International Journal of
Plant Biology

MDPI

Article

In Vitro Shoot Regeneration and Multiplication of Peruvian Rocoto Chili Pepper (*Capsicum pubescens* Ruiz & Pav.)

Angel David Hernández-Amasifuen, Alexandra Jherina Pineda-Lázaro, Jorge L. Maicelo-Quintana and Juan Carlos Guerrero-Abad *

Instituto de Investigación, Innovación y Desarrollo para el Sector Agrario y Agroindustrial (IIDAA), Facultad de Ingeniería y Ciencias Agrarias, Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas, Calle Higos Urco 342—Ciudad Universitaria, Chachapoyas 01000, Peru; angel.hernandez@untrm.edu.pe (A.D.H.-A.); sherina.97.19@gmail.com (A.J.P.-L.); jmaicelo@untrm.edu.pe (J.L.M.-Q.)
* Correspondence: juan.guerrero@untrm.edu.pe

Abstract: The rocoto (*Capsicum pubescens* Ruiz & Pav.) (Solanaceae) is an endemic herbaceous chili pepper from Peru. Low yields of rocoto production are due to the low availability of certified seeds or the production of superior plant seedlings. Therefore, the present study aimed to establish an in vitro protocol for the regeneration and multiplication of rocoto shoots. The multiplication was carried out on shoot tips excised from rocoto seedlings germinated under in vitro conditions, and then the explants were placed on Murashigue and Skoog (MS) medium supplemented with different concentrations of 6-benzylaminopurine (BAP) and Kinetin: 0.5, 1.0, 1.5 and 2.0 mg/L. For rooting, shoots were obtained from the multiplication phase and placed under different treatments made up of MS medium supplemented with different concentrations of indole butyric acid (IBA) and naphthalene acetic acid (NAA): 0.5, 1.0, 1.5 and 2.0 mg/L. In the multiplication phase, the best results were observed with MS medium supplemented with 1.0 mg/L BAP, with 82.22% shoot development, 2.93 shoots per explant and 2.75 cm shoot length. In the rooting phase, the best results were observed with MS medium supplemented with 1.5 mg/L IBA, with 91.11% root development, 9.73 roots per explant and 6.79 cm root length. Here, we show the first evidence and tool for the in vitro regeneration and multiplication of rocoto chili pepper, which could be used for the multiplication of superior genotypes, germplasm in vitro conservation and its use in plant breeding programs.

Keywords: *Capsicum pubescens*; plant growth regulators; indole butyric acid; 6-benzylaminopurine; shoot tip

check for updates

Citation: Hernández-Amasifuen, A.D.; Pineda-Lázaro, A.J.; Maicelo-Quintana, J.L.; Guerrero-Abad, J.C. In Vitro Shoot Regeneration and Multiplication of Peruvian Rocoto Chili Pepper (*Capsicum pubescens* Ruiz & Pav.). *Int. J. Plant Biol.* **2024**, *15*, 979–987. https://doi.org/10.3390/ijpb15040069

Academic Editor: Alena Gajdošová

Received: 1 August 2024
Revised: 22 August 2024
Accepted: 2 September 2024
Published: 4 October 2024

Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

The rocoto chili pepper (*Capsicum pubescens* Ruiz & Pav.) is a Solanaceous endemic plant from Peru, distributed from North America and Mexico to Chile in South America [1,2] and Asia [3]. It little studied, but some interesting botanical traits such as black seeds and purple corolla flowers have been documented. Its fruits have a smooth texture and a variety of pigmentation: green, orange and intense red have been found [4,5]. In Peru, the fruit is widely used in Peruvian gastronomy, consumed as a fresh fruit or in sauces in typical dishes such as "rocoto relleno" and "ceviche" [6]. Also, it is consumed as a condiment in dry powder form [7].

Previous studies have revealed several pharmacological properties of this genus, which has been widely used as an analgesic product. The genus Capsicum is a source of a variety of compounds, such as capsaicinoids, flavonoids and tocopherols [8]. The main phytochemical component of the genus Capsicum is the capsaicin, which acts by inhibiting the activity of the pain-related epidermal nerve cells, so lessening the sensation of pain [9]. Capsaicin is used as an anti-asthmatic, anti-microbial and anti-inflammatory agent. In addition to its healing properties, it also has antioxidant capacity [10].

The five most cultivated species of Capsicum in Peru are diploid, but four of them have a chromosome number n = 12, while wild species of Capsicum have been reported to have 13 chromosome pairs (2n = 26) [11,12]. This difference in chromosome number is also related to the difference in morphology, presenting a greater variability in wild species [13]. In the case of *C. pubescens*, it has been determined that it has 12 pairs of chromosomes (2n = 24); 1 pair of chromosomes is sub-metacentric and the remaining 11 are metacentric [14,15].

The plants of *C. pubescens* are affected by different diseases caused by bacteria, fungi or viruses in the plot fields' production [16,17]. As well as the difficulty in obtaining inbreeding lines for developing superior hybrid genotypes, because they are a self-incompatible species [18,19], they are also an allogamous pollination type, resulting in heterozygous populations with low yields in the following generations [20]. On the other hand, national production is also affected by the lack of improved seeds [21], which is the only way to propagate them, so farmers use seeds from previous crop seasons [22], thus maintaining very heterogeneous populations [23]. It is necessary to establish new methods of propagation of promising genotypes in relation to pathogen resistance, tolerance to abiotic stresses and higher yields [24,25].

The application of biotechnological tools, through plant tissue culture, to overcome the obstacles presented by the conventional propagation of this crop, has emerged as an effective alternative for the regeneration, ex situ conservation and mass multiplication of superior genotypes [26]. In vitro organogenesis regeneration presents difficulties in species of the genus Capsicum, but with few exceptions. In vitro regeneration studies have been conducted on some species of the genus Capsicum; the species that have been reported on are *C. annuum* [27], *C. baccatum* [28] and *C. chinense* [29], and regeneration and in vitro mass propagation protocol have been successfully standardized for them. In the case of *C. pubescens*, reports of callus induction [30], anther culture [31,32] and shoot regeneration response [33] have been presented. However, research on the in vitro multiplication of *C. pubescens* has not yet been conducted.

Considering the lack of scientific studies of the species, the present study was carried out to standardize a reproducible in vitro regeneration and multiplication protocol for *C. pubescens*.

2. Materials and Methods

2.1. Initiation of Aseptic Culture

Fruits of the rocoto genotype Serrano (Figure 1) were obtained from the nursery. The seeds were collected and washed with neutral detergent for 15 min under running tap water, and then soaked for 30 min in sterile distilled water. The seeds were then rinsed with sterile distilled water and surface-disinfected in 70% ethanol for 1 min, and then immersed in 2% NaClO solution for 15 min and rinsed three times with sterile distilled water. The seeds were then dried and cultured on half-strength MS medium [34] with 1.5% sucrose, 0.7% agar and pH 5.8. All processes were carried out under aseptic conditions in a laminar flow cabinet. Cultures were incubated in a growth room at 25 ± 1 °C under a light intensity of 45 $\mu molm^{-2}$ s^{-1} with a 16/8 h light/dark photoperiod.

2.2. Shoot Induction and Proliferation

Excised shoot tip explants (1.5 cm) were obtained aseptically from 20- to 30-day-old in vitro established seedlings. Explants were grown on MS medium without plant growth regulators (PGR) (control) and MS medium supplemented with different concentrations of 6-benzylaminopurine (BAP) and Kinetin: 0.5, 1.0, 1.5 and 2.0 mg/L. All treatments were also supplemented with 3% sucrose, 0.7% agar and pH adjusted to 5.8. Cultures were incubated at 25 ± 1 °C under a light intensity of 45 $\mu molm^{-2}$ s^{-1} with a 16/8 h light/dark photoperiod. The explants were subcultured after two weeks in fresh medium with the same compositions. After four weeks of culture, the percentage of explants with shoots, the number of shoots per explant and the shoot height were evaluated.

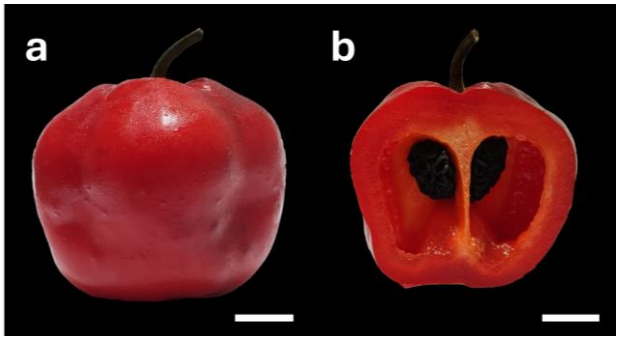

Figure 1. Fruit of rocoto (*Capsicum pubescens*) genotype Serrano prior to seed extraction for in vitro introduction: (**a**) front view of the fruit (bar = 2 cm); (**b**) longitudinal section of the fruit, showing the characteristic black seeds in the central part (bar = 2 cm).

2.3. Root Induction

Healthy regenerated shoot explants (2.0 cm in length) were excised and grown on root induction media, i.e., MS medium without PGR (control) and MS medium supplemented with different concentrations of indole butyric acid (IBA) and naphthalene acetic acid (NAA): 0.5, 1.0, 1.5 and 2.0 mg/L. All treatments were also supplemented with 3% sucrose, 0.7% agar and pH adjusted to 5.8. Cultures were incubated at 25 ± 1 °C under a light intensity of 45 μmolm^{-2} s^{-1} with a 16/8 h light/dark photoperiod. Explants were subcultured after two weeks in fresh medium with the same compositions. After four weeks of culture, the percentage of explants with roots, the number of roots per explant and the root length were evaluated.

2.4. Acclimatization of In Vitro Regenerated Plants

In vitro rooted seedlings were removed from their culture containers and gently washed with distilled water to remove the adhering medium. Seedlings were grown in small pots containing a sterile mixture of peat and sand in a 2:1 ratio, respectively. They were maintained at 25 ± 2 °C and 70% relative humidity under a light intensity of 80 μmolm^{-2} s^{-1} with a 16/8 h light/dark photoperiod. All plants were irrigated every two days. The survival rate was evaluated after four weeks of transplanting.

2.5. Experimental Design and Statistical Analysis

The experiment was set up with a completely randomized design (CRD). The experimental unit was one explant per culture medium, each treatment was carried out with 15 explants per treatment and all experiments were performed in triplicate. All percentage data were arcsine transformed prior to statistical analysis. Data were statistically analyzed by analysis of variance (ANOVA) using statistical analyses with R package software (Version 4.4.1 for Windows) [35,36]. Comparisons of means between treatments was determined by the 5% Tukey test.

3. Results and Discussion

3.1. Shoot Induction and Proliferation

The morphogenic responses of explants from excised shoot tips of *Capsicum pubescens* were obtained with MS medium with and without PGR, but at the same time callus formation was also observed in all treatments. A significant explant bud formation was observed in the BAP- and Kinetin-enriched culture media. A higher percentage of shoot development, with a maximum of 82.22%, was observed on the BAP-enriched medium (1.0 mg/L), and this was significantly ($p \leq 0.05$) higher than all other treatments (Table 1). For shoot formation, the medium enriched with 1.0 mg/L BAP was found to be optimal for shoot proliferation, with an average number of shoots per explant of 2.93 and a shoot length of 2.75 cm, respectively (Figure 2a). These results were significantly different ($p \leq 0.05$)

from the other treatments. Callus formation at the base of the explant cutting was observed in all treatments (Figure 2b).

Table 1. Effect of PGRs on shoot formation from shoot tip explants of *Capsicum pubescens*.

Plant Growth Regulator	Concentration (mg/L)	Percentage of Regeneration	No. of Shoots per Explant	Shoot Length (cm)
Control	0.0	15.56 ^{e} (15.63)	1.07 ± 0.07 ^{d}	1.06 ± 0.02 ^{e}
BAP	0.5	57.78 ^{c} (61.65)	1.80 ± 0.11 ^{c}	1.26 ± 0.01 ^{d}
	1.0	82.22 ^{a} (96.77)	2.93 ± 0.07 ^{a}	2.75 ± 0.02 ^{a}
	1.5	77.78 ^{a,b} (89.26)	2.23 ± 0.11 ^{b}	2.19 ± 0.01 ^{b}
	2.0	68.89 ^{b,c} (76.09)	1.87 ± 0.09 ^{c}	1.63 ± 0.01 ^{c}
Kinetin	0.5	17.78 ^{e} (17.88)	1.47 ± 0.13 ^{d}	1.10 ± 0.01 ^{e}
	1.0	26.67 ^{d,e} (27.04)	1.53 ± 0.13 ^{d}	1.20 ± 0.01 ^{d}
	1.5	35.56 ^{d} (36.37)	1.87 ± 0.19 ^{c}	1.22 ± 0.01 ^{d}
	2.0	22.22 ^{d,e} (22.42)	1.60 ± 0.13 ^{d}	1.22 ± 0.01 ^{d}

Figures in parentheses are the statistically transformed (arcsine) values of the percentage data. Means ± SE followed by different letters are significantly different according to Tukey's test ($p \leq 0.05$).

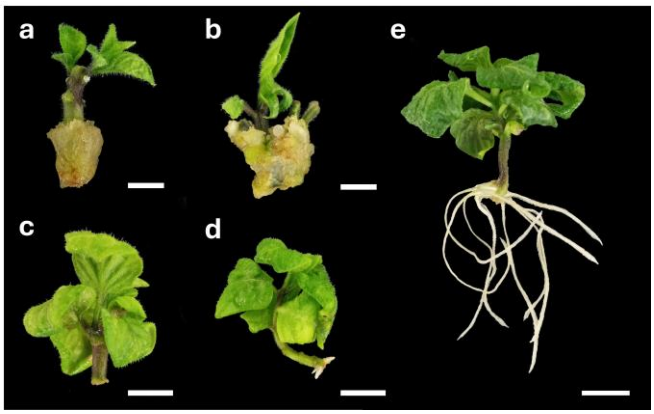

Figure 2. Multiplication and in vitro rooting of *Capsicum pubescens* shoots: (**a**) shoots formed on shoot tip explants in medium enriched with BAP (1.0 mg/L) (bar = 1 cm); (**b**) callus formation at the base of the explant cutting in medium enriched with BAP (1.5 mg/L) (bar = 1 cm); (**c**) shoots without roots in MS medium without PGR (control) (bar = 2 cm); (**d**) shoots with roots in medium enriched with NAA (2.0 mg/L) (bar = 2 cm); (**e**) plantlet with fully developed roots in medium enriched with IBA (1.5 mg/L) (bar = 2 cm).

C. pubescens is a small perennial shrub with different gastronomic properties and is widely used in the pharmaceutical industry due to the presence of capsaicin. It is a species of great genetic variety due to its incompatibility, which is why it does not have defined cultivars or certified seeds and farmers collect it with seeds of local genotypes from previous harvests. Therefore, plant propagation by tissue culture is a feasible alternative for the multiplication, conservation and use of superior genotypes for breeding programs [37,38].

MS medium enriched with the cytokinins BAP and kinetin in their different concentrations increased shoot formation from shoot tip explants of *C. pubescens*, with the best results being observed with the cytokinin BAP when enriching the MS medium, but callus

formation was also observed at the base of the explants, which affected the percentage of shoot induction in the explants. BAP-enriched MS medium increased the percentage of shoot development (82%) in shoot tip explants. The problem with callus formation was observed in all treatments and could be related to the potency of the MS medium used. The total or partial reduction in callus formation in explants is reported as a problem that can be overcome by reducing the nutrients making up the MS medium [30]. It has been reported that *Capsicum chinense* [39] and *Capsicum annuum* [40] overcame the problem of callus formation and thereby increased their shoot development rate by reducing the nutrient composition of the MS medium by half.

The in vitro multiplication of *C. pubescens* from shoot tip explants showed that the MS medium enriched with 1.0 mg/L BAP was optimal for shoot development, obtaining an 82.22% morphogenic response, with 2.93 ± 0.07 average shoots per explant and 2.75 ± 0.02 cm average shoot length. These results are lower than those reported in *Capsicum chinense* Jacq., where higher shoot development (12.0 shoots per explant) was observed when using 4.0 mg/L BAP in MS medium [41]. For the in vitro multiplication of *C. annuum* cv. Bharat, similar results to those obtained in the present investigation have been reported, where 87.65% shoot formation and 2.22 shoots per explant were observed on MS medium supplemented with 10.0 mg/L BAP [42]. The MS medium supplemented with 1.5 mg/L kinetin proved to be the optimum for shoot induction in *C. pubescens*, compared to the other concentrations of this cytokinin, allowing a 35.56% morphogenic response to be observed with 1.87 ± 0.19 shoots on average per explant and a 1.22 ± 0.01 cm average shoot length. These results are lower than those reported on *C. annuum* cv. Bharat, with 90.12% shoot regeneration and 2.72 shoots on average per explant on MS medium supplemented with 7.5 mg/L kinetin [42]. In the case of the report of in vitro multiplication of *C. frutescens*, similar results to those obtained in the present investigation have been reported, where 30% shoot regeneration was observed with 1.8 mean number of shoots per explant and 1.14 cm shoot length on MS medium supplemented with 2.0 mg/L kinetin [43].

3.2. Root Induction

The rooting induction of explants from healthy shoots of *Capsicum pubescens* was obtained with MS medium supplemented with PGR (IBA and NAA), while MS medium without PGR (control) did not produce a rooting response in shoot explants. A higher rooting percentage, with a maximum of 91.11%, was observed in the medium enriched with IBA (1.5 mg/L), which was significantly ($p \leq 0.05$) higher than all other treatments, while the effect of NAA in the enriched MS medium allowed a maximum rooting percentage of 64.44% (Table 2). For root formation, the medium enriched with 1.5 mg/L IBA was optimal for root proliferation, with an average number of roots per explant of 9.73 and a root length of 6.79 cm (Figure 2c–e). These results were significantly different ($p \leq 0.05$) from other treatments.

The in vitro rooting of *C. pubescens* from shoot explants showed that the MS medium enriched with 1.5 mg/L IBA was optimal for root development, obtaining a 91.11% morphogenic response, with 9.73 ± 0.43 average roots per explant and 6.79 ± 0.14 cm average root length. Meanwhile, the MS medium supplemented with 0.5 mg/L NAA proved to be the optimum for root induction in *C. pubescens*, compared to the other concentrations of this auxin, allowing a morphogenic response of 64.44% to be observed with 6.73 ± 0.30 average roots per explant and 2.17 ± 0.03 cm average shoot length. It is known that auxins play an important role in plant roots, and several studies have shown that auxins and their transport are essential for the production of lateral roots. It is reported that IBA and NAA, when administered, increase the length, volume and number of roots [44,45]. This is demonstrated in the results obtained in the present investigation, as well as in other investigations of the genus Capsicum where employing IBA produced the best results, such as the cases of *C. annuum* cv. Pusa Jwala [46], *C. chinense* [41] and *C. frutescens* [43]. Meanwhile, when NAA was used in the MS medium in other species of the same genus, good results were observed, superior to those obtained in the present investigation, as is the case in *C. annuum*,

with 12 roots per shoot in MS medium supplemented with 0.5 mg/L NAA [47], and in *C. chinense*, with the formation of 14 roots per shoot in MS medium supplemented with 0.25 mg/L NAA [41].

Table 2. Effect of PGRs on root formation from shoot explants of *Capsicum pubescens*.

Plant Growth Regulator	Concentration (mg/L)	Percentage of Rooting	No. of Roots per Shoot	Root Length (cm)
Control	0.0	0 ^{d} (0.00)	0 ± 0.00 ^{e}	0 ± 0.00 ^{f}
IBA	0.5	62.22 ^{c} (67.64)	3.73 ± 0.25 ^{c}	2.61 ± 0.03 ^{c}
	1.0	82.22 ^{a,b} (96.77)	6.60 ± 0.27 ^{b}	4.64 ± 0.05 ^{b}
	1.5	91.11 ^{a} (115.19)	9.73 ± 0.43 ^{a}	6.79 ± 0.14 ^{a}
	2.0	73.33 ^{b,c} (82.67)	6.47 ± 0.27 ^{b}	4.79 ± 0.05 ^{b}
NAA	0.5	64.44 ^{c} (70.52)	6.73 ± 0.30 ^{b}	2.17 ± 0.03 ^{c}
	1.0	57.78 ^{c} (61.65)	4.27 ± 0.27 ^{c}	0.73 ± 0.02 ^{d}
	1.5	57.78 ^{c} (61.65)	2.47 ± 0.26 ^{d}	0.69 ± 0.02 ^{d}
	2.0	55.55 ^{c} (58.95)	2.13 ± 0.19 ^{d}	0.17 ± 0.01 ^{e}

Figures in parentheses are the statistically transformed (arcsine) values of the percentage data. Means ± SE followed by different letters are significantly different according to Tukey's test ($p \leq 0.05$).

3.3. Acclimatization of In Vitro Regenerated Plants

Plants rooted in vitro during acclimatization had a 100% survival rate in the fourth week. The substrate used (peat and sand in a 2:1 ratio) proved to be quite effective for the ex vitro acclimatization of *C. pubescens* plants. None of the seedlings showed signs of pathogen infection during the acclimatization period.

Seedling acclimation is a crucial step that determines the efficiency of the whole process of in vitro regeneration and multiplication. During the acclimatization process, seedlings are exposed to a radically different environment than the one they experienced during their initial growth phase, which can trigger a series of both abiotic and biotic stress responses. Among the abiotic factors, one of the main challenges is rapid water loss, which can lead to significant tissue dehydration, compromising seedling viability. Likewise, in their new environment, seedlings are exposed to a diversity of microorganisms and invertebrates, which can cause biotic stress, further affecting their ability to adapt and survive. These combined factors can have an adverse impact on the development and long-term success of seedlings, underscoring the importance of careful management during the acclimatization stage [48]. The substrate used during acclimatization has a great impact on the survival and development of seedlings regenerated by in vitro culture [49–51]. The results of this study show that *C. pubescens* seedlings grown in a combination of peat and sand exhibit remarkable adaptation to ex vitro conditions. The characteristics of the substrate account for its substantial effects on plant development and survival, so a porous and well-drained substrate is required. It must also allow gas exchange and facilitate the obtaining of nutrients and water. This will favor the establishment of a strong root system, as well as plant growth and survival [52].

All seedlings grew well in the acclimatization stage, without disease symptoms, and the survival rate at four weeks was higher than expected and compared to that reported for other Caspsicum species with ranges between 80% and 95%, as is the case of *C. annuum*,

C. chinense and *C. frutescens* [27,41,42]. However, there has also been a report of 23.8% survival rate in *C. annuum*, *C. baccatum* and *C. chinense* [28].

The implications of these results are significant for genetic improvement and the germplasm conservation of *C. pubescens*. However, it is essential to expand this approach to include a larger number of *C. pubescens* genotypes, given that Peru harbors a wide genetic diversity of this species. Identifying and working with superior or more agronomically important genotypes is crucial to maximize the impact of this in vitro protocol. These genotypes could be selected and introduced in in vitro multiplication and regeneration programs, not only to improve agricultural production but also to preserve the genetic diversity of the species.

In future studies, a detailed characterization of *C. pubescens* genotypes is suggested to determine those with better yields and desirable traits. In addition, integrating advanced biotechnology techniques, such as gene editing and somatic embryogenesis, could further enhance the efficiency of the regeneration protocol and its application in breeding programs. This would contribute significantly to the sustainable production of *C. pubescens*, as well as to the conservation of its genetic diversity, ensuring the availability of superior cultivars adapted to local conditions and market needs.

4. Conclusions

The present research describes for the first time an efficient and reproducible in vitro shoot multiplication and regeneration protocol for *C. pubescens*, an endemic species of great nutritional, gastronomic and pharmaceutical value. This regeneration system guarantees the multiplication and rooting of the used explants of *C. pubescens*, which could also offer an alternative for the in vitro conservation of *C. pubescens* germplasm and its use in breeding programs.

Our research not only provides essential knowledge on the cultivation of *C. pubescens*, but also reveals the potential for applying advanced plant tissue culture techniques such as protoplast isolation and whole plant regeneration. These advances offer new tools for the study and improvement of this species. Likewise, the findings open up the possibility of developing somatic embryogenesis experiments in *C. pubescens*, which would provide a tool for gene editing research and the production of synthetic seeds. These techniques represent a promising avenue for the multiplication of elite genotypes and genetic improvement, which could accelerate the breeding of new cultivars with superior agronomic characteristics, with the potential to transform agricultural practices and contribute to the sustainability and efficiency of agricultural systems.

Author Contributions: Conceptualization, A.D.H.-A. and J.C.G.-A.; methodology, A.D.H.-A. and A.J.P.-L.; formal analysis, A.D.H.-A., A.J.P.-L. and J.C.G.-A.; investigation, J.L.M.-Q. and J.C.G.-A.; data curation, J.L.M.-Q. and J.C.G.-A.; writing—original draft preparation, A.D.H.-A. and A.J.P.-L.; writing—review and editing, J.L.M.-Q. and J.C.G.-A.; visualization, A.D.H.-A. and A.J.P.-L.; supervision, J.L.M.-Q. and J.C.G.-A. All authors have read and agreed to the published version of the manuscript.

Funding: Financially supported by the project "Creación de los Servicios de Investigación, Innovación y Desarrollo de Tecnología para el Sector Agrario y Agroindustrial de la UNTRM sede Chachapoyas, Departamento de Amazonas", C.U.I. N° 2313205.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets used and analyzed in this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Van Zonneveld, M.; Ramirez, M.; Williams, D.E.; Petz, M.; Meckelmann, S.; Avila, T.; Bejarano, C.; Ríos, L.; Peña, K.; Jäger, M.; et al. Screening Genetic Resources of *Capsicum* Peppers in Their Primary Center of Diversity in Bolivia and Peru. *PLoS ONE* **2015**, *10*, e0134663. [CrossRef] [PubMed]
2. Patel, K.; Calderon, R.; Asencios, E.; Vilchez, D.; Marcelo, M.; Rojas, R. Agro-Morphological Characteristics and Sensory Evaluation of Native Peruvian Chili Peppers. *J. Agric. Sci. Technol. B* **2016**, *6*, 180–187. [CrossRef]
3. Yamamoto, S.; Djarwaningsih, T.; Wiriadinata, H. *Capsicum pubescens* (Solanaceae) in Indonesia: Its History, Taxonomy, and Distribution. *Econ. Bot.* **2013**, *67*, 161–170. [CrossRef]
4. Meckelmann, S.W.; Jansen, C.; Riegel, D.W.; Van Zonneveld, M.; Ríos, L.; Peña, K.; Mueller-Seitz, E.; Petz, M. Phytochemicals in Native Peruvian *Capsicum pubescens* (Rocoto). *Eur. Food Res. Technol.* **2015**, *241*, 817–825. [CrossRef]
5. García-Díaz, R.F.; Castañeda-Garzón, S.L.; Valdéz-Hernández, E.F. Quality of Rocoto Pepper (*Capsicum pubescens* Ruiz & Pav.) Seeds in Relation to Extraction Timing. *Acta Agron.* **2018**, *67*, 246–251. [CrossRef]
6. Meckelmann, S.W.; Riegel, D.W.; Van Zonneveld, M.J.; Ríos, L.; Peña, K.; Ugas, R.; Quinonez, L.; Mueller-Seitz, E.; Petz, M. Compositional Characterization of Native Peruvian Chili Peppers (*Capsicum* spp.). *J. Agric. Food Chem.* **2013**, *61*, 2530–2537. [CrossRef] [PubMed]
7. Vera-Guzmán, A.M.; Chávez-Servia, J.L.; Carrillo-Rodríguez, J.C.; López, M.G. Phytochemical Evaluation of Wild and Cultivated Pepper (*Capsicum annuum* L. and *C. Pubescens* Ruiz & Pav.) from Oaxaca, Mexico. *Chil. J. Agric. Res.* **2011**, *71*, 578–585. [CrossRef]
8. Meckelmann, S.W.; Riegel, D.W.; Van Zonneveld, M.; Ríos, L.; Peña, K.; Mueller-Seitz, E.; Petz, M. Capsaicinoids, Flavonoids, Tocopherols, Antioxidant Capacity and Color Attributes in 23 Native Peruvian Chili Peppers (*Capsicum* spp.) Grown in Three Different Locations. *Eur. Food Res. Technol.* **2015**, *240*, 273–283. [CrossRef]
9. Naves, E.R.; De Ávila Silva, L.; Sulpice, R.; Araújo, W.L.; Nunes-Nesi, A.; Peres, L.E.P.; Zsögön, A. Capsaicinoids: Pungency beyond *Capsicum*. *Trends Plant Sci.* **2019**, *24*, 109–120. [CrossRef]
10. Pérez-Grajales, M.; Martínez-Damián, M.T.; Cruz-Alvarez, O.; Potrero-Andrade, S.M.; Peña-Lomelí, A.; González-Hernández, V.A.; Villegas-Monter, A. Content of Capsaicinoids and Physicochemical Characteristics of Manzano Hot Pepper Grown in Greenhouse. *Not. Bot. Horti Agrobot.* **2018**, *47*, 119–127. [CrossRef]
11. Mongkolporn, O.; Taylor, P.W.J. Capsicum. In *Wild Crop Relatives: Genomic and Breeding Resources*; Kole, C., Ed.; Springer: Berlin/Heidelberg, Germany, 2011; pp. 43–57, ISBN 978-3-642-20449-4.
12. Liu, F.; Zhao, J.; Sun, H.; Xiong, C.; Sun, X.; Wang, X.; Wang, Z.; Jarret, R.; Wang, J.; Tang, B.; et al. Genomes of Cultivated and Wild *Capsicum* Species Provide Insights into Pepper Domestication and Population Differentiation. *Nat. Commun.* **2023**, *14*, 5487. [CrossRef]
13. Eshbaugh, W.H. The Taxonomy of the Genus *Capsicum*. In *Peppers: Botany, Production and Uses*; Russo, V.M., Ed.; CABI: Wallingford, UK; Cambridge, MA, USA, 2012; pp. 14–28, ISBN 978-1-84593-767-6.
14. Guevara, M.; Siles, M.; Bracamonte, O. Análisis Cariotípico de *Capsicum pubescens* (Solanaceae) "Rocoto". *Rev. Peru. Biol.* **2000**, *7*, 134–141. [CrossRef]
15. Aguilera, P.M.; Debat, H.J.; Seijo, J.G.; Grabiele, M. A Reference Chromosomal Map of the Hot Chili Pepper *Capsicum pubescens* Cv. "Locoto" (Solanaceae). *Rodriguésia* **2021**, *72*, e01002020. [CrossRef]
16. López-Aranda, B.G.; Vásquez, E.F. *Fusarium* Sp. in *Capsicum pubescens* (Rocoto): Redefining Host-Pathogen Interaction. *Int. J. Plant Biotechnol.* **2020**, *6*, 18–22.
17. Vallejo-Gutiérrez, A.J.; Mejía-Carranza, J.; García-Velasco, R.; Ramírez-Gerardo, M.G. Respuesta de Genotipos de *Capsicum pubescens* al Daño Ocasionado Por El Complejo Fúngico de La Marchitez. *RMF* **2018**, *37*, 50–70. [CrossRef]
18. Saborío, M.; Da Costa, C. Autoincompatibilidad En Caspsicum Pubescens. *Agron. Costarric.* **1992**, *16*, 279–286.
19. Bo, M.L.; Carrizo García, C. Pollen Phenotyping and Performance in Rocoto Chili (*Capsicum pubescens* Ruiz et Pav., Solanaceae). *Grana* **2015**, *54*, 37–44. [CrossRef]
20. Zewdie, Y.; Bosland, P.W.; Steiner, R. Combining Ability and Heterosis for Capsaicinoids in *Capsicum pubescens*. *HortSci* **2001**, *36*, 1315–1317. [CrossRef]
21. Merino-Valdés, M.; Andrés-Meza, P.; Leyva-Ovalle, O.R.; López-Sánchez, H.; Murguía-González, J.; Núñez-Pastrana, R.; Cebada-Merino, M.; Serna- Lagunes, R.; Espinosa-Calderón, A.; Tadeo-Robledo, M.; et al. Influencia de Tratamientos Pregerminativos En Semillas de Chile Manzano (*Capsicum pubescens* Ruiz & Pav.). *Acta Agron.* **2018**, *67*, 531–537. [CrossRef]
22. Oliva, M.; Oliva, J.; Oliva, L.; Trauco, C.; Carranza, J. Selección de Ecotipos de Rocoto (*Capsicum pubescens*) Con Alta Productividad y Grado de Picor Manejados Bajo Sistema Agroforestal En El Distrito de Molinopampa, Amazonas. *Rev. De. Investig. Agroproducción Sustentable* **2019**, *3*, 78. [CrossRef]
23. Miyajima, I.; Shibasato-Takami, S.; Ichikawa, A.; Mizunoe, Y. Development of Tetraploid Rocoto Pepper (*Capsicum pubescens* R. & P.) by Colchicine Treatment to Achieve Self-Compatibility. *Hort. Res.* **2021**, *20*, 143–148. [CrossRef]
24. Marcelo, M.; Amasifuen, C. Conservación y Valoración De La Diversidad De Ajíes Del Perú. Available online: https://redagricola.com/conservacion-y-valoracion-de-la-diversidad-de-ajies-del-peru/ (accessed on 30 July 2024).
25. Serna-Lagunes, R.; Andrés-Meza, P.; Leyva-Ovalle, O.R.; Del Rosario-Arellano, J.L.; Merino-Valdes, M.; Murguía-González, J.; Galindo-Tovar, M.E.; Mejía-Carranza, J.; Sierra-Macías, M.; Espinosa-Calderón, A.; et al. Ecological Niche of Semidomesticated Populations of *Capsicum pubescens* Ruiz & Pav. Based on Accessions from Veracruz, Mexico. *Rev. Fitotec. Mex.* **2020**, *43*, 389. [CrossRef]

26. Radomir, A.-M.; Stan, R.; Florea, A.; Ciobotea, C.-M.; Bănuță, F.M.; Negru, M.; Neblea, M.A.; Sumedrea, D.I. Overview of the Success of In Vitro Culture for Ex Situ Conservation and Sustainable Utilization of Endemic and Subendemic Native Plants of Romania. *Sustainability* **2023**, *15*, 2581. [CrossRef]
27. Sanatombi, K.; Sharma, G.J. In Vitro Plant Regeneration in Six Cultivars of *Capsicum* Spp. Using Different Explants. *Biol. Plant.* **2008**, *52*, 141–145. [CrossRef]
28. Martínez-López, M.; García-Pérez, A.; Gimeno-Páez, E.; Prohens, J.; Vilanova, S.; García-Fortea, E. Screening of Suitable Plant Regeneration Protocols for Several *Capsicum* Spp. through Direct Organogenesis. *Horticulturae* **2021**, *7*, 261. [CrossRef]
29. Shu, H.; Zhang, Y.; He, C.; Altaf, M.A.; Hao, Y.; Liao, D.; Li, L.; Li, C.; Fu, H.; Cheng, S.; et al. Establishment of in Vitro Regeneration System and Molecular Analysis of Early Development of Somatic Callus in *Capsicum* Chinense and *Capsicum* Baccatum. *Front. Plant Sci.* **2022**, *13*, 1025497. [CrossRef]
30. Hernández Amasifuen, A.D.; Argüelles Curaca, A.; Cortez Lázaro, A.A.; Díaz Pillas, H.B. In Vitro Induction of Callus from Foliar Explants in Rocoto (*Capsicum pubescens* Ruiz & Pav.). *LGR* **2021**, *34*, 131–140. [CrossRef]
31. Hernández-Amasifuen, A.D.; Pineda-Lázaro, A.J.; Díaz-Pillasca, H.B. In Vitro Anther Culture of Rocoto (*Capsicum pubescens* Ruiz & Pav.). *Idesia* **2022**, *40*, 115–121. [CrossRef]
32. Pineda-Lázaro, A.J.; Hernández-Amasifuen, A.D.; Díaz-Pillasca, H.B. The Efficient Procedure of Embryogenic Callus Formation from Anther in *Capsicum pubescens* Ruiz & Pav. *Revis Bionatura* **2023**, *8*, 29. [CrossRef]
33. Pineda-Lázaro, A.J.; Argüelles Curaca, A.; Rojas-Chávez, J.A.; Díaz-Pillasca, H.B. Respuesta En El Establecimiento y Regeneración in Vitro de Rocoto (*Capsicum pubescens* Ruiz & Pav.). *Aporte Santiaguino* **2021**, *14*, 31–42. [CrossRef]
34. Murashige, T.; Skoog, F. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiol. Plant.* **1962**, *15*, 473–497. [CrossRef]
35. R Core Team. R: A Language and Environment for Statistical Computing. Available online: https://www.R-project.org (accessed on 7 June 2024).
36. De Mendiburu, F. Agricolae R, Versión 1.0-9. Available online: https://coesincelejo.files.wordpress.com/2011/01/manualpracticoagricolae.pdf (accessed on 7 June 2024).
37. Ochoa-Alejo, N.; Ramirez-Malagon, R. In Vitro Chili Pepper Biotechnology. *Vitr. Cell. Dev. Biol.-Plant* **2001**, *37*, 701–729. [CrossRef]
38. Valadez-Bustos, M.G.; Aguado-Santacruz, G.A.; Carrillo-Castañeda, G.; Aguilar-Rincón, V.H.; Espitia-Rangel, E.; Montes-Hernández, S.; Robledo-Paz, A. In Vitro Propagation and Agronomic Performance of Regenerated Chili Pepper (*Capsicum* spp.) Plants from Commercially Important Genotypes. *Vitr. Cell. Dev. Biol.-Plant* **2009**, *45*, 650–658. [CrossRef]
39. Hernández-Amasifuen, A.D.; Pineda-Lázaro, A.J.; Rojas-Chávez, J.A.; Díaz-Pillasca, H.B. In Vitro Regeneration of Arnaucho (*Capsicum chinense* Jacq.) from Apical Buds. *Manglar* **2021**, *18*, 71–75. [CrossRef]
40. Singh, H.; Dhillon, T.; Sidhu, A.; Gosa, S. Studies on in Vitro Propagation in Hot Pepper. *Indian. J. Hort.* **2011**, *68*, 201–205.
41. Kehie, M.; Kumaria, S.; Tandon, P. In Vitro Plantlet Regeneration from Nodal Segments and Shoot Tips of *Capsicum chinense* Jacq. Cv. Naga King Chili. *3 Biotech* **2012**, *2*, 31–35. [CrossRef]
42. Hegde, V.; Partap, P.S.; Yadav, R.C. In Vitro Regeneration of *Capsicum* (*Capsicum annuum* L.) from Cotyledon Explants. *Int. J. Curr. Microbiol. Appl. Sci.* **2017**, *6*, 225–237. [CrossRef]
43. Gururaj, H.B.; Giridhar, P.; Sharma, A.; Prasad, B.C.N.; Ravishankar, G.A. In Vitro Clonal Propagation of Bird Eye Chilli (*Capsicum frutescens* Mill.). *Indian. J. Exp. Biol.* **2004**, *42*, 1136–1140.
44. Arya, A.; Husen, A. Role of Various Auxins in Adventitious Root Formation. In *Environmental, Physiological and Chemical Controls of Adventitious Rooting in Cuttings*; Elsevier: Amsterdam, The Netherlands, 2022; pp. 213–238, ISBN 978-0-323-90636-4.
45. Lin, F.; Kang, Y.; Li, Y.; Guo, Y.; Wang, W.; Yang, G.; Yin, J.; Tang, F.; Eissa, M.A. In Vitro Propagation Technology for the Endangered Aquatic Species *Nymphoides coronata*. *Plants* **2024**, *13*, 1508. [CrossRef]
46. Ahmad, N.; Siddique, I.; Anis, M. Improved Plant Regeneration in *Capsicum annuum* L. from Nodal Segments. *Biol. Plant.* **2006**, *50*, 701–704. [CrossRef]
47. Ashrafuzzaman, M.; Hossain, M.; Ismail, M.; Haque, M.; Shahidullah, S.; Uz-zaman, S. Regeneration Potential of Seedling Explants of Chilli (*Capsicum annuum*). *Afr. J. Biotechnol.* **2009**, *8*, 591–596.
48. Oliveira, Y.D.; Anselmini, J.I.; Cuquel, F.L.; Pinto, F.; Quoirin, M. Pré-Aclimatização in Vitro de Abacaxi-Ornamental. *Ciênc. Agrotec.* **2010**, *34*, 1647–1653. [CrossRef]
49. Lone, A.B.; Barbosa, C.M.; Takahashi, L.S.A.; Faria, R.T.D. Aclimatização de Cattleya (Orchidaceae), Em Substratos Alternativos Ao Xaxim e Ao Esfagno. *Acta Sci. Agron.* **2008**, *30*, 465–469. [CrossRef]
50. Pospíšilová, J.; Tichá, I.; Kadleček, P.; Haisel, D.; Plzáková, Š. Acclimatization of Micropropagated Plants to Ex Vitro Conditions. *Biol. Plant.* **1999**, *42*, 481–497. [CrossRef]
51. Salgado Pirata, M.; Correia, S.; Canhoto, J. Ex Vitro Simultaneous Acclimatization and Rooting of In Vitro Propagated Tamarillo Plants (*Solanum betaceum* Cav.): Effect of the Substrate and Mineral Nutrition. *Agronomy* **2022**, *12*, 1082. [CrossRef]
52. Silva, K.B.D.; Reiniger, L.R.S.; Stefanel, C.M.; Rabaiolli, S.M.D.S. Sucrose and Substrates on the Acclimatization of Micropropagated *Luehea divaricata* Plants. *Floresta Ambient.* **2020**, *27*, e20171170. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.