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Somatic embryogenesis for agricultural improvement†

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Many important food and fibre crops have attained close to their maximum yields as a result of conventional breeding approaches and advances in agronomic and horticultural practices. The manipulation of cell and tissue cultures to produce somatic embryos efficiently is one of the keystones of the new technologies that will greatly alter the way crops are planted (as synthetic seed) and genetically altered in the future. Gene transfer into embryogenic plant cells is already challenging conventional plant breeding, and has become an indispensable tool for crop improvement. This review provides a current assessment of the impact of somatic embryogenesis in agriculture.

Key words: Genetic transformation, micropropagation, somaclonal variation, synthetic seed.

The 'green revolution' is considered to have resulted, at least in part, from the application of Mendelian genetics to crop improvement. This has resulted in the maximization of yields of many crops grown under conditions that reduce insect and disease pressure and on soils enriched with inorganic fertilizer. During the 1960s, it was realized that the production gains of the green revolution would be overcome by World population increases within a few decades. Therefore, the development of alternate strategies for increasing plant productivity were considered to be essential. *In vitro* procedures for manipulating plant differentiation, growth and development, including production of haploid plants from cultured anthers, regeneration of plants from cell cultures, and protoplast isolation, culture and fusion, were considered to be integral parts of this new technology. Cell culture coupled with molecular biology for crop improvement has been referred to as the 'genetic engineering revolution'.

One of the most important prerequisites for genetic manipulation of plants *in vitro* has been the ability to grow somatic cells in sterile plant growth medium and to regenerate plants from these cultures. The regeneration pathways of plants from somatic cell cultures have been defined as either organogenesis (Christianson 1987) or somatic embryogenesis (Ammirato 1985, 1987). Theoretically, the regenerants are derived from single, totipotent cells and this has been demonstrated with several species. However, under certain growth conditions (and particularly with organogenesis), morphogenesis can involve more than one cell (Christianson 1987). It is generally considered that somatic embryos are derived either from single cells or from single cells within a proembryonic mass. Somatic embryogenesis, therefore, is a more efficient pathway for studies involving production of genetically transformed plants. Since suspension culture is a highly efficient way to grow large numbers of cells, embryogenic suspension culture has great potential for *in vitro* propagation. The involvement of somatic embryogenesis as a modern tool for increasing agricultural productivity is the subject of this review.

What is Somatic Embryogenesis?

The production of somatic embryos was described independently by Steward (1958) and Reinert (1958), both using carrot callus, and by Stevenson (1956), using *Citrus* ovule cultures. Somatic embryos morphologically resemble zygotic embryos. They are bipolar and bear typical embryonic organs. However, they originate via a different pathway. Rather than developing from a zygote after fusion of the

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gametes, somatic embryos can theoretically be derived from cells within any type of tissue. They not only occur *in vitro* but can also be widely found in nature. For example, many plant species produce polyembryonic seeds, in which the adventitious embryos are produced either apogamously from the nucellus or integumental tissue, as in many types of *Citrus* and other tropical trees (Johri 1984), or in conifers, by cleavage polyembryony from the developing proembryo (Chamberlain 1935). Somatic embryogenesis has been documented as being genetically determined. There are major genotype or cultivar differences for this trait, and it has been shown to be inherited in alfalfa (Reisch & Bingham 1980), clover (MacLean & Nowak 1989), cucumber (Gavin *et al.* 1989) and many other species. The choice of donor tissue is critical, and is usually determined empirically. For many patterns of somatic embryogenesis embryonic or highly juvenile types of tissue have to be used as explants. Juvenile tissues are sometimes the only practical choice when culturing woody plants, in which the transition from juvenile to mature phases is associated with lignification. Haploid embryogenesis of many plant species is also possible from the gametes.

The pattern of development from cultured tissue is epigenetically determined and is influenced by the stage of development of the plant, the nature of the explant, basal medium, auxin etc. The physiological state of the explant can be limiting but can be influenced to some degree by pre-conditioning the stock plants (Christianson 1985). The appropriate explant at the correct developmental stage produces an embryogenic culture on a particular growth medium. The medium, normally based on those of Murashige & Skoog (1962), Schenk & Hildebrandt (1972) or Nitsch & Nitsch (1969) or B5 (Gamborg *et al.* 1968) is optimized using various supplements, including auxins or auxin-like substitutes: 2,4-dichlorophenoxyacetic acid (2,4-D), picloram, dicamba, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), sucrose and a source of either organic or inorganic ammonium. Other classes of plant growth regulators, particularly cytokinins, have sometimes been utilized in conjunction with auxins.

It is believed that induction of the embryogenic pathway of development can occur in one of two ways, by directive or permissive induction (Ammirato 1987; Christianson 1985, 1987). In directive induction, the presence of auxin and optimum culture conditions stimulate rapid cell division to form a callus. A change in cellular morphology associated with an asymmetric cell division, can occur (Kohlenbach 1978) and this results in a change of polarity. A large highly vacuolate cell is formed together with a small, avacuolate sister cell which is embryogenic (Figure 1). There is thus a redetermination of cell type and function (Christianson 1985). Simultaneously, there is a differential change in gene expression that is probably associated with increased demethylation of DNA (LoSchiavo *et al.* 1989).

Allowing the embryogenic potential of induced cells to be expressed is referred to as permissive induction (Christianson 1985; 1987). Certain plant tissues are known to have cells that already possess the characteristics associated with induced cells but the expression of their embryogenic potential is suppressed by the surrounding cells of the tissue. The embryogenic cells in the nucellus during the early stages of ovule development within polyembryonic seeds fall into this category. Following subculture of the nucellus (or embryogenic cells) onto medium that lacks an inductive agent such as 2,4-D, the cells divide and organize as somatic proembryos, freed from the inhibitory influence that occurs *in vivo*.

Embryogenic cultures can often be maintained for long periods by continued subculture in liquid media containing 2,4-D or another auxin. These embryogenic suspension cultures are composed of proembryonic masses (Halperin 1966) (Figure 2). Each proembryonic mass theoretically has the ability to form a single somatic embryo until it reaches a certain size, after which it loses the potential for integrated development (Williams & Maheshwaran 1986). The organization and maturation of singulated somatic embryos are inhibited so long as they are cultured in the presence of 2,4-D or another auxin. Cells of the proembryonic mass divide and continuously differentiate secondary somatic embryos in a repetitive cycle when in an inductive environment. It is possible to synchronize embryogenic suspension cultures to some degree by repeated sieving. Some of the proembryonic masses consist of only a few cells, whereas other masses can be 2 or 3 mm in diameter. The small proembryos can often develop as singulated embryos in medium free of auxin. These suspension cultures are used for many different *in vitro* procedures, including protoplast isolation and culture and *in vitro* selection, and are the basis for bioreactor micropropagation, because they are so highly embryogenic.

Normally, the media used for somatic embryo maturation are supplemented with additional amino acids and complex organic supplements; auxin is often omitted from the medium because of its inhibitory effect on the maturation of many species. Zygotic and somatic embryo development are generally similar, and the embryos pass through recognizable heart, torpedo and mature stages (Figure 3). Precocious or premature development, particularly germination, is a major problem during somatic embryo development, and this is controlled either by increasing the osmolarity of the maturation medium with additional sucrose (Lee & Thomas 1985; Carman 1989) or by incorporating abscisic acid into the medium (Ammirato 1974). At maturity, *in vitro*-grown embryos are significantly larger than zygotic embryos of the same species (Monnier 1978; Gray & Purohit 1991).

Embryos have been categorized according to their ability to withstand desiccation. Orthodox embryos can tolerate

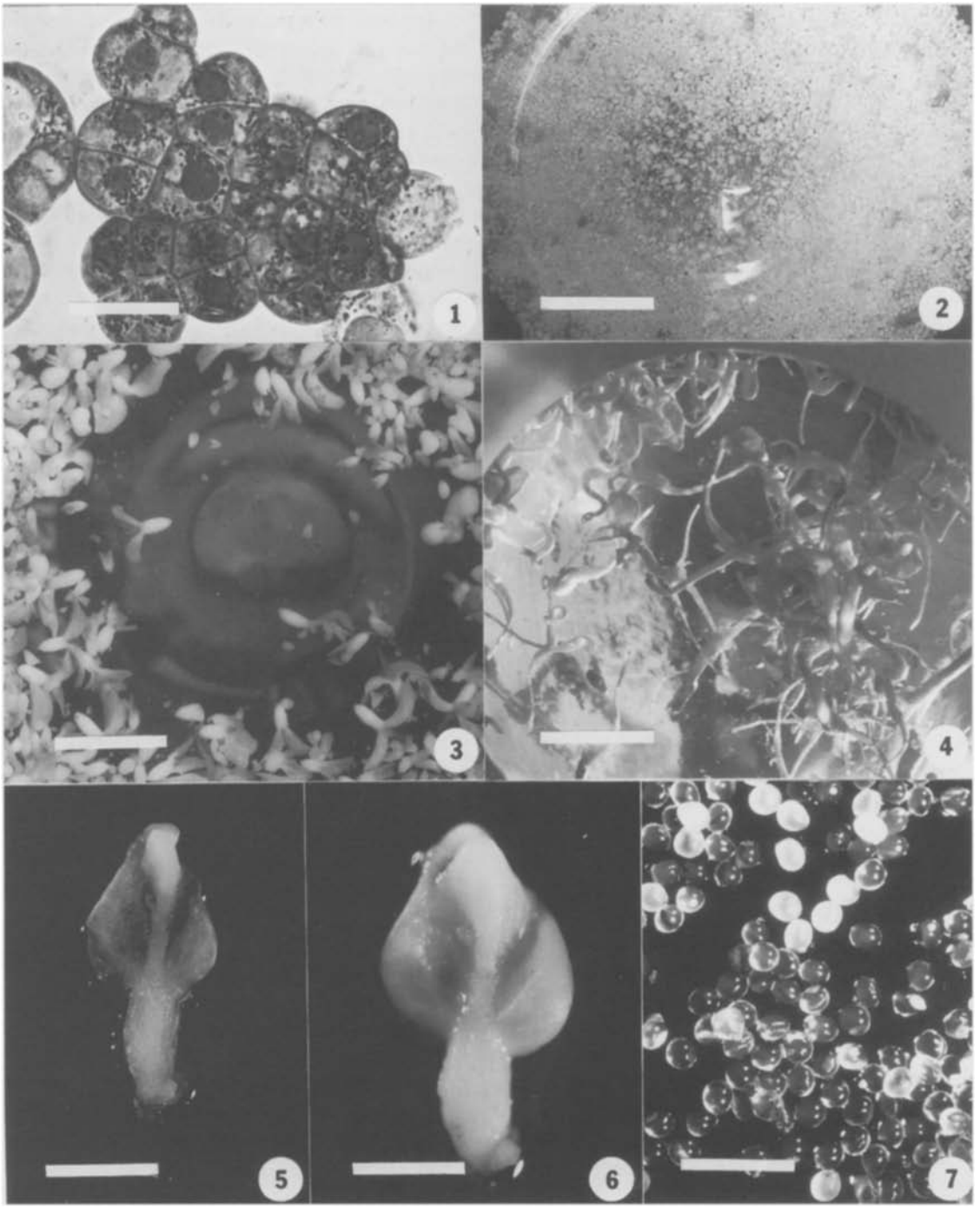

Figure 1. Cross section through a small embryogenic cell cluster of grape (*Vitis vinifera*). Bar = 11.6 μm.

Figure 2. Embryogenic suspension of culture of papaya (*Carica papaya*), consisting of proembryonic masses. Bar = 2.6 cm.

Figure 3. Mature papaya somatic embryos in suspension culture. Bar = 10.0 mm.

Figure 4. Precociously germinating papaya somatic embryos in suspension culture. Bar = 2.8 cm.

Figure 5. Dehydrated grape somatic embryo. Bar = 0.6 mm.

Figure 6. Grape somatic embryo in Figure 5, rehydrated. Bar = 0.6 mm.

Figure 7. Synthetic alfalfa seed: (somatic embryos encapsulated in sodium-alginate hydrogel), kindly supplied by Dr K. Redenbaugh, Calgene, Davis, CA, USA. Bar = 3.0 cm.

desiccation and, in doing so, enter a state of developmental arrest (Bewley & Black 1985). The evolution of quiescent embryos that were enclosed with a nutritive food supply—the endosperm in angiosperms and the megagametophyte in conifers—to form a seed was critical for the spread of higher plants and the survival of mankind. Seeds, which are dispersal structures which also enable short-lived annual plants to survive in a quiescent or dormant state, permitted higher plants to spread into new and sometimes inhospitable habitats at an unprecedented rate. Because of their special attributes, seeds are important items of commerce (Murray 1984). Their small size and quiescence mean that seeds can be stored and handled easily. These same qualities have allowed mankind to transport germplasm of many important crops from their centres of origin to suitable growing areas around the World. Seeds also represent the single most important source of human nutrition, because of their relatively high concentrations of important amino acids, proteins, lipids and polysaccharides. Orthodox seeds of some species can be stored dry for many years.

Recalcitrant embryos are unable to survive desiccation, and do not cease development during maturation. Recalcitrant seeds cannot be stored for more than a few days. Somatic embryos, regardless of whether they are of the recalcitrant or orthodox type, behave as recalcitrant embryos, and germinate when they have apparently reached their final state of development (Litz & Gray 1992) (Figure 4). Since embryo maturation and germination of recalcitrant embryos represent a continuum, without a period of developmental arrest, somatic embryos of the recalcitrant type—normally of large-seeded tropical species—would be expected to produce viable plants.

Somatic embryos of orthodox-type seed do not naturally enter a period of developmental arrest or quiescence *in vitro*. They often germinate precociously but the resulting plants are frequently unable to survive. Dehydration is an integral part of the developmental arrest of orthodox embryos (Kermode *et al.* 1986). During normal maturation, orthodox embryos accumulate storage compounds prior to dehydration. During a critical period in the final stage of development, orthodox embryos acquire desiccation tolerance (Senaratna *et al.* 1987, 1990; Koster & Leopold 1988; Gray 1989). Following rehydration, the storage compounds are consumed during germination. It is possible to induce quiescence experimentally in somatic embryos of the orthodox type, such as those of grape and orchard grass, by controlled dehydration to moisture levels of approx. 13%; they then remain viable for as long as 1 year in dehydrated storage (Gray 1987) (Figures 5 and 6).

Until 10 years ago, several groups of agriculturally important plant species were considered to be difficult to regenerate by somatic embryogenesis (Ammirato 1983). These included such important plants as the legumes, cereals, Solanaceous plants and woody plants, irrespective of plant family. Substantial progress has since been made with the legumes and cereals (Whelan *et al.* 1992), which constitute the most important staple foods. Although Solanaceous plants are still intractable, and appear to generally lack embryogenic potential, they are often very easy to regenerate via the organogenic pathway. Woody horticultural and forestry species continue to represent a major challenge. Horticulturally important trees represent mature-phase selections that have often been vegetatively propagated for several hundred years (Mullins & Srinivasan 1976). Although somatic embryogenesis of many tree species has often been reported, the defined pathway has generally involved either embryonic or juvenile explants (Litz & Gray 1992). Consequently, the regenerants have a very different and often inferior genetic composition compared with the cultivar.

Somatic Embryogenesis for Micropropagation

In vitro propagation is normally based on the stimulation of multiple-shoot growth from cultured shoot-tip and nodal explants (Murashige 1974; Brown & Thorpe 1995). It has not always been possible to adapt this approach to certain plant taxa, and in other instances the proliferation rate is too low for it to have practical utility and cost-effectiveness. Examples of the former would include most of the palms and orchids; the latter would include many of the cereals and other agronomic crops, such as soybean, alfalfa and canola. Ironically, the earliest commercialization of micropropagation involved the mass propagation of orchids, which involved the large-scale production of protocorms, (proembryonal masses) in suspension culture (Rao 1977). Micropropagation of exotic orchid hybrids enabled orchid breeders to release large numbers of propagules of choice plants relatively quickly to an appreciative market, and revolutionized the entire ornamental industry. The vast majority of date palm (*Phoenix dactylifera*) planting stock is also currently produced by somatic embryogenesis.

Individual orchid and date-palm plants have high intrinsic values. Somatic embryogenesis, as adapted for these plants has made little use of automation, and somatic embryo development of palms is generally incompletely understood. Consequently, the *in vitro* handling of the regenerants is often lengthy and labour-intensive. Somatic embryogenesis and maturation represent a developmental process that, if properly understood, could be automated for industrial production. This would permit the use of somatic embryos as synthetic seed for planting material of agronomic crops that have low intrinsic value as individual plants, but enormous value in terms of acreage and food value.

The production of high-quality seed has become an important business. However, with only a few exceptions (i.e. polyembryonic seed), plants that grow from seed represent meiotic recombinants of two parents, and as such,

they cannot be genetically identical. The possible production of clonal plants of many horticultural and agronomic species by somatic embryogenesis and the delivery of somatic embryos as some form of artificial seed are the subjects of much current research. This has focused on the control of somatic embryo maturation, to produce large numbers of high-quality embryos that will yield vigorous plants, and on different strategies for engineering a synthetic structure that has the handling properties of natural seed. Synthetic seeds are somatic embryos that have been specially processed for use in commercial propagation, the exact process depending on the plant species and application (Gray & Purohit 1991). According to Friend (1993 and Janick *et al.* 1993), four types of synthetic seed have been proposed: (1) uncoated, desiccated somatic embryos; (2) coated, desiccated somatic embryos; (3) encapsulated, hydrated somatic embryos (Figure 7); and (4) hydrated somatic embryos within a fluid gel. The synthetic seed coating must fulfil certain requirements. It must provide non-toxic protection for the naked somatic embryo that would facilitate handling. Ideally, it should permit the incorporation of nutrients and pesticides. In addition, the somatic embryos must be reversibly quiescent if they are of the orthodox type, so that they will germinate uniformly in response to an appropriate stimulus.

Various hydrogels have been proposed for encapsulation of hydrated somatic embryos: sodium and potassium alginate; carrageenan; guar gum; agar; Gel-Rite; tragacanth gum; and sodium pectate (Redenbaugh *et al.* 1993). Mixtures of hydrogel and somatic embryos can be added as small drops into a complexing bath, resulting in high-frequency encapsulation of singulated somatic embryos. Other approaches have involved the insertion of somatic embryos into preshaped moulds containing the hydrogel. Sodium alginate is the preferred hydrogel, and it complexes well with calcium. Encapsulated somatic embryos have been further coated with a water-impermeable hydrophobic layer to prevent water loss (Friend 1993).

Encapsulated somatic embryos represent an elegant approach to the problem of synthetic-seed development. Singulation of the somatic embryos is possible, and nutrients, pesticides and even mycorrhizal fungi (Strullu *et al.* 1989) can be incorporated into the gel mixture. However, the survival of encapsulated somatic embryos has often been reported to be lower than the unencapsulated (Zhong & Wang 1989; Deng *et al.* 1990; Rao & Singh 1991), probably due to poor respiration under the almost anaerobic conditions within the capsule.

There are other logistical problems associated with the large-scale production of hydrated somatic embryos as synthetic seed. Because somatic embryos of orthodox-seeded plants behave as recalcitrant embryos *in vitro*, the development of somatic embryos in a bioreactor would proceed to germination. In addition, encapsulated somatic embryos survive for only a brief time at room temperature, and have not been shown to survive for more than 60 days at 2°C (Liu *et al.* 1990). Therefore, co-ordination of somatic embryo production and planting would have to be very carefully organized and massive over-production of somatic embryos would be inevitable because of the losses due to precocious germination. Because of the need for quiescence for normal development and germination of orthodox-type embryos, somatic embryos that are delivered hydrated would probably have to be desiccated and rehydrated, thereby increasing the production costs.

Ideally, quiescent or dormant somatic embryos would be produced that would mimic the storage and handling characteristics of true seed. Although quiescence (Kitto & Janick 1985; Gray 1987) and dormancy (Rajasekaran & Mullins 1979) have both been documented in somatic embryos, long-term viability has seldom been reported (Senaratna *et al.* 1989; Attree & Fowke 1993). These studies indicated that quiescence was induced by dehydration. Therefore, traditional methods for inducing and maintaining quiescence in seeds [e.g. controlled drying followed by low-humidity storage (Barton 1961; Bewley & Black 1985)] may be applicable to somatic embryos.

Synthetic-seed technology will probably be exploited in different ways, depending on the crop, the embryo type (orthodox or recalcitrant) and the intrinsic value of each plant. For seed-propagated agronomic crops that have low intrinsic individual value, it would be attractive to produce somatic embryos in a bioreactor and to dehydrate them (to induce quiescence) prior to encapsulation. Certain vegetable crops that are grown from relatively expensive hybrid seed, such as tomato, bell peppers and seedless watermelons, could also be grown more efficiently from synthetic seed that is produced in the same manner.

For crop plants that are currently propagated vegetatively and that have a high intrinsic value (e.g. palms, some ornamental species and some fruit and nut rootstocks), the use of naked, hand-manipulated, non-quiescent somatic embryos could be cost-effective. As we have seen, orchids and date palms are currently being propagated in this manner. The high cost of ornamental crops that are painstakingly micropropagated by axillary-bud proliferation is primarily due to the labour-intensiveness of the cutting and subculture operations. However, Preil *et al.* (1988) and Preil (1991) have demonstrated that bioreactor production of the ornamental poinsettia (*Euphorbia pulcherrima*) could replace conventional propagation by cuttings and vastly increase the efficiency of conventional micropropagation.

Some crops, such as perennial fruits, nuts and some plantation crops (and their rootstocks), are vegetatively propagated in order to retain their unique genetic character. With a few exceptions, existing propagation methods are adequate, and the developmental costs of synthetic seed might not be justified. Noriega & Sondahl (1993) recently

indicated that somatic embryogenesis of coffee (*Coffea arabica*), a recalcitrant-seeded plant, was well suited to bioreactor culture. Other exceptions include plants that are difficult to propagate by other vegetative methods, such as clove (*Eugenia caryophylla*) and rootstock selections. However, the use of synthetic seed for germplasm conservation of crops which are normally propagated vegetatively could be advantageous, since germplasm of clonally propagated perennial plants must otherwise be maintained in field gene banks (Towill 1988; Withers 1989, 1992; Villalobos & Engelmann 1995). Field collections of clonal perennial plants are expensive to maintain, and plants can be lost due to environmental catastrophes and from the withdrawal of political will to support them. Synthetic-seed technology, coupled perhaps with cryopreservation of embryogenic cultures, could enable clonal germplasm to be conserved in seed repositories at reduced risk and expense (Engelmann & Dereuddre 1988; Redenbaugh 1990; Villalobos & Engelmann 1995). This method of germplasm conservation would be particularly useful for tropical species which are currently inadequately conserved and for collections threatened by disaster.

Certain obstacles remain to be overcome before the full potential of bioreactor production of synthetic seed can be realized: (1) somatic embryogenesis and the ability to grow embryogenic cultures in suspension are genetically determined (Litz *et al.* 1993) so many of the most valuable cultivars or hybrids of some important crops cannot be grown under optimized conditions in a bioreactor; (2) optimizing the production, growth and development of some somatic embryos has been associated with increased hyperhydricity (vitrification) (Monsalud 1994) and hyperhydric embryos are physiologically abnormal and unable to develop to maturity (DeBergh *et al.* 1992); (3) tissue-culture-induced variability (somaclonal variation) can occur in plants regenerated from somatic embryos. Somaclonal variants, such as those of coffee (Sondahl & Lauritis 1992) and oil palm (*Elaeis guineensis*) (Jones & Hughes 1989), are stable mutants, and can deviate from the clonal phenotype for one or more traits, thereby compromising the usefulness of somatic embryogenesis as a tool for propagation (McCoy *et al.* 1982; Orton 1983; Dennis *et al.* 1984; Brettel *et al.* 1986). Modifications in the *in vitro* protocol can minimize somaclonal variation but these often reduce the efficiency of the process. Factors that may influence somaclonal variation include the nature of the original explant (Murashige 1974; D'Amato 1975) and the length of the *in vitro* cycle (Barbier & Dulieu 1980; Skirvin & Janick 1976).

Somatic Embryogenesis for Cultivar/Variety Improvement

Strategies that have been adopted for *in vitro* improvement of agricultural crops are dependent upon the availability of a highly efficient and reliable regeneration system. Embryogenic cultures, which are considered to be totipotent, fulfill these requirements and so have been widely used in studies involving protoplasts, for *in vitro* selection, and increasingly for genetic transformation.

Somaclonal Variation

Somaclonal variation represents a paradox to those wishing to utilize *in vitro*-derived products. From the standpoint of micropropagation, the uncontrolled production of off-types is definitely undesirable. At one time, for example, it was believed that micropropagation of oil palm by somatic embryogenesis would revolutionize palm-oil production. However, some of the selected trees that were mass produced showed abnormal flower development in the tissue-culture progeny (Corley *et al.* 1986); the most serious condition seen, referred to as 'mantling', is believed to be caused by a single dominant gene (Beinaert & Vanderweyen 1941). From the plant breeders' perspective, such variation offers another method of generating genetic diversity. Recovery of discrete mutants with important agricultural traits from tissue cultures could lead to improvements in crops that are either highly inbred or extremely heterogeneous.

There have been relatively few studies involving identification of somaclonal variants from somatic embryo regenerants of species other than oil palm. According to Hanna *et al.* (1984), the rate of production of somatic mutations is much greater in regenerants derived from the organogenic pathway than from the somatic embryogenic pathway. Ozias-Akins & Vasil (1988) speculated that this was because somatic embryogenesis involves the expression of more developmental genes than organogenesis. Somaclonal variation can probably be affected by a number of factors, including species, genotype, explant type, length of time in culture and duration of the subculture period.

Sondahl & Lauritis (1992) identified about 40 different mutants from somatic embryos of *Coffea arabica*. Most of the characters affected were controlled by single dominant, partially dominant or recessive genes. In order to identify these mutants, large field plantings, of about 16,000 somatic embryo regenerants, were established. The cost of such a study would be prohibitive for all except a few crop species. The high efficiency of somatic embryogenesis could make it an ideal system for developing an *in vitro* selection scheme. This would depend upon the sensitivity of cell cultures to a selective agent, and the expression of the selected character at the whole-plant level. Many of the somaclonal variants that have been produced have shown heightened disease resistance. The *in vitro* selection of embryogenic cultures of peach (*Prunus persica*) for resistance to the toxins produced by *Xanthomonas campestris* pv *pruni*, the causative agent of bacterial leaf spot, and *Pseudomonas syringae* pv *syringae*, the cause of bacterial canker, has been

reported by Hammerschlag (1990) and Hammerschlag & Ognjanov (1990).

Somaclonal variation could be particularly useful for perennial crop plants. Since these plants are usually very heterogeneous and have long juvenile periods, conventional breeding has led to relatively little improvement. The ability to alter a valuable cultivar for a single genetic trait has great appeal.

Genetic Transformation

The transfer of foreign genes into plants is based upon the availability of an efficient *in vitro* regeneration system. Transformation can be achieved by several methods, including the direct insertion of DNA into protoplasts by microinjection (Crossway *et al.* 1986) or electroporation (Horn *et al.* 1988). However, the most significant breakthroughs have resulted from the development of microprojectile bombardment of regenerative tissues by DNA-coated tungsten or gold particles (Klein *et al.* 1987; Sanford 1988) and the use of genetically engineered avirulent strains of *Agrobacterium* as vectors (Herrera-Estrella *et al.* 1983; Herrera-Estrella & Simpson 1995). The early reports of genetic transformation by *Agrobacterium* generally involved organogenic tissues, such as the leaves of Solanaceous plants (Horsch *et al.* 1985). However, for species that are not easily regenerated by organogenesis, such as walnut (McGranahan *et al.* 1988) and mango (Mathews *et al.* 1992), embryogenesis has increasingly been preferred.

Agrobacterium tumefaciens-mediated genetic transformation has been successfully demonstrated with a wide range of important crop species, including both horticultural and agronomic dicotyledonous species. Most monocotyledonous crop species cannot be infected with *Agrobacterium*, so transformation of these species involves the bombardment of organogenic tissue or embryogenic cultures with DNA-coated microprojectiles. This method has been successfully used on wheat (Vasil *et al.* 1992), rice (Christou *et al.* 1991) and maize (Fromm *et al.* 1990).

Genetic transformation that is effected by *Agrobacterium* is dependent on the use of a disarmed Ti plasmid. The *Agrobacterium* genes that are responsible for tumour formation can be replaced with foreign genes that are expressed following infection of plant cells. The inserted genes normally include: (1) a selectable marker, such as the gene for neophosphatetransferase (NPT II), which confers resistance to the antibiotic kanamycin; (2) a gene that encodes a scorable marker, such as β-glucuronidase (GUS) which undergoes a useful histochemical colour reaction (Jefferson 1987); (3) a sequence that encodes a promoter, such as 35S from cauliflower mosaic virus, for expressing the different genes that have been introduced into the plasmid (Kuhlemeier *et al.* 1987); and (4) a limited menu of genes that have agricultural interest. The latter include, but are not restricted to, genes that are involved in pest and disease control, such as those encoding the coat protein of several important plant viruses (conferring virus resistance; Bevan *et al.* 1985; Powell-Abel *et al.* 1986; Pappu *et al.* 1995), cecropin (bacterial resistance; Jaynes *et al.* 1987), chitinase (resistance to insects and pathogenic fungi; Dunsmuir & Suslow 1989), the insecticidal crystalline proteins isolated from *Bacillus thuringiensis* (Fischhoff *et al.* 1987; Vaeck *et al.* 1987) and cowpea trypsin inhibitor (toxic to lepidopteran and coleopteran insects; Hilder *et al.* 1990). Herbicide resistance genes have been identified that provide specific immunity or detoxification (Padgette *et al.* 1989). Controlled ripening of fruit has been demonstrated in plants that have been transformed with 'antisense' constructs of genes that are implicated in ethylene synthesis (Hamilton *et al.* 1990; Oeller *et al.* 1991) and polygalacturonase activity (Sheehy *et al.* 1988; Smith *et al.* 1988). Other agricultural traits that are conferred by single genes are also being targeted.

Important plant varieties that have been modified with agriculturally useful genes are covered by patent laws that protect breeders' rights. Synthetic-seed technology would be an elegant method for producing clonal, genetically engineered planting material, that would safeguard the interests of the developers of the improved varieties.

Conclusions

Somatic embryogenesis has been documented in species from most taxonomic groups. It is presumed to be a universal, heritable trait of higher plants. The ability to revolutionize seed production and genetically enhance food and fibre crops through the manipulation of their isolated, cultured cells holds great promise for those crops that are difficult to improve by conventional means. However, details of the genetic control of somatic embryo development remain unclear, and the maturation process is still unacceptably inefficient. More and detailed study is necessary to unlock the full potential of somatic embryogenesis and make it fully accessible as a tool for plant breeding and production. Finally, growers and consumers must be convinced that agricultural products from somatic embryo-based technologies are both safe and necessary.

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