Nutri Horticulture

K.V. Peter

Nutri-Horticulture

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Editor

Professor K.V. Peter

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Devotion

The edited book NUTRI-HORTICULTURE is devoted to my wife Mrs. Vimala Peter who entrusted me to the safe and protective hands of Prof. P.I. Peter Chairman, NoniBiotech, Chennai to continue my aptitude for science writing and editing. In the process she suffered loneliness and solitude.

Acknowledgement

I acknowledge with gratitude Prof. P.I. Peter Chairman NoniBiotech, Chennai for support and facilities extended. The academic atmosphere he has created around me is highly motivating and stimulating. I have the highest sense of appreciation for all the 31 scientists from India, Australia, Indonesia and Thailand who toiled hard to produce excellent chapters within stipulated time. Dr. Kirti Singh, my teacher and now Chairperson World Noni Research Foundation is always supportive in my academic pursuits. I express my gratitude to the publisher Mr. Anil Mittal of Daya Publishing House, New Delhi for patience, quality printing and above all gentlemanliness.

Foreword

The transformation of horticulture from a way of rural life, art, practice and skill oriented endeavor to a front line science based hort-business is a milestone in the history of human development. Nutritional security through horticulture is the mantra. In earlier days it was a part of science of botany, later life sciences and now a symphony of genetics, soil science, soil physics, microbiology, virology, bacteriology, entomology, biotechnology, nanotechnology, molecular biology, micro and macroeconomics. Many areas of applied sciences are made use of to synthesize plant types to yield high under stressed conditions-biotic and abiotic. The growth of the Division of Horticulture into four scientific divisions-Vegetable Science, Fruit Science, Floriculture and Landscape Planning, Plantation crops and Medicinal Plants-is reflected in the high rate of growth in the above crops during the 11th plan period. Many Agricultural and Horticultural Universities have an additional Division of Post Harvest Technology and Processing. The financial allocation for the horticulture sector rose from Rs.24.7 crores in IVth plan to Rs 16,000 crores in XIth plan in addition to sizeable allocations to tea, coffee, rubber, coir, spice and coconut. The sector launched central sector schemes like National Horticultural Mission, Technology Mission for North Eastern and Himalayan States, Bamboo Mission, Construction expansion and modernization of cold storages, Micro irrigation, Use of Plastics, National Agriculture Insurance Scheme (NAIS) etc. The Horticultural Research and Development infrastructure in India today is one of the best in the world with ten Central Research Institutes, 13 Project Directorates, 13 All India Coordinated Research Projects in addition to separate Universities for Horticulture in Himachal Pradesh, Andhra Pradesh, Karnataka, Tamil Nadu and several Colleges of Horticulture. National Horticulture Board, National Horticulture Research and Development Foundation, Agriculture Produce Export Development Agency, State Farming Corporation of India, Institute for Organic Farming, Ghaziabad, Central Institute of Horticulture, Medziphema, Nagaland; International Horticulture Innovation and Training Centre, Jaipur are established to provide R and D support to Indian Horticulture. Many Scientific Societies were established to provide forum for organizing discussion and exchange of ideas and develop fraternity among Horticulture Scientists, farmers and extension workers. The premier ones are South Indian Horticulture Association, Horticulture Society of India, Indian Society of Vegetable Science, Orchid Society of India, Indian Society of Spices, Indian Society of Plantation Crops etc. Many journals of high impact factor now carry referred research

articles. Indian Journal of Horticulture, Vegetable Science and Indian Journal of Plantation Crops are journal much read and referred. India also witnessed emergence of a large number of books, series and monographs authored and edited by Indian Scientists of repute.

The present book NUTRI-HORTICULTURE carries 17 chapters with the first chapter on 'Improving nutrition security and health for all-the important role of horticulture' and authored by 31 scientists and teachers of high standing. The emphasis on a science based horticulture calls for books on horticulture which focus on the science behind the processes and practices of horticulture. Dr. K.V. Peter, a Former Professor of Horticulture; Director, Indian Institute of Spices Research, Calicut; Director of Research Kerala Agricultural University; Vice-Chancellor KAU and now Director World Noni Research Foundation, Chennai was successful in bringing together 31 eminent scientists even from FAO, Rome to contribute chapters to this edited book.

I congratulate the publisher Daya Publishing House, New Delhi for publishing the much valued book.

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Vice-Chancellor
Dr. Y.S. Parmar University of Horticulture & Forestry
Solan, H.P.

Preface

Horticultural crops-vegetables, fruits, tubers, mushrooms-are the nature's gift for nutritional security. Plantation crops and spices provide the much needed foreign exchange to the country and livelihood security by enhanced purchasing power to all the stakeholders. Ornamentals and aromatic crops lend much needed life to living to the inhabitants of the country. India sustains a large number of horticultural crops of considerable economic value. The costliest saffron in Kashmir, the cheapest curry leaf in the tarai forests to ghats of south India, the divine Noni plant in Andaman and Nicobar Islands; the high priced cardamom and pepper of South India and an array of ornamentals make India a distinct paradise on earth. Despite the numerous advantages India has in terms of biodiversity, climate, soil, water and above all the second largest domestic market, the annual growth rate in production is lesser than 4 per cent. Productivity of horticultural crops are the lowest except for a few crops like rubber, potato and cabbage. Many of the fruit trees are of seedling origin and less productive. Seed replacement ratio in vegetables are very low and spread of hybrids and high yielding varieties very thin. Water, fertilizer, pests and diseases are limiting factors to higher productivity. Post harvest losses range 20 to 40 per cent in fruits and vegetables. Marketing of perishable fruits, vegetables and cut flowers is still a gray area. Many initiatives have been launched by the Government of India, a few are National Horticultural Mission(NHM), National Horticulture Board(NHB), Rashtriya Krishi Vikas Yojana (RKVY), National Mission on Micro Irrigation(NMMI), National Bamboo Mission(NBM) and Technology Mission for North Eastern and Himalayan States. The National Information Commission is mandated with reaching information to all in the country including the vast unreached. Use of ICT in the transfer of knowledge in horticulture would empower farmers, students, scientists, trade, processors and consumers as well to acquire nutritional security. The present book NUTRI-HORTICULTURE edited by Prof. K.V. Peter focuses on Nutritional security, science behind horticultural practices, water requirement of crops, rainwater harvesting, biological control of pests and diseases and related subject areas. Thirty one eminent scientists have contributed to the 17 chapters. I recommend the book to all the stakeholders in Horticulture.

Dr. Brahma Singh
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Introduction

Science based Horticulture encompasses and targets increased productivity and enhanced income to farmers at optimum and efficient use of space, time, water, energy and information, communication technology. Fruits, vegetables, ornamentals, plantation crops, spices, herbs, tuber crops, medicinal and aromatic plants, mushrooms and processing into value added products come under the preview of general horticulture. Majority of crops are non-calorific and provide the much needed protective and curative foods. The distinction between food security and nutrition security takes into cognizance the availability of fruits, vegetables and spices to diet of common man. In 2010, FAO estimated that a total of 925 million people were undernourished. These numbers indicate that millions of people who do not have access to sufficient quantities of food to meet their energy needs, let alone meet their nutrient requirements (Janice Albert-Chapter 1). The World Health Organization (WHO) categorized the major health risks and found that high blood pressure, high blood glucose, overweight and obesity are the leading causes of mortality irrespective of income levels. Underweight and micronutrient deficiencies cause millions of child death each year in poor communities. Infectious diseases are another major cause of death and they are often associated with low nutritional status consequent to negligible intake of fruits and vegetables. Horticulture can play a vital role in preventing the above diseases and promoting health and wellness. Community level projects in Africa and Asia demonstrated that promotion of nutrition/kitchen garden led to availability and consumption of vegetables and fruits which improved the nutritional status of children.

All the Horticultural crops-for that matter all the members of plant kingdom-are associated with micro and macro organisms both beneficiary and parasitic. Being an integral part of the biosphere, there is no independent and exclusive living for horticultural crops. Endophytes-prokaryotic bacteria, eukaryotic fungi and yeasts-have been isolated from a diverse group of plants and different plant organs, more frequently from roots. There is an emerging interest in endophytes in view of their potential significance as agents of plant growth promotion, stress alleviation, phytoremediation and as sources of bio-molecules and novel genes (Pious Thomas-Chapter 2). Of the nearly 3 lakh plant species which exist on earth, each individual plant is host to one or more endophytes. The capability

of colonizing internal host tissues has made endophytes valuable for horticulture as a tool to improve crop performance.

Productivity of majority of horticultural crops is low in developing countries like India except seasonal crops like cabbage and perennial tree crops like rubber. After the discovery of heterosis-hybrid vigour-, attempts are made to exploit the phenomenon in vegetables, fruits, ornamentals, spices, tubers and plantation crops. Hand emasculation and pollination being costly, male sterility was employed to develop female lines. Genetic, cytoplasmic and genic cytoplasmic male sterility were reported. Cytoplasmic male sterility being maternal in inheritance, its utility is enormous keeping the female characters intact in the progenies. Cytoplasmic male sterility was reported in tomato, chilli, brinjal, onion, chive, radish, carrot, Chinese cabbage, cabbage, sugar beet, potato, cucumber, plantago, alfalfa and petunia (Tembhune-Chapter 3). The cytoplasmic male sterility is affected by temperature as the responsible genes are temperature sensitive.

Another matter of concern for low productivity and crop loss is incidence of pests and diseases. In India alone annual crop loss due to pests, diseases and weeds are estimated to Rs. 600, 000 million in 2005. (Parvatha Reddy-Chapter 4). Integrated pest management is an important principle on which sustainable crop protection can be based. IPM is defined as "a pest management system that in the context of the associated environment and the population dynamics of the pest species utilizes all suitable techniques and methods, in a compatible manner as possible and maintains the pest populations at levels below causing economic injury". Biointensive IPM incorporates ecological and economic factors into agricultural system design and decision making and addresses public concerns about environmental quality and food safety. It is defined as a systems approach to pest management based on an understanding of pest ecology. It begins with steps to accurately diagnose the nature and source of pest problems and then relies on a range of preventive tactics and biological controls to keep pest populations within acceptable limits. Reduced risk pesticides are used if other tactics have not been adequately effective, as a last resort and with care to minimize risks".

Quality of seeds/planting materials governs upto 20 per cent of crop productivity. One reason for poor total production is low seed/planting material replacement ratio. One reason may be high cost of seed coupled with preference for local seeds for consumption. Micropropagation through tissue culture has come as an economically sustainable practice and TC plants are available in several fruits, vegetables, ornamentals, spices, tubers, aromatic and medicinal plants and plantation crops. Homogeneinity, earliness, resistance to biotic and abiotic stresses are a few desirable characters. *In vitro* breeding has been now standardized to develop recombinants and variants with marker and distinguishing traits (Zul Zulkarnain *et al.*-Chapter 5). Clonal micro propagation, somatic embryogenesis, somaclonal variation, *in vitro* micro grafting, haploid plant production, embryo rescue technology, *in vitro* flowering, *in vitro* pollination and fertilization, protoplast technology, plant genetic engineering and transformation etc. are areas of applied science of much relevance to Horticulture.

In perennial plants like fruit trees, root stock breeding has come to stay. Use of seedlings as root stocks has several disadvantages as the seedlings are heterozygous, heterogeneous and many times incompatible at late stage. Polyembryonic seedlings consisting of zygotic and nucellar can be easily identified by the faster growth of zygotic seedlings and they can be culled out at first sight. First reported in citrus by Leeuwenhoek (1719) and then confirmed by Strasburger (1878), polyembryony was described as formation of more than one embryo in a seed. Nucellar seedlings are true to types, they form genetically uniform rootstocks, more vigourous seedlings, virus free seedlings and budwood and above all have a tap root developing into a better root system(Roshan and Nongallei Pebam-Chapter 6).

Computational biology is a recent application of bioinformatics in scientific research. Applicable in all branches of science, its utility in plant science was realized lately; medical, space and atomic-nano sciences being the early users. Biological databases available online can be mainly classified into nucleotide sequence data bases, protein sequence databases, protein structural databases, protein-protein interaction databases, metabolic pathway databases, microarray databases and specialized databases which are developed for some very specific applications (Aimy Sebastian and Vibin Ramakrishnan-Chapter 7). Genome Sequencing and annotation-sequencing techniques; genomic annotation; Proteomics applications-protein structure prediction; protein function prediction; Evolutionary studies in plants; Microarray data analysis; Mass spectrometric Analysis; Computational Primer Designs and a glossary of computational terms would be reader friendly to biological scientists now introduced to Computational Biology.

Horticultural crop improvement is a continuing scientific pursuit. Floral biology reveals the nature of pollination-self, cross, often cross-. Methods of crop improvement through conventional breeding depend largely on the system of pollination. Self pollination is defined as intra floral, intraplant –interfloral, interplant pollen transfer when plants are apomictic and homozygous. Hermaphroditism, cleistogamy, absence of anaemophily and entomophily make self pollination inevitable. Introduction, selection-pure line, mass-;hybridization and selection-pedigree, bulk, single seed descent, back cross, multiple cross, dihaploidy-are the common methods of crop improvement in self pollinated crops.(Vanaja-Chapter 8).

Natural rubber is one of the industrial crops introduced to India from the tropical rain forests of Central and South America and now the second largest producer and the highest productivity recorder. The Rubber Research Institute of India, Kottayam has done pioneering research on introduction and evaluation of clones; management of fungal diseases like abnormal leaf fall and physiology and biochemistry of rubber. There are not many literature available to readers in plantation crops pertaining to biochemistry and physiology of latex production in rubber (Usha Nair *et al.*-Chapter 9). Latex is extracted by tapping the trunk of tree before sun rise. It is coagulated into semi solid mass by adding acid; crushed to remove water; smoked and dried. The chemistry of latex is interesting to read.

Water is one of the most important inputs in crops production. Each cell and tissue has free water and bound water. Free water is the ideal solvent of water soluable compounds and maintains turgour pressure so vital for the posture. Free water movement through osmosis has been dealt extensively elsewhere. Water constitutes 80 to 90 per cent of most plant cells and tissues in which there is active metabolism. Soil serves as the storage reservoir for water to be used by plants. Soil is a three phase system comprising of the solid phase made of mineral and organic matter, the liquid phase called the soil moisture and the gaseous phase called the soil air. Water is retained by a soil particle in the form of a thin film around it and is contained in the numerous small pores of the soil matrix with forces such as surface tension capillary, cohesion and adhesion. Hygroscopic, Capillary and Gravitational are the three forms in which water is held in the soil. Information on water requirement of horticultural crops are limited. The Chapter 10 by Joseph gives basic information on soil-plant-water relationship and methods of predicting water requirement of crops.

Rainwater is the main source of water for human domestic use, irrigation, hydro-energy generation, eco-biological diversity maintenance and sustenance. Water is the most precious and unique natural resource in our planet. A few statistics mention loss to the extent of 90 per cent flowing down to sea and ocean;4 per cent to fauna use and only 6 per cent to irrigation in hilly to semi-hilly terrain. In scanty rain fall areas like Africa; Middle East Countries; deserts of India rainwater is the only source of drinkable water. Desalinised water is costly and unavailable for irrigation. All these indicate the

need for scientific methods of rainwater harvesting (Visalakshi-Chapter 11). The chapter describes rainwater harvesting for direct use and for augmenting the groundwater storage.

Perennial trees are propagated vegetatively for uniformity in stand, fruiting and more tree density in high density orcharding. Dicots rather than monocots are more amenable for grafting. On planting grafted portion is held above ground to prevent rooting of scion. Biology of Grafting in dicot plants is elaborated with details in diagrams (Parameswaran and Renish Jayaraj-Chapter 12). Factors influencing the success of graft union are plant species and type of grafts; environmental conditions following grafting; growth activity of the root stock and virus contamination, insects and diseases. Graft incompatibility results from adverse physiological responses between grafting partners; virus transmission and anatomical abnormalities of the vascular tissues in the callus bridge.

Among biotic stresses affecting horticultural crops, fungi and bacteria cause significant damage and loss. Attempts to screen crop germplasm to sort out resistant genotypes are carried out in many laboratories. Among bacterial diseases wilt by *Ralstonia solanacearum* is devastating in tomato, brinjal and tomato. Among fungal diseases *Phytophthora infestans* causes leaf fall in many crops. The interdisciplinary research with plant breeders joining plant pathologists has yielded useful results in many crops. The screening techniques for disease resistance against fungal and bacterial diseases of vegetables are elaborated by Naik *et al.* (Chapter 13). The diseases covered are dieback and anthracnose, Phytophthora fruit rot and leaf blight, fusarium wilt in chilli and bell pepper; buck eye rot, late blight, early blight and bacterial wilt in tomato; foliar and fruit diseases, fusarium wilt in cucurbits; black rot and stalk rot in cruciferous vegetables and alternaria blight in cabbage.

There is a school of thought that the present estimate of post harvest loss of 30-40 per cent if brought down to an achievable 10-15 per cent, the horticultural crops availability can be increased to 20-25 per cent, not a mean achievement in any scale. Pre-harvest treatment, stage of harvest, genotype of cultivars, environmental parameters like temperature, humidity and rainfall, methods of packing, pre-packing preparations and above all controlled atmospheric treatments determine the extent of loss till the produce reaches the consumer in edible form. Shelf life is a European terminology emanated from the wooden cabinets in British homes to keep fruits and vegetables. The period range in which the cosmetic appeal of the produce keeps satisfying to the consumer was called shelf life. Post-harvest factors affecting shelf life are increased metabolic activity resulting from high humidity, high temperature and pre-contamination with fungi, bacteria and insect eggs/larvae. Hot water treatments are recommended to control different pathogens in fruits. Vapour treatment, fumigation, chemical treatments-washing with ozonized water, chlorine solution, calcium application and use of growth regulators-can minimize post harvest loss (Ram Asrey and Kalyan Barman-Chapter 14).

It is observed that scientists, teachers and students are not kept informed about the history of both basic and applied sciences and resultant innovations. A knowledge on history will throw light on the men, methods and materials, background information and the way results were interpreted and later applied for human welfare. Two chapters (Nair *et al.*-Chapter 15 and Jijii Rajmohan-Chapter 16) deal with history of coconut breeding and history of Nematology Research. Coconut provided thirst quenching drink to early mankind. It is called tree of life for its diverse uses of every part of the palm with more than 100 different products including for food and drink, fodder for livestock, fibre, cosmetics and timber. The root has medicinal properties, leaf has many uses from thatching to fuel to composting etc. Organized coconut breeding started in India in 1916 at the erstwhile Central Coconut Research Station, Kasargod (presently CPCRI) and Research Stations now under Kerala Agricultural University. In Sri Lanka, research work on coconut began with the inception of the Coconut Research Institute in 1928 (Chapter 15-Nair *et al.*). Breeding new varieties and hybrids is a long drawn and slow process

because of its floral biology, heterozygous nature, low rate of sexual propagation with one seedling per nut, lack of selection procedures for isolation of superior hybrid seedlings, lack of reproducible asexual methods for rapid multiplication, prolonged interval between generation and long juvenile phase before flowering. Methods of breeding followed are selection-motherpalm selection, seedling selection, identification of prepotent palm-and heterosis breeding. Tall x Dwarf hybrids and Dwarf x Tall hybrids were developed. Dwarf x Tall hybrids are found efficient in point of view of hybrid production. Breeding for special characteristics like disease resistance, drought resistance and nut water quality have made much headway.

Chapter 16 (Jijii Rajmohan) elaborates history of nematode research. Nematodes are triploblastic, bilaterally symmetrical, unsegmented, pseudoelomate and vermiform animals. They exist almost every where in nature. The different groups of nematodes are fungal feeders, bacterial feeders, predators, animal parasites, algal feeders, omnivores and plant parasites. Landmarks in the History of Nematology Research are reviewed from literature since 1873 when Butschli described the morphology of free-living nematodes. In India Barber, then the economic botanist at Coimbatore, studied on root knot infesting tea in South India in 1901. He identified root knot nematode in black pepper in 1906. Extent of crop loss due to Nematode attack is estimated 5 per cent in oil seeds, 8 per cent in cereals and pulses, 10 per cent in fruit crops and 12 per cent in vegetables. Symptoms of attach vary from leaf discolouration, dead or devitalized buds, seed galls, twining of leaves and stem and lesions on leaves and stem. Above ground symptoms are stunting, discolouration of foliage, decline or dieback and wilting.

The 17th Chapter by Ashok and Mani pertains to marketing management of crops. It deals with marketing environment, marketing mix, marketing segmentation, marketing information system and marketing potential. Forecasting in marketing is also dealt with.

The edited book NUTRI-HORTICULTURE carries 17 chapters authored by 31 scientists from 15 Universities/Research Institutes.

K.V. Peter

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Chapter 5

In vitro Breeding Strategies in the Development of Australian Native Plants

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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 sciences.

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 are reviewed in countless books. Some of the major landmarks in the evolution of *in vitro* techniques are summarised in Table 5.1.

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

Clonal Micropropagation

The general term tissue culture is used to refer to the *in vitro* culture of various types of plant parts including stems, leaves, roots, flowers, callus, cells, protoplasts and embryos. These parts, known as explants, are isolated from the *in vivo* condition and cultured on an artificial sterile medium so that they regenerate and differentiate into new intact plants (Street, 1973). A more specific term, micropropagation, is applied to indicate the use of tissue culture techniques to the plant propagation system started with very small plant parts (explants) grown aseptically in a test tube or other similar

containers (Hartmann *et al.,* 1990). However, it is often found in practice that these two terms are used interchangeably to describe any plant propagation technique involving aseptic culture.

Table 5.1: 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. 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 (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.* (1958b) and Reinert (1958a).
- ☆ 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 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 (Schell,1987 and Schell and Vasil,1989).

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) are successfully propagated using tissue culture techniques. Generally speaking success is relatively rapid with herbaceous plants. Many Australian native plants, including numerous rare woody plant species, are successfully propagated using *in vitro* micropropagation techniques (Speer, 1993; Johnson and Burchett, 1996; Taji and Williams, 1996; Taji *et al.,* 1997)

In vitro multiplication involves three main pathways. These are axillary shoot formation, adventitious shoot production and somatic embryogenesis. Axillary shoot formation is the true-to-type multiplication of plants from pre-existing meristems (axillary and apical). This type of tissue culture involves an expanded shoot of terminal and lateral growing points where axillary shoot proliferation is promoted and the growth of terminal shoot is suppressed. Hartmann *et al.* (1990) stated that this condition enables the multiplication of microshoots, which can be excised and rooted *in vitro* to produce microplants, or which can be cut into microcuttings to be rooted outside the *in vitro* system.

The advantage of using axillary shoot proliferation from meristem, shoot tip or bud as a means of regeneration is that the incipient shoots have already been differentiated *in vivo.* Only shoot elongation and root differentiation are required to establish a complete plant. *In vitro* organogenesis and embryogenesis, on the other hand, must undergo developmental changes which usually involve callus formation (Hu and Wang, 1983) that frequently causes genetic mutation in regenerated propagules.

The term adventitious shoot refers to the shoots arising from any plant parts other than the leaf axil or shoot apex (Bhojwani and Razdan, 1983). In other words, adventitious shoot production 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 other species. Hartmann *et al.* (1990) stated that adventitious shoot induction includes initiation of adventitious shoot development either directly

from the explant or indirectly from the callus that is produced on the explant as a result of wounding and growth regulator treatment.

Although adventitious shoot induction offers a great potential for rapid plant propagation, Boulay (1987) claimed that there are several problems associated with this technique. The first is the problem of obtaining true-to-type genetic copies which is the main purpose of clonal propagation. This particularly appears when the adventitious shoot proliferation passes through an intervening callus stage. There is also variation in growth behaviour of resulted plants. It is found that some copies are not always juvenile. Some clones manifest plagiotropic growth and poor vigour, whereas others exhibit orthotropic growth and vigour typical of young seedlings. These problems may arise from damage to meristems of clonal stock during the propagation process.

Somatic Embryogenesis as a Mean of Plant Regeneration

Somatic embryogenesis was first demonstrated by Steward *et al.* (1958c; 1958b) and Reinert (1959) in cultures of carrot tissues. Somatic embryogenesis is a remarkable developmental process by which non-sexual cells undergo a developmental sequence, similar to that seen in zygotic embryos, without the need for sexual reproduction. Somatic embryogenesis is influenced by the genotype, explant source, developmental stage, culture medium and other inductive factors, like the mineral composition of the media, type and concentration of carbon sources, amino acids, heavy metal ions and manipulation of environmental conditions (Tapingkae *et al.,* in press).

Somatic embryogenesis has become one of the most desired pathways in the regeneration of plants via tissue culture because it bypasses the necessity of time-consuming and costly manipulation of individual explants, which is a problem with organogenesis (Folta and Dhingra, 2006; Carneros *et al.,* 2009). To date somatic embryoids are obtained in a large number of monocotyledonous and dicotyledonous plant species including some Australian plants (Lakshmanan and Taji, 2000; Taji and Williams, 2005).

Application of somatic embryogenesis to Australian plants was reported for *Eucalyptus* species (Watt *et al.,* 1991; McComb *et al.,* 1996; Prakash and Gurumurthi, 2010), Australian cotton cultivars (Zhang *et al.,* 2009; Yan *et al.,* 2010), sorghum (Sargent *et al.,* 1997), papaya (Sieler *et al.,* 1993; Ernawati *et al.,* 1997), sedges and rushes (Sieler *et al.,* 1993).

Somatic embryogenesis provides a possible *in vitro* system for a number of difficult to propagate Australian native species including *Lysinema ciliatum* (Senaratna, 2000), *Leucopogon verticillatus* (Anthony *et al.,* 2004), *Swainsona formosa* (Sudhersan and AboEl-Nil, 2002), fanflower *Scaevola aemula* (Wang and Bhalla, 2004; 2006), Koala Fern *Baloskion tetraphyllum* (Panaia *et al.,* 2004; Ma *et al.,* 2006) and Black Kangaroo Paw *Macropidia fuliginosa*, *Stirlingia latifolia* and *Lepidosperma squamatum* (Panaia *et al.,* 2005).

Embryogenic callus was established for Australian monocots genera like *Anigozanthos*, *Blandfordia* and *Thysanotus* which are used as cut flowers or pot plants. However, the process of somatic embryogenesis was not fully utilised and applied to these plants (Johnson, 1997). In the study on waxflowers, a plant regeneration system via somatic embryogenesis of leaf and immature seed tissues was developed for *Chamelaucium repens* and *C. uncinatum* (Ratanasanobon, 2007).

Extensive *in vitro* research and their success are achieved for a number of hardwoods including the *Pinus*, *Eucalyptus*, and *Acacia* species (Midgley and Turnbull, 2003; Merkle and Nairn, 2005; Nehra *et al.,* 2005). If somatic embryos are encapsulated to produce artificial or synthetic seeds, it has enormous potential to reduce the cost of production, reduce loss of biodiversity, increase the efficiency of

rehabilitation programs and increase the supply of many Australian native species to the horticultural industry.

Somaclonal Variation in Micropropagation

Genetic stability during micropropagation is controlled by many factors including genotype, presence of chimeral tissue, explant type and origin, culture medium, culture conditions and duration of culture (reviewed by Debnath and Teixeira da Silva, 2007). The occurrence of variation in plants regenerated from *in vitro* cultures was named as 'somaclonal variation' by Larkin and Scowcroft (1981). Somaclonal variation is one of the most important concerns in commercial micropropagation. It can be distinguished by their morphological, biochemical, physiological and genetic characteristics. Molecular markers are powerful tools in genetic identification of somaclonal variation.

Rapid and efficient *in vitro* regeneration methods which minimise somaclonal variation are critical for the genetic transformation and mass propagation of commercial varieties. Although somaclonal variation is undesirable for commercial micropropagation, it is useful to crop breeders, where variation in tissue culture regenerated plants from somatic cells can be used in the development of crops with novel traits.

The mechanism of somaclonal variation is still not completely understood; however, both genetic and epigenetic mechanisms are believed to play a role (Gao *et al.,* 2009). Somaclonal variation has been associated with changes in chromosome number and structure, point mutations and alteration in DNA methylation (reviewed by Baenziger *et al.,* 2006; Bartoszewski *et al.,* 2007; Santana-Buzzy *et al.,* 2007). Somaclonal variation frequency is determined by a number of factors, including genotype, explant source, medium composition and duration of culture (reviewed by Predieri, 2001). Turner *et al.* (2001) found that genetic fidelity and shoot apex viability (for cryopreserved material) of *Anigozanthos viridis* subsp. *terraspectans* (Haemodoraceae), a threatened plant from south west Australia, were maintained following tissue culture, cold storage and cryostorage of *A. viridis* subsp. *terraspectans* for 12 months. Lakshmanan *et al.* (2006) developed the rapid and efficient *in vitro* regeneration methods which minimise somaclonal variation for the genetic transformation and mass propagation in commercial varieties of sugarcane (*Saccharum* spp. interspecific hybrids).

In vitro 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 biotic stresses and pest resistances or growth controlling properties. Reduction in vigour, yield and quality are usually attributed to various virus, bacteria and fungi infections leading to loss of productivity and commercial usage of plants. There are methods like thermotherapy and meristem culture available to overcome pathogen infection problems. However, their involvement in recovering pathogen free in tree species is very limited. Micrografting, on the other hand, offers an alternative strategy of producing pathogen free plants (Tangolar *et al.,* 2003; Naz *et al.,* 2007).

Micrografting may involve either scions or rootstocks taken from *in vitro* stock plant banks utilising the phenomenon of rejuvenation that occurs *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 (Taji *et al.,* 2002). Since it was first introduced by Murashige *et al.* (1972) and followed by the success of Navarro *et al.* (1975), this technique is now routinely used by several commercial fruit nurseries for

eliminating graft-transmitted viral diseases, for rejuvenation of parent stock and for mass production. Standardized procedures exist for citrus, cherry, apple, grape and avocado (Obeidy and Smith, 1991; Tangolar *et al.,* 2003; Suarez *et al.,* 2005). Micrografting applied to woody species shows rather slow progress (Detrez, 1994; Reid *et al.,* 2001), the kinds of plants which can be propagated by micrografting will diversify in the future. In our own laboratory, Kawaguchi and Taji (2005) and Kawaguchi *et al.* (2008) were successful in micrografting *Swainsona formosa* to a number of rootstocks improving the plant's resistance to a number of soil borne diseases.

There are a number of benefits in *in vitro* micrografting system. It may be of great value in studying stock-scion relationship, because with *in vitro* system it is easier to isolate and identify the special compounds produced from grafted plants and to screen new scion-rootstock combinations. All types of *in vitro* grafting can be used to evaluate incompatibility, since symptoms of incompatibility under *in vivo* conditions are the same as under *in vitro* conditions. In addition, virus elimination in fruit trees has become the major application of *in vitro* micrografting (Taji *et al.,* 2002; Suarez *et al.,* 2005; Naz *et al.,* 2007).

Factors affecting the success of *in vivo* micrografting are also known to influence the success of *in vitro* micrografting. Obeidy and Smith (1991) suggested that variables associated with proper attachment of stock and scion, protection of the union during healing and removal of grafting implements after healing had significant influence on success of the procedure. In addition, Tangolar *et al.* (2003) claimed that different factors like the age of plant materials and grafting technique could affect the success rate of micrografting. There are also many reports on the success of *in vitro* micrografting under different environmental culture conditions and growth hormones used (Naz *et al.,* 2007).

Table 5.2: A Few Examples of Species which are Successfully Used as Rootstocks and Scions in *in vitro* Micrografting

Rootstocks	*Scions*	*References*
Acacia tortilis subsp. *raddiana*	*Acacia tortilis* subsp. *raddiana*	Detrez (1994)
Citrus jambheri Lash	*Citrus nobilis* Lour x *C. deliciosa* Tenora	Naz *et al.* (2007)
Citrus limon L. Burm. f.	*Citrus paradisi* Macf.	Obeidy and Smith (1991)
Citrus limon L. Burm. f.	*Citrus limon* L. Burm. f.	Obeidy and Smith (1991)
Citrus limon L. Burm. f.	*Citrus sinensis* L. Osbeck	Obeidy and Smith (1991)
Citrus paradisi Macf.	*Citrus limon* L. Burm. f.	Obeidy and Smith (1991)
Citrus sinensis L. Osbeck	*Citrus limon* L. Burm. f.	Obeidy and Smith (1991)
Malus domestica Borkh.	*Malus domestica* Borkh.	Obeidy and Smith (1991)
Persea americana Mill.	*Persea americana* Mill.	Suarez *et al.* (2005)
Prunes cerasus L.	*Prunes cerasus* L.	Obeidy and Smith (1991)
Swainsona formosa (G. Don) J. Thompson	*Clianthus puniceus*	Kawaguchi and Taji (2005)
Vitis vinifera L.	*Vitis vinifera* L.	Tangolar *et al.* (2003)

The benefits of micrografting are reported by many authors, particularly on the elimination of viral diseases (Murashige *et al.,* 1972; Navarro *et al.,* 1975; Suarez *et al.,* 2005; Naz *et al.,* 2007). To date, *in vitro* micrografting is extensively used to recover disease-free plants from the commercial citrus

varieties and is becoming a potential tool for separating virus and virus like agents (Navarro, 1981). The apical meristem contains little or no virus titre and meristematic cells grow faster than all viruses. Therefore, production of disease-free foundation plants by micrografting remains the only means to supply disease-free bud stocks to the growers. The challenge of successfully grafting the very small and delicate materials from *in vitro* plants has warranted development of novel techniques to facilitate the process. Furthermore, this technique is useful in shortening the juvenile phase in tree crops, and considerably reduces the time in breeding programs (Taji *et al.,* 2002).

On the other hand, however, *in vitro* micrografting is a cumbersome, tedious and extremely time-consuming exercise. The refinements in media composition and techniques as well as growth factors in micrografting process may result in viable means of producing high yielding and pathogen-free plants. More studies are required to evaluate the degree of juvenility re-established by such techniques with the objectives of obtaining true-to-type propagation.

Haploid Plant Production

Obtaining homozygous lines from highly outcrossing species would take a long time (Williams and Taji, 1992). The process starts with cross-pollination to combine desirable parental traits resulting in heterozygous but genetically uniform offspring. Reproduction of these offspring is frequently accompanied by the separation of homologous chromosomes and genes from different parents at meiosis and production of genetic variability within the population of the next generation (Croughan, 1995). Therefore, the overall process of producing homozygous lines as predicted by Ferrie and Keller (1995) may take 10 years or even more, depending on the plant species. In contrast, use of microspore embryogenesis *via* anther or microspore culture is valuable for the detection of recessive gene traits and the exploitation of gametoclonal plants (Christou, 1992). More importantly, this technique offers the opportunity to generate pure homozygous lines more rapidly and efficiently than with conventional ways (Tomasi *et al.,* 1999). Taji *et al.* (2002) claimed that such lines could be generated in only one generation, while conventional methods need at least five generations.

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 like colchicine or oryzalin. Through chromosome doubling treatment, it is possible to produce homozygous, fertile, doubled-haploid and pure breeding lines (Ferrie and Keller, 1995). Thus, microspore embryogenesis makes mutational breeding and selection of beneficial traits possible.

Since it was first demonstrated by Guha and Maheshwari (1964) on *Datura inoxia* and Nitsch and Nitsch (1969) on tobacco, haploids were used to produce homozygous genotypes in a number of economically important monocotyledonous and dicotyledonous species (Table 5.3). Production of haploid plants through anther culture has not been exploited extensively with Australian native plants. However, in our own laboratory Tade (1992), Olde (1994) and Zulkarnain (2003) have some success in anther culture of Sturt's Desert Pea (*Swainsona formosa*).

Haploid technology is of significant interest for developmental and genetic research, as well as for plant breeding and biotechnology. Haploid plants are useful in understanding cellular totipotency because they develop from single male or female gametes without fertilization (Powell, 1990). Haploid individuals also provide an excellent example when studying induced mutagenesis, where recessive traits can be easily detected (Seguí-Simarro and Nuez, 2008b). Tolerance to unfavourable conditions

like drought, cold, heavy metals or low nutrients, are amongst recessive traits which can be detected promptly in haploid plants. The problems associated with outcrossing and self-incompatibility in some species may also be solved by microspore embryogenesis (Taji *et al.,* 2002).

Table 5.3: Examples of Haploid Plants which are Successfully Regenerated *via* Microspore Embryogenesis

Species	*References*
Albizzia lebbeck	Crosser *et al.* (2006)
Anemone sp.	Custers *et al.* (2001)
Brassica napus	Maluszynsky *et al.* (2003)
Cajanus cajun	Crosser *et al.* (2006)
Delphinium sp.	Custers *et al.* (2001)
Glycine max	Crosser *et al.* (2006)
Hordeum vulgare	Maraschin *et al.*(2005)
Lupinus spp.	Bayliss *et al.* (2002)
Malus domestica	Höfer *et al.* (1999)
Medicago sativa	Zagorska *et al.* (1997)
Nicotiana tabacum	Maluszynsky *et al.* (2003)
Oryza sativa L.	Aryan (2002),
Peltophorum pterocarpum	Crosser *et al.* (2006)
Populus sp.	Hyun *et al.* (1986)
Psophocarpus tetragonolobus	Crosser *et al.* (2006)
Triticum aestivum	Maluszynsky *et al.* (2003)
Vigna unguiculata	Crosser *et al.* (2006)
Zantedeschia sp.	Custers *et al.* (2001)

However, haploid individuals tend to be smaller in size, less vigorous, more sensitive to diseases and environmental stresses and most importantly, they are sterile (Seguí-Simarro and Nuez, 2008b). Therefore, for practical purposes, it is usually desirable to obtain doubled haploids. Regardless of the mode of development, doubling of haploid plants either spontaneously via endoreduplication (DNA duplication without mitosis), nuclear fusion (merging of coalescing nuclei into a larger nuclei, mixing both DNA contents), endomitosis (mitosis in the absence of mitotic spindle and nuclear envelope breakdown) or by chemical means like colchicine or oryzalin treatments, leads to a homozygous doubled haploid individual with two identical copies of each chromosome (Crosser *et al.,* 2006). For many families of angiosperms, doubled haploidy is used as a routine tool in breeding programs because this technique provides pure lines in a single generation, which may save considerable time in the breeding of new cultivars (Tuvesson *et al.,* 2000; Maluszynsky *et al.,* 2003).

Embryo Rescue Technology

Embryo rescue techniques like embryo culture, ovule culture and ovary culture, are used in interspecific and intergeneric hybridisation programs. Embryo rescue holds great promise not only for effecting distant crosses, but also for obtaining plants from inherently weak and immature embryos, obtaining haploid plants as well as for shortening the breeding cycle. Moreover, embryo rescue can

provide a means of overcoming dormancy of recalcitrant seeds (Raghavan, 2003; Taji and Williams, 2005).

A good example of the application of embryo rescue is the work of Drew *et al.* (2006a; 2006b) who developed a successful embryo-rescue and culture protocol for use with the intergeneric hybrids between *Carica papaya* L. and *Vasconcellea quercifolia*, which is papaya ringspot virus type P resistant. Palmer *et al.* (2002) obtained many of the new hybrid plants from the crosses between indigenous *Vigna* species and mungbean cultivars grown in Australia. By employing embryo rescue, a hybrid between *Sorghum bicolor* and *S. macrospermum* was retrieved and cultured *in vitro* by Dillon *et al.* (2007).

The successful production of plants from the cultured embryos largely depends on parental genotype, maturation stage and medium composition (Palmer *et al.,* 2002). Liu *et al.* (2008) reported that culture medium, genotype and year of cross affected the embryo development and recovery from *in vitro* cultured ovules in breeding stenospermocarpic seedless grape varieties for the Australian table and dried grape industries. Early embryo rescue of wax flower hybrids is the major research activity in the tissue culture laboratory in Western Australia (Shan, 2007). It is impossible to imagine modern plant breeding without embryo rescue technique.

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. The *in vitro* technique is a useful strategy for studying flowering physiology in vascular plants (Van Staden and Dickens, 1991). 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 *et al.,* 2002; Lakshmanan and Taji, 2004).

Factors controlling *in vivo* flowering are also found to be important in the induction of *in vitro* flowering. In addition, studies on the *in vitro* flowering have unravelled many aspects of flowering more than *in vivo* investigations have. The involvement of factors like photoperiod (Singh *et al.,* 2006), light intensity (Sheeja and Mandal, 2003), light quality (Victorio and Lage, 2009) and plant growth regulators (Singh *et al.,* 2000; Britto *et al.,* 2003; Amutha *et al.,* 2008; Kanchanapoom *et al.,* 2009) are clearly critical for the induction of flowering *in vitro.*

To date, the significant aspect of *in vitro* flowering is that the capability of producing fertile flowers is more quickly than *in vivo* system. This is important in breeding of plants which are difficult to flower during *in vivo* culture like orchids, or produce flowers once in several years like bamboo. An example of the success of producing orchid fertile flower under *in vitro* system was reported in *Dendrobium* hybrids (Hee *et al.,* 2007; Sim *et al.,* 2007; Sim *et al.,* 2008). Using one of the hybrids, *Dendrobium* Chao Praya Smile, as a model system, flowers were produced following 5-6 months *in vitro* seed sowing by appropriate benzyladenine treatment. This duration is much shorter than the 2-

3 years required using conventional growing method. As a result, early evaluation of the characteristics of flowers is possible. In addition, following *in vitro* flowering, pollination is also performed *in vitro* with formation of viable seeds. Hence, the breeding cycles of orchid were reduced significantly.

Another example illustrating the significant contribution of *in vitro* flowering is the breeding strategy of bamboos. Bamboos are one of groups of plants which have a peculiar behaviour of flowering and seeding at the end of a very long vegetative growth phase. In nature, flowering of bamboo takes place after 12 to 24 years of growth and then the plants dying at the end of fruiting season (Taji *et al.,* 2002). Because of the peculiar flowering habits, it was almost impossible to breed for superior traits in bamboos. This situation, however, was changed following Nadgauda *et al.* (1990) reports on the first success of *in vitro* flowering in bamboos *Bambusa arundinacea* and *Dendrocalamus brandisii,* and subsequently in a few more species of bamboos (Chambers *et al.,* 1991; Rout and Das, 1994). Since then *in vitro* flowering is observed in many types of bamboos (Gielis *et al.,* 2002).

Table 5.4: Examples of Species which are Successfully Induced to Flower *in vitro*

Species	*References*
Ammi majus L.	Pande *et al.* (2002)
Bambusa edulis	Lin *et al.* (2004)
Basilicum polystachyon (L.) Moench	Amutha *et al.* (2008)
Ceropegia bulbosa Roxb. var. *bulbosa*	John Britto *et al.* (2003)
Citrus limon	Tisserat *et al.* (1990)
Citrus nobilis x *C. deliciosa* Tenora	Singh *et al.* (2006)
Cymbidium ensifolium var. *misericors*	Chang and Chang (2003)
Dendrobium hybrids	Hee *et al.* (2007), Sim *et al.* (2007,2008)
Dendrocalamus strictus	Singh *et al.* (2000)
Eulophia graminea Lindl.	Chang *et al.* (2010)
Gentiana triflora	Zhang and Leung (2000), Zhang and Leung (2002)
Hypericum brasiliense	Abreu *et al.* (2003)
Kniphofia leucocephala	Taylor *et al.* (2005)
Lycopersicon esculentum Mill.	Sheeja and Mandal (2003)
Murraya paniculata (L.) Jack.	Jumin and Ahmad (1999)
Nicotiana tabacum L.	Peeters *et al.* (1991)
Pharbitis nil	Galoch *et al.* (2002)
Psygmorchis pusilla	Vaz *et al.*(2004)
Rosa hybrida L.	Kanchanapoom *et al.* (2009)
Streptocarpus nobilis	Floh and Handro (2001)
Swainsona formosa (G. Don) J. Thompson	Tapingkae *et al.* (2009)

More than 10 years after the first report on *in vitro* flowering in bamboo, some positive results are obtained on a wide range of monocotyledonous and dicotyledonous species (Table 5.4), but practical and commercially exploitable results were not reported yet. Many hurdles still need to be overcome before the methods really become applicable at agricultural and commercial scales. The major challenge

at the moment is to establish reliable methods which allow continuous production of fertile flowers and viable seeds of desired species. Taji *et al.* (2002) suggested that the physiological state of donor plant, the properties of culture medium, as well as environmental and endogenous signals are among other factors which need to be considered to induce *in vitro* flowering reliably. Undoubtedly, the *in vitro* flowering system capable of producing viable seeds would be a valuable approach in enhancing the breeding programs of those species with a long juvenile growth phase.

In vitro Pollination and Fertilisation

In vitro pollination and fertilisation is a method in which male and female gametes are isolated and introduced to each other under optimum conditions for zygote development. It involves pollen tube penetration of the embryo sac by manipulation of maternal tissue and by methods other than the normal *in situ* process. Initially developed to bypass pre-zygotic incompatibility barriers, this technique was used for the production of hybrids, the induction of haploid plants, overcoming sexual self-incompatibility and in the study of pollen physiology and fertilization (Taji and Williams, 2004).

Various *in vitro* methods are developed to overcome incongruity barriers in a number of plant species (Taji *et al.*, 2002; Taji and Williams, 2005). Sexual barriers preventing interspecific hybridisation are divided into pre- and post-fertilization barriers (Stebbins, 1958). Pre-fertilization barriers are bypassed using *in vitro* pollination and fertilization. *In vitro* pollination enhances the possibility of pollen tube penetration into the egg apparatus and central cell. Once fertilization has occurred, hybrid embryo growth is restricted by post-fertilization barriers. Post-fertilization barriers could be overcome by using embryo rescue followed by *in vitro* culture techniques (Van Tuyl *et al.*, 1997). The technique of *in vitro* pollination and fertilization was first reported in 1962 in poppy, *Papaver somniferum* (Kanta *et al.*, 1962). Since that time, several species were pollinated successfully and fertilized *in vitro* including *Swainsona laxa*, a rare Australian plant (Taji and Williams, 1987).

Interspecific hybridisation is an important research area in the genus *Sorghum* because of the possibility of transferring the genes for resistance to important insects and pathogens from wild Australian *Sorghum* species to the grain sorghum (*Sorghum bicolor*) genome (Price *et al.*, 2006). Several diseases limit pawpaw (papaya) production in Australia but the main concern is papaya ringspot virus-type P (PRSV-P). Protocols were developed to produce large number of intergeneric hybrids between *Carica papaya* L. and *Vasconcellea quercifolia*, which is PRSV-P resistant. A very efficient protocol was developed for both the rescuing and the germination of the embryos. Most of the rescued embryos produce embryogenic callus and multiple plantlets. The results demonstrate that efforts in wide hybridization to transfer PRSV-P resistance to *C. papaya*, are better directed towards crosses between *C. papaya* and *V. quercifolia* than with other *Vasconcellea* species (Drew *et al.*, 2006a; Drew *et al.*, 2006b).

The success of *in vitro* pollination, fertilization and subsequent production of viable seed depend on a number of exogenous and endogenous factors (Dusi *et al.*, 2010; Skalova *et al.*, 2010). The main exogenous factor is the composition of culture media. The most important endogenous factor seems to be ovule and pollen grain maturity.

Protoplast Technology

The plant protoplast consisting of cytoplasm and nucleus with the cell wall removed, provides a unique single cell system to underpin several aspects of modern biotechnology. Protoplasts can be isolated mechanically by cutting or breaking the cell wall, and by digesting it away with enzymes or by a combination of mechanical and enzymatic separation (Davey *et al.*, 2005). Isolated protoplasts

serve as the field of somatic cell cloning and development of mutant lines. Teulieres and Boudet (1991) reported preliminary attempts to isolate protoplasts from *Eucalyptus globulus.*

Reliable procedures are available to isolate and culture protoplasts from a range of plants (Debnath and Teixeira da Silva, 2007). Several factors especially the source tissue, culture medium and environmental factors, influence the ability of protoplasts and protoplast-derived cells to express their totipotency and to develop into fertile plants. Recently, direct differentiation of globular embryo structures from mesophyll protoplast cultures of *Scaevola aemula* (fan flower) was reported (Wang, 2010). This is the first achievement of direct differentiation of embryo structure in protoplast culture of Australian native plants. It will enhance the study of embryo development in Australian native plants which are well known for their low seed viability and germination.

Protoplast fusion comprises of removal of cell wall and then fusion of cell contents. Somatic hybridization by protoplast fusion and plant transformation could overcome many of the barriers to interspecific hybridization among plant species or genera. The hybrid plants are then assessed for desirable new traits. Traits of interest range from disease resistance, increased nutritional value (in the case of food crops), seedlessness, improved vigour to other stress factors, etc.

Plant cell and protoplast isolation and fusion techniques have become important and well-accepted methods for studying the physiology, biochemistry and breeding of plants. Since the first successful report on somatic hybridization with tobacco (Carlson *et al.,* 1972), the potentials of somatic fusions are exploited for crop improvement in many crops including rice, wheat, rapeseed, canola, tobacco, tomato, potato and citrus (Guo *et al.,* 2004; Tapingkae *et al.,* in press).

Successes with somatic hybridization 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 genome of one parent and either the cytoplasmic genome of the other parent or a combination of both parents, and their potential use has been a common approach in plant improvement (Guo *et al.,* 2004; Grosser and Gmitter, 2005). Successful somatic cell hybridization depends totally on development of the optimum conditions to regenerate whole plants from hybrid protoplasts. Each species requires its own unique regeneration conditions which need to be determined (Ratanasanobon, 2007).

Within Australia, breeding programs have focused upon producing new varieties exhibiting increased adaptations to the Australian environment, with additional objectives towards overcoming a range of undesirable traits. Australian native plant species like *Grevillea* (family Proteaceae) are of increasing commercial importance in the horticulture and revegetation industries. Tissue degradation and low protoplast yields were investigated to provide a basis for breeding with the aim of creating a salt tolerant plant (Kennedy and De Filippis, 2004). Chikkala *et al.* (2009) studied adventitious shoot regeneration and protoplast isolation and culture of several cauliflower (*Brassica oleracea* var. *botrytis*) cultivars, sourced from Europe and Australia to develop improved nuclear and plastid transformation protocols. He *et al.* (1997) obtained transgenic wheat (*Triticum aestivum*) through protoplast electroporation.

In forest trees, protoplast fusion and gene transfer are methods which potentially can be used to enhance wood quality and oil, along with tolerance to salt, drought, temperature and disease (Sartoretto *et al.,* 2008).

There is great demand for Australian native flowers of different colours. Fused protoplasts from two different *Chamelaucium* species were achieved at the floriculture laboratory at the Department of

Agriculture and Food, Western Australia (Shan, 2007). Employing protoplast technology to create new colour variants may enable the industry to compete in the domestic and international markets.

Plant Genetic Engineering and Transformation

The isolation and addition of specific foreign genes to a plant species to enhance its properties is a technique with almost unlimited potential. This technique allows us to investigate the evolution, structure, function or regulation of a particular gene, which can be manipulated for production of clones with enhanced economic characteristics (Daggard, 1996). Karp *et al.* (1998) introduced the term "molecular breeding" to describe the development and application of molecular genetic techniques to introduce novel and desirable characteristics with high value to plant breeding program.

Genetic engineering may also involve the transfer of genes - and thus the characteristics governed by those genes - from one species to another. The objectives of plant genetic engineering include improving flower quality in ornamentals and crop production in agriculture as well as introducing new traits like enhanced nutrient intake, resistance to environmental stress or better post harvest quality. Certain characteristics allow a genetically modified crop to be grown, harvested, or shipped at a lower cost.

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 the 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. Another recent advanced tool for plant genetic engineering, developed by Krichevsky (2008), is the modular satellite (pSAT) vector system. This molecular tool was first developed to provide N- and C-terminal fusions of the genes under investigation to five different autofluorescent tags, EGFP, EYFP, Citrine-YFP, ECFP, and DsRed2. However, this plasmid system also allows cloning of untagged ORFs, or genes marked with different tags, for simultaneous expression in the plant cell.

Whilst extensive research was 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 were 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 (canola), cotton and tomato (Vasil, 1991). Due to the high cost, this technology is presently limited to crop species with potential for high economic returns (Table 5.5). It is anticipated that these molecular tools will be used routinely for the improvement of ornamental species and in the development of crops for biofuel production in the foreseeable future.

The application of molecular biology technique in improving plant quality is increasingly important in both commercial and research purposes in Australian native species. Though publication on the application of molecular based biotechnology in Australian native plants is still limited, there is no doubt that access to this technology will be of increasing importance in studies of plant systematics, conservation biology as well as commercialisation of Australian native species.

Table 5.5: Examples of Transgenic Crop Plants with their Advantages and Modification Methods

Plants	*Advantages*	*Modification Methods*	*References*
Rice	Rice plants containing high provitamin A (β-carotene).	Genes from narcissus, maize and *Erwinia* bacteria were inserted into rice chromosome.	Gupta (2004)
Maize, cotton, potato	Resistance to pests	Toxic gene Bt was transferred from *Bacillus thuringiensis*	Gupta (2004)
Tobacco	Resistance to cold climate	Genes regulating the resistance to cold climate from *Arabidopsis thaliana* or cyanobacteria (*Anacyctis nidulans*) were inserted into tobacco chromosomes.	Gupta (2004)
Tomato	Fruits remain firm and fresh for a long time	Antisenescens gene was transferred into tomato chromosome to inhibit the production of polygalacturonase enzyme.	Gupta (2004)
Soybean	Plants containing high oleic acid and resistant to herbicide glyphosate	Herbicide resistant gene from *Agrobacterium* line CP4 was introduced to soybean chromosome. Molecular technology was used to increase oleat acid formation	Gupta (2004); Heller (2007)
Sweet potato	Plant resistance to viral disease	Gene from certain virus was transferred into sweet potato by the aid of gene silencing technology	Loebenstein and Thottappilly (2009)
Canola	Plants producing canola oil high in lauric acid content	Gene *FatB* from *Umbellularia californica* was transferred into canola chromosome to increase laurate acid content.	Scarth and Tang (2006)
Papaya	Plant resistance to certain virus such as *Papaya Ringspot Virus* (PRSV)	Gene encoding PRSV was transferred into papaya chromosome	Gonsalves (2004)
Rockmelon	Slow ripening of fruits	New gene from bacteriophage T3 was taken out to reduce the production of ethylene in rockmelon.	ILSI Research Foundation (2010)
Sugar beet	Plant resistance to glyphosate and gluphosinate herbicides	Genes from *Agrobacterium* line CP4 and *Streptomyces viridochromogenes* were transferred into sugar bit chromosomes	Haim D. Rabinowitch and Currah (2002)
Plum	Plant resistance to plum pox virus	Gene encoded plum pox virus was transferred into plum chromosomes.	Scorza *et al.* (1994)
Wheat	Plant resistance to *Fusarium graminearum*	Gene encoding chitinase enzyme from barley was transferred into wheat chromosome.	Shin *et al.* (2008)

Current efforts in genetic engineering in Australian native plants are focussed primarily on species of eucalyptus, because of their commercial importance (Chandler, 1995). Field trials of genetically engineered *Eucalyptus grandis* carrying selectable marker genes was reported by Edwards *et al.* (1995) following the availability of lasting tomato (Flavr Savr, *Lycopersicon esculentum*) on market in 1994. This genetically modified tomato produces less of the substance that causes tomatoes to rot, so remains firm and fresh for longer time. Strawberries, pineapples, sweet peppers and bananas have also been genetically modified by scientists to remain fresh for longer. In 2008, the first transgenic canola was harvested in Australia.

In future, research and development of transgenic crop should focus on creating species with improved production stability; better nutritional value; resistant to environmental impacts of intensive and extensive agriculture; as well as developing protocols and regulations which ensure transgenic crops designed for purposes other than food, such as pharmaceuticals, industrial chemicals, biofuels, vaccines, etc.

Conclusion

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 recent book (de Fossard, 1993) provides 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 aid the domestication and development of Australian native plant species as potential ornamental crops. Further advances and applications of plant biotechnology require more basic research. 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. Furthermore, the endless possibilities for improvements in plants through biotechnology have the potential to help solve world hunger and problems in agriculture as a result of climate change.

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