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Callus Induction and Cellular Suspensions from Murtilla (*Ugni molinae* Turcz.) for *trans*-resveratrol Production

[1]Salazar Carolina, [2]Bustos Evelyn, [2]Perez Claudia, [2]Becerra Jose, [3]Bru Roque and [4]Uribe Matilde

[1]Department of Plant Production, Faculty of Agriculture, University of Concepción, Chillán, Chile
[2]Laboratory of Natural Products Chemistry, Department of Botany,
Faculty of Natural Sciences and Oceanography, University of Concepción, Concepción, Chile
[3]Department of Agrochemistry and Biochemistry, Faculty of Science, University of Alicante, Alicante, Spain
[4]Laboratory of Tissue Culture, Biotechnology Center,
Faculty of Forestry Sciences, University of Concepción, Concepción, Chile

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Corresponding Author:
Salazar Carolina
Department of Plant Production, Faculty of Agriculture, University of Concepción, Chillán, Chile
Email: carolinasalazar@udec.cl

Abstract: The present study reports for the first time the quantification of resveratrol and the use of biotechnological techniques applied to stilbene productions in species from genus *Ugni* and calli production from adult explants (fruits). Resveratrol is synthesized by a series of families of higher plants, which has generated much interest in recent years for its antioxidant, anticancer and antitumor properties, which would allow longevity of cells to be prolonged. In this study, leaves and mature fruits were collected from three sites in Southern Chile and correspond to three ecotypes of the Chilean endemic species *Ugni molinae* Turz. (murtilla). These were established *in vitro* to prepare the callus and subsequent development of cellular suspensions for *trans*-resveratrol production. Our results showed that these stilbenes are present in murtilla and that their concentrations vary between ecotypes and tissues, reaching up to 553.5 μg g^{-1} of *t*-resveratrol produced in ecotype 3 callus. These values are relatively higher than those found in other plant species. Under optimum culture conditions, extraction of resveratrol from *Ugni molinae* is scalable to industrial levels, which makes it a viable alternative for obtaining stilbenes.

Keywords: Cell Aggregates, Stilbenes, Phytoalexins, Cellular Lines, Resveratrol

Introduction

Chile is a country with high biological diversity. Here high concentrations of antioxidants provided by natural compounds such as anthocyanins, flavonoids and phenolic acids, have been identified in native and/or endemic plant species (Ruiz *et al*., 2010; Rubilar *et al*., 2011). However, resveratrol, an important antioxidant compound, mainly foundin berries, has not been detected in native Chilean plants yet. One such species, *Ugni molinae* Turcz. (murta or murtilla in Chile), a perennial plant of the Myrtaceae family is known for its aromatic and sweet berries fruits. The, contents of flavonoids and other polyphenols has been found in these fruits with properties that indicate presence of compounds such as phytoalexins (Aguirre *et al*., 2006; Rubilar *et al*., 2006; Avello *et al*., 2009). Resveratrol (3,5,4'-trihydroxystilbene; *t*-R) a phytoalexin that belongs to the stilbene family, is naturally occurring in its isomeric forms *cis* and *trans*, or bound to a glycoside (piceid). The *trans* isomer is a bioactive compound with several health benefits (antioxidant, anti-inflammatory, anticancer and anti-diabetic) prolonging cells longevity (Baur and Sinclair, 2006). These compounds have been identified in fruits of commercial interest, such as peanuts, pistachios, grapes and some berries (e.g., blueberries) (Rocha-González *et al*., 2008). *t*-R is typically obtained from these sources by conventional extraction and separation techniques that involve costly chemical methods and large volumes of plant tissue. This has a number of disadvantages, such as its content in raw material is low and accumulates slowly, there is a wide variability among individuals and abusive use may lead to eradication of plant species (Trejo-Tapia and Rodríguez, 2007; Rocha-González *et al*., 2008). Given these factors, callus culture and the selection of highly productive lines have attracted much interest in recent years to produce high contents of alkaloids, saponins,

polyphenols and terpenes like shikonin, taxol and berberin, which are widely used in the pharmaceutical, agrochemical and food industries (Karuppusamy, 2009). Cellular suspensions for resveratrol production have been established from species such as *Vitis* spp. (Bru *et al*., 2006; Santamaria *et al*., 2011a; 2011b; Belchi-Narravo *et al*., 2012), *Arachis hypogaea* L. (Ku *et al*., 2005) and *Gossypium hirsutum* L. (Kouakou *et al*., 2006). The level of resveratrol is shown to depend not only on the speciesor genotype of donor plant, but also on culture conditions and type of elicitor used (Morales *et al*., 1998; Donnez *et al*., 2009; Halder and Jha, 2015; Xu *et al*., 2015). Of these, the highest content has been found in cellular suspensions of *Vitis vinifera* with values up to 1,257 mg L^{-1} per day (Bru *et al*., 2006). Nevertheless, stilbenes have not yet been quantified and/or determined in a variety of plants and fruits, especially wild ones. This plant has never been studied in the biotechnology processes or for resveratrol quantification to select the most productive stilbenes lines. Given this situation, this study aimed to assess for the first time the *t*-R content in three ecotypes of *Ugni molinae*.

Material and Methods

Plant Material

Three ecotypes aged 1 and 1.5 years, grown under nursery conditions by the Chilean National Institute of Agricultural Research (INIA, Carillanca), the Araucanía Region, were used for this study. These ecotype were obtained from three different sites; Porma (38º08, 73º16, 70 masl), the Araucanía Region (E1); Aucar-Quemchi (42º09, 73º29, 20 masl) the Los Lagos Region (E2) and Mehuin (39º26, 73º12, 10 masl) the Los Ríos Region (E3). Leaves, immature and mature fruits, were randomly collected by hand from each mother plant. Samples were taken to the laboratory under cold conditions and washed with tap water.

In vitro Establishment of Leaves and Fruits for Theinduction of Callus

Leaves were washed with sterile distilled water for 5 min, followed by a superficial aseptic in a laminar flow chamber with 70% ethanol (v/v) for 5 min and three rinses with sterile distilled water (3 min each). 0.1% mercury (II) chloride $HgCl_2$ solution (v/v) was applied as a disinfectant for 1 min, followed by five washes with sterile distilled water (3 min each), following the method by Liu *et al*. (2010). Explants segmented in sizes of approximately 50 mm^2, keeping a part of the petiole, were arranged in an abaxial position on the culture medium. Both mature and immature fruits were washed with tap water and 2% (v/v) commercial dish soap (Quix™) for 5-10 min and continuously immersed under running water for 6 h. Explants were sterilized with 1.25% sodium hypochlorite NaClO for 30 s and rinsed five times with sterile distilled water in a laminar flow chamber (Liu *et al*., 2010). Then the exocarp was extracted and segmented into 50 mm^2 pieces and established with the outer exocarp surface on the culture medium.

Media and Culture Conditions

The growth medium used was BTM (Chalupa, 1983), supplemented with 30 g L^{-1} sucrose, 0.5 g L^{-1} Polyvinylpyrrolidone PVP (Calbiochem™), 2 ml L^{-1} Plant Preservative Mixture PPM (Nalgene™) and different concentrations and combinations of 2,4-D, KIN and NAA as growth regulators (Table 1). The pH of the media was adjusted to 5.8 with HCl and NaOH (1M) prior to solidification with 7 g L^{-1} bacteriological agar (Merck™). Media were autoclaved for 20 min at 1 atm and 121°C and then arranged in Petri dishes. Six explants were established, with five replicates per treatment for leaves and six replicates for fruits. Trials were incubated in a growth chamber at 25±1°C and 55% relative humidity in constant darkness. During callogenesis, six subcultures were performed to increase biomass. In the last subculture, cell growth by means of Fresh Weight (FW) and the contents of *t*-resveratrol and *t*-piceid in the formed calli (µg g^{-1} FW) were assessed.

Cellular Suspensions

Friable calli (2 g FW) of selected lines were used to initiate cellular suspensions in 250 mL flasks, with 100 mL of the respective culture medium without agar and supplemented with 20 g L^{-1} sucrose, 0.5 g L^{-1} PVP, 2 mL L^{-1} PPM, 2 g L^{-1} potassium nitrate KNO_3 and 0.250 g L^{-1} hydrolyzed casein. Suspensions were maintained in an orbital shaker at 110 rpm in a growth chamber at 25±1°C and in constant darkness for 21 days. Three replicates were performed per each cell line. Cell growth was quantified every 2 days and expressed as packed cell volume (%PCV) after centrifugation at 4,000 rpm for 10 min in a swing bucket rotor. The viability of these aggregates was assessed every 7 days with 100 µL Trypan Blue (0.1%) under an Olympus CX31 optic microscope.

Table 1. Callus induction treatments of *Ugni molinae*

Treatments	2,4 D (mg L^{-1})	KIN (mg L^{-1})	NAA (mg L^{-1})
T0	-	-	-
T1	1.0	1.0	1.0
T2	5.0	1.0	1.0
T3	1.0	0.5	1.0
T4	5.0	0.5	1.0

Extraction of Stilbenes from Calli and Cell Aggregates

One g of calli was maintained in the darkness with 4 mL of 80% ethanol for 12 h at 4°C with continuous stirring. Samples were centrifuged at 3,000 rpm for 10 min. Finally, the supernatant was collected and filtered through a 0.45 µm Millipore™ membrane and stored at -20°C until analyzed in HPLC (Martínez-Esteso *et al*., 2009).

Extraction of T-Resveratrol from Plant Material

Fresh leaves (10-15 g) were extracted over 5 d in 95% ethanol at 4°C in the dark following the methodology of Rubilar *et al*. (2006) with some modifications. For the exocarps of the mature and immature fruits, 20-50 g of fruits were extracted for 7 d in methanol:acetone:water:formic acid (40:40:20:0.1 v/v) at 4°C in the dark, according to the methodology of Rimando *et al*. (2004) with some modifications. The obtained filtrates were homogenized in ultrasound equipment (Branson 1210™) and the organic solvent was removed by a rotary evaporator (Heidolph OB2000™) at 30°C. This method was performed in triplicate and samples were stored at -20°C until analyzed.

HPLC Analysis of T-Resveratrol and T-Piceid

Stilbenes were analyzed in a SPD-M10 Avp model chromatographer (Shimadzu™) equipped with a C18 reverse phase column (Kromasil, 250×4.6 mm, 5 µm 100 A C18, Phenomenex), a multifocal diode arrangement detector (190-800 nm) and an LC-10ATvp Shimadzu™ pump. The Class-vp software was run for data acquisition and instrument control. Methanol solutions of 1 mg mL^{-1} *t*-R (Calbiochem™) and 1 mg mL *t*-P (Sigma-Aldrich™) were used as standards which were detected at a fixed wavelength of 306 nm. Separation of previously filtered *in vitro* material (Millipore 0.22 µm), was prepared at 40°C using a mixture of 0.1% acetic acid in water (solvent A) and acetonitrile (solvent B) at a flow rate of 1.0 mL min^{-1}, in a 30 min segmented gradient consisting of 5-70% B (25 min), 95% B (0.1 min), 95% B (2 min) and 5% B (0.1 min). Under these conditions retention times were 17.4 min for *t*-R and 14.1 min for *t*-P. The plant material extracts were analyzed by HPLC according to the methodology by Vitrac *et al*. (2002), using 0.1% TFA in water as solvent A and of 0.1% TFA in acetonitrile:water (80:20 v/v) as solvent B at a flow rate of 0.7 mL min^{-1} and an injection volume of 20 µL.

Experimental Design and Statistical Analyses

An analysis of variance (ANOVA) with factorial arrangement using Tukey's multiple comparison test, was used to analyze the statistical differences for the concentrations of growth regulators. The results were statistically significant at $P \leq 0.05$, The SAS System 9.2 software for Windows™ was used.

Results

Callus Induction and Growth

Callus formation from fruit exocarp occurred 60 days after explants were established on induction media. Significant differences were observed among the callus induction treatments in mature exocarps, where all the explants of ecotypes 2 and 3 formed calli with T1. In the immature fruits, the best response in ecotype 2 was obtained in T4 and in ecotype 3 both in T3 and T4 (Table 2). Induction was uneven among ecotypes because not all explants were able to de-differentiate and produce a response. The morphological appearance of the formed calli was strikingly similar between explants; a friable cell mass with whitish coloration and brown tones (Fig. 1).

Selection of Highly Productive Lines from the Established Calli

T-Rproduction in the callus lines from the immature fruits was higher than those from mature fruits. The highest *t*-R concentrations in the immature fruits were found in cellular line T3EI3 with 553.4 µg *t*-R. g^{-1} FW, as opposed to the most productive line of the mature exocarp line T1EM3, with 76.19 µg *t*-R g^{-1} FW (Fig. 2). The highest *t*-P values were found in the cell line T3EI2, with 52.6 µg *t*-P g^{-1} FW; followed by line T1EI3 with 47.8 µg of *t*-P g^{-1} FW.

Cell Aggregates from the Suspensions Culture

The *t*-R concentration at the beginning of suspensions (day 0) was 1.89 µg *t*-R g^{-1} FW for line T4EI3 and 2.70 µg *t*-R g^{-1} FW for line T3EI3, which increased with a maximum of 54.26 µg *t*-R g^{-1} FW on day 14 in line T4EI3 and of 30.56 µg of *t*-R g^{-1} FW on day 21 in line T3EI3. The *t*-P concentration was higher than *t*-R in both lines, particularly on day 14, when it reached 54.03 µg of *t*-P g^{-1} FW for line T3EI3 and up to 95.47 µg of *t*-P g^{-1} FW in line T4EI3. The results agree with the growth profile of the cell aggregates, where an exponential phase was present between days 4 and 14 for line T4EI3 and between 6 and 12 days for line T3EI3. These remained steady between days 14 and 16, with 82% maximum cell viability for T3El3 and 70% for T4El3.

Quantification of t-R and t-P in the Plant Material

The *t*-R and *t*-P concentrations were also assessed in plant material, particularly in leaf and exocarp of the mature and immature fruits analyzed by HPLC, thus being the first publication to report resveratrol levels in different *Ugni molinae* ecotypes. The highest *t*-R concentrations were detected in fruits, with biggest differences found between ecotypes. The same occurred with calli. The best results were obtained from the exocarp of the immature fruits from ecotype 3, with a total of 5,100 µg *t*-R g^{-1}, followed by the exocarp of the mature fruits of ecotype 2 and 3 (2,750, 2,600 and 1,400 µg *t*-R g^{-1}).

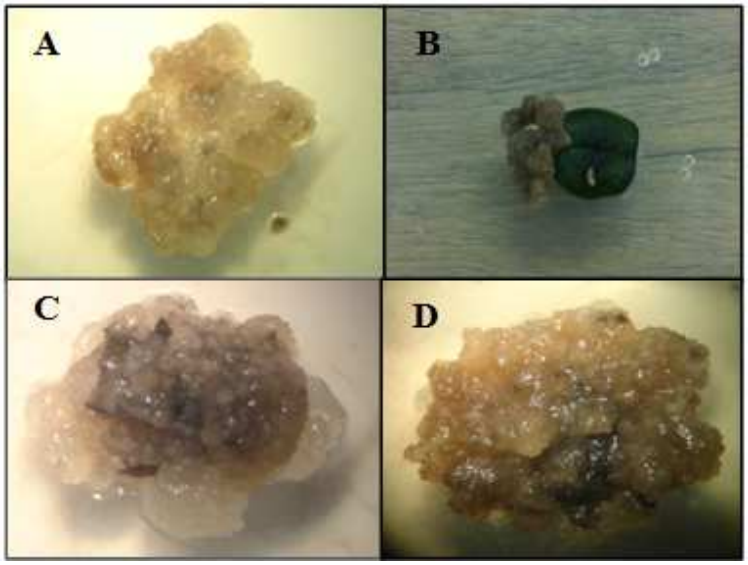

Fig. 1. Morphological appearance of calli. A. B. Friable calli of the leaves from ecotype 3 in treatment T2 C. Calli of the immature fruit exocarp from ecotype 3 treatment in T3. D. Calli of the mature fruits exocarp from ecotype 3 in treatment T2

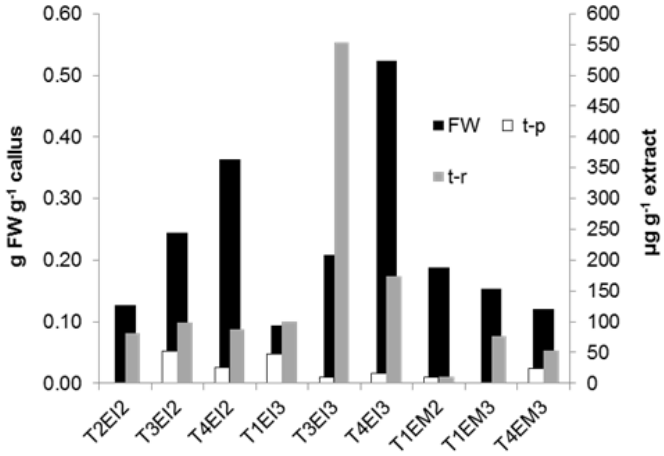

Fig. 2. Biomass and concentration of t-R and t-P from the calli obtained from the fruit exocarp after 3 months of induction. T1-T2-T3-T4: Callus induction treatments. EI2: Calli from the immature fruit ecotype 2 EI3: Calli from the immature fruit ecotype

Table 2. Percentage of callus induced from the leaves and fruit exocarps of *Ugni molinae* after establishing explants. Givenmeans in a column with different letters represent statistically significant differences at $P \leq 0.05$, as determined by Tukey's test. T0: Control without growth regulators. T: Treatments, TE: Type of explant, L: Leaves, MFE: Mature fruit explant, IFE: Immature fruit explant. T1: 1 mg L^{-1} 2,4-D + 1 mg L^{-1} KIN + 1 mg L^{-1} NAA. T2: 5 mg L^{-1} 2,4-D + 1 mg L^{-1} KIN + 1 mg L^{-1} NAA. T3: 1 mg L^{-1} 2,4-D + 0.5 mg L^{-1} KIN + 1 mg L^{-1} NAA.T4: 5 mg L^{-1} 2,4-D + 0.5 mg L^{-1} KIN + 1 mg L^{-1} NAA

	Callogenesis (%)								
	Ecotype 1			Ecotype 2			Ecotype 3		
T									
TE	L	MFE	IFE	L	MFE	IFE	L	MFE	IFE
T0	0± 0	0±0	0±0 a	0±0	0±0 a	0±0 a	0±0	0±0 a	0±0 a
T1	7±0.9	0±0	8±0.7 a	3±0.5	100±0 b	0±0 a	3±0.5	100±0 b	83±0 c
T2	3±0.5	0±0	0±0 a	3±0.5	50±0.7 c	50±2.8 b	3±0.5	0±0 a	50±2.8 b
T3	7±0.9	0±0	50±0.7 b	0±0	0±0 a	50±0.7 b	0±0	50±0 c	100±0 d
T4	0±0	0±0	0±0 a	0±0	50±0.7 c	67±1.4 c	0±0	61±1.4 c	100±1 d

These values were higher than the *in vitro* material. Nevertheless, the values obtained from the mature and immature calli were higher than those found in leaves (60 μg *t*-R g^{-1}), especially in the calli formed from the immature fruit (245.9 *t*-R μg g^{-1} callus).

Discussion

Callus Induction and Growth

The combination of 1 mg L^{-1} of ANA + 1 mg L^{-1} of 2,4-D and 0.5 mg L^{-1} of KIN as a growth regulator for callus induction, has been used in other berry cultures; e.g., *Vaccinium macrocarpon* Ait. and *Vaccinium phalae* (ohelo) (Madhavi *et al*., 1995; Fang *et al*., 1999). This allowed the subsequent development of suspension culture to produce anthocianins. However, this has been the only species reported for callus induction from berry plants, thus, our study becomes the third report of callus culture from berries. Furthermore, is one of a few that developed callus using a fruit explant from immature and mature exocarps (adult material). As shown here, the synthesis and location of secondary metabolites may vary among plant tissues by using different plant parts. In the study by Liu *et al*. (2010), three plant tissues in four genotypes of *Vitis vinifera* were used to successfully form calli. However, the best results occurred in young leaves and seeds rather than in the exocarps of fruits, where the response was slower and even showed necrosis in some genotypes. This response is expected in almost all occasions, because calli (undifferentiated) is best formed from young material such as leaves and nodal segments. Instead our research form friable calli from adult material (fruits explants and leaves), noting that the use of the hormonal combination was adequate.

Selection of Highly Productive Lines from the Established Calli

Vitis vinifera has been the most widely studied plant species for tissue culture-mediated production of stilbenes and phenolic compounds (Cai *et al*., 2011), being *trans-piceidthe stilbene* with the highest concentration found, from the Red Globe cultivar the most productive line (69.9 μg *t*-P g^{-1} FW) (Santamaria *et al*., 2011b). However, very few studies have reported stilbene content in callus. In the *Arachis hypogaea* 'Tainan' cultivar, callus elicited with ultraviolet light and microorganisms showed *t*-R contents between 1.03 and 7.08 μg g^{-1} FW and *t*-P contents from 0.71 to 9.61 μg g^{-1} FW (Ku *et al*., 2005; Yang *et al*., 2010). When comparing these results with those obtained here (cell line T3El2, with 52.6 μg *t*-P g^{-1} FW), *Ugni molinae* gave very similar *t*-P values compared the most productive line from the Red Globe cultivar and in addition, much higher *t-R* content values (line T3El3 with 553.4 μg *t*-R g^{-1} FW) compared to other plant species. These results are very promising and revealed that highly productive lines can be obtained from calli.

Cell Aggregates from the Suspensions Culture

The biomass growth of *U. molinae* cell aggregates in liquid culture followed a behavior similar to that reported for cellular suspensions of *Vitis* spp., starting with an initial growth phase between days 2-4 of culture until day 12-14, when plant cells finally entered a steady state that ends day 16-18 (Martínez-Esteso *et al*., 2009; Santamaria *et al*., 2011a; 2011b; Belchi-Narravo *et al*., 2012). The end of the cell division phase and the start of cell expansion characteristic of the steady phase, are associated with the synthesis and accumulation of specific secondary metabolites, including some alkaloids, anthocyanins and other phenolic derivatives (Lindsey and Jones, 1989). This may explain the increase in the piceid and *t*-Rcontents in aggregates as they primarily accumulate in cellular compartments as a storage form. Piceid has also been assessed in 'Gamay' *Vitis vinifera*cell suspensions with the highest values found of 280 mg L^{-1} (Aumont *et al*., 2004). It is the most abundant stilbenoid and accumulates with the biomass in normal growth medium (Martínez-Esteso *et al*., 2011). When elicitors are added, resveratrol becomes highly abundant and is released into the culture medium (Morales *et al*., 1997; Bru *et al*., 2006; Donnez *et al*., 2009; Martínez-Esteso *et al*., 2009; 2011). Cellular suspensions of *Gossypium hirsutum* have been developed as well for the *in vitro t*-R production, with values of 7.2 μg g^{-1} FW and also in roots of *Arachis hipogea*, with values of 2 μg g^{-1} and between 0.81 and 1.5 mg g^{-1} FW (Medina-Bolivar *et al*., 2007; Kouakou *et al*., 2006; Kim *et al*., 2008). Cellular suspensions in *Ugni molinae* or Myrtaceas species have never been reported, thus, this quantification is the first to be published. The results obtained are comparable with those reported for other species and could be further improved if elicitation strategies are implemented in the future.

Quantification of t-R and t-P in Plant Material

In Chile, resveratrol from *in vivo* material only has been quantified in *Vitis vinifera* (0.007 and 0.26 μmol g^{-1} DW) and in *Berberis buxifolia* (0.98 μmol g^{-1} and 3.87 μmol g^{-1} FW) (Ruiz *et al*., 2010). The results obtained here from murtilla fruits, exceeded the values of the genotypes formerly studied in grapes, that ranged from 39.71 to 300 μg g^{-1} (Tobar-Reyes *et al*., 2009; Liu *et al*., 2013), in blueberries (32 ng g^{-1} DW), peanut (1.92 μg g^{-1} DW) and pistachios (1.67 μg g^{-1} DW) (Lyons *et al*., 2003; Tokusoglu *et al*., 2005), being these values interesting for further research. In all these studies, concentrations varied depending on genotype and sample collection area. Some authors have explained that this

phenomenon may be due to the attribution of abiotic or biotic stress factors from the surrounding environment, time of harvesting, climatic conditions, geographic origin, plant development and even crop type (organic, wild or agricultural) (Vitrac *et al*., 2002; Li *et al*., 2006). The comparative studies between *in vivo* and *in vitro* material (calli) to quantify secondary metabolites have been conducted for species like *Vaccinium macrosporum* and *Buddleja cordata* (Madhavi *et al*., 1995; Estrada-Zuniga *et al*., 2009). Presently, however, very few successful examples of the commercial application of cellular suspensions exist due to the low biosynthesis of the compound, differentiation and compartmentalization of cells, lack of developing organelles, unstable cellular lines and the difficulty of scaling production (Kolewe *et al*., 2008; Estrada-Zuniga *et al*., 2009).

Even when *t*-resveratrol production in *U. molinae* did not increase when cell suspensions were used, our results demonstrated that native species from Chile could be a biological reserve of resveratrol, which remains unknown and could be highly competitive with other currently used extraction sources. Further research is required to identify the variables that affect final *t*-resveratrol and *t*-piceid production.

Conclusion

This study is the first to report presence of resveratrol from *in vitro* cultures of *Ugni molinae* from different explants. The callus formed from the fruit exocarp was used to establish cell suspensions, where amounts of *t*-resveratrol and *t*-piceid were identified in cell aggregates. The most productive line was ecotype 3 when 5 mg L^{-1} of 2,4-D + 0.5 mg L^{-1} KIN and 1 mg L^{-1} of NAA were used, obtaining 553.4 µg *t*-R g^{-1}. The *t*-R production in immature fruits was higher than in mature callus. The highest *t*-P values were found in cell line T3EI2, with 52.6 µg *t*-P g^{-1} FW, followed by line T1EI3 with 47.8 µg of *t*-P g^{-1} FW. Differences among ecotypes were observed in all stages. The amounts of resveratrol found in the *in vitro* callus and field material from the fruit exocarp exceeded those reported for other plant species. This indicates that *Ugni molinae* is a productive source of resveratrol that was not known until now.

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Author's Contributions

Carolina Salazar: Performed all the experiments and worked on the study conception and design, data acquisition, data analysis and interpretation and wrote the manuscript.

Evelyn Bustos: Data acquisition, sample preparation, analysis and interpretation.

Claudia Perez: Data analysis and interpretation, sample preparation, wrote the manuscript and scientific discussion.

Jose Becerra: Advised in the experimental methods, critical revision.

Roque Bru: Read and improved the manuscript, Critical revision.

Matilde Uribe: Coordinated the study, data analysis and interpretation, integrated all the data and was involved in writing the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

Aguirre, M.C., C. Delporte, N. Backhouse, S. Erazo and M.E. Letelier *et al*., 2006. Topical anti-inflammatory activity of 2α-hydroxy pentacyclic triterpene acids from the leaves of *Ugni molinae*. Bioorganic Med. Chem., 14: 5673-5677. DOI: 10.1016/j.bmc.2006.04.021

Aumont, V., F. Larronde, T. Richard, H. Budzinski and A. Decendit *et al*., 2004. Production of highly ^{13}C-labeled polyphenols in *Vitis vinifera* cell bioreactor cultures. J. Biotechnol., 109: 287-294. DOI: 10.1016/j.jbiotec.2004.01.004

Avello, M., R. Valdivia, M.A. Mondaca, J.L. Ordoñez and M. Bittner *et al*., 2009. Actividad de *Ugni molinae* Turcz. frente a microorganismos de importancia clínica. Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas, 8: 141-144.

Baur, J. and D. Sinclair, 2006. Therapeutic potential of resveratrol: The *in vivo* evidence. Nature, 5: 493-506. DOI: 10.1038/nrd2060

Belchi-Narravo, S., L. Almagro, D. Lijavetzky, R. Bru and M. Pedreño, 2012. Enhanced extracellular production of *trans*-resveratrol in *Vitis vinifera* suspension cultured cells by using cyclodextrins and methyljasmonate. Plant Cell Reports, 31: 81-89. DOI: 10.1007/s00299-011-1141-8

Bru, R., S. Sellés, J. Casado-Vela, S. Belchí-Navarro and M.A. Pedreño, 2006. Modified cyclodextrins are chemically defined glucan inducers of defense responses in grapevine cell cultures. J. Agric. Food Chem., 54: 65-71. DOI: 10.1021/jf051485j

Cai, Z., A. Kastell, I. Mewis, D. Knorr and I. Smetanska, 2011. Polysaccharide elicitors enhance anthocyanin and phenolic acid accumulation in cell suspension cultures of *Vitis vinifera*. Plant Cell Tissue Organ. Culture, 108: 401-409. DOI: 10.1007/s11240-011-0051-3

Chalupa, V., 1983. Micropropagation of conifer and broadleaved forest trees. Commun. Inst. Forestalis Cechosloveniae, 13: 7-39.

Donnez, D., P. Jeandet, C. Clément and E. Courot, 2009. Bioproduction of resveratrol and stilbene derivatives by plant cells and microorganisms. Trends Biotechnol., 27: 706-713. DOI: 10.1016/j.tibtech.2009.09.005

Estrada-Zuniga, M.E., F. Cruz-Sosa, M. Rodríguez-Monroy, J.R. Verde-Calvo and E.J. Vernos-Carter, 2009. Phenylpropanoid production in callus and cell suspension cultures of *Buddleja cordata* Kunth. Plant Cell Tissue Organ. Culture, 97: 39-47. DOI: 10.1007/s11240-009-9496-z

Fang, Y., M.A.L. Smith and M.F. Pépin, 1999. Effect of exogenous methyljasmonate in elicited anthocyanin-producing cell cultures of ohelo (*Vaccinium pahalae*). Vitro Cellular Develop. Biol. Plant, 35: 106-113. DOI: 10.1007/s11627-999-0019-8

Halder, M. and S. Jha, 2015. Enhanced *trans*-resveratrol production in genetically transformed root cultures of peanut (*Arachis hypogaea* L.). Plant Cell Tissue Organ. Culture, 124: 555-572. DOI: 10.1007/s11240-015-0914-0

Karuppusamy, S., 2009. A review on trends in production of secondary metabolites from higher plants by *in vitro* tissue, organ and cell cultures. J. Med. Plants Res., 3: 1222-1239. DOI: 10.5897/JMPR

Kim, J.S., S.Y. Lee and S.U. Park, 2008. Resveratrol production in hairy root culture of peanut. *Arachis hypogaea* L. transformed with different *Agrobacterium rhizogenes* strains. Afr. J. Biotechnol., 7: 3788-3790. DOI: 10.5897/AJB08.499

Kolewe, M., V. Gaurav and S. Roberts, 2008. Pharmaceutically active natural product synthesis and supply via plant cell culture technology. Molecular Pharmaceut., 5: 243-256. DOI: 10.1021/mp7001494

Kouakou, T.H., P.W. Téguo, J. Valls, Y.J. Kouadio and A. Decendit *et al*., 2006. First evidence of *trans*-resveratrol production in cell suspension cultures of cotton (*Gossypium hirsutum* L.). Plant Cell Tissue Organ. Culture, 86: 405-409. DOI: 10.1007/s11240-006-9136-9

Ku, K.L., P.S. Chang, Y.C. Cheng and C.Y. Lien, 2005. Production of stilbenoids from the callus of *Arachis hypogaea*: A novel source of the anticancer compound piceatannol. J. Agric. Food Chem., 53: 3877-3881. DOI: 10.1021/jf050242o

Li, X., B. Wu, L. Wang and S. Li, 2006. Extractable amounts of *trans*-resveratrol in seed and berry skin in *Vitis* evaluated at the germplasm level. J. Agric. Food Chem., 54: 8804-8811. DOI: 10.1021/jf061722y

Lindsey, K. and M.G.K. Jones, 1989. Biotecnología Vegetal Agrícola. 1st Edn., Acribia S.A, ISBN-10: 9788420007250, pp: 276.

Liu, C., L. Wang, J. Wang, B. Wu and W. Liu *et al*., 2013. Resveratrols in *Vitis* berry skins and leaves: Their extraction and analysis by HPLC. Food Chem. 136: 643-649. DOI: 10.1016/j.foodchem.2012.08.017

Liu, W., C. Liu, C. Yang, L. Wang and S. Li, 2010. Effect of grape genotype and tissue type on callus growth and production of resveratrols and their piceids after UV-C irradiation. Food Chem., 122: 475-481. DOI: 10.1016/j.foodchem.2010.03.055

Lyons, M.M., C. Yu, R.B. Toma, S.Y. Cho and W. Reiboldt *et al*., 2003. Resveratrol in raw and baked blueberries and bilberries. J. Agric. Food Chem., 51: 5867-5870. DOI: 10.1021/jf034150f

Madhavi, D.L., M.A.L. Smith and M.D. Berber-Jiménez, 1995. Expression of anthocyanins in callus cultures of cranberry (*Vaccinium macrocarpon* Ait.). J. Food Sci., 60: 351-355. DOI: 10.1111/j.1365-2621.1995.tb05671.x

Martínez-Esteso, M.J., S. Sellés-Marchart, J.C. Vera-Urbina, M.A. Pedreño and R. Bru-Martínez, 2009. Changes of defense proteins in the extracellular proteome of grapevine (*Vitis vinifera* cv. Gamay) cell cultures in response to elicitors. J. Proteom., 73: 331-341. DOI: 10.1016/j.jprot.2009.10.001

Martínez-Esteso, M.J., S. Sellés-Marchart, J.C. Vera-Urbina, M.A. Pedreño and R. Bru-Martinez, 2011. DIGE analysis of proteome changes accompanying resveratrol production by grapevine (*Vitis vinifera* cv. Gamay) cell cultures in response to cyclodextrin and methyl jasmonate elicitors. J. Proteom., 74: 1421-1436. DOI: 10.1016/j.jprot.2011.02.035

Medina-Bolivar, F., J. Condori, A. Rimando, J. Hubstenberger and K. Shelton *et al*., 2007. Production and secretion of resveratrol in hairy root cultures of peanut. Phytochemistry, 68: 1992-2003. DOI: 10.1016/j.phytochem.2007.04.039

Morales, M., J. Alcántara and A.R. Barceló, 1997. Oxidation of *trans*-resveratrol by a hypodermal peroxidase isoenzymes from Gamay rouge grape (*Vitis* V*inifera*) berries. Am. J. Enol. Viticulture, 48: 33-38.

Morales, M., R. Bru, A. García-Carmona, R. Barceló and M.A. Pedreño, 1998. Effect of dimethyl-β-cyclodextrins on resveratrol metabolism in Gamay grapevine cell cultures before and after inoculation with shape *Xylophilus ampelinus*. Plant Cell Tissue Organ. Culture., 53: 179-187. DOI: 10.1023/A: 1006027410575

Rimando, A., W. Kalt, J. Magee, J. Dewey and R. Ballington, 2004. Resveratrol, pterostilbene and piceatannol in *Vaccinium* berries. J. Agric. Food Chem., 52: 4713-4719. DOI: 10.1021/jf040095e

Rocha-González, H., M. Ambriz-Tututi and V. Granados-Soto, 2008. Resveratrol: A natural compound with pharmacological potential in neurodegenerative diseases. CNS Neurosci. Therapeut., 14: 234-247. DOI: 10.1111/j.1755-5949.2008.00045.x

Rubilar, M., C. Jara, Y. Poo, F. Acevedo and C. Gutiérrez *et al*., 2011. Extracts of Maqui (*Aristotelia chilensis*) and Murta (*Ugni molinae Turcz.*): Sources of antioxidant compounds and α-glucosidase/α-amylase inhibitors. J. Agric. Food Chem., 59: 1630-1637. DOI: 10.1021/jf103461k

Rubilar, M., M. Pinelo, M. Ihl, E. Scheuermann and J. Sineiro *et al*., 2006. Murta leaves (*Ugni molinae* Turcz.) as a source of antioxidant polyphenols. J. Agric. Food Chem., 54: 59-64. DOI: 10.1021/jf051571j

Ruiz, A., I. Hermosín-Gutiérrez, C. Mardones, C. Vergara and E. Herlitz *et al*., 2010. Polyphenols and antioxidant activity of Calafate (*Berberis microphylla*) fruits and other native berries from southern Chile. J. Agric. Food Chem., 58: 6081-6089. DOI: 10.1021/jf100173x

Santamaria, A., N. Mulinacci, A. Valletta, M. Innocenti and G. Pasqua, 2011a. Effects of elicitors on the production of resveratrol and viniferins in cell cultures of *Vitis vinifera* L. cv Italia. J. Agric. Food Chem., 59: 9094-9101. DOI: 10.1021/jf201181n

Santamaria, A.R., D. Antonacci, G. Caruso, C. Cavaliere and R. Gubbiotti *et al*., 2011b. Stilbene production in cell cultures of *Vitis vinifera* L. cvs Red globe and *Michele palieri* elicited by methyljasmonate. Natural Products Res., 24: 1488-1498. DOI: 10.1080/14786410903421446

Tobar-Reyes, J., O. Franco-Mora, E. Morales-Rosales and J. Cruz-Castillo, 2009. Contenido de resveratrol en hojas de vides silvestres (*Vitis* spp.) mexicanas. Rev. Facultad Ciencias Agrarias UNCuyo. Tomo, 41: 127-137.

Tokusoglu, O., M.K. Unal and F. Yemis, 2005. Determination of the phytoalexin resveratrol (3,5,4′-trihydroxystilbene) in peanuts and pistachios by high-performance liquid chromatographic diode array (HPLC-DAD) and gas chromatography mass spectrometry (GC-MS). J. Agric. Food Chem., 53: 5003-5009. DOI: 10.1021/jf050496+

Trejo-Tapia, G. and M. Rodríguez-Monroy, 2007. La agregación celular en la producción de metabolitos secundarios en cultivos vegetales *in vitro*. Interciencia, 32: 669-674.

Vitrac, X., J.P. Monti, J. Vercauteren, G. Deffieux and J.M. Mérillon, 2002. Direct liquid chromatographic analysis of resveratrol derivatives and flavanonols in wines with absorbance and fluorescence detection. Analytica Chimica Acta, 458: 103-110. DOI: 10.1016/S0003-2670(01)01498-2

Xu, A.I., J.C. Zang and W.D. Huang, 2015. Effects of ultraviolet C, methyl jasmonate and salicylic acid, alone or in combination, on stilbene biosynthesis in cell suspension cultures of Vitis vinifera L. cv. Cabernet Sauvignon. Plant Cell Tissue Organ. Culture., 122: 197-211. DOI: 10.1007/s11240-015-0761-z

Yang, M.H., C.H. Kuo, W.C. Hsieh and K.L. Ku, 2010. Investigation of Microbial Elicitation of *trans*-Resveratrol and *trans*-Piceatannol in Peanut Callus Led to the Application of Chitin as a Potential Elicitor. J. Agric. Food Chem., 58: 9537-9541. DOI: 10.1021/jf1022725