Use of In Vitro Breeding Strategies in the Development of Australian Native Plants

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Abstract

Plant biotechnology has emerged as a powerful tool of crop improvement and has aroused a great deal of interest in many countries because of its application to agriculture and horticulture.

We report here the application of a number of in vitro plant breeding techniques such as micropropagation, in vitro flowering, in vitro pollination and fertilisation, anther culture, somatic embryogenesis, and gene technology in the development of Australian native plants. The report covers an overview of our experience working with Australian plants in the past 25 or so years. Many of these plants have the potential to become future floricultural products.

INTRODUCTION

Plant tissue culture is a technique that exploits the ability of many plant cells to revert to a meristematic state. Although originally developed for botanical research, plant tissue culture has now evolved into important commercial practices and has become a significant research tool in agriculture, horticulture and in many other areas of plant science.

Plant tissue culture is the sterile culture of plant cells, tissues or organs under aseptic conditions leading to cell multiplication or regeneration of organs or whole plants. The steps required to develop reliable systems for plant regeneration and their application in plant biotechnology have been reviewed in countless books. Some of the major landmarks in the evolution of in vitro techniques are summarised in Table 1.

In this paper the current applications of this technology to agriculture, horticulture, forestry and plant breeding is briefly described with specific examples from Australian plants where applicable.

RAPID CLONAL PROPAGATION AND PATHOGEN ELIMINATION THROUGH MICROPROPAGATION

A major commercial application of plant tissue culture methods has been rapid clonal propagation of many plants. Micropropagation has changed from a laboratory curiosity to a commercial industry, and offers many advantages over the conventional methods of plant propagation. In vitro multiplication involves three main pathways:

(i) Axillary shoot formation is the true-to-type multiplication of plants from pre-existing meristems (axillary and apical).

(ii) Adventitious shoot prodcution is de novo bud formation on structures without a pre-existing meristem, for example leaf segments of *Begonia rex*, leaf petiole of *Saintpaulia* sp. or root segments of many species. In this system some off-types may be produced.

(iii) Callus production and somatic embryogenesis. Although much more productive than the previous two systems it often results in the production of "somaclonal variation" (Larkin and Scowcroft, 1981). This de novo pathway has enormous potential in the area of plant breeding, artificial seeds and genetic engineering.

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Micropropagation is widely used by many private and publicly funded companies around the world for mass production of plants. Many ornamental plants (orchids, gerberas, ferns, roses, carnations, lilies, etc), vegetables (tomato, carrot, celery, etc) food crops (cassava, potato, sugarcane), fruits (banana, pineapple, apple, strawberry, cherries), plantation crops (coconut, tea, cocoa) and spices (clove, cinnamon, ginger, turmeric) have been successfully propagated using tissue culture techniques. Generally speaking success has been relatively rapid with herbaceous plants. Many Australian native plants, including numerous rare woody plant species, have been successfully propagated using in vitro micropropagation techniques (Speer, 1993; Taji and Williams, 1996; Johnson and Burchett, 1996; Taji et al., 1997).

HAPLOID PLANTS AND ANTHER CULTURE

The cells of haploid plants contain a single complete set of chromosomes, and these plants are useful in plant-breeding programs for selection of desirable characteristics. Because they possess only a single set of chromosomes, even recessive mutations are phenotypically expressed. The purpose of anther and pollen culture is to produce haploid plants by induction of embryogenesis from repeated division of monoploid spores, either microspores or immature pollen grains. Plant breeders are especially interested in haploid plants as fertile double haploid homozygous plants could be obtained either by spontaneous doubling of the chromosome or by the application of ploidy inducing chemicals such as colchicine or oryzalin.

Although the number of successful pollen culture systems is relatively small, this technique has resulted in several improved varieties of crop plants in China (Hu and Yang, 1986).

Production of haploid plants through anther culture has not been exploited extensively with Australian native plants. However, in our own laboratory Olde (1994) and Zulkarnain (2003) have had some success in anther culture of Sturt's Desert Pea (*Swainsona formosa*).

SOMATIC EMBRYOGENESIS

The capacity of flowering plants to produce embryos is not restricted to the development of the fertilised egg. Embryos or embryoids can be produced under certain conditions in cultured plant tissues. This phenomenon was first observed by Steward et al., in 1958. To date somatic embryoids have been obtained in a large number of species including some Australian plants (Tetu et al., 1990; Lakshmanan and Taji, 2000).

Somatic embryogenesis is obtained either directly from the epidermis of immature or mature zygotic embryos or indirectly on callus or cell suspensions from the cultured explants. Frequently, the primary embryo is accompanied by direct secondary embryogenesis which may cause difficulty in the production of true bipolar somatic embryos (Sangwan-Norreel et al., 1991).

Although currently commercial micropropagation is mostly done by shoot-tip cultures, which requires a great deal of labour, somatic embryogenesis could be employed for large scale propagation with relatively little labour and time. In addition, if somatic embryos are encapsulated to produce artificial seed, most of the advantages found in seed propagation can be retained (Redenbaugh et al., 1986; McKersie and Bowley, 1993).

Successful production of Australian plants through somatic embryogenesis has been reported for *Eucalyptus* species (McComb et al., 1989; Watt et al., 1991), for a number of difficult to propagate Australian plants including *Lysinema ciliatum* a plant from Epacridaceae family (Senaratna, 2000) and for *Swainsona formosa* (Sudhersan and AboEl-Nil, 2002). Formation of embryos followed by their encapsulation to produce the artificial seed has enormous commercial potential especially in plantation crops such as *Eucalyptus* and pine.

IN VITRO FLOWERING

The transition from the vegetative to reproductive phase in plants, is an important developmental process with considerable practical importance in plant breeding. Despite decades of research and rapid advances in technology, our understanding of this important developmental process is still fragmentary. From the results of previous research, it is evident that the majority of plants use environmental cues to regulate flowering. Environmental variables with regular seasonal patterns such as temperature, photoperiod and irradiance are the key signals in floral induction. These factors are perceived by different plant parts, and strong and diverse interactions between the environmental variables are required for floral induction to occur in many species.

Classical physiological, genetic and grafting experiments, though invaluable in deciphering various aspects of flowering, have failed to unravel the true nature of the flowering stimulus or the mechanism(s) by which various environmental cues induce flowering. Novel approaches involving in vitro flowering and molecular techniques offer unique opportunities to investigate flowering process from new perspectives especially in species which are difficult to flower or produce flowers only once in several years (Taji, Kumar and Lakshmanan, 2002; Lakshmanan and Taji, 2003).

EMBRYO RESCUE

Embryo culture is one of the oldest applications of in vitro techniques, first applied to mature embryos 100 years ago (Hannig, 1904). The main use of embryo culture has been for the rescue of hybrid embryos where incompatibility often leads to embryo abortion. These embryos can often be excised before they abort and cultured to maturity in vitro. It can also provide a means of overcoming seed dormancy, particularly where the dormancy is induced by the surrounding seed tissues. A good example of the application of embryo rescue is the work of Drew et al. (1995) who have made extensive crosses between papaya and related species and have selected a variety of useful traits.

IN VITRO POLLINATION AND FERTILISATION

In vitro pollination and fertilisation is a technique in which male and female gametes are isolated and introduced to each other under conditions suitable for zygote development. It involves pollen tube penetration of the embryo sac via manipulation of maternal tissue, by methods other than the normal in situ process. Initially developed to bypass pre-zygotic incompatibility barriers, this technique has been used for the production of hybrids, the induction of haploid plants, overcoming sexual self-incompatibility, and in the study of reproductive processes, and pollen physiology. The diversity of applications is mirrored by the diversity of species to which this technique has been applied (Taji and Williams, 2003)

Embryo rescue is most applicable where in situ pollination and fertilisation are possible. However, in some cases where incompatibility prevents in situ pollination or fertilisation it may be possible to carry out in vitro fertilisation.

The point of incompatibility between genotypes may be anywhere along the developmental pathway from formation of compatible pollen to embryo growth. A common point of incompatibility is at the stigmatic surface. The incompatibility reaction between the stigma and the pollen grain either prevents pollen germination or it inhibits pollen tube growth down the style to the embryo. In these cases it may be possible to culture the female floral organs (ovary plus stigma), remove the stigmatic surface, then apply the pollen to the remaining surface (Taji and Williams, 1987). In extreme cases it is possible to inject the male nuclear material directly into the egg cell. This may lead to normal development of the embryo and seed within the ovarian tissues or embryo rescue may be necessary.

MICROGRAFTING

There is also scope for blending the traditional horticultural practice of grafting with micropropagation. Grafting enables the combination of selected stocks or scions

which are not successful on their own roots as well as providing various stress and pest resistances or growth controlling properties. Micrografting may involve either scions or rootstocks taken from in vitro stock plant banks utilising the phenomenon of rejuvenation that occur in vitro to enable adventitious rooting of mature rootstock selections. Micrografting may be carried out aseptically using in vitro rootstocks with either in vitro or disinfested ex vitro scion material, or on micropropagated rootstocks after transfer to the nursery. In our own laboratory, Kawaguchi (2002) was successful in micrografting *Swainsona formosa* to a number of rootstocks improving the plant's resistance to a number of soil borne diseases.

PROTOPLAST CULTURE AND FUSION

A protoplast is a plant cell from which the cell wall has been removed. After the removal of the rigid cell wall, the cell is surrounded by a thin plasmalemma membrane. Many uses of protoplasts are now being explored. The interest in protoplast fusion technique is related to the prospect that wider crosses than are possible by sexual means, due to incompatibility, may be achieved with protoplast fusion.

The fusion of plant protoplasts is not a particularly new phenomenon. Küster in 1909 described the process of random fusion in mechanically isolated protoplasts. However, the work of Cocking in 1960 and the enzymatic removal of cell wall have made protoplast isolation and fusion a practical reality.

Takebe et al. (1971) were the first group to successfully obtain divisions and regeneration of whole plants in tobacco mesophyll protoplasts. Carlson et al. (1972) obtained the first somatic hybrid in *Nicotiana tabacum*. Melchers et al. (1978) developed the fusion of protoplasts of potato and tomato and obtained the so called "pomatoes" and "topatoes" which were intergeneric hybrids.

Successes with somatic hybridisation have mainly involved the production of useful and fertile hybrid plants as a result of simple additive combination of the complete genomes of two unrelated species. However, production of cybrids which contain the nuclear and cytoplasmic genome of one parent and only the cytoplasmic genome of the second parent, and their potential use has been described by Gleba and Shlumukov (1990) and Galun (1993).

Use of protoplast technology is in its infancy with Australian native plants. Many laboratories are attempting the use of this technology for direct gene transfer. Teulieres and Boudet (1991) reported some preliminary attempts to isolate protoplasts from different species of *Eucalyptus.*

PLANT GENETIC ENGINEERING AND TRANSFORMATION

The addition of specific foreign genes to a plant species in order to enhance its properties is a technique with almost unlimited potential.

There are several approaches to inserting foreign genes into plants. These include direct DNA uptake by protoplasts (electroporation, polyethylene glycol (PEG)- induced uptake, micro-injection or sonication), direct DNA delivery by biolistic methods or indirectly via vectors (viral or bacterial). The system which has proved most successful is based on the T_i plasmid of a soil bacterium, *Agrobacterium tumefaciens*. Whilst vectors based on viral genome have also been extensively studied, none have yet been developed for general use in plant transformation. This is mainly due to the pathogenic nature of the virus, the restrictions on genome size and the fact that virus DNA is not stably transmitted to the progeny of infected plants.

Whilst extensive research has been undertaken in the area of genetic transformation of dicotyledonous species since the production of the first transgenic plants in 1983, only a limited number of agronomically important genes have been successfully transferred to crop plants. These genes confer resistance to certain herbicides, insects or viruses and have all been stably integrated into the genome of several species, including maize, soybean, oilseed rapes, cotton and tomato (Vasil, 1991). Due to the high cost, this technology is presently limited to crop species with potential for high economic

returns. It is anticipated that these molecular tools will be used routinely for the improvement of ornamental species in the foreseeable future.

Current efforts in genetic engineering in Australian native plants have been focussed primarily on species of *Eucalyptus,* because of their commercial importance (Chandler, 1995).

CONCLUDING REMARKS

Development of Australian plants using the in vitro technology has come a long way since the first comprehensive publication of Ron deFossard in 1976. His more recent book (deFossard, 1993) provides the reader with protocols developed for a large number of plants including many Australian native species.

Biotechnology is a very powerful tool to further advance the various fields of plant sciences. In practice it should be combined with "classical" breeding strategies and with conventional plant propagation practices. These tools are aiding the domestication and development of Australian native plant species as potential ornamental crops. Further advances in, and application of, plant biotechnology also require more basic research and indeed biotechnology provides some powerful tools by which we can extend our comprehension of the physiology, metabolism and developmental biology of plants. This is particularly important with Australian plants for which we still know very little about their unique biology.

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Table

Table 1. Historical landmarks in the evolution of plant cell and tissue culture technology.

* Gautheret, Nobecourt and White achieved the first success in developing plant tissue culture, e.g. Gautheret (1934) obtained callus formation from cultured explants of tree cambium and phloem tissue.
* After the discovery of cytokinins by Skoog and co-workers, Skoog and Miller in 1957 observed that the shoot and root formation are controlled by the auxin / cytokinin balance.
* In vitro somatic embryogenesis was first described by Steward et al. (1958) and Reinert (1958).
* Anther culture and production of haploid plants was achieved by Guha and Maheshwari (1964, 1966) and by Bourgin and Nitsch (1967).
* Protoplast culture, fusion, and development of somatic hybrids were described in the 1960s and 1970s (Cocking, 1960; Belliard et al., 1979; Gleba and Sytnik, 1984).
* During 1980s recombinant DNA technology and production of transgenic plants were achieved. For example Schell, 1987; Schell and Vasil, 1989.