Application of Artificial Seed to Tropical Crops

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PRODUCTION OF ARTIFICIAL SEEDS

Artificial seeds, consisting of somatic embryos enclosed in a protective coating, have been proposed as a low-cost, high-volume propagation system (Redenbaugh et al., 1986). The inherent advantages of artificial seeds are the production of many somatic embryos and the use of conventional seed-handling techniques for embryo delivery. The objective is to produce clonal "seeds" at a cost comparable to true seeds. Current vegetative propagation practice is limited to crops that have a high economic value for individual plants, but would have much wider application if the costs were reduced considerably. Research on artificial seeds has increased significantly, as demonstrated by increased attention from several laboratories, (Kim and Janick, 1987; Obendorf and Slawinska, 1986; Slawinska and Obendorf, 1987; Redenbaugh et al., 1988) and by a special symposium at the 22nd International Horticultural Congress held in 1986 (Gray, 1987a).

Two types of artificial seeds have been developed, hydrated, and desiccated. Hydrated artificial seeds consist of somatic embryos individually encapsulated in a hydrogel, such as calcium alginate (Fig. 1). Embryos are mixed with sodium alginate and dropped into a calcium salt solution to form calcium alginate capsules (Redenbaugh et al., 1986). The capsules are washed in water and then planted. Conversion frequencies, i.e., percent somatic embryos that form complete plants, of alfalfa artificial seeds were 60% to 90% in vitro (Table 1) and 2% to 10% when planted directly in the greenhouse (Redenbaugh et al., 1987a).

Desiccated artificial seeds were produced by coating a mixture of carrot somatic embryos, roots, and callus in polyoxyethylene glycol. The coated mixture was allowed to dry for several hours on a Teflon surface in a sterile hood. The dried mixture was then placed on culture medium in vitro, allowed to rehydrate, and then scored for embryo survival. "Embryo survival" was not defined. Only coated embryos survived desiccation. Further results on plant recovery were not reported (Kitto and Janick, 1985a, 1985b).

Table 1. Progress in alfalfa somatic embryo conversion (Redenbaugh et al., 1987a).

Year	Percent in vitro conversion ^a of randomly selected ^b somatic embryos
1982	<0.5
1983	8.0
1984	30.0
1985	40-50
1986	50-70
1987	60-90

^aConversion frequency is defined as the percentage of somatic embryos forming complete plants (Redenbaugh et al., 1987a).

^bAll embryos >1 mm in diameter.

At present, neither embryo encapsulation method is completely satisfactory. Hydrated capsules are more difficult to store because of the requirement for embryo respiration. A second problem is that capsules dry out quickly unless kept in a humid environment or coated with a hydrophobic membrane (Redenbaugh et al., 1987b). For both encapsulation methods, an appropriate artificial endosperm has not been developed. For albuminous species, exogenous carbohydrates and nutrients will probably be required (Redenbaugh et al., 1987b). Desiccated artificial seeds have other problems that need resolution. The desiccation process itself damages the embryos. One exception to this was reported by Gray (1987b), who found that desiccation appeared to release grape so-

matic embryos from a quiescent state and increase conversion (20% to 28% for desiccated embryos stored 7 to 21 days and 5% for non-desiccated embryos). However, no coating method was used. Gray (1987b) also observed that embryo conversion decreased rapidly with storage.

A more limiting problem than the type of coating for artificial seeds is the difficulty in producing high-quality, vigorous somatic embryos. For commercial applications to be developed, somatic embryos must germinate rapidly and develop into plants at rates and frequencies similar to true seed. Until recently, most reports on somatic embryogenesis focused only on the production of embryos and recovery of a few plants. Research emphasis is shifting toward embryogeny and improving embryo quality (Carman et al., 1987; Gray, 1987b; Obendorf and Slawinska, 1986; Redenbaugh et al., 1986; Redenbaugh et al., 1987a, 1987b; Redenbaugh et al., 1988) as applications for using somatic embryos for vegetative propagation are identified. Considerable progress has been made with alfalfa somatic embryogeny. Embryogenesis can be highly controlled and uniform; high-quality embryos can be produced routinely from several genotypes (Brown and Atanassov, 1987; Strickland et al., 1987). Research on conversion of embryos to plants has progressed to where 60% to 90% of random-picked (diameter > 1 mm) embryos will form plants (Table 1). It is anticipated that improvements in embryo qual-

Fig. 1. Alfalfa somatic embryos encapsulated in calcium alginate beads. Embryos are ≈4 mm long.

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ity will alleviate many of the problems related to encapsulation and desiccation.

STATUS OF SOMATIC EMBRYOGENESIS IN TROPICAL CROPS

Tropical horticultural crops consist of a broad range of species, including beverage and drug plants (cacao, coffee, opium), cereals (millets, rice, sorghum), fibers (cotton, hemp, jute), fruits (date palm, mango, papaya), grain and forage legumes (alfalfa, soybean, winged bean), oil and fat producers (coconut, safflower, soybean), ornamental plants (cymbidium, orchids, petunia), root and tuber crops (cassava, sweetpotato, yam), spices (coriander, ginseng, vanilla), rubber, sugarcane, trees (mahogany, sandalwood, teak), and vegetables (bean, cucumber, tomato). Many of the major temperate crops have sources of origin in the tropics and, although still grown in the tropics, have a much greater economic value in the temperate zone. The number of tropical plant species ranges from 112,000 in the western hemisphere to 50,000 in Asia and 40,000 in Africa (Graf, 1974).

Somatic embryogenesis has been achieved in a wide range of tropical crops (Table 2). However, in most cases, either none or just a few plants were produced. In some reports, such as for sorghum, conversion frequencies were given as number of plants per callus culture. For most artificial seed applications, the percentage of embryos forming plants will be required for a production system. Although not all crops will need to have singulated artificial seeds, an expectation of performance from any given encapsulated unit will be necessary.

To date, little somatic embryogenesis research has been conducted with ornamental crops. Because of their relatively high per-unit value and requirement for clonal fidelity, ornamentals have been propagated using tissue culture methods via an axillary shoot proliferation. Stimart (1986) listed 90 floral crops propagated using tissue culture methods, but only one (*Asclepias tuberosa*) was reported to use "embryoids from callus" as a multiplication method. Zimmerman (1985) discussed somatic embryogenesis in woody ornamentals and concluded that "[s]omatic embryogenesis will be little used for woody plants important in horticulture for some time," because of lack of basic research and the necessity for years of field testing to ensure clonal fidelity. Although somatic embryos can be produced from another important crop, *Elaeis guineensis* (oil palm), the embryos are not used directly for plant regeneration. Rather, somatic embryos are placed on a culture medium to induce shoot formation, followed by transfer of the shoots to another medium for root development. After plants are produced, they are transplanted into greenhouses (Corley, 1982).

In general, plants were produced from somatic embryos for many species, but the embryogenesis systems were not optimized for plant production and conversion frequencies were not reported. A systems optimization

approach will be critical for commercial development of any crop. Several authors did report conversion frequencies, but failed to indicate sample size and whether the frequency was typical or due to selection of only visually high-quality embryos. For commercial operations, embryo selection may be restricted to size classes that can be sorted using methods such as screens or density gradients.

As somatic embryogenesis and conversion performance for many crops improve, species-specific characteristics may be critical for development of artificial seeds. One example is the potential requirement of an artificial endosperm for albuminous plants. For some species, such as orchardgrass, a starch-rich artificial endosperm that mimics the natural one may be essential, while, for others, such as grape, a lipid endosperm may be needed (Gray 1987b).

Other important criteria relate to the type of artificial seeds that can be produced. Seeds of some tropical crops are recalcitrant (unorthodox) in that they have short viability and must be stored at relatively high moisture content to maintain viability (Table 3). Desiccation below 20% to 40% (fresh weight) or temperatures below 0 to 10C generally damage recalcitrant species (Bewley and Black, 1985). One of the greatest problems with recalcitrant crops is the difficulty in storing seeds and maintaining a broad genetic base. For artificial seed production, a hydrogel encapsulation system may be required for recalcitrant crops because of this sensitivity to desiccation.

Seeds of other tropical fruits, such as *Avicennia caribaea* (starfruit), *Carica papaya* (papaya), *Psidium guajava* (guava), and *Passiflora edulis* (passion fruit), show a high degree of tolerance to desiccation (Teng and Hor, 1977). For these and other orthodox seed crops, a desiccated system may have specific advantages, particularly if desiccation improved embryo maturation. However, our understanding and control of somatic embryogenesis are insufficient at this time to determine the optimum encapsulation system for any specific crop.

COFFEE ARTIFICIAL SEEDS

Although plant tissue culture methods have been used to propagate tropical crops, such as banana and oil palm, for large-scale field plantations, such propagules are relatively expensive (Zimmerman 1986). Costs of clonal propagation must be lowered for widespread commercialization. As discussed previously, artificial seeds have the potential of providing vegetative propagation at a cost structure similar to seeds.

Coffee (*Coffea* spp.) is produced in more than 50 countries, with Brazil being the largest grower. More than 60 million bags of coffee are produced yearly at a value of \$12 billion. Although there are many coffee species, only two, *C. arabica* and *C. canephora*, are of major importance (Marshall, 1975).

Coffee propagation is generally through seeds, with seedlings grown in a shaded nur-

sery for 6 to 24 months before transplanting to the field (Cobley, 1976). *Coffea arabica* is primarily a self-pollinating species, and, consequently, its progeny have a high degree of uniformity. Other species, such as *C. canephora*, *C. excelsa*, and *C. liberica*, are usually self-sterile and must be cross-pollinated, thereby resulting in progeny with greater variation. Coffee has been reported as a recalcitrant species without a dormancy phase. Seeds are damaged when the moisture content drops to 8% to 35% and temperature is <10C. At ambient temperatures, coffee seeds can be stored between 2 and 6 months, depending on species. Increased shelf life, up to 10 months, can be achieved by storing seeds at 25C with 92% to 98% RH (Bewley and Black, 1985). Extended viability up to 30 months has been achieved with some species by storing the seeds wet at 40% moisture content in sealed bags at 15 to 19C (Van der Vossen, 1975). More recently, Ellis (unpublished data) indicated that coffee was not a recalcitrant species. Rather, seed drying techniques for coffee had not been optimized, thereby causing decline in seed viability. Using appropriate methods, coffee seeds could be desiccated without decreasing subsequent germination frequency. This change in classification of coffee to an orthodox species is indicative of the type of information needed to develop artificial seeds, as will be discussed below.

Vegetative propagation was first used for coffee in 1887, and several methods are in use today, including cuttings, budding, and grafting (Willson, 1975). Van der Vossen (1975) indicated that vegetative propagation has been used mainly for breeding purposes and to preserve germplasm. However, as disease-resistant hybrids are produced and superior genotypes identified, vegetative propagation for large-scale plantings is being considered. At present, hybrid coffee can only be produced vegetatively.

Traditional cloning techniques are limited in that only few plants can be produced because orthotropic shoots must be used. Although tissue culture methods have been recognized for some time as potential propagation tools for coffee, no commercial application has been achieved. Several advantages of tissue culture propagation for coffee are a) juvenility and vigor can be restored, b) a much higher propagation rate is possible, c) disease-indexed plants can be produced, and d) the problem of plagiotrophism that occurs with other vegetative propagation techniques can be overcome.

Somatic embryogenesis was first reported in coffee (*C. canephora*) in 1970 (Staritsky, 1970) and plant formation from *C. arabica* embryos in 1977 (Sondahl and Sharp, 1977a, 1977b). These and other early papers on coffee somatic embryogenesis focused primarily on the production of embryos and did not present information on embryo yields and conversion frequencies. Yasuda et al. (1985) reported production of 186 ± 90 somatic embryos per leaf callus after 8 weeks, but did not quantitate plant production from the embryos. Recently, Garcia and Menendez

Table 2. Somatic embryogenesis in tropical crops.

Species	Common name	Germination ²	Conversion ³	Reference
<i>Ammi majus</i>	Bishop's weed	Yes	Yes	Grewal et al., 1976
<i>Anethum graveolens</i>	Dill		Yes	Ratnamba and Chopra, 1974
<i>Antirrhinum majus</i>	Snapdragon	Yes	Yes	Sangwan and Harada, 1975
<i>Carica candamarcensis</i>	Mountain papaya		Yes	Jordan et al., 1982-83
<i>Carica papaya</i>	Papaya		Yes	Litz and Conover, 1983
<i>Chamaedorea costaricana</i>	Palm	No	No	Reynolds and Murashige, 1979
<i>Citrus aurantifolia</i>	Lime		Yes	Mitra and Chaturvedi, 1972
<i>C. aurantium</i>	Sour orange	No	No	Kochba et al., 1982
<i>C. limon</i>	Lemon		Yes	Moore, 1985
<i>C. medica</i>	Citron	No	No	Tisserat and Murashige, 1977; Tisserat, 1982
<i>C. paradisi</i>	Grapefruit		Yes	Moore, 1985
<i>C. reticulata</i>	Mandarin		Yes	Moore, 1985
<i>C. sinensis</i>	Sweet orange		Yes	Moore, 1985
<i>Cocos nucifera</i>	Coconut	No	No	Gupta et al., 1984; Pannetier and Buffard-Morel, 1982
<i>Coffea arabica</i>	Coffee		Yes	Sondahl and Sharp, 1977b; Yasuda et al., 1985
<i>C. arabica</i> cv. Catimor	Coffee		20% to 27%	Garcia and Menendez, 1987
<i>C. canephora</i>	Coffee		Yes	Staritsky, 1970
<i>Coriandrum sativum</i>	Coriander		Yes	Zee, 1981
<i>Cucumis sativus</i>	Cucumber		Yes	Lazarte and Sasser, 1982
<i>Digitalis lanata</i>	Foxglove	No	No	Diettrich et al., 1986
<i>Dioscoria deltoidea</i>	Yam	No	No	Singh, 1982
<i>Elaeis guineensis</i>	Oil palm	Yes	Yes	Corley, 1982
<i>Eugenia jambos</i>	Rose apple		Yes	Litz, 1984a
<i>E. malaccensis</i>	Malay apple		Yes	Litz, 1984a
<i>Glycine max</i>	Soybean		25% to 66%	Ranch et al., 1986
<i>Gossypium hirsutum</i>	Cotton		Yes	Price and Smith, 1984; Gawal and Robacker, 1987
<i>Howeia forsteriana</i>	Paradise palm	No	No	Reynolds and Murashige, 1979
<i>Ipomoea batatas</i>	Sweetpotato		Yes	Jarret et al., 1984
<i>Mangifera indica</i>	Mango	No	No	Litz, 1982, 1984c
<i>Manihot esculenta</i>	Cassava		25%	Stamp and Henshaw, 1982, 1987
<i>Medicago sativa</i>	Alfalfa		60% to 90%	Redenbaugh et al., 1987b
<i>Musa</i> sp. "ABB"	Plantain	No	No	Cronauer and Krikorian, 1983
<i>Myrciaria cauliflora</i>	Jaboticaba	No	No	Litz, 1984b
<i>Nigella sativa</i>	Fennel flower		38% to 49%	Banerjee and Gupta, 1975
<i>Oryza sativa</i>	Rice	Yes	Yes	Wernicke et al., 1981; Abe and Futsuhara, 1985
<i>Panax ginseng</i>	Ginseng		Yes	Chang and Hsing, 1980
<i>Panicum maximum</i>	Guinea grass		Yes	Lu and Vasil, 1981
<i>P. miliaceum</i>	Proso millet		Yes	Heyser and Nabors, 1982
<i>Papaver somniferum</i>	Opium poppy	Yes	Yes	Nessler, 1982
<i>Pennisetum purpureum</i>	Napier grass		Yes	Wang and Vasil, 1982
<i>Phaseolus vulgaris</i>	Bean	No	No	Martins and Sondahl, 1984
<i>Phoenix dactylifera</i>	Date palm		Yes	Reynolds and Murashige, 1979; Tisserat, 1982
<i>Saccharum officinarum</i>	Sugar cane		Yes	Ho and Vasil, 1983
<i>Santalum album</i>	Sandalwood		Yes	Bapat and Rao, 1979
<i>Sapindus trifoliatus</i>	Soapnut		Yes	Desai et al., 1986
<i>Sassafras randaianse</i>	Sassafras	Yes	8%	Chen and Wang, 1985
<i>Solanum melongena</i>	Eggplant		25%	Gledlie et al., 1986
<i>Setaria italica</i>	Millet	Yes	Yes	Xu et al., 1984
<i>Sorghum arundinaceum</i>	Sudan grass		Yes	Boyes and Vasil, 1984
<i>S. bicolor</i>	Sweet sorghum		132 plants/ g of callus	MacKinnon et al., 1986
<i>Thalictrum urbaini</i>	Meadow rue		Yes	Yang and Chang, 1980
<i>Theobroma cacao</i>	Cacao	No	No	Pence et al., 1979
<i>Trifolium repens</i>	White clover		Yes	Maheswaran and Williams, 1984
<i>T. rubens</i>	Clover	Yes	Yes	Parrott and Collins, 1982-83
<i>Urginea indica</i>	Indian squill		2%	Jha and Sen, 1986
<i>Veitchia merrilli</i>	Christmas palm		Yes	Srinivasan et al., 1985
<i>Vicia faba</i>	Horse bean	No	No	Griga et al., 1987
<i>Zea mays</i>	Corn		Yes	Armstrong and Green, 1985; Lu et al., 1982

²Germination frequency is defined as the percentage of somatic embryos that have formed roots, shoots, leaves, or a combination of these.

³Conversion frequency is defined as the percentage of somatic embryos forming complete plants (Redenbaugh et al., 1987a). In some reports, the percentage of callus cultures producing plants was given; however, this is not a useful measurement for artificial seed development and was not recorded. One exception exists for species, such as sorghum, where singulation is unimportant and clumps of embryos are adequate for propagation. For these species, the number of plants per gram of callus is a useful measurement for a production system.

Table 3. Recalcitrant tropical crops.*

Species	Common name
<i>Achras zapota</i>	Sapodilla
<i>Aleurites</i> spp.	Candlenut, tung oil tree
<i>Artocarpus</i> spp.	Breadfruit
<i>Blighia sapida</i>	Akee
<i>Calocarpum sapota</i>	Sapote
<i>Chrysophyllum cainito</i>	Star apple
<i>Citrus</i> spp.	Orange, lemon, grapefruit
<i>Cocos nucifera</i>	Coconut
<i>Cola nitida</i>	Kola nut
<i>Diospyros virginiana</i>	Persimmon
<i>Dovyalis hebecarpa</i>	Kitembilla
<i>Eugenia uniflora</i>	Pitanga
<i>Flacourtia indica</i>	Ramontchi
<i>Garcinia mangostana</i>	Mangosteen
<i>Hevea brasiliensis</i>	Rubber
<i>Litchi chinensis</i>	Litchi
<i>Malpighia</i> spp.	Barbados cherry
<i>Mangifera indica</i>	Mango
<i>Myrciaria cauliflora</i>	Jaboticaba
<i>Nyssa</i> spp.	Tupelo
<i>Nephelium lappaceum</i>	Rambutan
<i>Swietenia</i> spp.	Mahogany
<i>Theobroma cacao</i>	Cacao
<i>Trapa natans</i>	Water chestnut

*Based on Bewley and Black, 1985; Teng and Hor, 1977; Roberts, 1975.

(1987) reported on production of high-quality *C. arabica* somatic embryos that had an in vitro conversion frequency ranging from 20% to 27%.

Somatic embryogeny of coffee is more advanced than that for most tropical crops, based on the availability of repeatable somatic embryogenesis protocols for producing high-quality embryos and on the recently established base-line conversion frequency (20% to 27%). As a consequence, research can now begin on identifying and developing an artificial seed delivery system. High-quality somatic embryos can be produced, coated, and tested in various planting environments. Whether the coating system will use hydrated or desiccated embryos can not be determined at this time. Although the effect of desiccation on zygotic embryo development is not known, non-desiccated seeds are apparently fully matured, as they are used for plant propagation. The change in viewpoint that coffee is not recalcitrant suggests that additional research on zygotic embryogeny may provide other information on how to produce somatic embryos that have the thriftiness and quality of zygotic embryos.

Various steps are needed for commercialization of coffee artificial seeds. The quality and conversion frequency of embryos must be increased to provide a minimum level of performance. The conversion environment must be identified, whether it will be in vitro, incubator, greenhouse, or nursery. The issue of somaclonal variation must also be resolved. One potential method to minimize variation is to induce somatic embryos directly from explants, such as leaves (Dublin, 1981). Once initial embryos are produced, then an embryo-off-embryo propagation system may be appropriate for scale-up (Michaux-Ferrière et al., 1987). However, if variation is controllable, a callus-to-embryo

system will be more desirable because of greater scale-up potential. Finally, field testing will be required to determine the efficacy of the propagation system and to calculate the total costs and benefits of coffee artificial seeds. Perhaps the greatest advantage of coffee artificial seeds over traditional clonal propagation will be the cost per propagule. Although estimates as low as \$0.00026 per unit have been presented for alfalfa (Redenbaugh et al., 1987b), costs that are 10 or 100 times greater will remain attractive for coffee.

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