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Ex Vitro Simultaneous Acclimatization and Rooting of In Vitro Propagated Tamarillo Plants (*Solanum betaceum* Cav.): Effect of the Substrate and Mineral Nutrition

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Abstract: Plants propagated by seed do not ensure genetic uniformity and are sometimes infected with diseases. In Vitro micropropagation techniques are an alternative method to traditional cloning approaches for producing true-to-type and pathogen-free plants. However, due to the particularities of the in vitro environment, these plants face many challenges, often critical to their survival, to adapt to ex vitro conditions. In this context, four substrates and two types of mineral nutrition (quick-release (QRF) and controlled-release (CRF) fertilizers), as well as their absence were evaluated in the process of acclimatization of *Solanum betaceum* plants. Stomatal conductance (g_s), chlorophyll content index (CCI), and dry biomass of roots, shoots, and entire plants were the parameters analyzed during the acclimatization. The best crop performance (g_s, CCI, and dry biomass) were observed in substrates consisting of vermiculite plus the application of mineral nutrients through a CRF, proving that mineral nutrition has the greatest positive impact on the acclimatization process. In these conditions, plants were obtained with a total dry biomass being significantly higher (515.0 mg (QRF) and 635.9 mg (CRF) when compared to the total dry biomass of untreated plants (119.9 mg). The best conditions for this first experiment were replicated in a second test in order to assess the best fertilizer amount suited for plantlet growth. In this case, the best results were obtained with 0.4 g of CRF, in which plants showed a dry biomass of roots (542.7 mg) and a total dry biomass (594.5 mg), which was significantly higher than in the control (183.2 mg and 165.9 mg, respectively) or with other concentrations of CRF (0.8 and 1.6 g). A similar trend was found concerning the CCI (5.3) and g_s (72.5 mmol m^{-2} s^{-1}) in which 0.4 g CRF gave also the best results when compared with the control (without CRF) or with 0.8 g (4.7 and 56.2 mmol m^{-2} s^{-1}) and 1.6 g (4.7 and 52.2 mmol m^{-2} s^{-1}) treatments. In general, it was found that tamarillo plantlets acclimatized to 0.4 g of CRF had a faster initial growth and better performance (CCI and g_s), with plants ready to go to the greenhouse/field more quickly, thus reducing the time to obtain suitable plants for the market and shortening the production cycle.

Keywords: chlorophyll content; controlled-release fertilizer; dry biomass; in vitro micropropagation; quick-release fertilizer

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1. Introduction

Tamarillo (*Solanum betaceum* Cav.), commonly called tree tomato or "tomate de la paz", in Spanish [1], is native to the Andean region of South America [2,3], where it grows wild or in orchards. It is cultivated for its juicy edible fruits, and has interesting nutritional properties due to low caloric levels [4] and relatively high protein content, vitamins, minerals [5], and chemicals with important antioxidant, therapeutic, and preventive properties [6–8].

Tamarillo can be propagated by seeds or conventional methods of vegetative propagation, such as cutting and grafting [4]. However, propagation through seeds does not

assure the genetic uniformity of plants, besides being a slower process thus compromising productivity. Traditional cloning methods applied to this species have proven to be difficult given that the propagated plants are sometimes infected with diseases [4]. Production of pathogen-free material is the first step of controlling diseases [9]. In Vitro micropropagation techniques, as an alternative method of cloning, have been applied to many crops to propagate selected genotypes or hybrids and to scale-up cloning [10].

Although in vitro cloning is nowadays a set of techniques of common use, the transfer of plants to the field is usually a crucial step of the micropropagation process that quite often limits the success of these techniques [11–13]. A critical step in micropropagation, is the adaptation of in vitro produced plantlets to ex vitro environments, due to the shock resulting from the transfer from a high humidity environment to much drier conditions [14,15]. In general, plants need several weeks to adapt to the new ex vitro conditions and correct anomalies in their morphology, anatomy, and physiology, induced by the special conditions of in vitro culture [15–17]. In other words, they need an acclimatization period.

Acclimatization refers to the climatic adaptation of an organism that has been placed in a new environment that is different from the previous one [18]. Thus, an effective protocol for acclimatization of in vitro propagated plants must be developed to assure that losses during this process are residual.

The functionality of the adventitious root system is essential to plant survival during the acclimatization process, as it affects the absorption of water and nutrients, which were previously guaranteed by the in vitro medium [17]. Therefore, factors such as substrate and mineral nutrition may also affect the success of the acclimatization process.

According to Moreira et al. [19], choosing a suitable substrate reduces plant mortality during acclimatization. Several authors have witnessed different results in the performance of the cultures according to the type of substrate used. For example, the use of high amounts of vermiculite [20], a mixture of peat and vermiculite [21], or a combination of soil and vermiculite [22] have resulted in better crop performance in terms of plant growth [20,22], root length, as well as root growth and penetration into the subsoil [20]. Moreover, some substrates facilitate the functional link between soil/root microbiota and subsequent increase in crop yield [21]. In the same way, according to Betancourt-Osorio et al. [23] plant nutritional status may significantly influence plant acclimatization, and according to Oliet et al. [24], the way nutrients are made available to plants can substantially influence seedling development.

The most commonly used commercial fertilizers are water soluble quick-release fertilizers (QRF) that are predictively readily available for plants [25]. QRF, also called conventional fertilizers, are ideal for pre-plant applications and are the most used in nurseries in the fertigation form [25]. However, the application of solid fertilizers to the substrate, particularly fertilizers that are not immediately available to plants, have proven to be a good alternative [24,26], because conventional fertilizers, due to their immediate release, do not follow the dynamic needs of crop growth [25,27].

An alternative are the controlled-release fertilizers (CRF) or slow-release fertilizers (SRF).The release rate of CRFs is designed in a way that allows for synchronized nutrient release that matches the needs of plants at different stages of development. In these kind of fertilizers, granules are coated or encapsulated by organic or inorganic materials that control the rate, pattern, and duration of the nutrient release [25,28]. On the contrary, in SRFs, nutrient release is not controlled because it depends on microbial activity, which, in turns, depends on environmental conditions such as temperature and humidity [25,29–31].

There are several protocols for tamarillo cloning using proliferation of axillary shoots [32], organogenesis [33,34], and somatic embryogenesis [35,36]. However, a detailed study concerning the effects of substrate and mineral nutrition on the acclimatization of the micropropagated plants has not been performed yet. The objective of this work was the comparative analysis of four substrates and two types of mineral nutrition (QRF and CRF) as well as their absence, and their influence on the growth and development of tamarillo micropropagated plantlets during the acclimatization process. The evaluation of these

factors was carried out through the study of photosynthetic parameters such as the chlorophyll content index and stomatal conductance, as well as through plant growth parameters such as the quantification of the biomass of the acclimatized plants.

2. Materials and Methods

2.1. In Vitro Culture

Tamarillo plants were established from in vitro germinated seedlings of a red variety. Briefly, seeds were collected from mature fruits, gathered from trees growing at the Botanic Garden of the University of Coimbra, coordinates 40°12′22.43″ N and 8°25′32.44″ W; altitude 74 m. They were rinsed in running water and placed in a 20% sodium hypochlorite solution, containing two to three drops of Tween 20, for about 15 min. and after disinfection were subjected to three washes with sterile distilled water. Germination occurred in Petri dishes and the developing seedlings were transferred to test tubes with 12 mL of MS medium [37] supplemented with 3% sucrose, and the pH adjusted to 5.7. In Vitro multiplication was achieved by axillary shoot proliferation in test tubes with 12 mL of MS medium supplemented with 3% sucrose, 0.2 mg L^{-1} of benzylaminopurine (BAP), and pH adjusted to 5.7, and placed in growth chambers at 25 °C, with a photoperiod of 16 h light and 8 h dark with white light and 15–20 µmol m^{-2} s^{-1} of intensity.

2.2. Rooting, Acclimatization and Treatment with Fertilizers

Rooting was carried out ex vitro, placing the shoots directly on the substrate without any additional treatment [38]. Rooting occurred simultaneously with the acclimatization process. Plants were transplanted to 34.3 cm × 21.3 cm × 9 cm alveoli trays, with 40 compartments of about 170 cm^3. The substrates consisted of peat, peat and perlite (1:1), peat and vermiculite (1:1), and peat, perlite, and vermiculite (1:1:1). For each substrate, three treatments were tested: (i) plants were not nutritionally supplemented (control treatment—C); (ii) plants were supplemented with controlled-release fertilizer (CRF); (iii) plants were supplemented with a water soluble quick-release fertilizer (QRF). Since there is no information about the reference values for the use of fertilizers in tamarillo shoots during the acclimatization phase, it was decided to use approximately 0.8 g of CRF per alveoli, and a dilution of 1.5 mL/L of QRF, according to the manufacturers' instructions. These fertilizers were chosen because fertigation is the most common procedure in nurseries (QRF) [24,25], vegetative growth was intended at this stage, and these were the ones with the highest and similar nitrogen content (CRF = 10% N; QRF = 12% N) available in the market. In this first experiment, a total of 192 plants were used (16 plants × 4 substrates × 3 treatments).

A second test was also carried out in which the substrate consisted of a mixture of peat and vermiculite (1:1) and to which CRF (10% N) was added. This fertilizer was chosen because it showed good results in the first assay (see Results and Discussion). Four treatments were applied. In the control, the plants were not nutritionally supplemented and, in the remaining treatments, three amounts of CRF were tested: 0.4, 0.8, and 1.6 g/alveoli. A total of 64 plants were used (16 plants × 4 treatments).

In both tests, rooting and acclimatization occurred simultaneously and fertilizers were applied in the substrate. CRF was applied only once, at the time plantlets were transferred to the alveoli, whereas QRF was applied with watering. The alveoli trays containing the plants were covered with a hood with an air filter, the opening of which occurred gradually in order to keep the humidity values relatively high, at least during the first days after transplantation, in order to facilitate the adaptation to ex vitro conditions. The total opening of the air filters and the removal of the hoods occurred 1.5 and 3 weeks after transfer to ex vitro conditions, respectively.

After four weeks, the acclimatization was evaluated by calculating plant survival rates, according to the equation [38]:

$$\text{Survival rate } (\%) = \frac{\text{number of survival plants}}{\text{number of acclimatized plants}} * 100$$

2.2.1. Dry Biomass

By the end of the assay the biomass was quantified by weighing the dehydrated plants previously kept for 3 days in a drying oven at 57.5 °C (when the weight stabilized).

2.2.2. Stomatal Conductance

Stomatal conductance was registered with a *Decagon Devices* leaf porometer, model SC-1 (NE Hopkins Court, Pullman, WA 99163, USA). A total of 15 measurements was performed during the acclimatization period, after the fourth week of ex vitro transplantation, 8 and 7 times before irrigation, in the first and second experiments, respectively. Before this period, the plants were too small and it was not possible to position the leaf porometer clamp without extracting them from the substrate. Measurements were made in 8 of the 16 acclimatized plants in a total of 120 records. Results are presented as the average of all measurements ± standard error.

2.2.3. Chlorophyll Content Index

Chlorophyll content index (% transmission at 940 nm/% transmission at 620 nm) was registered in the same conditions as described for stomatal conductance. A Hansatech chlorophyll content meter, model CL-01 (Hansatech Instruments, Norfolk UK) was used, and the measurements took place 8 and 7 times in the first and second experiments, respectively, in the same way that the stomatal conductance measurements occurred.

2.3. Statistical Analysis

Values are given as mean ± standard error (SE). Data were analyzed and comparisons between treatments were made using One-way ANOVA test on IBM SPSS Statistics 26 software (Armonk, NY, USA), followed by a Tukey's multiple comparison test, for a significance level of 0.05.

3. Results

Ex vitro simultaneous acclimatization and rooting of tamarillo plants occurred with survival rates of 75.0, 81.3, and 93.8% for plants acclimatized with 1.6, 0.8, and 0.4 and 0 g/alveoli of CRF (second test), respectively. In the first test, there was a survival rate of 100% in all treatments.

It was not possible to perform measurements of g_s and the CCI in the plantlets that were not nutritionally supplemented (in both tests) because they were too small to correctly position the measuring equipment on leaves (Figure 1). The results clearly show (Figure 1) that the mineral nutrition has a considerable impact on plant growth during ex vitro acclimatization, making it possible to shorten the duration of this period and have plants ready to proceed to the greenhouse/field faster.

There were no significant differences at the level of g_s, regarding the type of substrate (Table 1). However, in relation to the type of fertilizer, it was found that g_s was significantly ($p = 0.02$) higher in plants supplemented with CRF comparatively to QRF (Table 1). Similar results were obtained for the CCI, which was also significantly ($p = 0.00$) higher in plants treated with CRF (Table 1).

Table 1. Effect of substrates (P: peat; PP: peat with perlite; PV: peat with vermiculite; PPV: peat with perlite and vermiculite—without distinguishing between fertilizers); and the effect of controlled-release (CRF) and quick-release (QRF) fertilizers (without distinguishing between substrates), on stomatal conductance (g_s—mmol m^{-2} s^{-1}) and chlorophyll content index (CCI). Each value is the average ± SE (standard error) of 120 records taken in 8 plants, 3 days each, during the last 2–3 weeks of the acclimatization period.

	Substrates				Fertilizer	
	P	**PP**	**PV**	**PPV**	**CRF**	**QRF**
gs	74.2 ± 28.8 ^{a}	72.5 ± 38.4 ^{a}	61.9 ± 29.4 ^{a}	88.1 ± 72.9 ^{a}	91.6 ± 54.1 ^{a}	56.7 ± 27.0 ^{b}
CCI	6.4 ± 2.1 ^{b,c}	5.8 ± 2.1 ^{c}	8.3 ± 2.8 ^{a,b}	9.5 ± 3.1 ^{a}	9.7 ± 2.3 ^{a}	5.3 ± 1.5 ^{b}

For the same row, averages with the same letter are not significantly different ($p > 0.05$).

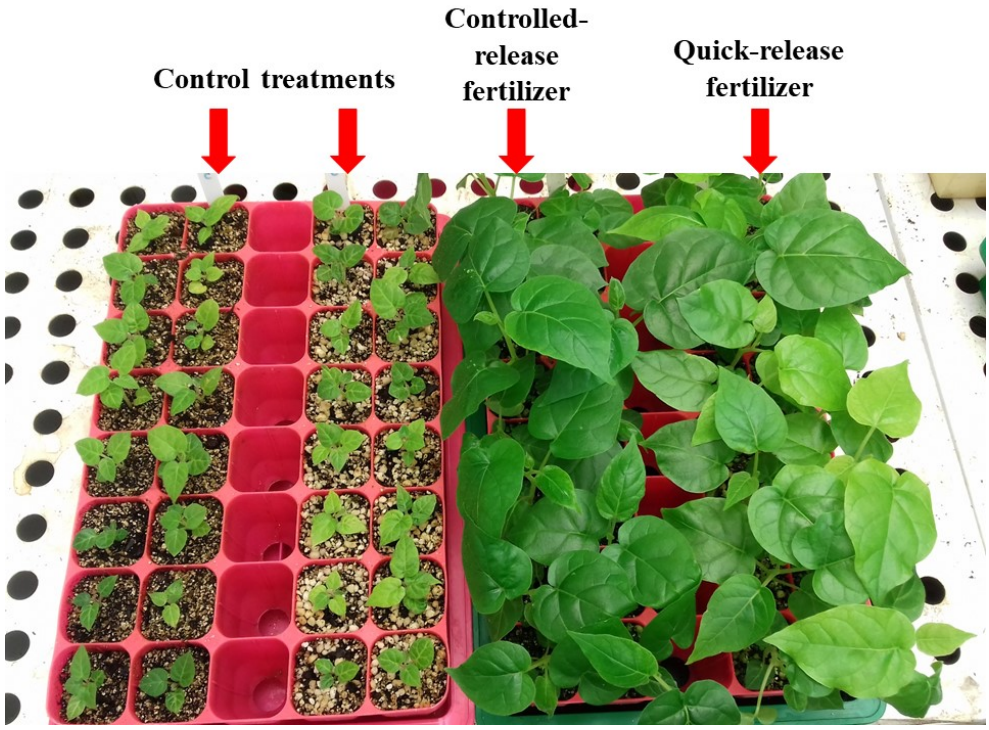

Figure 1. Effect of nutritional supplementation on plant growth during the acclimatization period (3 weeks after ex vitro transplantation).

The CCI was also significantly higher in plants whose substrate consisted of a mixture with vermiculite when compared to those without vermiculite (Table 1).

Table 2 shows how dry biomass is affected by fertilization. The results indicate that the amount of biomass was significantly higher in plants supplemented with mineral nutrients. Such as for CCI, the dry biomass of roots and shoots was significantly higher in plants whose substrate consisted of a mixture with vermiculite when compared to those without vermiculite, as shown in Table 3.

Table 2. Effect of the type of fertilization (C: control treatment; QRF: quick-release fertilizer; CRF: controlled-release fertilizer) on the biomass of the acclimatized plants. Each value is the average ± SE (standard error) of 120 records taken in 8 plants, for 3 days, during the last 2–3 weeks of the acclimatization period.

Fertilizer	Dry Biomass		
	Root	Shoot	Total
C	80.6 ± 30.4 ^{c}	159.2 ± 51.1 ^{b}	119.9 ± 57.5 ^{b}
QRF	210.7 ± 57.6 ^{b}	820.3 ± 287.2 ^{a}	515.0 ± 370.7 ^{a}
CRF	361.8 ± 111.3 ^{a}	910.0± 319.2 ^{a}	635.9 ± 364.5 ^{a}

For the same column, averages with the same letter are not significantly different ($p > 0.05$).

Table 3. Effect of the substrate (P: peat; PP: peat with perlite; PV: peat with vermiculite; PPV: peat with perlite and vermiculite) on dry biomass (mg) of roots, shoots, and entire plantlets.

Substrate	Dry Biomass		
	Root	Shoot	Total
P	196.3 ± 82.3 ^{a,b}	591.6 ± 312.8 ^{a,b}	393.9 ± 301.3 ^{a}
PP	176.9 ± 82.7 ^{b}	485.4 ± 370.4 ^{b}	331.1 ± 306.1 ^{a}
PV	251.3 ± 191.0 ^{a}	702.8 ± 500.4 ^{a,b}	477.1 ± 436.4 ^{a}
PPV	246.2 ± 160.3 ^{a}	739.6 ± 464.0 ^{a}	492.9 ± 422.9 ^{a}

For the same column, averages with the same letter are not significantly different ($p > 0.05$).

The interaction between substrates (P, PP, PV, and PPV) and fertilizer (C, QRF, and CRF) did not have a significant effect on g_s, CCI, shoots' dry biomass, and total dry biomass. Therefore, the factors act independently on these variables (Table 4), but the interaction substrate/fertilizer had a significant effect for the roots dry biomass (Table 4). Thus, root growth is promoted by a substrate containing a mixture with vermiculite (Table 3) and supplemented with CRF (Table 2).

Table 4. Analysis of variance for the interaction between substrates (peat; peat with perlite; peat with vermiculite; peat with perlite and vermiculite) and fertilizer (control treatment; quick-release fertilizer; controlled-release fertilizer) in all parameters analyzed.

		Sum of Squares	df	Mean Square	F	*p*-Value
g_s	substrate	5578.9	3	1859.6	0.975	0.411
	fertilizer	19,496.3	1	19,496.3	10.219	0.002
	substrate × fertilizer	964.8	3	321.6	0.169	0.917
CCI	substrate	138.9	3	46.3	31.706	0
	fertilizer	311.8	1	311.8	213.455	0
	substrate × fertilizer	7.1	3	2.4	1.615	0.196
Roots dry biomass	substrate	48,799.8	3	16,266.6	5.794	0.002
	fertilizer	633,841.6	2	316,920.8	112.878	0
	substrate × fertilizer	99,655.7	6	16,609.3	5.916	0
Shoots dry biomass	substrate	476,560.2	3	158,853.4	3.169	0.036
	fertilizer	5,380,192	2	2,690,096	53.664	0
	substrate × fertilizer	524,065.8	6	87,344.3	1.742	0.139
Total dry biomass	substrate	410,249.5	3	136,764.8	1.505	0.219
	fertilizer	4,664,187	2	2,332,094	25.671	0
	substrate × fertilizer	440,497.2	6	73,416.2	0.808	0.566

The second assay was established in a mixture of peat and vermiculite, to which different amounts of CRF were added. The results displayed in Table 5, show that the total dry biomass (entire plantlets) is significantly higher in plants acclimatized in alveoli containing 0.4 g CRF. This means that the CRF has a positive impact on the growth and development of the tamarillo plantlets during the acclimatization process (low values of total dry biomass in not treated plantlets), but only when a certain amount of fertilizer is supplied. In fact, 0.8 and 1.6 g CRF/alveoli considerably reduce all the parameters analyzed. These results were corroborated by the evaluation of the physiological parameters (stomatal conductance and chlorophyll content) presented in Table 6, and in Figures 2 and 3, in which the regression lines slopes are negative for total biomass as well as for stomatal conductance and chlorophyll content.

Table 5. Effect of different concentration of CRF on dry biomass of roots, shoots, and entire plantlets (total).

CRF Amount	Dry Biomass		
	Roots	Shoots	Total
0 g	183.2 ± 52.0 [b]	148.5 ± 55.3 [a]	165.9 ± 51.7 [b]
0.4 g	542.7 ± 138.7 [a]	646.3 ± 168.6 [a]	594.5 ± 149.3 [a]
0.8 g	63.3 ± 44.2 [b]	392.2 ± 265.7 [a]	227.7 ± 248.0 [b]
1.6 g	35.7 ± 36.6 [b]	282.9 ± 258.3 [a]	159.3 ± 213.4 [b]

For the same column, averages with the same letter are not significantly different ($p > 0.05$).

Table 6. Effect of different concentration of CRF on stomatal conductance (g_s) and chlorophyll content index (CCI).

CRF Amount	gs	CCI
0 g *	—	—
0.4 g	72.5 ± 12.9 [a]	5.3 ± 0.5 [a]
0.8 g	56.2 ± 8.2 [b]	4.7 ± 0.3 [b]
1.6 g	52.2 ± 12.7 [b]	4.7 ± 0.3 [b]

For the same column, averages with the same letter are not significantly different ($p > 0.05$). * Due to the small dimensions of the leaves measurements could not be done.

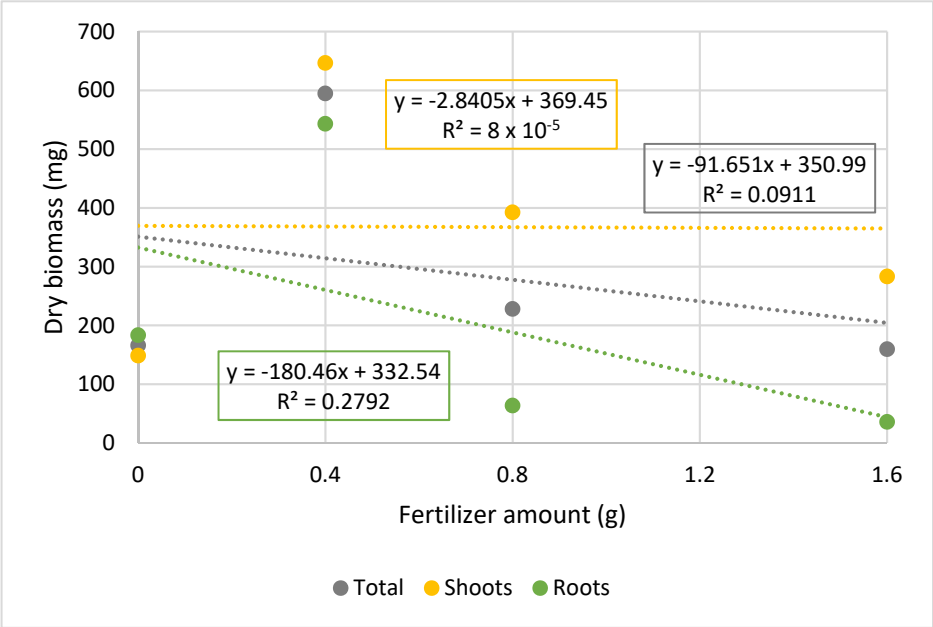

Figure 2. Linear regressions established between the dry biomass of the acclimatized tamarillo plants and the applied CRF doses.

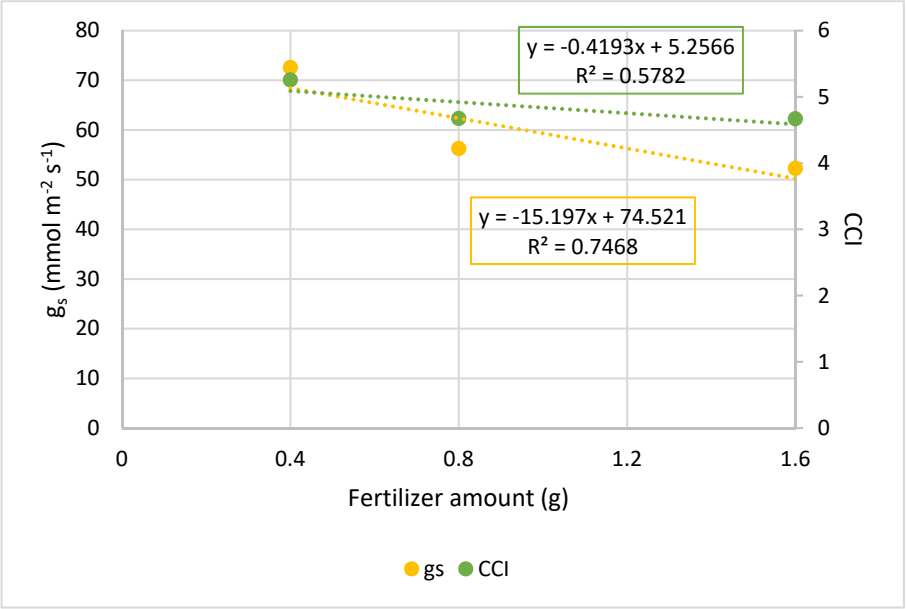

Figure 3. Linear regressions established between the stomatal conductance (g_s) and the chlorophyll content index (CCI), and the applied CRF doses.

4. Discussion

The results obtained revealed survival rates above 75%, which demonstrates the high capacity of this species to adapt to ex vitro conditions [38,39]. However, the survival rate results obtained in the first (100%) and second (between 75 and 93.8%) experiments are somewhat different. Even so, from the observation of Figure 1, and Tables 1 and 2, it is evident that CRF has a positive influence on tamarillo plant development during the acclimatization/rooting period, although there may be some variability in the results [38]. As no bibliographic references concerning this aspect were found, the second test was carried out in an attempt to find a reference dose of CRF for tamarillo during the acclimatization/rooting phase.

In addition to the survival rate, the g_s and the chlorophyll content were evaluated since, according to Mielke and Schaffer [40], the ability of a given species to acclimatize in different environments can be evidenced by the evaluation of the initial growth and the plasticity of the photosynthetic system.

A critical aspect for plant survival during the acclimatization process is the development of an effective stomatic regulation of transpiration [15,16,41,42], because the main problem after the transfer from the in vitro to the ex vitro environment, it is the high rate of water losses by the plants. The reason why it was decided to test the acclimatization of *S. betaceum* on different substrates, which has no direct influence on the plants' physiology, was because this could condition water availability until they achieved effective control of stomatal activity. The substrates were made with mixtures of perlite, a very porous material, and vermiculite, which has a high water-holding capacity. Thus, it was assured that tamarillo plantlets could overcome its known waterlogging problems [23] and the moisture necessary for plants adjusting to the ex vitro environment, leading to it being considerably dryer than in vitro conditions. However, no significant differences in the values of g_s, in relation to the type of substrate were found. Perhaps this is because the mechanisms that regulate stomatal activity are similar at this stage of plant development, since stomatal activity tends to be less variable in very young or older plants [43]. However, when the effect analyzed was the type of fertilizer, it was found that the g_s was significantly higher in plants supplemented with CRF comparatively to QRF. In the same way, the CCI was also significantly higher in plants that received CRF.

Between 50 to 70% of the nitrogen (N) in the leaves is associated with enzymes present in chloroplasts, pointing to a direct relationship between the N and chlorophyll contents [44–46]. However, our results showed that the plants treated with the fertilizer that provides 10% nitrogen (CRF) had a significantly higher CCI when compared to the fertilizer that provides 12% nitrogen (QRF). Apparently, these results are not due to the N content of the fertilizers (which is still similar), but to their release characteristics.

According to Oliet et al. [24] the form of nutrient delivery can substantially influence the development of the plants. Thus, the application of gradual release fertilizers has been a promising alternative to conventional water soluble quick-release fertilizers, commonly used in nurseries and for pre-plant applications [24,25], because as CRF release nutrients over time they replenish nutrients in the soil solution as nutrients are released from the fertilizer granules [47].

Considering that chlorophyll content is crucial for photosynthetic efficiency and, consequently, to growth and adaptability to different environments, the use of gradual release fertilizers can contribute to obtain plants of better potential quality, such as those observed by Oliet et al. [24], Almeida et al. [30], Rossa et al. [31], and Neto et al. [48].

The CCI was also significantly higher in plants whose substrate consisted of a mixture with vermiculite, compared to those without vermiculite, but according to Afonso et al. [49], it is possible the photosynthetic pigments show both qualitative and quantitative changes according to the substrate composition, as found by these researchers in *Albizia niopoides*. Thus, in our experiments, it seems plausible to assume that, due to the greater water retention capacity of vermiculite [20], which in this case would be a nitrogen solution (QRF

and CRF), and given the relationship between N and chloroplasts, chlorophyll was superior in plants developing in substrates with this element.

According to Costa [50], the nutritional status of plants can be determined by analyzing their composition, and the best way to do this is, in a first approach, is to evaluate dry matter. From Table 2 it is evident that the amount of biomass was significantly higher in plants supplemented with mineral nutrients. These results are in agreement with observations [51] showing that plants from nutrient-rich sites tend to produce more biomass per unit nutrient in the plant. Similar results were observed by other researchers. Thus, Ramírez-Soler et al. [52], reported that unfertilized tamarillo plants showed a decrease in the dry matter of roots, shoots, and total, in relation to well-nourished plants. Clark and Richardson [53] recorded that there is a relationship between the accumulation of biomass in the tamarillo plants and the mineral nutrients, and Betancourt-Osorio et al. [23] found that well-nourished tamarillo plants showed a significantly higher shoot length than poorly nourished plants.

Our data showed that the dry biomass of roots and shoots was higher in plants acclimatized in substrates made up of mixtures with vermiculite (PV; PPV). Similar results were obtained by Jacobs et al. [20] in *Pseudotsuga menziesii*, wherein peat and vermiculite facilitated the penetration of roots into the substrate, resulting in greater root volume and length, and greater average height. Thus, considering the first test performed, the substrates were made up of mixtures with vermiculite and supplemented with mineral nutrients through the application of CRF, which seems to favor the ex vitro acclimatization of tamarillo plants, being evident that it is mineral nutrition that has the greatest impact in this process.

In this framework, the second test was established in a mixture of peat and vermiculite and applying different concentrations of CRF, with the objective of determining which are the best amounts that should be used in tamarillo. The results showed that CRF has a positive impact on tamarillo growth during the acclimation process, given the low values of total dry biomass of non-treated plants, which reinforces the results obtained in the first test. However, this positive effect was only observed when relative low amounts (0.4 g/alveoli) were used. According to Larcher [44], having satisfied the needs of the plants, an increase in nutrients can confer advantages in terms of competition for increasing resistance to pathogens or adverse climatic conditions. However, this can also not promote any improvement, just luxury consumption [51]. Additionally, when the concentrations are too high, they can even become toxic [51]. In our experiments, it seems that luxury consumptions may have occurred for the two highest concentrations of fertilizer (0.8 and 1.6 per alveoli). Because of that, these plants might not be stimulated to expand the root system in search of nutrients. Supporting this assumption are the data in Table 5, showing the difference between the dry biomass of the roots and shoots of the non-fertilized plants when compared with those treated with 0.4 g/alveoli of CRF; the last ones display more proportional values, also comparatively with the plants treated with 0.8 and 1.6 g/alveoli of CRF. The development and architecture of the root system can interfere with the greater or lesser success of plant establishment in the field, since the root biomass is an indicative of the better performance of the plants when transferred to the field, because they present greater capacity of support and absorption of water and nutrients, increasing the productive potential and the ability to adapt to adverse environmental conditions [54,55].

The regression analyses of these results show that the root system is the most sensitive to variations in concentration of CRF. It can also be seen (Figure 2) that the amount of dry biomass (total, roots, and shoots) is greater for a smaller concentration of CRF (0.4 g/alveoli), and that all slopes of the regression lines are negative, which suggests that with this concentration of CRF, toxicity phenomena may also have occurred. Similar results were obtained by Silva et al. [56] who found that CRF had a significant influence on the growth of *Acacia mangium*; however, there was a reduction in seedling gain after a specific dose, which varied according to the parameters evaluated.

The results showed in Figure 3 also indicate that, similar to dry biomass, the slopes of the regression lines established between the amount of CRF and the physiological parameters of g_s and CCI are negative, and therefore, the greater the amount of CRF, the lower the g_s and the chlorophyll content of the plants. Moreover, g_s and CCI were significantly higher in plants treated with 0.4 g/alveoli of CRF, compared to those treated with the highest amounts, thus supporting the idea that the 0.8 and 1.6 g/alveoli fertilizer's concentrations would be too high for these plants in these conditions, becoming toxic.

Taken together, the results indicate that in tamarillo, after the ex vitro transplantation, supplementation with nutrients promotes plant development and reduces the acclimatization period, allowing plants to be ready to go to the greenhouse/field more quickly, and giving them competitive advantages due to their more developed root systems and stem growth, and therefore, greater photosynthetic capacity. It was also found that the amount of CRF used in the first test would have been too high, given the results of the second test (under the same conditions), but as the comparison term was the application of fertilizer or its absence, and as it was found that supplementation with mineral nutrients has a positive impact on the acclimatization process; that specific dose of CRF was always better than nothing. However, this test revealed that these plants are very small (seedlings) and fragile, their nutritional needs are low, and toxicity phenomena can easily arise at the same time that resources are being consumed unnecessarily.

Several researchers have focused their research on the crucial issue of acclimatization of micropropagated plants, and there are several works describing methodologies that allow improving the performance of these plants in vitro, giving them greater resistance to later ex vitro conditions, such as through manipulation of the culture medium composition [57], the use of bioreactors [58], or selenium nanoparticles [59]. Perez-Jiménez et al. [60] went further and studied the effect of different CO_2 concentrations on the ex vitro acclimatization process in *Cynara scolymus* plants. Our study is a further contribution, advancing the use of mineral nutrients that were usually only used by nurseries, when the plants are already perfectly adapted to the ex vitro environment, and showed potential for the future development of a protocol for ex vitro acclimatization, in the sense to make the process faster and losses during this phase minimal.

Thus, CRFs are interesting alternatives, as they can reduce the time to obtain suitable plantlets for the market and shorten the production cycle, reducing labor and mainly offering effective measures to be implemented to improve the quality of plantlets and optimize production costs [26], when testing/knowing the specific needs of each plant species at each stage of its state of growth and development.

5. Conclusions

Considering the results obtained, it can be concluded that: (i) the tamarillo plants showed great capacity to the ex vitro environment adaptation; (ii) mineral nutrition has a strong positive impact on plant growth during the acclimatization process; (iii) the type of substrate had no influence on the g_s, but this parameter was significantly higher in the plants to which CRF was administered when compared to QRF; (iv) the CCI was significantly higher in plants whose substrate consisted of a mixture with vermiculite when compared to those without vermiculite, and significantly higher in plants that were administered CRF when compared to QRF; (v) the amount of dry biomass was significantly higher in plants supplemented with mineral nutrients, but was not affected by the type of substrate; (vi) the total dry biomass, g_s, and CCI were significantly higher in tamarillo plants supplemented with 0.4 g/alveoli of CRF when compared to 0 g/alveoli and/or 0.8 and 1.6 g/alveoli.

The data also showed that mineral nutrition promotes plant growth when transplanting ex vitro, as they have a more developed root system that is indicative of the better performance of the plants when transferred to the field, as they have a greater capacity for sustaining and absorbing water and nutrients, increasing the productive potential and the

ability to adapt to adverse environmental conditions, and resulting in greater vegetative expression, therefore, greater potential photosynthetic capacity.

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