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Tissue Culture of Date Palms—A New Method to Propagate an Ancient Crop—and A Short Discussion of the California Date Industry

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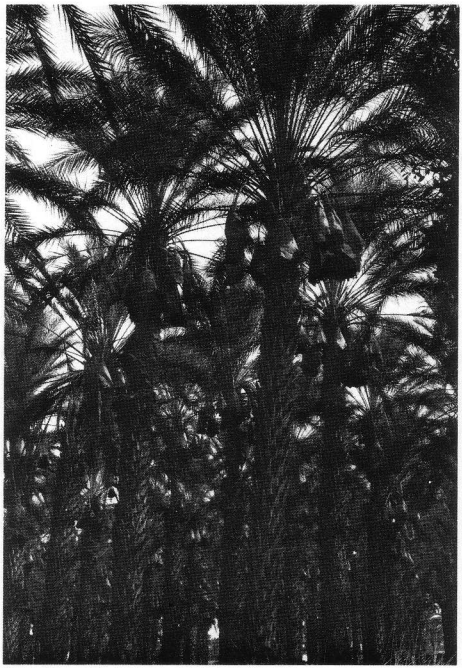
The date palm, *Phoenix dactylifera* L., is one of the economically most important species of the Arecaceae (Palmae). Date palms provide a staple food crop for several North African and Middle East countries (Fig. 1). Records reveal that the date palm has the distinction of being one of the oldest cultivated tree crops beginning at least as early as 4,000 B.C. (Zohary and Spiegel-Roy 1975).

Extent of the U.S. Date Industry

The date industry in California is restricted to the hot arid, inland desert regions of Riverside and Imperial counties. About 99% of the dates produced in the United States are grown in the Coachella valley of Riverside county. The total date acreage of Riverside county has remained relatively constant since the 1950's and today consists of 3,868 acres (Anon. 1978, Mitchell 1973). Also, some insignificant date acreage is located in adjacent Imperial and San Bernardino counties and the Phoenix and Yuma regions in Arizona. Dates are a specialty crop in California and in 1978 yielded 24,317 tons of fruit worth $19,130,000 from 3,542 bearing acres (Anon. 1978).

Cultural Innovations in the U.S. Date Industry Since the 1960's

The California date industry began around the turn of the 20th century through the introduction and multiplication of proven date palm clones which were obtained from North Africa and the Middle East. Excellent reviews of date palm industry's establishment and history are available (Nixon 1971). Prior to 1964, the date industry was highly labor-intensive, such as that of other date producing countries in the world. An integration of mechanical and hand operations has occurred in the U.S. industry based on economic necessity (Wright 1975). The termination of the Mexican National Program in 1964, and with it cheap agricultural labor, has resulted in the introduction of successful new mechanical methods to pollinate, dust, and harvest dates. These techniques were developed by USDA and University of California agricultural engineers (Perkins and Brown 1964). Mechanical harvesting has been successful because the cultivar "Deglet Noor" comprising 85% of the date trees in the Coachella Valley is amenable to this method. Deglet Noor is a naturally semi-dried date and can be allowed to ripen on

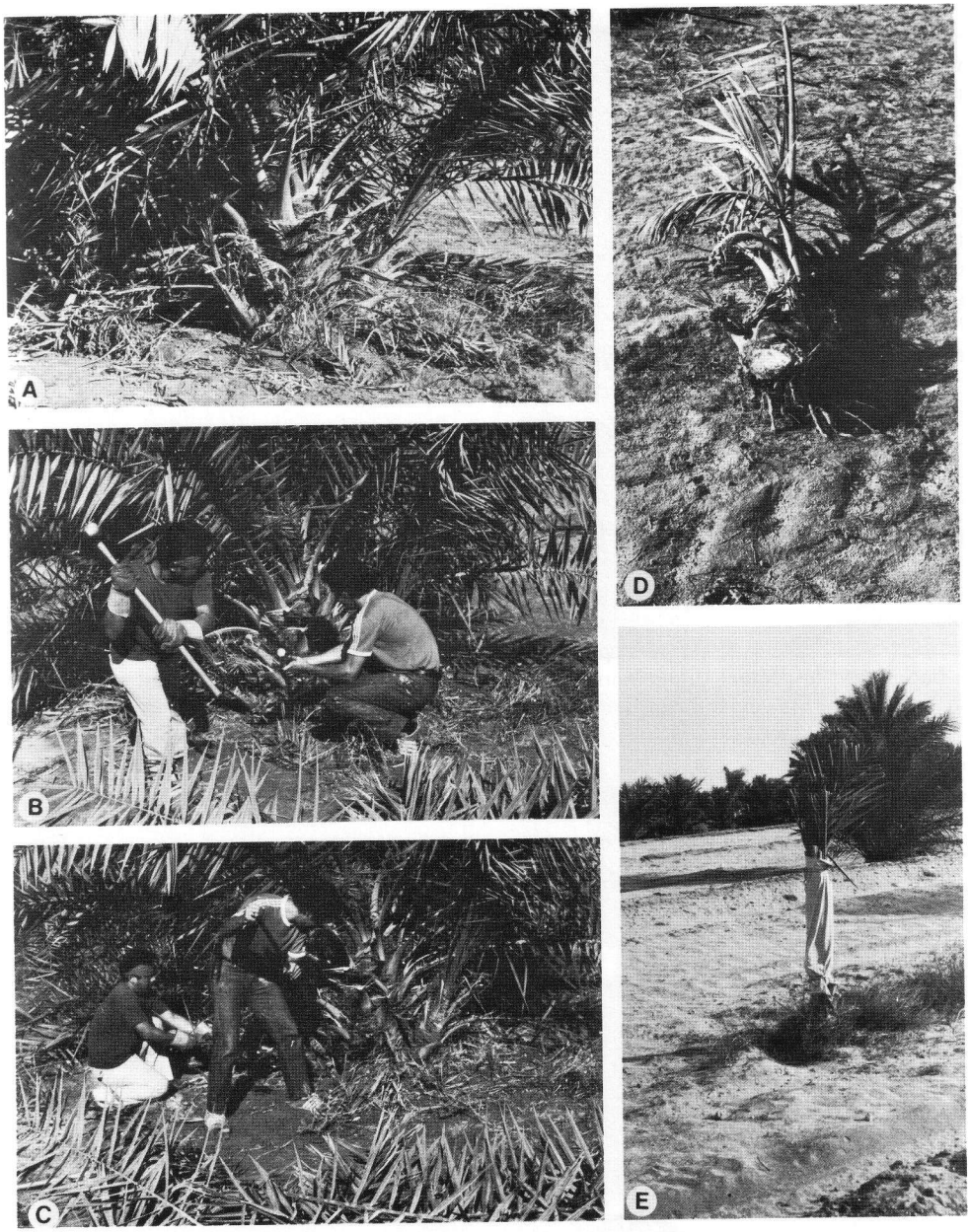


1. Date palm (variety "Deglet Noor") commercial garden located in Indio, California, with ripe fruit bunches. Approximately 300 pounds of fruit can be harvested from each of these 30 year-old trees.

the bunch. The whole bunch may be removed, eliminating individual hand picking. Picked whole fruit bunches may be harvested and processed in a uniform manner (Huxsoll and Reznik 1969). Naturally soft dates such as the Medjools and Barhees are not amenable to mechanical harvesting and must be hand picked and processed. Successful mechanical pollination of trees using a ground applicator has been achieved (Perkins and Burkner 1973). These applicators can also be employed in dusting operations. However, some growers prefer both hand pollination and harvesting. Mechanical pollination, probably accounts for only 20–25% of the crop fruit set. Other attempts to devise methods to mechanize further cultural practices such as pruning, bagging and fruit bunch tie-down have not been adopted because they are economically not feasible. The date industry has drastically reduced the labor force necessary to perform its cultural practices to only 20–25% of its former level since the introduction of these techniques (Wright 1975). Any substantial increase in soft date acreage is not probable because of increased labor demands that would result in their maintenance. New improved cultivars must compare favorably with Deglet Noor in order to be planted and no foreseeable replacement is apparent (Carpenter 1979).

Methods of Date Palm Propagation

The key to the success and viability of the California date palm industry has been the large uniform quality fruit production, usually 12,000 to 14,000 pounds of fruit are produced per acre. A single Deglet Noor tree may yield 200 to 300 pounds of fruit a year. Such uniform yields have only been possible because of the foresight of the original date growers to procure and propagate desirable clonal varieties by offshoots (Nixon 1971). Offshoots are lateral buds located at the base of the trunk above the point of leaf attachment. These buds grow out producing a tree which is an exact copy of the parent. Only a limited number of offshoots are produced by vegetative buds during the juvenile life cycle of the palm, the first five to seven years of growth. Thereafter, bud development is usually devoted to the generation of fruit bunches (Fig. 1). Occasionally, high offshoots will develop in the fruit producing region of the tree. Production of high offshoots is infrequent and unpredictable and the reason for their origin is not known. Less than a dozen offshoots are usually produced during the life of a palm, and the number may vary considerably among different varieties. Commercially acceptable date palms cannot be propagated from seed. Half the progeny will be male and half will be female. The



2. Field techniques to propagate the date palm vegetatively. Newly initiated leaves are growing out from crown. (a) Example of 7 year-old date palm bearing offshoots. (b) Positioning chisel to sever offshoot from parent tree. (c) Detachment of roots and fiber to obtain offshoot. (d) Freshly cut offshoot with preformed adventitious roots. (e) Offshoot wrapped with burlap bag planted in field after 1 year.

seedling female palms usually produce fruit which is commercially inferior in quality to the clonal parent.

Offshoots should have roots initiated prior to the time of their detachment from the parent tree to ensure survival when planted. Roots are produced naturally from the base of the offshoots when they are in contact with soil. Offshoots slightly above ground level can be induced to form roots by mounding soil around their bases; in the case of higher offshoots air layering will suffice. Prior to the time of detachment from the parent trees offshoots are severely pruned (Fig. 2a). The offshoot is removed from the tree in a procedure involving two men (Fig. 2b, 2c). One man positions a large chisel between the offshoot and the parent tree while the second man drives the chisel with a sledge hammer to sever the vascular connection. The crown portion of the shoot is wrapped with a burlap bag to protect the growing plant against excessive cold and heat during the early years of growth. The transplanted offshoot will produce its own foliage, offshoots and fruit bunches in three to five years (Fig. 2d, 2e). Generally, 40 palm offshoots are planted in one acre and are spaced about 20 feet from each other.

World Demand for Offshoots

Adult fruit bearing trees are denuded of offshoots to facilitate easier cultural handling and increased fruit yields per tree (Mitchell 1973). Thereafter, adult date palms seldom, if ever, produce more offshoots. Available offshoots are either employed in new local plantings or are sold to foreign buyers for planting overseas. For example, Israel has developed a vigorous date industry patterned after that of the U.S., employing imported offshoots. Offshoots from the Coachella valley are a world-wide source of commercially desirable disease-free trees. The prices of an offshoot may vary from $25 to $50 each, depending on the size and type of cultivar.

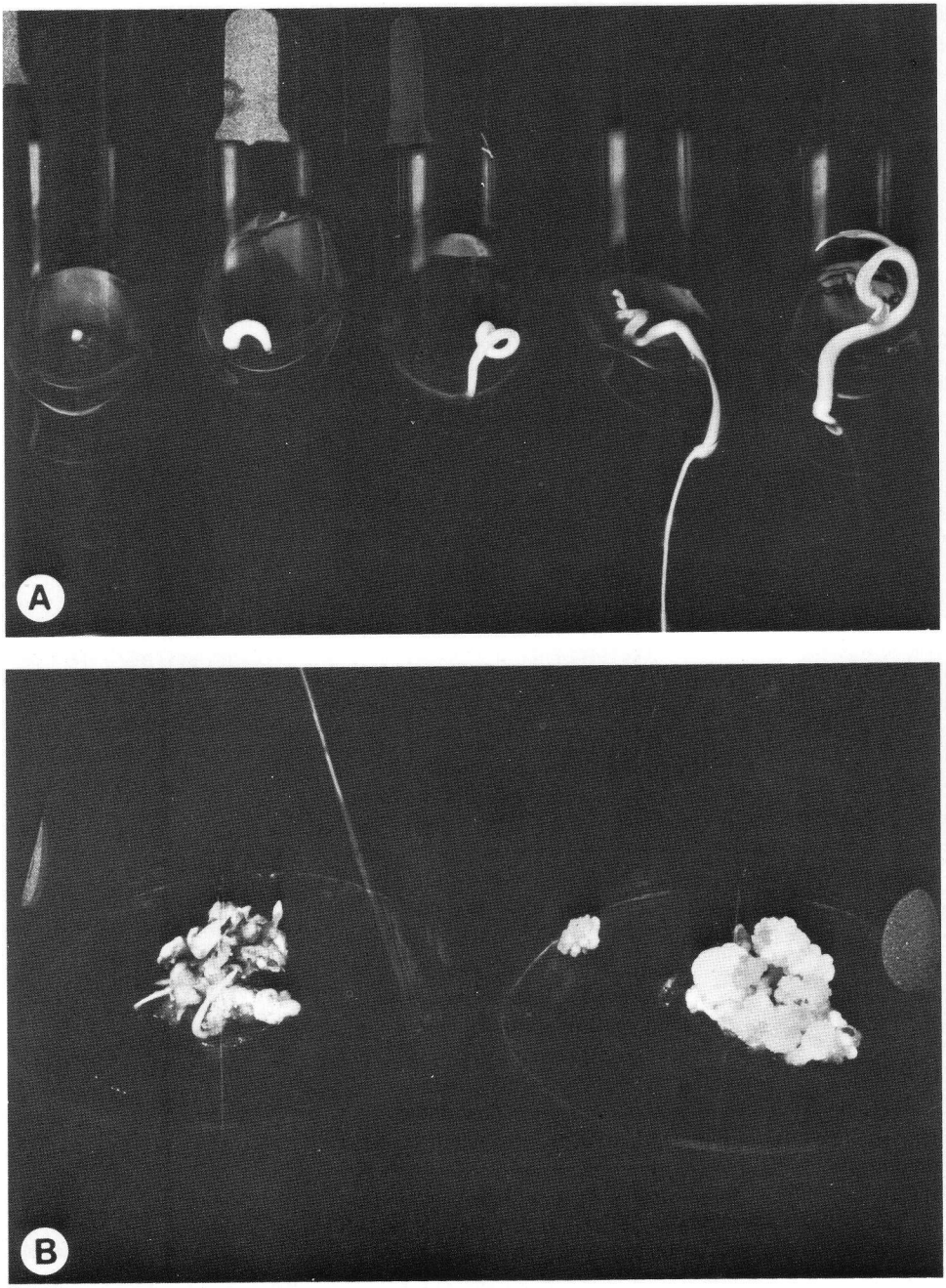
The existing method of vegetative propagation of date palms is reliable but slow. Should the California industry attempt to expand date plantings using proven clonal varieties, only 100–200 acres with 40 date palms per acre could be planted each year. Presently, about 4,000 to 6,000 offshoots are produced annually in the Coachella valley (Mitchell 1973). It would require decades to replace the existing date acreage in the Coachella valley with a superior variety.

In Morocco and Algeria, the bayoud disease (causal agent—*Fusarium oxsporum* Schlect. var. *albedinis*) has devastated the date industries destroying 10 million palms since the turn of the century (Carpenter 1973). It would require several decades perhaps centuries to repopulate devastated areas with resistant cultivars propagated clonally using conventional methods. Further, modernization of the Old World date industry similar to that of the U.S. could not readily be applied since many presently employed date varieties produce fruit which are not amenable to mechanical harvesting and processing. Deglet Noor and other commercially comparable cultivars are not available on a large scale.

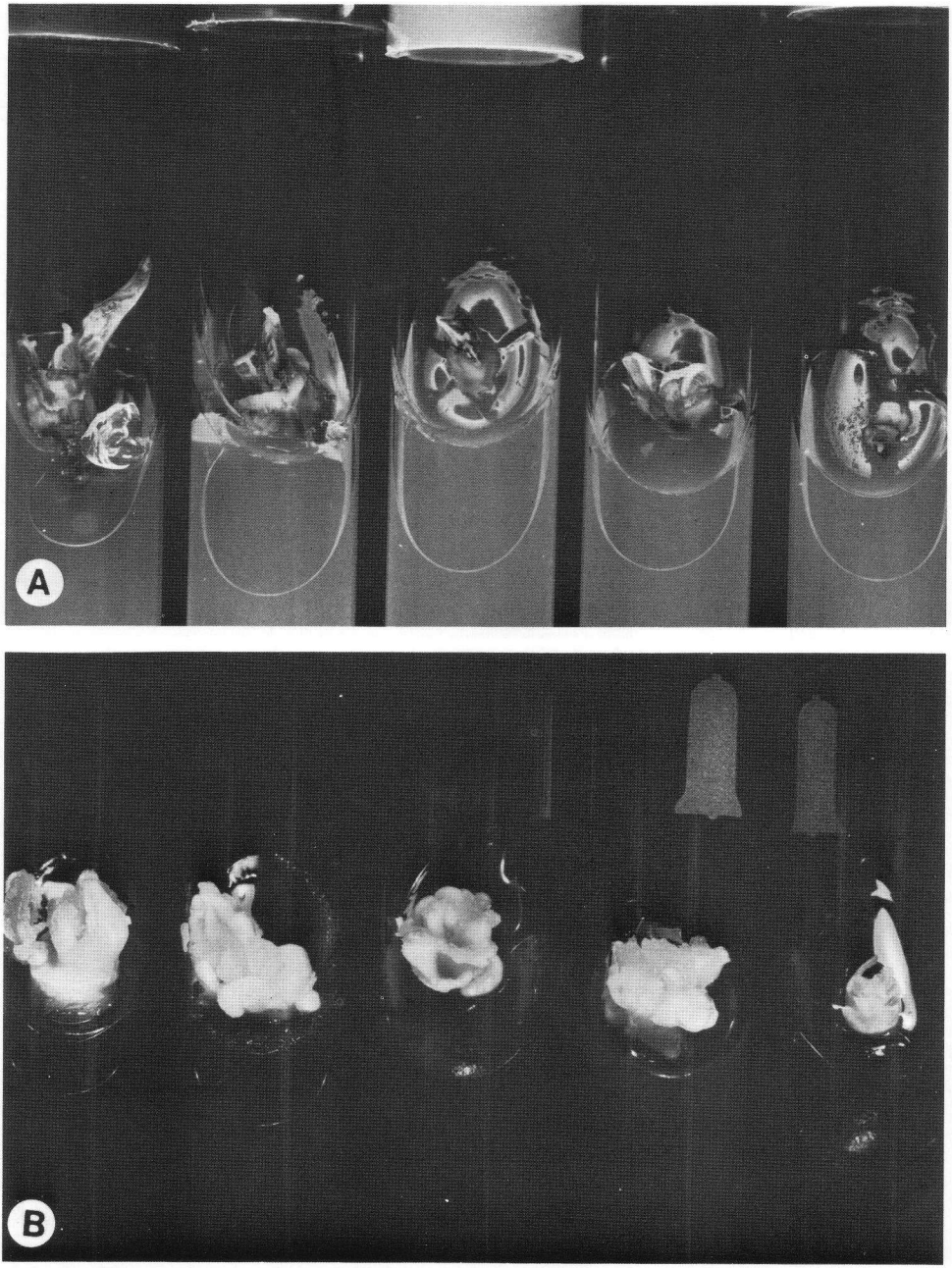
Tissue Culture as a New Means to Produce Clones

Tissue culture micropropagation is a term used to describe the cloning of plants under artificial sterile conditions. Tissue culture cloning is often economically more feasible and faster than using existing vegetative propagation methodology to increase rapidly large numbers of a desired clone. Since the early 1960's, several plant species have been commercially propagated using tissue culture techniques including orchids, ferns and an ever increasing multitude of herbaceous ornamentals (De Fossard 1976, Murashige 1976, Reinert and Bajaj 1977). However, propagation techniques for woody species are notably less developed.

Interest in the development of suitable



3. Morphogenetic potential of excised date palm embryos from Halawy fruit cultured *in vitro*. (a) Germination sequence, from left to right: freshly excised embryo, elongation of cotyledon after 2 weeks in culture, continued cotyledon elongation after 3 to 4 weeks in culture, germination of the primary root after 4–6 weeks in culture and emergence of the first foliar leaf, after 4–6 weeks in culture. (b) Totipotency of embryos cultured on nutrient medium containing 100 mg/l 2,4-dichlorophenoxyacetic acid. Left: callus producing organized structures after 4 months in culture. Right: nodular callus devoid of any organogenesis.



4. Effect of charcoal on date palm lateral bud explants. (a) Lateral buds cultured on nutrient medium devoid of charcoal. (b) Lateral buds cultured on nutrient medium containing 0.3% activated charcoal. Note that buds cultured on medium without charcoal are exhibiting growth inhibition compared to buds cultured on medium containing charcoal.

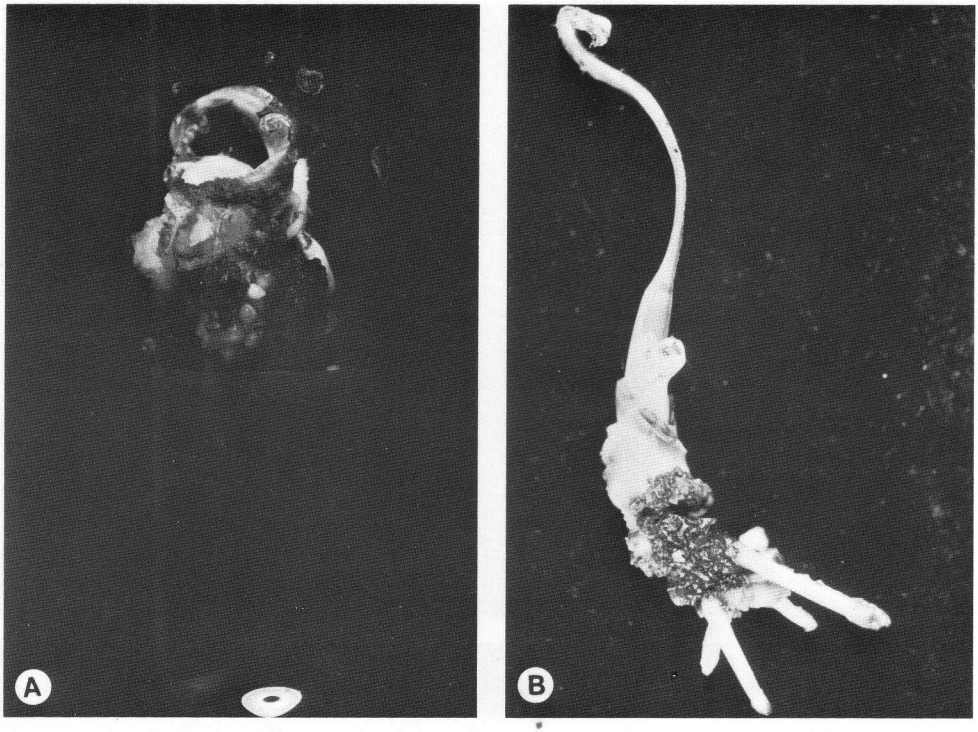
procedures to clone palms *in vitro* began in the 1960's and early 1970's (De Guzman and Del Rosario 1964, Rabéchault, Ahée and Guénin 1970, Schroeder 1970). Embryo culture, a type of tissue culture procedure in which the embryo is excised from the seed and grown separately, may have several potential applications in palm research and breeding studies. Rare palm hybrid embryos such as that of the "Makapuno" coconut cultivar which normally does not germinate in nature may be propagated *in vitro* (Balaga and De Guzman 1970, De Guzman and Del Rosario 1964, Ventura, Zuniga, Figueroa and Lazo 1966). Also, Hodel (1971) suggested that embryo culture could aid in the germination of other incompatible interspecific and intergeneric palm hybrids or slowly germinating palm seeds (Fig. 3a). Host-pathogen relationships such as that for "Lethal Yellowing" disease common in coconut may be studied through the inoculation of growing embryos in sterile culture (Fisher and Tsai 1979). Further, embryo explants may also serve as a tissue source to rapidly multiply palms *via* callus (Fig. 3b). Embryogenetic callus that has given rise to plantlets or miniature tissue cultured plants has been obtained from excised embryos of several palm species such as coconut (De Guzman, Del Rosario and Ubalde 1974), oil (Corley, Barrett and Jones 1976, Rabéchault, Ahée, and Guénin 1970) and date palm (Ammar and Benbadis 1977, Reynolds and Murashige 1979; Tisserat 1979) (Fig. 3b). Unfortunately, embryo culture is not useful for multiplication of desired clones due to the highly heterozygous nature of the embryo.

Clonal propagation using tissue culture must involve the culture of somatic tissues or organs from the vegetative and/or reproductive structures of the clone. Two striking factors regulate the growth of palm tissue and organ explants *in vitro*: 1) the omnipresent occurrence of explant and medium browning, and 2) the inherent explant totipotentiality, or its ability to regenerate an entire plant from cultured cells.

Browning is a wound response resulting when the explant is cut or damaged. Browning substances released by such injuries may be inhibitory or lethal to the development of the cultured tissue. Several early investigators noted this phenomenon and attributed their negative results to its occurrence (Reuveni and Lilien-Kipnis 1974, Schroeder 1970). The browning phenomenon was overcome by addition of adsorbants to the nutrient medium (Fisher and Tsai 1979, Poulain, Rhiss and Beauchesne 1979, Reynolds and Murashige 1979, Tisserat 1979). Applications of activated charcoal (0.1 to 1%) has been found to reduce browning for palm embryos (Fisher and Tsai 1979, Tisserat 1979) and other vegetative tissue sources (Reuveni and Lilien-Kipnis 1974, Tisserat 1979, Wang and Huang 1976) (Fig. 4). Likewise, addition of polyvinylpyrrolidone (200 mg/l) to the nutrient medium has been found to retard successfully browning in date palm shoot tip cultures (Poulain, Rhiss and Beauchesne 1979).

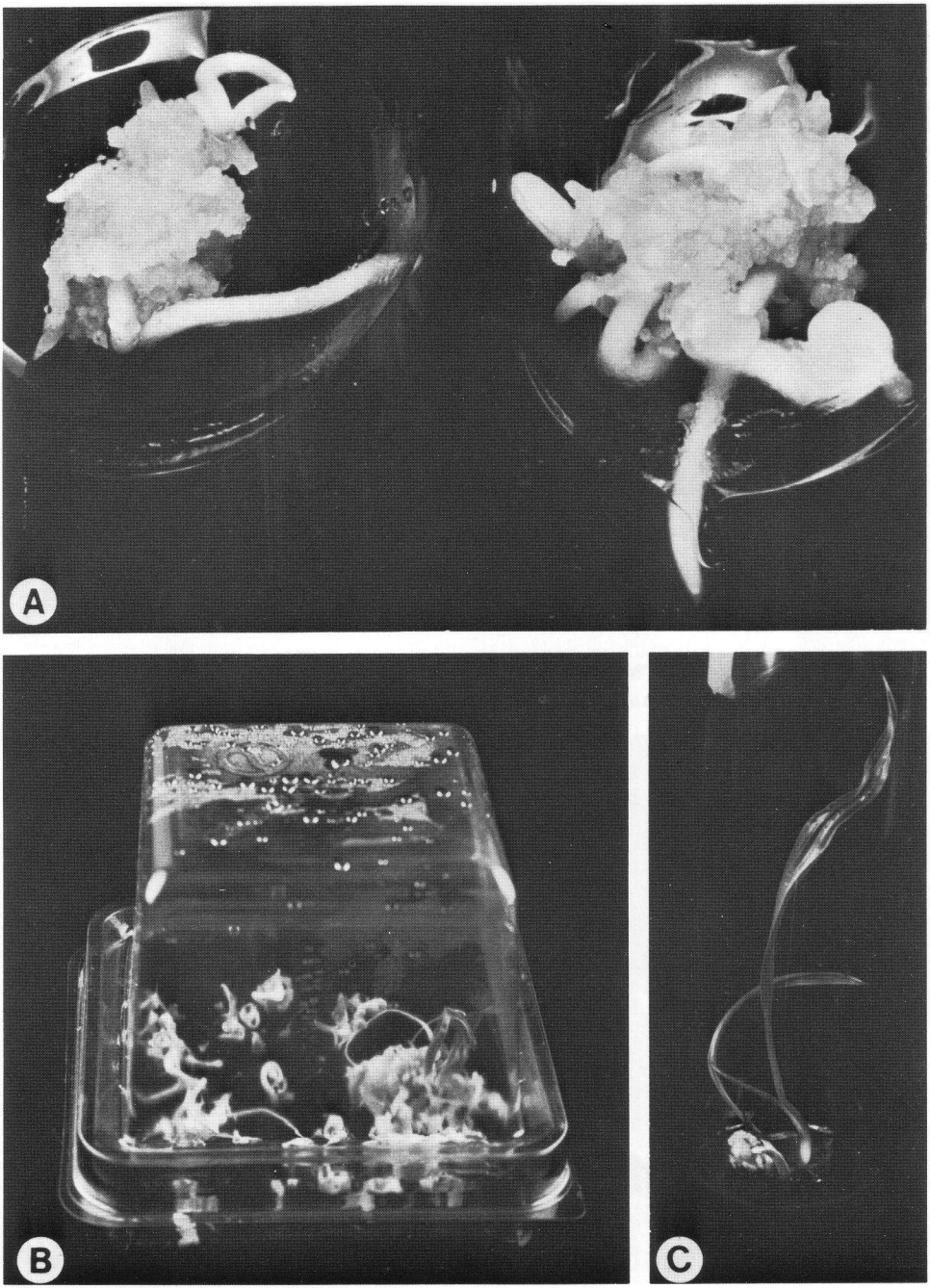
Plantlets have been derived from the rooting of cultured shoot tips in oil palm (Staritsky 1970) and date palm (Poulain, Rhiss and Beauchesne 1979, Reuveni and Lilien-Kipnis 1974, Tisserat 1979) (Fig. 5). Rooted shoot tips will only provide one plant per explant; while an almost unlimited number of plantlets are available from embryogenetic callus.

The best type of somatic explant source to produce a callus capable of giving rise to plantlets is from meristematic regions of the plant. Mature leaves, fruits, inflorescences, stems, roots and other specialized plant organs and tissues will have the least chance to grow *in vitro* because they are composed mostly of cells which are highly differentiated and usually incapable



5. Examples of rooted date palm shoot tips. (a) Early initiation of adventitious root primorida at the base of the tip after 4 months in culture. (b) Shoot tip with several elongated adventitious roots after 6 months in culture.

of undergoing further cell divisions. In contrast, prolific embryogenetic callus has been obtained from date palm shoot tips (Tisserat 1979), lateral buds (Tisserat 1979, Tisserat and DeMason 1980) and immature inflorescences (Reynolds and Murashige 1979) (Fig. 6). In date palm, explants were cultured on nutrient medium containing Murashige and Skoog salts, 3% sucrose, 100 mg/l *i*-inositol, 0.4 mg/l thiamine·HCl, 3 mg/l N^6-(Δ^2-isopentyl) adenine, 100 mg/l 2,4-dichlorophenoxyacetic acid, 0.3% neutralized activiated charcoal and 0.8% Phytagar. After several transfers at 8 week intervals the explants enlarge and eventually produce a white nodular callus. This callus may be subdivided and proliferated almost indefinitely. The author has had several callus clones of date palm in culture for up to 3 years and no diminished embryogenetic capacity has been observed. Histological sections of this callus reveal that it consists of microscopic proembryonic bodies and meristematic centers (Tisserat and DeMason 1980). Transfer of callus to a nutrient medium devoid of hormones allows visible asexual embryos and plantlets to develop (Fig. 6). Palm plantlets produced from callus arise from a somatic embryo that germinates through a sequence of events called asexual embryogenesis. The process of asexual embryogenesis is analogous to the germination of the normal zygotic embryo within the seed (Corley, Barrett and Jones 1976; Tisserat and DeMason 1980). These plantlets may be transferred to soil where they assume a growth pattern comparable to that of a seedling (Figs. 6, 7, 8). Assuming that no



6. Examples of embryogenetic date palm callus derived from lateral buds. (a) Callus cultures producing several asexual embryos cultured on nutrient medium without hormones. (b) Plantlets, asexual embryos and callus produced from "Medjool" cultivar callus plated on agar medium within a PlantCon. (c) Isolated plantlet derived from callus.

7. Examples of date palm plantlets derived from callus in high humidity transition containers. These plantlets have been in soil for $\frac{4}{6}$ months.

8. Examples of free-living tissue cultured date palms varying in age from 1 to 2 years. These plantlets may now be transferred into the field.

9. Cryopreservation of date palm clones through micropropagation.

genetic change has occurred during the differentiation process, these plants will produce uniform clonal trees that are copies of the parent tree. A single individual date palm tree or offshoot may theoretically be developed into a million or more genetically uniform individuals. A note of caution should be mentioned regarding this clonal process; aberrant plantlets derived from plant tissue cultures are not uncommon (Reinert and Bajaj, 1973).

Cloning through plant tissue culture may be coupled with cryogenic storage in order to accumulate and preserve a clonal palm repository (Tisserat, Ulrich and Finkle 1981). The National Date Palm Germplasm Repository was established in 1977 at the U.S. Date and Citrus Station, Indio, California. Numerous living trees of Old World and New World varieties are retained in this collection which could serve as a source of future date palm breeding material. Maintenance of such living germplasm collections is expensive and involves large spatial requirements. Cryobiology may be implemented as an alternative method to store crop germplasm (Fig. 9). Date palm calli may be frozen to the temperature of liquid nitrogen (−196° C) and kept indefinitely without genetic change. Thawed calli readily produce plantlets (Tisserat, Ulrich and Finkle, 1981). Such a procedure could serve to preserve endangered fruit tree clones with a minimum amount of expense, maintenance and space.

Two methods exist to produce plantlets using tissue culture techniques: 1) asexual embryogenesis in which somatic embryos germinate, and 2) organogenesis, the sequential production of roots from shoots

or shoots from roots. Date palm plantlets may be derived using either process.

Future research should now be directed at developing a quality-control test to determine the clonal purity of tissue cultured plantlets. Vegetative and fruiting characteristics of clonal plantlets should be evaluated and compared with the parent. Electrophoretic tests involving protein, isoenzyme and nucleic acid banding patterns could be developed as a preliminary test to eliminate any potentially aberrant tissue cultured palms from being cultivated further.

Refinement of the plantlet production process is necessary in order to make this technique commercially feasible. Understanding and control of the mechanism to mass produce axillary shoots from cultured tips and buds, in date palms at least, would alleviate such concern regarding the clonal nature of these plantlets. Many palms do not exhibit any natural method of vegetative propagation such as production of offshoots (e.g., African oil palm, betel palm, and coconut palm). Plantlets derived from callus would appear to be the only viable method to clone these palms.

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