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Somatic embryogenesis for crop improvement

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SUMMARY

Conventional breeding and advances in agronomic and horticultural practices, most of the crops have attained close to their maximum yields. The manipulation of cell and tissue cultures to produce somatic embryos efficiently is one of the milestone of new technologies that will greatly alter the way crops are planted(as artificial 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.

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With the increase in world population, the agricultural yield have often stagnated and even declined in some areas. In many parts of the world rural poverty has increased and the natural resource base has degraded. Conventional approach to modernization of agriculture on the principle of intensification through specialization (as in green revolution) has not adequately addressed these problems. During 1960's it has been realized that grain production of green revolution would not be sufficient to overcome because of increasing world population in the coming few decades. Therefore, development of alternate strategies for increasing plant productivity were considered to be of utmost importance. *In vitro* procedures for manipulating plant differentiation, growth and development, regeneration of plants from cell culture 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 pre-requisite for genetic manipulation of plant is the regeneration of plants under aseptic condition on a culture medium from somatic cell, either *via* organogenesis (Christianson,1987) or somatic embryogenesis(Ammirato,1985;1987). In organogenesis root and shoot development are often mutually exclusive and a sequence of media changes is necessary to generate an entire plant. Since cell or tissue transforms are expensive in terms of material and personal time and increase the chance for contamination. Many researchers regard somatic embryogenesis as the *in vitro* system of choice for mass propagation of super and genetically engineered genotypes (Gupta *et al.,* 1991). Somatic embryogenesis has a number of advantages over other micropropagation techniques, namely axillary shoot proliferation and adventitious shoot production. The advantages most commonly cited includes very high multiplication rate and the potential for scale up in liquid culture (*i.e.* bioreactors) and for direct delivery to the green house or field as artificial seed (Markle *et al.*,1990). Such features make it likely that clonal propagules produced *via* somatic embryogenesis will have significantly low that clonal propagules produced using other micropropagation system due to lower labour costs. Further more, embryogenic cultures have also been shown to make excellent target material for gene transfer *via Agrobacterium* Ti plasmid mediated and biolistic transformation (Mc Granahans *et al.*, 1989; Parrot *et al.,*1988). Thus it is widely believed that embryogenic cultures will eventually be employed for commercial scale production of clonal propagules. The involvement of somatic embryogenesis as a modern tool for increasing agricultural productivity is the subject of this review.

What is somatic embryogenesis:

Since 1958 when the first plant embryos were obtained from somatic tissues of carrot (*Daucus carota*) cultured *in vitro* (Reinert, 1958; Steward, 1958) ever increasing number of species have been induced to form somatic embryos. Somatic embryos resembles their sexual counterparts and presumably the result from expression of genes regulating the same development pathway. They are bipolar structure having root and shoot apices.

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However, they originate *via* a different pathway rather than developing from a zygote after fusion of the 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 adventive embryo develop from sporophytic cells, embryos have been fostered from generative cells such as in *Datura innoxia* (Guha and Meheshwari, 1964 and *Nicotiana tabaccum* (Nitsch,1969). Triploid embryos have also been observed in endosperm cultures of *Santalum album* (Lakshmi Sita *et al*., 1980). Adventive or asexual embryos are produced from nucellus or integument tissue again the members of the Rutaceae, especially Citrus species are perhaps best known for nucellar derived embryos (Esan,1973). Cell within the embryos sac such as syngerids or antipodal, may also develop into embryos bearing the gametic chromosome number. The proembyo, embryo or its suspensor may also give rise to multiple embryos. In addition, there are examples of embryos arising naturally from endospermic cells, as in the case of *Brachiaria setigra* (Muniyamma, 1977). More unusual embryos have formed within anther of *Narcissus biflorus* (Koul and karihaloo,1977). Somatic embryogenesis has been documented as being genetically determined. This phenomenon has been widely reported in several species including *Cicer arietinum* (Barna and Wakhlu, 1993;1995), *Citrus* (Carimi *et al*., 1999), *Capsicum annuum* (Binzel *et al.,* 1996), *Cucumis sativus* (Cade,1988), *Brassica juncea*(Kumari *et al*., 1988) and *Bunium persicum* (Wakhlu *et al*.,1990).

The choice of the donor plant tissue is critical and is usually determined empirically. For many pattern of somatic embryogenesis, embryonic or highly juvenile types of tissue have to be used as explant. Juvenile tissues are sometimes the only practical choice when culturing woody plants, in which the transition from juvenile to mature phases is associated with lignifications. The pattern of development from cultured tissue is epigenetically determined and is influenced by the stage of development of the plant, nature of the explant, basal medium, auxin etc. The physiological status of the explant can be limiting but can be influenced to some degree by pre-conditioning the stock plant (Roussy *et al.,*1996). The appropriate explant, the correct developmental stage produces an embryogenic culture on a particular growth medium. The medium normally based on Murashige and Skoog (1962), Schenk and Hildebrandt(1972) or Nitsch and Nitsch (1969) or B_5(Gamborg *et al.,* 1968) is optimized using various supplements including auxins or auxin like substitutes 2,4-dichlorophenoxyacetic acid (2,4-D), picoram, dicamba, 2,4,5-trochlorophenoxyacetic acid (2,4,5-T), sucrose and a source of either organic or inorganic ammonium. Other classes of plant growth regulators cytokinins and gibberellins have sometimes been utilizes in combination with auxins. Sharp *et al.*(1980) described two routes to somatic embryogenesis. The first is direct embryogenesis where embryos initiate directly from tissue in the absence of callus proliferation. This occurs through pre-embryogenic determined cells (PEDC) but the expression in their embryogenic potential is suppressed by the surrounding cells of the tissue and need to be released. The embryogenic cells in the nucellus during the early stages of ovule development within polyembryogenic seeds fall in to this category. Following subculture embryogenic cells onto medium that lacked inducing growth regulators, the cell divide and organize as somatic proembryo freed from the inhibitory influence that occurs *in vitro*. The second is indirect embryogenesis where some cell proliferation is required to form callus. A change in cellular morphology associated with an asymmetric cell division, can occur (Kohlenbach, 1978) and this results in a change of the polarity. A highly vacuolated cell is formed together with a small, avacuolate sister cell which is embryogenic. Thus there is a predetermination of cell type and function (Christiansan, 1985). Simultaneously, the ability to form somatic embryos is in most cases, not merely an intrinsic property of a species. Instead, it is a property under genetic control such that individual genotypes within a species can fifer in their ability to undergo somatic embryogenesis. This phenomenon has been widely documented in several species such as *Sorghum bicolour* (Misra and khurana, 2003), Maize (Green and Phillips, 1975) and Wheat (Sears and Dickand,1982; Maddocle *et al*.,1983; He *et al*., 1988)

Embryogenic cultures can often be maintained for long periods by continuous subculture in liquid media containing 2,4-D or another auxin. These embryogenic suspension cultures are composed of proembryonic masses (Halperin, 1966). Each proembryonic mass theoretically has the ability to form a single embryo until it reaches a certain size, after which it loses the potential for integrated development (Williams and Maheshwaran,1986). The organization and maturation of somatic embryo are inhibited so long as they are cultured in the presence of 2,4-D or another auxin. Cells of the proembryogenic mass divide and continuously differentiate secondary somatic embryos in a repetitive cycle when in an inductive environment and it is possible to synchronize embryo suspension cultures to some degree by sieving. Some of the proembryonic masses consists of only a few cells, whereas other masses can be 2-3 mm in diameter.

The small pre-embryos can often develop as singulated embryos in the medium free of auxins. The suspension cultures are used for many different *in vitro* procedures, including protoplast isolation and cultures, *in vitro* selection and are the basis for bioreactors micro propagation because they are highly embryogenic.

Normally, the media used for somatic embryo maturation are supplemented with additional amino acids and complex organic supplements, auxins is often omitted from the medium because of inhibitory effect on the maturation of somatic embryos in many species (Halperin and Wetherell,1964; Fujimura and Komamine,1975,1980; Wakhlu and Sharma,1988). Zygotic and somatic embryos development are generally similar and the embryos pass through recognizable heart, torpedo and mature stages. Precocious or premature embryos germination can be control either by increasing the osmolarity of the maturation medium with addition of sucrose (Ammirato and Steward,1971 and Steward *et al.*, 1975) or by incorporating abscisic acid into the medium (Ammirato, 1974). At maturity, *in vitro* grown embryos are significantly larger than zygotic embryos of some species (Monnie,1978 ; Gray and Purohit, 1991).

Embryos have been categorized according to their ability to withstand desiccation. Orthodox embryos can tolerate desiccation and in doing so, enter a state of development arrest (Bewley anf Black, 1985). The evolution of quiescent embryos that were enclosed with a nutritive food supply, the endosperm in angiosperms and megagametophyte in conifers to form a seed was critical for the spread of higher plants and the survival of mankind. Seeds which have dispersal structures, enables short lived annual plants to survive in 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 have allowed mankind to transport germplasm of many important crops from their centre 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 costs of important amino acids, protein, lipids and polysaccharides. Orthodox seeds of some species can be stored for many years. The 2nd type of embryos, recalcitrant embryos are unable to tolerate desiccation and do not cease development during maturation. Recalcitrant seeds can't be stored for more than few days. Somatic embryos, regardless of whether they are of the recalcitrant or orthodox type, behave as recalcitrant embryos and germinate when they are apparently reached their final state of development (Litz and Gray, 1992). Since embryos maturation and germination of recalcitrant embryos represent a continuum, without a period of developmental arrest, somatic embryos of the recalcitrant types 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 under *in vitro*. They often germinate precociously but the resulting plants are 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 this critical period in the final stage of development, orthodox embryos acquires desiccation tolerance (Senaratna *et al.*, 1987, 1990; Koster and Leopold,1988; Gray, 1989). It is possible to induce quiescence experimentally in somatic embryos of the orthodox type, such as those of grapes and orchard grass, by controlling dehydration to moisture level (13% approximately) they then remain viable as long as 1 year in dehydrated storage (Gray,1987).

Several groups of agricultural important plant species were considered to be difficult to regenerate *via* somatic embryogenesis (Ammirato, 1983). These includes such important plants as legumes, cereals, solanceous plants and woody plants, irrespective of plant family. Substantial progress has been made with legumes and cereals (Whelan *et al.*, 1992), which constitutes the most important staple foods. Although solanaeous plants are still intractable and appear to generally lack embryogenic potential, they are often very easy to regenerate *via* caulogenesis. Woody horticultural and forestry species continue to represent a major challenge. Horticultural important trees represent mature phase selection that have been vegetatively propagated for several hundred years (Mullins and Srinivasan, 1976). Although somatic embryogenesis of many tree species have been reported, the defined pathway has generally involved other embryonic or juvenile as explants (Litz and Gray,1992). Consequently the regenerants have a very different and often inferior and genetic composition as compared with the cultivars.

Somatic embryogenesis for micropropagation:

The large scale cloning of crop plants potentially has broad application in agriculture(Murashige,1974). The possibility of producing large number of plants of a single genotype evoke vision of application to breeding programmes, new seed production methods and even an alternative to normal crop production from seeds. Tissue

culture techniques have found wide use in commercial propagation of horticultural plants. Particularly ornamental and in the elimination of specific pathogen for the production of pathogen free plants (Rao,1977). Micropropagation of exotic orchid hybrids enable orchid breeders to release large scale propagules of choice plant relatively quickly to an appreciative market and revolutionized the entire orchid industry. The methods commonly used for the production of horticulture propagules are relatively labour intensive, low volume and have high unit costs compared with current agricultural seed practices. Highly mechanized cultural systems able to efficiently produce large number of propagules must be developed if high frequency cloning id to be a variable concept. The generally accepted method for multiplication and regeneration are diagrammed in Fig. 1. The most widely used commercial method of regeneration is axillary shoot formation. This method is very labour intensive due to number of individual manual manipulations involved and low multiplication rates. Another method of regeneration is adventitious bud formation, a form of organogenesis.

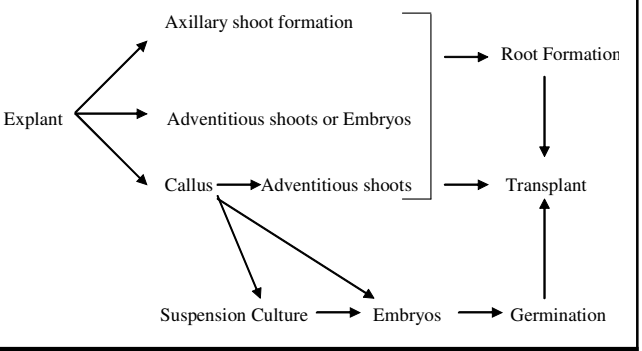

Fig. 1 : Different methods for *in vitro* plant regeneration

Clonal method by this method includes two types. In some systems adventitious shoots can be induced directly on the original explants. These adventitious shoots must then be rooted, transplanted to soil and gardened off. This method require fewer manipulations than shoot tip culture but potentially requires a large explant source depending upon the manipulation rate. In another system adventitious buds formed on callus cultures. Again these shoots have to be rooted, hardened and transplanted to soil.

Finally the third method of regeneration is somatic embryogenesis. Clonal multiplication by this method involve the induction of embryos either on the surface of explant, callus or in a suspension culture. In term of their utility the formation of embryos on callus or explant may present little advantage over adventitious shoots. Isolation of individual embryos still have to be done manually. However, in suspension culture, embryos can be isolated to produce populations if single propagule which should be highly amenable to large scale handling and mechanized planting system. This would permit the use of somatic embryos as synthetic seed for planting material of agronomic crops that have low value in terms of acreage and food value.

The production of high quality seed has become an important business. However, with only few exceptions (*i.e.* polyembryonic seed) plants that grow from seed representation of two parents, and as they can not 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 of the subject of much current research. This has focused on the control of somatic embryo maturation, to produce large number of high quality embryos that will yield vigorous plants and on different strategies for engineering a synthetic seed that has the handling properties of natural seed. Synthetic seed comprising of somatic embryo enclosed in a nutritive and protecting coat (Redenbaugh *et al.*,1986; Mukunthakumar and Mathur,1992; Gray and Purohit, 1991). According to Friend(1993) and Janick *et al.* (1993), four types of synthetic seeds have been proposed : 1) uncoated desiccated somatic embryos, 2) coated desiccated somatic embryos, 3) encapsulated hydrated somatic embryos, 4) hydrated somatic embryos within gel. It must provide non toxic protection for the naked somatic embryos that would facilitate handling. Ideally, it should permit the incorporation of nutrients and pesticides. In addition, the somatic embryos must be reversible quiescent if they are of orthodox type, so that they will germinate uniformly in response to an appropriate stimulus. Various hydro gels have been proposed for encapsulation of hydrated somatic embryos: sodium and potassium alginate, carrageen, guar gum, gel rite, tragacanth gum and sodium pectate (Redanbaugh *et al.*, 1993). Mixture of hydro gel and somatic embryos can be added as small drops into a complex bath resulting in high frequency encapsulation of sigulated somatic embryos. Other approach have involved the inseration of somatic embryos into pre-shaped moulds containing hydro gel. Sodium alginate is the preferred hydro gel 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 represents an elegant approach to the problems of synthetic seed development. Singulation of somatic embryos and in

corporation of nutrients, pesticides and mycorrhizal fungi into the gel is possible (Strullu *et al.,* 1989). However, the survived encapsulated somatic embryos has often been reported to be lower than the encapsulated (Zhang and Wang, 1989; Deng *et al.*, 1990; Rao and Singh,1991) probably due to poor respiration under the almost anaerobic conditions within the capsule. There are also other problems associated with the large scale production of hydrated somatic embryos as synthetic seeds. Because somatic embryos of orthodox seeded plant behave as recalcitrant embryos *in vitro.* The development of somatic embryos in a bioreactor would proceed to germination. In addition somatic embryos survive for only a brief time at room temperature and have not been shown to survive for more than 60 days at 20^0 C (Liu *et al.*, 1990). Therefore, co-ordination of somatic embryos production and planting would have to be very carefully organized and massive overproduction of somatic embryos would be inevitable for normal development and germination of orthodox type embryos. Somatic embryos that are delivered hydrated would probably have to be desiccated and dehydrated ,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 (Kitti and Janick, 1985; Gray,1987) and dormancy (Rajasekaran and mullins,1979) have both been documented in somatic embryos, long-term viability has seldom been reported (Senaratna *et al.*, 1989; Attree and Fowke,1993). These studies indicated that quiescence was induced by dehydration. Therefore, traditional methods for inducing and maintaining quiescence in seeds may be applicable to somatic embryos (Barltin,1961; Bewley and Black,1985).

Synthetic seed technology will probably be exploited in different ways, depending on the crop, the embryo types and intrinsic value of each plant. For seed propagated agronomic crop that have low intrinsic individual value, it would be attractive. To produce somatic embryos in a bioreactor and to dehydrate them prior to encapsulation, certain vegetable crop that are grown from relatively expansive hybrid seeds 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 plants, ornamental species and fruit and nut rootstocks etc., 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 cuttings and subculture operations. However, Preil *et al.*(1988) and Priel (1991) have demonstrated that bioreactor production of the ornamental poinsettia (*Euphorbia pucherima*) 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 are vegetatively propagated to retain their genetic characters. With a few exceptions, existing propagation methods are adequate and developmental costs of synthetic seed might not be justified. Noriega and Sondahl (1993) indicated that somatic embryos of coffee (*Coffea arabica*)a recalcitrant seeded plant are difficult to propagate by other vegetative methods, such as clove (*Eugenia caryophylla*) and rootstock selection. However, the use of synthetic seed for germplasm conservation of crops which are normally propagated vegetatively could be advantageous, since germplam of clonally propagated perennial plants must otherwise be maintained in the field gene-banks (Towtill, 1988; Withers, 1989, 1992; Villalobos and Engelmann, 1995). Field collections of clonal perennial plants are expensive to maintain and plants can be lost due to environmental catastrophes and from the withdrawl of political will to support them. Synthetic seed technology coupled perhaps with cryopreservation of embryogenic cultures,could enable clonal germplasm in seed repositions at reduced risk and expense (Engelmann and Dereuddre,1988; Redenbaugh, 1990; Villalobos and 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 remains to be overcome before the full potential of bioreactor production of synthetic seed can be realized: 1) Somatic embryogenesis is the ability to grow embryogenic cultures in suspension is genetically determined (Litz *et al.*, 1993), so many of the valuable cultivars or hybrids of some important crops can not be grown under optimized conditions in a bioreactor, 2) optimizing the production, growth and development of somatic embryos has been associated with increased hyperhydricity (Monsalud,1994) and hyperhydric embryos are physiologically abnormal and unable to develop to maturity (De Bergh *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 (Sondhal and Laurtis,1992) and oil palm (*Elacis guineesis*) (Jones and Huges,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 (MaCoy *et al.*, 1982; Ortan,1983; Dennis *et al*., 1984). Modification 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 explants (Murashige,1974; D'Amato,19750 and the length of the *in vitro* cycle (Barbies and Dulieu,1980; Skirvin and Janick,1976).

Synchrony:

Populations of somatic embryos typically show a wide range of size and stages of development because 1) at the time of transfer from the maintenance medium to the medium that will allow development, there is a range of pro embryogenic cell cluster from those with just a few cells to those substantially large, 2) somatic embryogenesis is to some extent repetitive (varies from species to species) so that new embryogenic centre may arise from clusters or maturing embryos. Having all the embryos pass through each stage simultaneously would be very valuable for both theoretical studies (biochemical analysis) and for practical applications as such as mechanical planting or artificial seed production. Attempts must address the problems of uniformity of inoculum and preventing adventive/accessory embryogenesis.

The most common method for attaining some degree of uniformity, at least in terms of the starting population, is by means of sieving at the time of transfer to the secondary medium. A graded series of stainless steel mesh sieves (Halperin,1966; Ammirato,1974) or nylon mesh (Fujimura and Lomamina,1975) have proved adequate. Passing the suspension through glass beads has also been effective (Warren and Flower,1978). Sieving followed by centrifugation in 16% ficoll solution containing 2% sucrose has isolated a population of *Daucus* cell aggregates from 3 to 10 cells each which develop synchronously when moved to growth regulator free medium (Fujimura and Kmomine,1979; 1980b). For controlling 'repetitive embryogenesis' ABA has proven effective in *Carum carvi* (Ammirato,1974) and *Pennisetum americanum* (Vasil and Vasil, 1981).

Genetic stability:

Changes in the ploidy level have been noted in many cultures leading to mixed populations of polyploids and aneuploids and plants regenerated from such cultures often show a range of chromosome complements (Sunderland, 1977; Ammuato, 1978). The embryogenic capacity of cultures has been seen to decrease and disappear during progressive subculture (Syono,1965) and this loss of potential has been traced at least in certain cases, to the change in chromosome complement where aneuploids gradually replace diploid cells (Smith and Street,1974). This loss of potential may not necessarily be permanent in habituated as in *Citrus sinensis* cultures, embryogenesis was restored by eliminating sucrose or aging tissue (Kochba and Button, 1974). By changing the sequence of growth regulators with each subculture, a non embryogenic line of *Daucus carota* gradually regained embryogenic capacity (Chandra, 1981). Whether this was due to a selective enrichment of a small number of embryogenic cells remaining in the suspension or the re-induction of cells that were epigenetically changed was not determined. However, there is evidence that chromosomal, genetic and epigenetic changes occur as cells are cultured. Although some *Daucus* suspension showed a range of chromosomeal abnormalities, the regenerated plants were almost entirely diploid (Mitra *et al*., 1960; Mok *et al*., 1978). At that time it was thought that only cells with an unaltered chromosome complement could develop into somatic embryos and plants. However, in studies of somatic embryogenesis in long term *Daucus carota* cultures, embryos and plants could be grown, but they were often sterile (Sussex and Frie, 1968). Changes in the chromosome karyotype rather than complement have been seen in somatic embryos and plants e.g. *Hemerocallis* (Krikorian *et al.,* 1981). In *Bromus inermis* (Gamborg *et al.*, 1970), the resulting plants from somatic embryos were albino and *Lalium multiform* more than 50% of the regenerated plants were albino (Dale, 1980).

The maintenance of chromosomal and genetic integrity is essential if the goal of somatic embryogenesis is clonal multiplication. There are a number of studies demonstrating that frequent subculturing can effectively minimize the extent of chromosomal change in cell culture (Bayliss,1977; Sunderland,1977; Evans and Gamborg, 1982). Krikorian (1982) reported that the phenotype of *Daucus carota* plants raised from somatic embryos was normal provided the suspensions from which they are derived were maintained for relatively short period *i.e.* less than a year. Thus the frequent establishment of fresh cultures from plant material and careful attention to the subculture regime may help to maintain genetic and chromosomal stability.

Somaclonal variation:

Somaclonal variation represent a paradox to those wishing to utilize *in vitro* derived products. From the standpoint of micropropagation, the uncontrolled product of off-type is definitely undesirable. At one time, it was

believed that micropopagation 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). From the plant breeding perspective, such variation offer another method of generating genetic diversity. Recovery of discrete mutants with important agriculture trait from tissue culture could lead to improvement in crops that are either highly inbred or extremely heterogenous. There have been relatively few studies involved identification of somaclonal variants from somatic embryo regenerants of species other than oil palm. The rate of production of somatic mutants is much greater in regenerants derived from the organogenic pathway than the somatic embryogenic pathway (Ozias-Akin and Vasil,1988). Somaclonal variations 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.

Sondhal and Lauritis (1992) identified 40 different mutants from somatic embryos of *Coffea arabica*. Most of the characters affected were controlled by single dominant, partially or recessive genes. 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 toxin produced by *Xanthomonas compestris* p.v. compestris causing bacterial leaf spot and Pseudomonas syringe, the cause of bacterial canker has been reported by Hammerschlag (1990) and Hammerschlag and Ognjanov (1990). Somaclonal variations could be particularly useful for perennial crop plants since these are usually very heterogeneous and have long juvenile periods and 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:

In the last decade and half, the development of novel tools of direct gene transfer collectively termed as genetic engineering and has added new dimensions to plant improvement programmes. Genetic engineering provides the breeder with new tools which complements and supplement sexual hybridization for improvement of existing varieties or creation of totally new germplasm, by insertion of genes encoding for useful agronomic traits. It was only in 1983 that chimerical genes were first expressed in genetically transformed plant tissues (Bevan *et al.*, 1983; Herrera-Estrella *et al.*,1983). It is widely believed that the transgenic technology may revolutionize plant improvement programmes. The results obtained so far points in that direction (Borlaug,1997; Swaminathan *et al.*, 1999; Cook,2000; Jauhar and chibber,1999; Repellin *et al.*, 2001).

The transfer of foreign gene 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 micro-injection (Crossway *et al.*, 1986) or electroporation (Horn *et al.*,1988). However, the most significant breakthroughs have resulted from the development of micro-projectile bombardment of regenerative tissue by DNA- coated tungsten or gold particles (Klein *et al.*, 1987; Sanford,1988) and the use of genetically engineered avirulent strains of *Agrobacterium* as vector (Herrera-Estrella and Simpson,1995). The early reports of genetic transformation by *Agrobacterium* generally involves organogenic tissues, such as the leaves of solaneous plants (Horsch *et al.,* 1985). However, for species that are not easily regenerated by organogenesis such as walnut (Mc-Granahan *et al.*,1988) and mango (Methews *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 can not be infected with *Agrobacterium*, so transformation of these species involves the bombardment of organogenic or embryogenic cultures with DNA-coated micro particles. This method has been successfully used on wheat (Vasil *et al.*,1992), rice (Christou *et al.*,1991) and maize (Fromm *et al.*,1990). The Bt (*Bacillus thuringiensis*) gene has been successfully bioengineered into corn genome, confirm resistance to European corn borer (ECB), largely eliminating damage from this serious pest. Recently, several seed companies, such as Ciba seeds and Mycogen have produced superior hybrids. This is an efficient method of eliminating the pest damage without adversely affected grain yield (Jauhar, 2001). Another major break through in providing built-in genetic resistance to disease is the discovery that expression of a virus coat protein gene as a transgene in plant confers resistance to that virus is direct proportion to the amount of coat protein produced by the transformed plant (Beachy *et al.*, 1990). This novel approach has opened up new avenues of control of viral diseases in plants. Thus, papaya ring spot in Hawaii has been controlled by coat- protein mediated resistance (Gonsalves, 1998). Transgenic approach have also been employed to combat fungal diseases in cereal crops including wheat and barley (Dahleen *et al.*, 2001; Muthukrishnan *et al.*, 2001). Production of the first

transgenic durum wheat by micro projectile bombardment and standardization of technology of durum wheat transformation (Bommineni *et al.*, 1997) opened up avenues of engineering with antifungal genes, Mi gene that confers resistance to the root knot nematode in tomato which when introduced into potato confers resistance to the aphid, *Macrosiphum euphorbiac* (Milligan *et al*., 1998; Rossi *et al.*,1998). Controlled ripening of fruits has been demonstrated in plants that have been transformed with antisense constructs of genes that are implicated in ethylenesynthesis (Hamilton *et al*.,1990; Oeller *et al*., 1991) and polygalacturonase activity (Smith *et al.*, 1988). Other agricultural traits that are conferred by single gene are also targeted.

Important plant varieties that have been modified with agricultural useful genes are covered by patent laws that protect breeder's right. 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 many potential advantages for mass propagation and genetic improvement of crops. A number of limitations remains to be overcome before embryogenic system can be applied for operational production of propagules by taking advantages of such features as repetitive embryogenesis and pre-embryogenic meristemoids production, somatic embryo production can be scaled up. This ability hold great promise for those crops that are difficult to improve by conventional means. Somatic embryogenesis also offer potential for efficient production of transgenic populations in a range of plant species. Through somatic embryogenesis it should be possible to maximize the number of transformed cells from which regenerants can ultimately be produced by repetitive embryogenesis, such independent transformation event should be recoverable as one to many whole non chimeric plantlets. However, details of the genetic control of somatic embryos development remains unclear and the maturation process is still unacceptable inefficient. More detailed studies are needed for exploiting full potential of somatic embryogenesis. A close interaction and collaboration between biotechnologist, plant breeder, pathologist and agronomist will be essential to transfer the laboratory findings to the field. Finally growers and consumers must be convinced that agricultural products from somatic embryo based technology are both safe and necessary.

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