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Propagation of Chilean Wine Palm (Jubaea chilensis) by Means of in Vitro Embryo Culture

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ABSTRACT

It has been demonstrated that *in vitro* culture of embryos of the Chilean wine palm (*Jubaea chilensis* (Mol.) Baillon) accelerated and considerably increased their growth and development. Traditionally, fruits of this species sown in conventional ways have exhibited poor germination. Embryos were cultured in solid medium, Murashige-Skoog (MS), without hormones and with activated charcoal (4 gr/l) in the first stage of culture. After 30 to 40 days 25% of the embryos initially cultured were ready to be transplanted into soil.

The Chilean wine palm *Jubaea chilensis* (Mol.) Baillon is native in continental Chile. It is a very long-lived plant and due to its morpho-anatomy is well adapted to xerophytic climatic zones. In Chile it is distributed from south of the Limarí River (31° latitude South) to the mouth of the Maule River (35°18′ latitude South). Nevertheless, the greatest concentration of specimens appears in the Valleys of Ocoa (V región) and Cocalán (VI región). This plant is used for reforestation since oil is obtained from its fruits and honey from the sap. The Chilean wine palm is propagated exclusively from seeds; their poor germination and slow growth constitute the principal difficulties to its culture. The percentage of germination obtained in nurseries with traditional methods is less than 2% (from 36,000 fruits sown after a year only 600 germinated (Trobok, personal communication)).

One of the methods currently used to accelerate or solve the difficulties in germination is the culture of embryos *in vitro* (Figs. 2,3,4). This allows the development of the plant under controlled conditions, eliminating such possible causes of dormancy as impermeability of the outer cover of fruit or seed and or the presence of inhibitors or immature embryos.

The present work investigates the possibility of propagating the Chilean wine palm rapidly and efficiently by means of *in vitro* embryo culture.

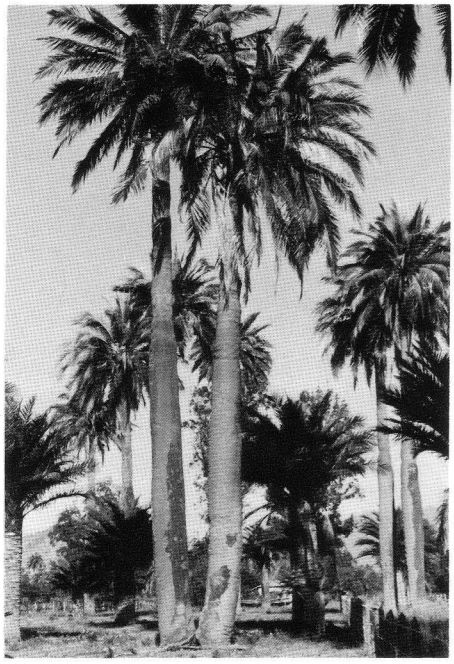
Materials and Methods

In the autumn of 1984 ripe fruits* were gathered from 400 year-old Chilean wine palms situated in the palm farm of Cocalán (Fig. 1).

Previous to *in vitro* culture, fruits and embryos were classified into two groups according to size and weight (Table 1). This was to facilitate comparison studies of these two groups in their subsequent growth and development.

Extraction of embryos involved cracking seeds (Figs. 2,3) and sterilizing their embryos in a 3% sodium hypochlorite solution for 15 minutes followed by rinsing three times in sterile water. The medium used for culture was Murashige and Skoog (MS) (1) with naphthalene acetic acid (NAA, 2 mg/l); (2) MS with benzylaminopurine (BAP, 2 mg/l) and without hormones. All the media was supplied with saccharose (30 g/l), adjusted to a pH of 7.

* We call "fruit" the seed plus endocarp which is the traditional structure used in propagation.



1. Specimens of the Chilean wine palm from which the fruits for this research were obtained.

Liquid and solid media (agar 7 g/l) were used. Later attempts included activated charcoal (4 g/l) and polyvinylpyrrolidone (4 g/l) in the MS liquid and MS/2 solid media without hormones. All media were sterilized in an autoclave at 110° C for 15 minutes.

Five embryos were cultured in each 125 ml erlenmeyer flask containing 25 ml of nutrient medium. Preliminary attempts to determine the most favorable medium and temperature for growth and development were done using three erlenmeyers (15 embryos total for each treatment). Later, fifty erlenmeyers with solid MS media and without hormones were cultured, 25 with small embryos and 25 with big embryos. Cultures were kept in a climatic chamber at 30° C ± 3 with an intensity of 54 uE (PAR) and a photoperiod of 16 hours of light.

Evaluation of different media was done eight days after culturing. Embryos that managed to develop were subcultured to a fresh medium of the same chemical composition and a definitive evaluation took place 20 days after the transplant.

Simultaneously and for comparison, fruits were sown in a sandy substrate under the same conditions as embryos *in vitro*.

Table 1. Average size and weight of 20 embryos of Chilean wine palm.

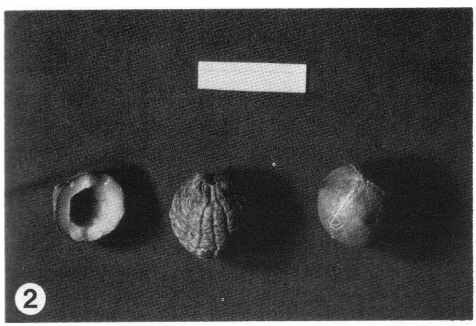
	Weight (gr)	Diameter (mm)	Length (mm)
Big fruits	8.85	27.05	—
Small fruits	4.91	20.81	—
	(mg)		
Big embryos	3.78	1.24	3.13
Small embryos	3.09	1.08	2.98

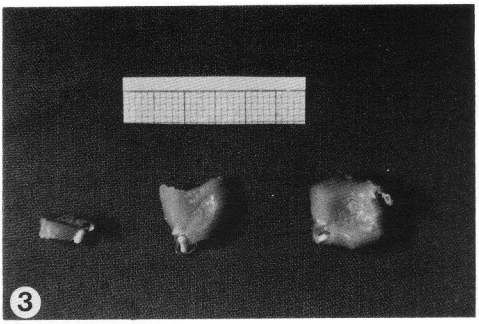
Results

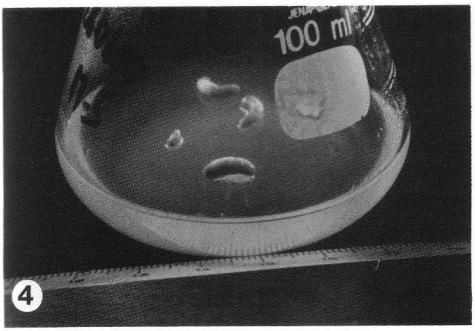
The results of the preliminary experiments showed that in a solid medium (MS) without hormones, the embryos began to grow after five to eight days of culture. Growth was initiated on 79% and 85% of the big and small fruits respectively. Also, the size of the embryos was three to five times larger than their initial size (Fig. 4). All were kept at a temperature of 30° C since it was observed that at temperatures lower than 25° C there was very little or no growth.

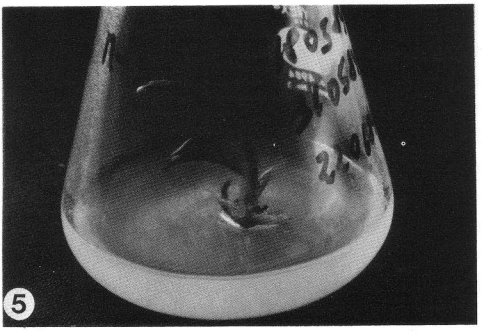
Embryos cultured in a liquid MS medium without hormones only increased in size without showing further development. Embryos put in a solid MS medium with different combinations of growth regulators did not develop and slowly decayed.

Eight days after culturing, embryos that began to germinate were subcultured in a fresh medium of equal chemical composition. After thirty days 58% of them had developed shoots, 8% had roots, and only 6% had shoots and roots (Figs. 5). This low percentage of development was overcome by a new culture of embryos in the same nutrient medium but adding acti-





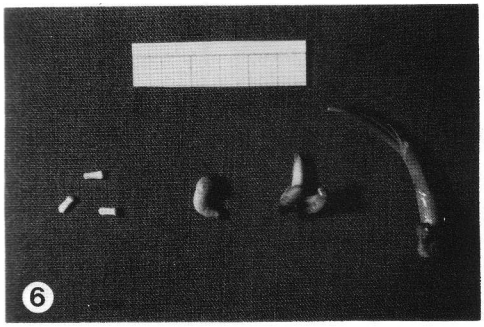


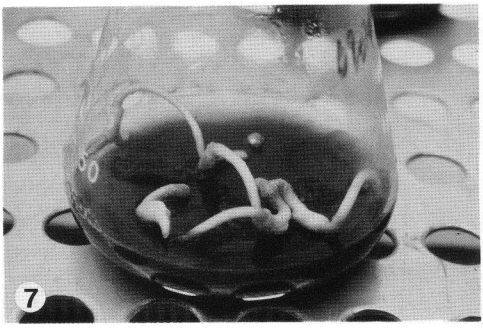


2. A fruit of a Chilean wine palm with and without mesocarp. 3. Position of the embryo in the fruit of a Chilean wine palm. 4. Initiation of germination *in vitro*. 5. Seedling 30–40 days after being sown.

vated charcoal (4 g/l). This resulted in 45% of the embryos developing radicles (Fig. 7). After 15 days in this medium embryos were removed to the same nutrient medium but without activated charcoal where approximately 50% of them developed shoots.

Thirty to forty days after initial culturing, 25% of the embryos were ready to be transplanted to a sand : vermiculite (60: 40) substrate. Preliminary results of this phase of the investigation give low percentage of survival, about 20% after seven months.





6. Development of embryos of Chilean wine palm in a solid MS nutrient medium without hormones. After only 30–40 days development of shoots was obtained. 7. Initiation of root development ten days after sowing when activated charcoal is added to MS medium; later they will be removed to a nutrient medium without activated charcoal where the development of shoots will take place.

Contrary to previous results, fruits shown under the same environmental conditions did not show any germination after three months. Notwithstanding, when embryos were removed from the fruits they were completely hydrated and when cultured *in vitro* they began to germinate normally.

Discussion

In the present research it is suggested that seed of the Chilean wine palm does not germinate under natural conditions due to various possible factors: 1, mechanical impediment of part of the endocarp or endosperm for expansion of the embryo; 2, inhibition caused by chemical stimuli on part of the endocarp, endosperm, or seed coat during imbibition; 3, accumulation of natural growth inhibitors in the endosperm which once imbibed cannot be overcome by the embryo; and 4, surrounding conditions inappropriate for natural germination that differ from those required for growth *in vitro*.

Later observations showed that accumulation of natural growth inhibitors was not a determining factor since when ten embryos were cultured in only 3. ml of the nutrient medium mentioned above, they began to develop without any difficulty even under this condition of crowding.

While finishing this work several attempts are taking place to establish in pots plants produced *in vitro* as well as the propagation by direct sowing with different treatments and environmental conditions.

Acknowledgments

I wish to sincerely express my gratitude to Mrs. Claudia Botti, Ing. Agr. MS. Professor of Botany, Facultad CS. Agraria y Forestales, U. de Chile, for her help in revising and correcting the text of this publication.

RESULTS OF THE WRITING CONTEST

Nine papers were submitted to the contest to write an article about growing palms or on a favorite palm. The papers were all very interesting and the four judges had difficulty reaching decisions. Four winners were finally chosen:

First Prize: **Garrin Fullington,** "*Parajubaea*—an unsurpassed palm for cool, mild areas"

Second Prize: **Charles J. Reynolds,** "An unappreciated native: *Sabal palmetto*"

Third Prize: **Leonard Goldstein,** "Growing palms in cold areas of South Florida"

Fourth Prize: **Ralph Velez,** "My forty-two years as a palmophile"

All articles submitted will appear eventually in *Principes*, beginning with Garrin Fullington's in this issue (see pp. 172–176). The winners can expect their prizes in due course.

THE EDITORS