks were evaluated: 1 L and 2 L. We chose 30 explants per flask as this quantity produced a greater number of shoots. The cultures were grown for 60 days (45 days for proliferation and 15 days for rooting), at a temperature of 23 °C under 16 h of light per day and white LED illumination. Subsequently, the resulting plants were acclimated in a greenhouse. Four parameters were measured prior to acclimation: fresh plant weight (total weight of shoots/number of shoots), number of shoots, percentage of non-sprouting explants (explants that did not germinate), and proliferation rate (number of shoots/number of initial explants).

2.3.3. Evaluation of Light Spectra

Considering the available data from previous research, we used 30 explants in 2 L flasks for this study. Three different sources were used for light treatments: white LED, red:blue LED 1 (RB1, model LX-GLT8), and red:blue LED 2 (RB2, PARALED system PARALED_GRB_20W, Ciencia Pura SpA. Santiago, Chile). Each light source had different proportions of Blue (420–460 nm) and Red (660 nm). These wavelengths are consistent with the known maximum absorptions for chlorophyll an and b, indicating optimal photosynthetic efficiency [22]. The culture was illuminated from the top of the culture vessel and cultured in a 16 h photoperiod (long day). The experimental conditions and parameters were the same as in the previous experiment. In addition, Yield Photon Flux Density (YPFD, μmol m^{-2} s^{-1}), Red:Blue ratio, and Daily Light Integral (DLI) (mol m^{-2}) were measured using a compact spectrophotometer (Lighting Passport Standard Pro model, Allied Scientific Pro^{TM}, Gatineau, QC, Canada). To avoid light contamination, all assays were separated from each other by white screens made by a local manufacturer.

2.4. Statistical Analysis

Comparisons were made using one-way ANOVA with Tukey multiple comparison tests or Mann–Whitney test analysis. A value of $p < 0.05$ was considered as statistically significant difference. All statistical analyses were calculated using GraphPad Prism 9 computer software (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Evaluation of the Number of Explants per Flask

The number of *C. longa* explants per flask was evaluated. After 90 days of incubation, plants were removed and fresh weight, the number of shoots, percentage of non-sprouting explants, and proliferation rate were determined. The results are shown in Figure 2, where the assay with 30 explants yielded an average fresh weight of 13.8 g/plant, which was higher than the other treatments (Figure 2a). The same number of explants also yielded the highest number of shoots, with green leaves and roots, averaging 135.8 plants per flask (Figure 2b). In the assay using 20 and 30 explants, the percentage of non-sprouting explants was comparable. However, in the assay with 40 explants, this parameter increased twofold (Figure 2c). Figure 2d shows the proliferation rate, which decreased as the number of explants used per flask increased.

Figure 3 is a photographic record of the plants produced. These are representative of the assay. At the end of proliferation and rooting, all shoots had green leaves and roots (Figure 3a), with an average size of 10 cm (Figure 3b). The non-sprouting explants had no green leaves and oxidation and vitrification were observed (Figure 3c). Subsequently, all the obtained shoots were successfully acclimated in a temperature- and humidity-controlled greenhouse (Figure 3d).

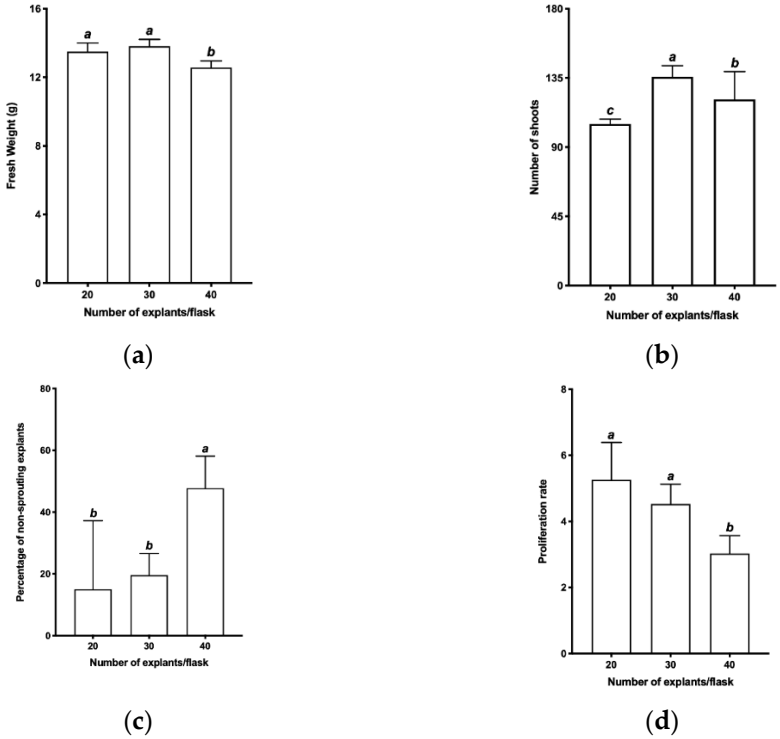


Figure 2. Effect of the number of initial explants per culture flask on the propagation of *C. longa* by TIS. (**a**) Fresh weight per plant. (**b**) Number of shoots. (**c**) Percentage of non-sprouting explants. (**d**) Proliferation rate. g: gram. Average ± SD was plotted for all replicates analyzed. Tukey's multiple comparison tests were applied at the 95% confidence level to compare the differences. Bars with different letters show significant differences at $p < 0.05$ for each parameter studied.

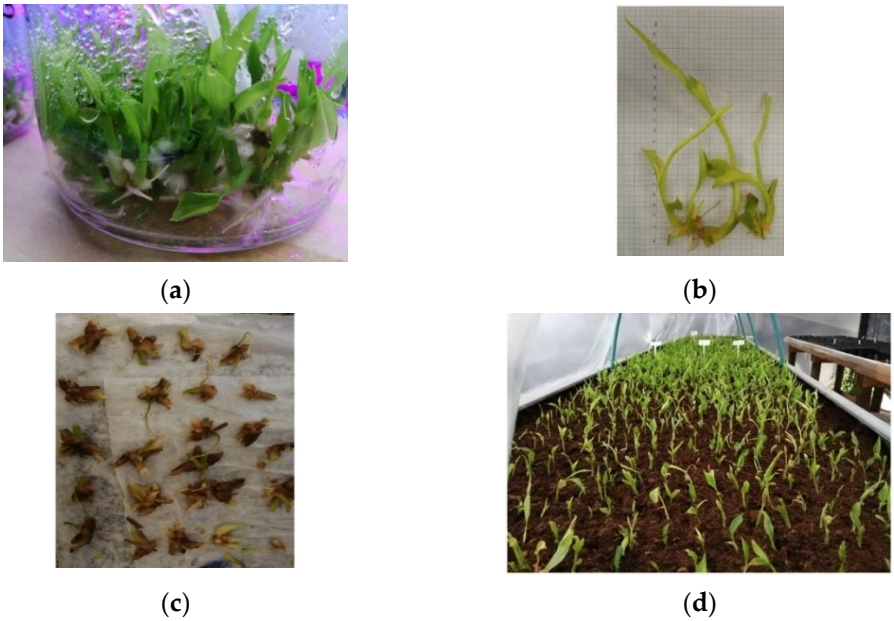


Figure 3. Plants obtained through the process of proliferation and rooting by TIS. (**a**) Shoots obtained by TIS ten days before acclimation. (**b**) Shoots. (**c**) Non-sprouting explants. (**d**) Acclimated plants from each treatment in a greenhouse.

In all the tests carried out, it was observed that in the middle of the proliferation phase (day 30 of 60) and the rooting phase (day 15 of 30), the plants reached maximum

development, so in the following experiments, the incubation time for both phases were reduced by half.

3.2. Evaluation of the Volumetric Capacity of the Flasks

In the second assay, we evaluated the effect of flask volumetric capacity on plant growth. The results showed that the volumetric capacity of the flask affected the proliferation and rooting of *C. longa* in vitro (Figure 4). In the 2 L flasks, the average fresh weight of plants was 5.7 g/plant, which was greater than the value in the 1 L flasks (4.7 g/plant) (Figure 4a). The system produced an average of 127 plants in the 2 L flask, and 117 plants in the 1 L flask (Figure 4b). No significant differences were observed in the percentage of non-sprouting explants during the experiment (Figure 4c). For the proliferation rate, we obtained values of 4.2 for the 2 L flask and 3.9 for the 1 L flask (Figure 4d). Thus, it was concluded that the use of a 2 L flask would produce a greater number of plants with a higher fresh weight than the use of a 1 L flask.

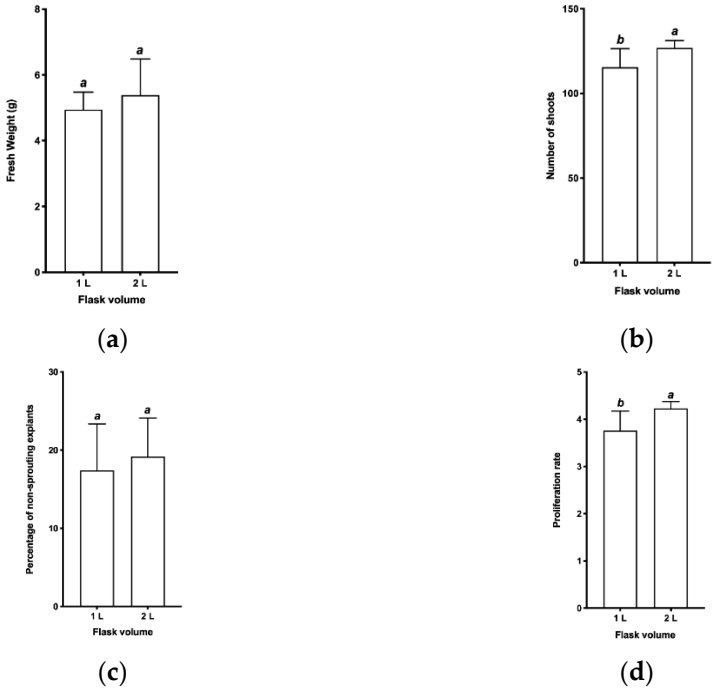
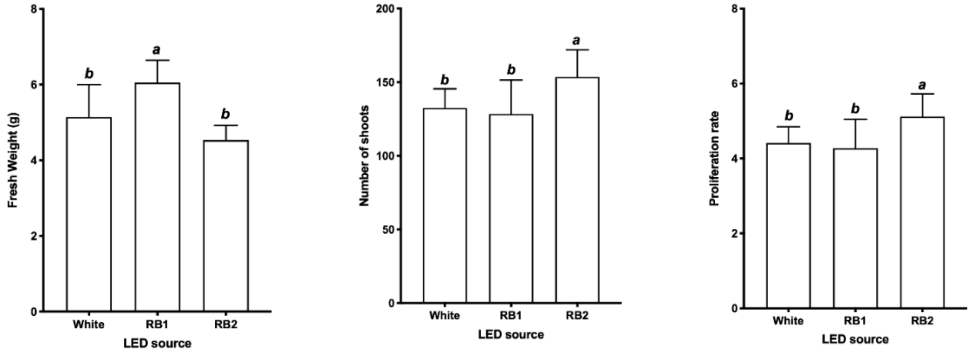


Figure 4. Effect of the flask volume on the production of *C. longa* by TIS. (**a**) Fresh weight of plants obtained at the end of the 45 days of TIS. (**b**) Number of shoots. (**c**) Percentage of non-sprouting explants. (**d**) Proliferation rate. Average ± SD was plotted for all replicates analyzed. Mann-Whitney U test was used.

3.3. Evaluation of Light Spectra

To evaluate the last parameter, we used three different LED light sources: white LED, red:blue LED 1 (RB1) and red:blue LED 2 (RB2). The plants obtained were extracted for analysis and acclimated in a greenhouse. The results, summarized in Figure 5, show that the red:blue LEDs had a positive effect on the in vitro proliferation and rooting of *C. longa*. When RB1 was used, an average fresh weight of 6.05 g/plant was obtained, which is higher than that of white LED and RB2 (5.14 and 4.53 g/plant, respectively) (Figure 5a). In most of the assays performed under any sets of conditions, the percentage of non-sprouting explants at the end of the experiment was minimal or zero. However, in terms of productivity, the RB2 spectrum was the most productive, yielding 154 plants (Figure 5b). Similarly, RB2 gave a proliferation rate of 5.12 plants per explant, while white LED and RB1, produced 4.4 and 4.3 plants per explant, respectively.



(**a**) (**b**) (**c**)

Figure 5. Effect of the three different LED light sources on the production of *C. longa* by TIS. (**a**) Fresh weight of plants. (**b**) Number of shoots. (**c**) Proliferation rate. g: gram. RB1: red:blue LED 1, RB2: red:blue LED 2. Average ± SD was plotted for all replicates analyzed. Tukey's multiple comparison tests were applied at the 95% confidence level to compare the differences. Bars with different letters show significant differences at $p < 0.05$.

For each LED light source analyzed, the Yield Photon Flux Density (YPFD) for red and blue photons, the ratio of red:blue photons, and the Daily Light Integral (DLI) were determined. The results are summarized in Table 1. The white LED had the lowest photosynthetically active radiation (PAR) output performance compared to other light sources; RB1 had ten times more red photons and two times more blue photons than the white LED light source. As for the RB2 ratio, the difference in red photons is similar to the ratio of RB1. However, the number of blue photons is two and five times higher than that of the RB1 and the white LED, respectively. The other parameter analyzed, DLI, shows a similar pattern to that of YPFD. The DLI represents the number of photosynthetically active radiations (PARs) delivered to a specific area over a 24 h period. This variable is particularly useful for describing the light environment of plants; RB2 is the light source with the most photons per area (high DLI).

After the proliferation and rooting period, the plants were acclimated in a greenhouse. The plants exhibited green leaves, abundant rootlets and no vitrification was observed (Figure 6a). After four months of greenhouse cultivation, the plants were about 20 cm in size, had developed the characteristic orange-colored rhizomes (Figure 6b,c), and were ready for shipment back to Rapa Nui.

(**a**) (**b**) (**c**)

Figure 6. Plants from the study of the red:blue LED spectral ratio using TIS. (**a**) Recently sprouted explants taken from the TIS. (**b**) *C. longa* rhizome after four months of greenhouse acclimation. (**c**) *C. longa* plants after four months of greenhouse acclimation.

Table 1. Characteristics of each light source used. s: second, m: meter.

LED Source	YPFD (μmol m^{-2} s^{-1})		Ratio Red: Blue	DLI (mol m^{-2})
	Red	Blue		
White	2.2092	2.7178	≈1:1	0.8609
RB1	25.531	5.4207	≈5:1	3.0226
RB2	25.975	11.733	≈2:1	3.8047

4. Discussion

The success of micropropagation by TIS depends on the volume of the culture vessel and liquid medium container compared to the amount of explant biomass in the early and late stages of cultures, the immersion regime to which the culture is exposed, the type and composition of light, and the effect of each of these parameters on the others [12]. Liquid media cultivation with TIS is considered a technology with great potential in terms of automation and, therefore, reduction of labor costs. However, to optimize the in vitro production of *C. longa*, it is necessary to establish the appropriate experimental conditions.

The first two variables analyzed are closely related. As the number of explants per flask increases, the area available for growth declines. In addition, the movement of the explants during the immersion cycle in the liquid medium is also reduced. Likewise, the flask volumetric capacity had a similar effect. The number of shoots was proportional to the number of explants initially used per TIS flask. The use of greater volumetric capacity flasks provides a larger distribution area for explants during proliferation and rooting in TIS. The round bottom area of a 1 L flask is half that of a 2 L flask, and the use of flasks with smaller round bottom areas may limit propagation. Using a flask with a small bottom area will limit plant growth and result in underdeveloped plants. Therefore, if the number of explants per flask is high (small distribution area) or the capacity is low (small round bottom area), the percentage of *C. longa* explants that do not sprout will increase and TIS productivity will drop.

Another factor to consider is the amount of headspace air available to in vitro plants. In studies on the Musa system [7], it was shown that the supply of air in TIS improved the shoot quality compared to growing in solid media. This may be due to the increase in O_2 (oxygen) concentration and the simultaneous decrease in CO_2 (carbon dioxide) and C_2H_4 (ethylene) concentration by the supply of fresh air during immersions. There are few studies on the effect of the immersion frequency on the micropropagation of *C. longa* in vitro. Previous reports reveal that decreasing ventilation through low number of immersions produces a high fresh weight of plants [23]. In addition, in another study, the authors compared different methods for micropropagation of *Curcuma zedoaria*. They concluded that using TIS with a low immersion frequency generates a better shoot proliferation and could be achieved with less labor input than other systems [24]. Although our results were satisfactory and the immersion conditions were effective, this may be a variable that should be considered in future studies.

Next, three different LED light sources were tested. The main wavelengths required for photosynthesis are in the blue and red regions of visible light. The LEDs used in this study have a spectral energy distribution with an emission peak at 420 and 440 nm for the blue LEDs and at 660 nm for the red LEDs. These wavelengths coincide with the known peak absorption wavelengths for chlorophyll a (430 and 663 nm) and b (453 and 642 nm), indicating optimal photosynthetic efficiency [22]. Plants have photoreceptors to control their responses to these physiologically important wavelengths. Phytochromes are primarily red-light photoreceptors that distinguish between red and far-red wavelengths to control physiological responses such as node elongation [25], floral transition [26], and flowering [27]. Blue light, on the other hand, inhibits cell growth. Blue light photoreceptors can regulate and alter gene expression to inhibit stem elongation [28,29] or control chloroplast movements to maximize light absorption [30]. White LEDs have a complex absorption spectrum that includes blue, green, yellow, orange, red, and far-red wavelength

photons (Supplementary Figure S1a). However, because the photon composition is more complex, the PAR value per day, i.e., the daily light integral (DLI) that the plant receives in 24 h, is significantly lower than that of other light sources. Similarly, the red and blue YPDF values of the white LED (Table 1) are the lowest of all light sources, prioritizing survival over proliferation and multiplication of *C. longa* in vitro.

For RB1, the absorption spectrum peaks at blue, red, and far-red wavelengths, a simpler configuration than that of the white LED (Supplementary Figure S1b). In addition, the DLI value of RB1 was four times higher than that of the white LED. The total number of photons is distributed in a limited number of wavelengths that are more useful to plants during the growth process, resulting in plants with higher fresh weight (Figure 5a) compared to other light sources. However, the number of plants and the proliferation rate were lower than those of other light sources (Figure 5b,c), suggesting that red light promotes plants growth and not the multiplication of initial explants. Furthermore, *C. longa* leaves exposed to RB1 showed progressive etiolation, which is consistent with what has been described in the literature. This is because red LEDs have a more pronounced effect on the plant, acting similar to a growth regulator [31]. Therefore, to improve in vitro propagation of *C. longa* and obtain a large number of plants, RB1 LED light is not sufficient.

On the other hand, blue photons also showed an effect on the multiplication and growth of *C. longa* in vitro. The RB2 light source had more blue photons, which promoted explant multiplication rather than growth. There was an increase in plant number and a decrease in fresh weight compared to the other light sources. The DLI value was the highest among all the light sources studied, which could be attributed to the composition of the absorption spectrum. RB2 has a limited spectral width with only three peaks, two in the blue (420 y 460 nm) and one in the red (660 nm) of the visible spectrum (Figure S1c). Kosell et al. reported that blue light can modulate the concentration of nutritionally important secondary metabolites, such as chlorophyll [32]. These pigments in leaf tissue absorb the maximum amount of light, especially at 430 and 453 nm, and higher concentrations of chlorophyll pigments are produced in response to light with stronger blue wavelengths [32,33]. The RB2 LED light generated more shoots and higher proliferation rates. However, the mechanism by which RB2 and other red and blue photon ratios promotes the multiplication of explants in vitro remains unknown. Therefore, future studies on the effect of LED light on the in vitro culture of *C. longa* by TIS will have to examine secondary metabolism in detail.

5. Conclusions

The number of explants per flask, flask volume, and light source affect in vitro propagation of *C. longa*. The use of 30 explants in 2 L flasks will promote gas exchange (e.g., CO_2, O_2, and C_2H_4) and explant movement, resulting in a greater number of plants (higher proliferation rate). The use of RB2 had a positive effect on the in vitro propagation of *C. longa*. However, other factors such as leaf photosynthetic activity and the synthesis of secondary metabolites produced by *C. longa* should be taken into account in future studies.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11112121/s1, Figure S1. Absorption spectra in the visible region of the light sources used. (a) White LED, (b) Red:Blue LED, and (c) Red:Blue LED 2. The *y*-axis corresponds to the photons flow and the *x*-axis to the absorption of these photons. The black line corresponds to the McCree reference spectrum, which represents the action spectrum of photosynthetically active photons in photosynthesis.

Author Contributions: Conceptualization, M.J.M. and M.C.; methodology M.C., C.F., L.G., C.B., P.M. and M.M.; investigation, P.M., M.M. and M.J.M.; resources, M.C., C.F. and L.G.; writing—original draft preparation, M.J.M. and M.C.; writing—review and editing, M.J.M., M.C., L.G. and C.F.; funding acquisition, M.C., L.G., C.F. and C.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by ANID-FONDEF IT18I0015 "Massive propagation for the sustainable development of medicinal species in Rapa Nui".

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Holdsworth, D.K. A Preliminary Study of Medicinal Plants of Easter Island, South Pacific. *Int. J. Pharmacogn.* **1992**, *30*, 27–32. [CrossRef]
2. Kocaadam, B.; Şanlier, N. Curcumin, an active component of turmeric (*Curcuma longa*), and its effects on health. *Crit. Rev. Food Sci. Nutr.* **2017**, *57*, 2889–2895. [CrossRef] [PubMed]
3. Balada, C.; Castro, M.; Fassio, C.; Zamora, A.; Marchant, M.J.; Acevedo, W.; Guzmán, L. Genetic diversity and biological activity of Curcuma longa ecotypes from Rapa Nui using molecular markers. *Saudi J. Biol. Sci.* **2021**, *28*, 707–716. [CrossRef]
4. Nasirujjaman, K.; Uddin, M.S.; Zaman, S.; Reza, M. Micropropagation of Turmeric (*Curcuma longa* Linn.) through in vitro Rhizome Bud Culture. *J. Biol. Sci.* **2005**, *5*, 490–492. [CrossRef]
5. Etienne, H.; Berthouly, M. Temporary immersion systems in plant micropropagation. *Plant Cell Tissue Organ* **2002**, *69*, 215–231. [CrossRef]
6. Berthouly, M.; Etienne, H. Temporary immersion system: A new concept for use liquid medium in mass propagation. In *Liquid Culture Systems for In Vitro Plant Propagation*; Springer: Dordrecht, The Netherlands, 2005; pp. 165–195.
7. Roels, S.; Noceda, C.; Escalona, M.; Sandoval, J.; Canal-Villanueva, M.J.F.; Rodriguez, R.; DeBergh, P. The effect of headspace renewal in a Temporary Immersion Bioreactor on plantain (Musa AAB) shoot proliferation and quality. *Plant Cell Tissue Organ* **2005**, *84*, 155–163. [CrossRef]
8. El-Hawaz, R.F.; Bridges, W.C.; Adelberg, J.W. In Vitro Growth of Curcuma longa L. in Response to Five Mineral Elements and Plant Density in Fed-Batch Culture Systems. *PLoS ONE* **2015**, *10*, e0118912. [CrossRef]
9. Aragón, C.E.; Escalona, M.; Rodríguez, R.; Canal, M.J.; Capote, I.; Pina, D.; González-Olmedo, J.L. Effect of sucrose, light, and carbon dioxide on plantain micropropagation in temporary immersion bioreactors. *Vitr. Cell. Dev. Biol. Anim.* **2010**, *46*, 89. [CrossRef]
10. Kevers, C.; Franck, T.; Strasser, R.J.; Dommes, J.; Gaspar, T. Hyperhydricity of Micropropagated Shoots: A Typically Stress-induced Change of Physiological State. *Plant Cell Tissue Organ* **2004**, *77*, 181–191. [CrossRef]
11. José, M.C.S.; Blázquez, N.; Cernadas, M.J.; Janeiro, L.V.; Cuenca, B.; Sánchez, C.; Vidal, N. Temporary immersion systems to improve alder micropropagation. *Plant Cell Tissue Organ* **2020**, *143*, 265–275. [CrossRef]
12. Hesami, M.; Jones, A.M.P. Application of artificial intelligence models and optimization algorithms in plant cell and tissue culture. *Appl. Microbiol. Biotechnol.* **2020**, *104*, 9449–9485. [CrossRef]
13. Adelberg, J.; Driesse, T.; Halloran, S.; Bridges, W.C. Relationships between nutrients and plant density in liquid media during micropropagation and acclimatization of turmeric. *Vitr. Cell. Dev. Biol. Anim.* **2013**, *49*, 724–736. [CrossRef]
14. Gupta, S.D.; Jatothu, B. Fundamentals and applications of light-emitting diodes (LEDs) in plant growth and morphogenesis. *Plant Biotechnol. Rep.* **2013**, *7*, 211–220. [CrossRef]
15. Miler, N.; Kulus, D.; Woźny, A.; Rymarz, D.; Hajzer, M.; Wierzbowski, K.; Nelke, R.; Szeffs, L. Application of wide-spectrum light-emitting diodes in micropropagation of popular ornamental plant species: A study on plant quality and cost reduction. *Vitr. Cell. Dev. Biol. Plant* **2018**, *55*, 99–108. [CrossRef]
16. Wangchuk, K.; Manochai, B.; Chulaka, P.; Wongchaochant, S.; Chintakovid, W.; Pumprasert, J. Monitoring of active constituents of turmeric (*Curcuma longa* L.) rhizome stored under supplemented white LED-light with different light intensities. *Acta Hortic.* **2019**, 131–138. [CrossRef]
17. Mendonça, E.G.; Stein, V.C.; De Carvalho, H.H.; Santos, B.R.; Beijo, L.; Paiva, L.V. The Use of Continuous, Temporary Immersion Bioreactor System and Semisolid Culture Medium for the Production of Eucalyptus camaldulensis CLONES. *Ciência Florest.* **2016**, *26*, 1211–1224. [CrossRef]
18. Escalona, M.; Lorenzo, J.C.; González, B.; Daquinta, M.; González, J.L.; Desjardins, Y.; Borroto, C.G. Pineapple (Ananas comosus L. Merr) micropropagation in temporary immersion systems. *Plant Cell Rep.* **1999**, *18*, 743–748. [CrossRef]
19. Carvalho, L.S.O.; Ozudogru, E.A.; Lambardi, M.; Paiva, L.V. Temporary Immersion System for Micropropagation of Tree Species: A Bibliographic and Systematic Review. *Not. Bot. Horti Agrobot.* **2019**, *47*, 269–277. [CrossRef]
20. Grzegorczyk-Karolak, I.; Grąbkowska, R.; Piątczak, E. Plant Liquid Cultures as a Source of Bioactive Metabolites. *Plant Cell Tissue Differ. Second. Metab. Fundam. Appl.* **2021**, 743–771. [CrossRef]
21. Murashige, T.; Skoog, F. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiol. Plant.* **1962**, *15*, 473–497. [CrossRef]

22. McCree, K. The action spectrum, absorptance and quantum yield of photosynthesis in crop plants. *J. Agric. Meteorol.* **1971**, *9*, 191–216. [CrossRef]
23. Adelberg, J.; Cousins, M. Thin Films of Liquid Media for Heterotrophic Growth and Storage Organ Development: Turmeric (Curcuma longa) as a Model Plant. *HortScience* **2006**, *41*, 539–542. [CrossRef]
24. Stanly, C.; Bhatt, A.; Keng, C.L. A comparative study of Curcuma zedoaria and Zingiber zerumbet plantlet production using different micropropagation systems. *Afr. J. Biotechnol.* **2010**, *9*, 4326–4333.
25. Schaer, J.A.; Mandoli, D.F.; Briggs, W.R. Phytochrome-Mediated Cellular Photomorphogenesis. *Plant Physiol.* **1983**, *72*, 706–712. [CrossRef] [PubMed]
26. Boss, P.K.; Bastow, R.M.; Mylne, J.S.; Dean, C. Multiple Pathways in the Decision to Flower: Enabling, Promoting, and Resetting. *Plant Cell* **2004**, *16*, S18–S31. [CrossRef] [PubMed]
27. Runkle, E.; Heins, R.D. Specific Functions of Red, Far Red, and Blue Light in Flowering and Stem Extension of Long-day Plants. *J. Am. Soc. Hortic. Sci.* **2001**, *126*, 275–282. [CrossRef]
28. Lin, C. Plant blue-light receptors. *Trends Plant Sci.* **2000**, *5*, 337–342. [CrossRef]
29. Banerjee, R.; Batschauer, A. Plant blue-light receptors. *Planta* **2005**, *20*, 498–502. [CrossRef] [PubMed]
30. Fraikin, G.Y.; Strakhovskaya, M.G.; Rubin, A.B. Biological photoreceptors of light-dependent regulatory processes. *Biochemistry* **2013**, *78*, 1238–1253. [CrossRef] [PubMed]
31. Wang, H.; Gu, M.; Cui, J.; Shi, K.; Zhou, Y.; Yu, J. Effects of light quality on CO2 assimilation, chlorophyll-fluorescence quenching, expression of Calvin cycle genes and carbohydrate accumulation in Cucumis sativus. *J. Photochem. Photobiol. B Biol.* **2009**, *96*, 30–37. [CrossRef] [PubMed]
32. Kopsell, D.; Sams, C.E.; Morrow, R.C. Blue Wavelengths from LED Lighting Increase Nutritionally Important Metabolites in Specialty Crops. *HortScience* **2015**, *50*, 1285–1288. [CrossRef]
33. Brazaitytė, A.; Miliauskienė, J.; Vaštakaitė-Kairienė, V.; Sutulienė, R.; Laužikė, K.; Duchovskis, P.; Małek, S. Effect of Different Ratios of Blue and Red LED Light on Brassicaceae Microgreens under a Controlled Environment. *Plants* **2021**, *10*, 801. [CrossRef]