Biotechnology for Sustainability

Achievements, Challenges and Perspectives

Editors
Subhash Bhore,
K. Marimuthu &
M. Ravichandran

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Cover image: A diagram showing the 17 Sustainable Development Goals (Credit: www.un.org/)

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Dedication

This book is dedicated to all researchers working in various domains of biotechnology and to all stakeholders those are working for the global sustainable development to improve the health of the people and planet.

Preface

World Environment Day (WED) is a biggest global annual event celebrated each year on June 5 to create the positive awareness to preserve the environment and planet earth. This year, the theme for WED-2017 was "Connecting people to nature". Our environment should be healthy for our growth, development and to achieve the sustainable development goals (SDGs) adopted by the international community to transform the world.

Most recently, António Guterres (United Nations Secretary General) precisely highlighted that "Without a healthy environment we cannot end poverty or build prosperity. We all have a role to play in protecting our only home: we can use less plastic, drive less, waste less food and teach each other to care". In fact, to achieve the SDGs by protecting environment, everyone needs to do their part.

We strongly believe that biotechnology can play an important role directly or indirectly in achieving various SDGs. Hence, we had decided to publish a book, "Biotechnology for Sustainability" to commemorate the WED and to highlight the achievements, challenges and perspectives in various domains of the biotechnology. In response to our call for articles, we had received 50 manuscripts. The selected articles published in this book are highlighting various issues, achievements, challenges and perspectives for the viable development and sustainability. The World Commission on the Environment and Development defined sustainability as the "development that meets the needs of the present without compromising the ability of future generations to meet their own needs". The United Nations recent estimate suggest that the world's food supply needs to be doubled by the year 2050 to keep up with the growing demand. To achieve this is a huge challenge; because, the amount of arable land is continuously decreasing as a result of rising urbanization, saline soils and desertification. Biotechnologists (and plant breeders) around the world are working persistently to produce crops which will boost the food production to meet the growing demand. Genetically engineered crop varieties do offer many promising possibilities to boost nutritive value of the food, sustain farming on marginal lands, and to minimize the loss by creating pests and disease resistant varieties.

The articles published in this book are going to be useful in creating awareness about the environmental issues, natural resources, biodiversity conservation, sustainable development and various biotechnological approaches that could be used to alleviate the respective challenges.

We would like to express our sincere gratitude and thanks to Dato' Seri Utama Dr. S. Samy Vellu, Chancellor and Chairman, AIMST University for his support in publishing this book.

We wish to thank all contributing authors for making a common cause with us. This book publication project could not have been completed without the courteous cooperation of the authors to highlight achievements, challenges and or perspectives in using biotechnological approaches for the sustainability.

We are confident that this book will serve as a reference to various researchers, scientists, academicians and graduate students involved in biodiversity conservation, environmental protection and various fields of biology and biotechnology.

It is hoped that a prudent use of biotechnology in the biodiversity conservation, environmental protection, and production of more and better quality of food, fiber, fuel and drugs will contribute in accomplishing SDGs and to promote peace in the world.

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Plant Tissue Culture for Sustainability

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Abstract: The United Nations has placed great emphasis on sustainability. Three of the most important requirements of sustainable development are: eradicating extreme poverty and hunger, protecting the environment, and conserving biodiversity. Because of human activities the stable functioning of earth's life support system – which includes the atmosphere, oceans, forests, waterways, biodiversity and biogeochemical cycles, is at risk. One of the major contributing factors is the large scale destruction of natural forests. Deforestation had many adverse effects; most importantly, the effects on climate, environment, and biodiversity. The three pillars of sustainable development are: sustainable agriculture, conserving biodiversity, and protecting the environment through reversing the effects of deforestation by large scale afforestation. Plant Tissue Culture can greatly contribute in all the three.

***Keywords*:** Afforestation; biodiversity conservation; micropropagation; plant tissue culture; sustainable agriculture

1. Introduction

The United Nations Summits and Commission Reports from the 1987 Brundtland Commission (World Commission on Environment and Development) report onwards have placed added emphasis on sustainability of all development efforts. Three of the most important requirements are: 1. Eradicating extreme poverty and hunger, 2. Protecting the environment, and 3. Conserving biodiversity. To eradicate extreme poverty and hunger two things are essential: first, sustainable agriculture which makes food available/affordable and second, creation of jobs which translates to purchasing power. One of the major factors in protecting the environment is reversing the loss of natural forests. Conserving biodiversity is of great relevance now than ever before for the reason that our world is fast changing. To have crop varieties suitable for this changing environment is to preserve as much natural variation in plant varieties as possible. Plant tissue culture can contribute to all the three. In this paper I will elaborate on how Plant Tissue Culture, my area of research, can contribute to Sustainable Agriculture, Protecting Forests, and Conserving Biodiversity.

2. Sustainable development

In 1987 it was the Brundtland Commission (World Commission on Environment and Development) report "Our Common Future" which brought the concept of "Sustainable Development" into common use. The World Commission on Environment and Development was set up by the UN General Assembly in 1983. Brundtland Commission Report defined Sustainable Development as "Development that meets the needs of the present without compromising the ability of the future generations to meet their own needs". According to the Brundtland Commission Report, the needs, in particu-

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lar the essential needs of the world's poor, to which overriding priority should be given, and the limitations imposed by the State of Technology and Social organization on the Environment's ability to meet present and future needs should be addressed. The Brundtland Commission Report emphasized the need to integrate economic and ecological factors in decision-making at all levels for sustainable development. These factors include, reviving growth, changing quality of growth, meeting essential needs for jobs, food, energy, water and sanitation, ensuring the resource base, reorienting technology and managing risks. In its broadest sense, the strategy for sustainable development aims to promote harmony among people and between human beings and environment.

In 1992, at the Earth Summit (Rio, 1992) there was consensus that environment, and economic and social development cannot be considered in isolation, and in addition to treaties and agreements on climate change, biological diversity, deforestation, and desertification, the Rio Declaration contains fundamental principles on which nations can base their future decisions and policies, considering the environmental implications of socio-economic development.

In 2000 the Millennium Summit of the United Nations, following the adoption of the United Nations Millennium Declaration, established the eight Millennium Development Goals (MDGs) to be achieved by the year 2015. The MDGs are: 1. to eradicate extreme poverty and hunger, 2. to achieve universal primary education, 3. to promote gender equality and empower women, 4. to reduce child mortality, 5. to improve maternal health, 6. to combat HIV/AIDS, malaria, and other diseases, 7. to ensure environmental sustainability, and 8. to develop a global partnership for development. In the present context Goal 7: Ensuring environmental sustainability is very important. Two of the important targets of MDG 7 are: Integrating the principles of sustainable development into country policies and programs, reversing loss of environmental resources, and reducing biodiversity loss.

In 2012, the United Nations Rio+20 summit in Brazil committed governments to create a set of "Sustainable Development Goals" (SDGs). On September 25th 2015, countries adopted a set of goals to end poverty**,** protect the planet**,** and ensure prosperity for all as part of a 2030 Sustainable Development Agenda. Each goal has specific targets to be achieved in 15 years. The 17 Sustainable Development Goals (SDGs), otherwise known as the Global Goals, are a universal call for action to end poverty, protect the planet and ensure that all people enjoy peace and prosperity always. The goals are interconnected. The key to success on one will involve tackling issues associated with another. The SDGs work in the spirit of partnership and pragmatism, to make the right choices now to improve life, in a sustainable way, for future generations. They provide clear guidelines and targets for all countries to adopt in accordance with their own priorities and the environmental challenges of the world at large. The SDGs are an inclusive agenda. They tackle the root causes of poverty and unite all nations together to make a positive change for both people and planet (UNDP).The 15th SDG of UN relates to Life on land, and involves protecting, restoring and promoting sustainable use of terrestrial ecosystems, sustainably managing forests, combating desertification, and halting and reversing land degradation and halting biodiversity loss.

The stable functioning of Earth's life support system – which includes the atmosphere, oceans, forests, waterways, biodiversity and biogeochemical cycles, is a prerequisite for future human development. However, as per recent research findings this functioning is at risk (Rockström *et al*., 2009). Further human pressure may lead to large-scale, abrupt, and

potentially irreversible changes to Earth's life support system (Lenton 2011; Barnosky *et al*., 2012). Likely impacts on humanity include: diminishing food production, water shortages, extreme weather, ocean acidification, deteriorating ecosystems, and sea-level rise. In this backdrop Griggs *et al*. (2013) suggested that we redefine sustainable development as "Development that meets the needs of the present while safeguarding Earth's life-support system, on which the welfare of current and future generations depends." Without economic, technological, and societal transformations, chances of large-scale humanitarian crises exist. Such crises could undermine any gains made by meeting the MDGs. A re-evaluation of the relationship between people and planet is necessary (Griggs *et al*., 2014).

3. Three pillars of sustainable development

In the second half of the 20th century there was intensification of agriculture in most parts of the world. Intensive agriculture involved: (i) expanding farm lands, by removing natural forests, (ii) better irrigation, by constructing big dams, which again submerged vast forests in their catchment areas (iii) use of chemical fertilizers and pesticides, to produce high yields. Destruction of natural forests had many adverse effects; most importantly, the effects on climate, environment, and biodiversity. Extensive use of chemical fertilizers and pesticides also had their own adverse effects. Excessive use of chemical fertilizers has resulted in nitrate accumulation, increased soil salinity, and water eutrophication. High use of pesticides has resulted in development of resistance in many pest species. In recent years there is much concern about environmental contamination by fertilizers and pesticides.

Sustainable Development, that meets the needs of the present while safeguarding Earth's life-support system, on which the welfare of current and future generations depends, stands on three pillars:

i. Sustainable agriculture
ii. Conserving biodiversity
iii. Protecting the environment

Increasing food production must involve, developing/ introducing better (efficient, high yielding, insect-pest resistant) varieties of crop plants, conserving biodiversity, and protecting environment. Plant Tissue Culture can greatly contribute in all these.

4. Plant tissue culture

Plant tissue culture is the *aseptic growing of whole plants or parts (cells, tissues/ organs) in/ on defined (synthetic) nutrient media under controlled (environmental) conditions (temperature, light, humidity)*. Usually in glass vessels (test tubes, conical flasks, jam bottles etc.) - for a review see John *et al*. (1997).

Plant tissue culture is based on cellular 'totipotency', the inherent potential of a plant cell to regenerate a whole plant. Unlike animal cells, most plant cells retain the capacity to regenerate the whole organism even after undergoing the final differentiation. In plants, as long as the cells have an intact membrane system and a viable nucleus, even highly mature and differentiated cells retain the ability to regenerate to a meristematic state. Though initially, in the first two decades of the 20th Century progress was slow, standardization of universal plant tissue culture media - White's (White, 1933), Gamborg's (Gamborg *et al.*, 1975) and MS (Murashige and Skoog, 1963) changed the scene. Plant tissue culture media contain minerals, growth factors and a carbon source (usually sucrose). Controlled environmental factors are light (intensity and length – photoperiod), temperature, relative humidity. On/ in a custom standardized medium, and controlled environmental conditions, the explant (starting plant material) - usually young,

undifferentiated tissue, regenerate into whole plants.

4.1. Types of cultures

Different types of cultures are possible: (i) culture of whole plants, (ii) embryo culture (embryo rescue), (iii) organ culture (shoot tip culture, root culture, leaf culture, anther culture etc.), (iv) callus culture, (v) cell suspension and single cell culture, (vi) protoplast culture.

4.2. Callus culture

Callus is an amorphous mass produced by cell proliferation, occurring in an unorganized manner. In nature it is a wound response, or a plant reaction to the presence of micro-organisms, insects, or to some kind of stress. Under *in vitro* conditions callusing is a response to endogenous or exogenous growth regulators. The potential for callus formation is dependent on the tissue (explant) type. Meristematic tissues are more suitable for callus induction than mature tissues. Callus cultures can be maintained for long by sub-culturing the primary callus (callus established originally from the explant), at periodic intervals.

4.3. Somaclonal variations

Long term callus cultures can however, suffer from spontaneously arising genetic variations, reflected in the phenotype of plants regenerated from such calli. These variations are known as *somaclonal variations. Somaclonal variations* are reported in many species. The basis of somaclonal variations is not well understood. Chromosomal rearrangements, activation of endogenous transposons, and changes in the status of DNA methylation, are considered to be the contributing factors.

4.4. Suspension cultures

Culture of unorganized plant cells, as single cells/ cell aggregates, in liquid medium. Friable callus when cultured in agitated liquid medium, the cells separate and form a suspension of single cells/ aggregates of few cells. These cells/ cell aggregates grow/ divide/ separate as a result of agitation, and can be continually maintained in this state. Growth of cells in suspension culture can be more easily manipulated in liquid medium than on semi-solid medium. Slowly agitating the liquid medium on a rotary shaker is necessary for the growth of the cultures, which can be sub-cultured. Growth in single isolated cells can be induced by culturing them in hanging drops in microchambers. Suspension cultures are useful in plant production by somatic embryogenesis form single cells. In regeneration of plants from callus established on semi-solid media from small cell aggregates, and for the production of secondary metabolites. Suspension cultures can also be initiated from tissue other than callus (Geile and Wagner, 1980).

4.5. Protoplast cultures

Protoplasts are plant cells without cell walls. In 1882, Klercker isolated protoplasts mechanically for the first time. The yield of protoplasts was very low. In 1960, Cocking using enzymes for the first time could isolate protoplasts in large numbers. Protoplasts can be isolated from different plant parts, or from tissues already in culture. Enzymatic isolation is now the most commonly used method. A combination of these two can also be used.

One of the important applications of protoplasts is in somatic hybridization. Many agents like $NaNO_3$ (Power *et al.*, 1990), a higher pH, and a higher concentration of calcium ions in the medium (Melchers and Labib, 1974), polyethylene glycol (Kao and Michayuluk, 1974; Wallin *et al.*, 1974), and a high strength electric field (Zimmermann and Scheurich, 1981), are used for obtaining fusion between protoplasts. Protoplasts, when placed in appropriate media regenerate cell walls and form calli, from which plants can be regenerated. Protoplasts are used for producing somatic hybrids (parasexual hybrids), for genetic manipulation,

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and for basic studies on a variety of aspects. Regenerating plants from protoplasts is difficult in some species. Conventional hybridization depends on affinity of gametes. Wide crosses are not possible because of well-established cross breeding barriers. Protoplast fusion makes such hybridizations possible.

4.6. Anther (isolated microspore) cultures

Guha and Maheshwari (1964) obtained haploid embryos, directly from anther cultures of *Datura innoxia*. The origin of these embryos was traced to the pollen grains. The potential of anther culture for obtaining haploid plants, and from them by chromosome doubling of homozygous diploid plants was apparent. In 1974, Nitsch had reported regeneration of haploids and homozygous diploids by chromosome doubling, from isolated microspore culture (Nitsch, 1974a; 1974b). Culturing the microspores along with anther wall is essential for success. In isolated microspores, pollen embryogenesis is induced only rarely.

This technique has great potential in plant breeding. Normally it takes selfing for many generations to obtain homozygosity in parental lines required in breeding programmes. This time can be considerably reduced by haploid culture techniques.

4.7. Meristem culture and shoot tip culture

When growing points (meristems) of shoots are cultured they continue their organized growth. The shoots/multiple shoots produced can be rooted to produce plantlets. This capacity has practical application and economic significance for plant propagation.

Culture of the meristemic zones /extreme shoot tip is known as *meristem culture*, and culture of small segments (5-10 mm in size) from the shoot tip is known as *shoot tip culture*. It was known that meristems of virus infected roots are free of the pathogen (White, 1933; 1934). Limasset and Cornuet (1949) found that shoot tips of virus infected plants are also virus-free. Morel and Martin (1950; 1955) could produce healthy plants from virus-free plants through shoot-tip culture from infected mother plants. This is possible because the pathogen concentration is not uniform in the infected plants, and apical buds of rapidly growing shoots are often not invaded by the virus. Morel (1960) used shoot apices of orchids to obtain their rapid clonal multiplication. Shoot tip culture has two important practical applications: (i) virus eradication and (ii) micro-propogation. These developments were followed by *in vitro* propagation of plants from shoot tip culture. Initially most of the species micropropogated were herbaceous (Morel, 1964; Murashige, 1974). Now methods are available for the micropropagation of a large number of species belonging to a wide range of plant groups.

4.8. Embryo culture

Very young to mature embryos can be cultured *in vitro.* Embryo culture is one of the oldest applications of plant tissue culture in plant breeding. It has many practical applications, and very useful in obtaining hybrid plants from crosses in which post-zygotic incompatibility exists. In post zygotic incompatibility, fertilization and zygote formation occur on cross pollination. The zygote grows, but is not accepted by the endosperm. This results in embryo abortion at some stage of development before maturing of the seed. In such instances when the ovary/ ovule/ embryo with a part of the maternal tissue is excised and cultured on a suitable medium and under optimum culture conditions, it matures to produce a seedling. This procedure is hence called *embryo rescue*. Sharma *et al.* (1980) obtained few hybrids between *Solanum melongena* and *S. khasianum* by this method. Embryo culture is useful also in overcoming seed dormancy and for obtaining seed germination in some vegetatively propagating species in which seeds are produced but

normally do not germinate (e.g. some wild bananas).

4.9. Invitro pollination and fertilization

Pre-zygotic incompatibility is one of the major limitations in obtaining hybridization between many plant species and varieties. In pre-zygotic incompatibility, the zygote is not formed on cross pollination. The pollen either do not germinate on the stigma of the female parent or the pollen tube gets arrested at some point of its growth on the stigma/ in the style.
A variety of methods used *in vivo* to overcome this barrier.

Kanta *et al*. (1962) developed an *in vitro* technique for overcoming pre-zygotic incompatibility. In this method, the mature/nearly mature ovaries/ovules are cultured on suitable media and pollinated *in vitro* with cross pollen to obtain hybrids (Zenktler, 1980; Raghavan, 1990).

4.10. Root cultures

Tip portions from primary and secondary roots of many plants can be cultured. In 1922, Kotte and Robbins independently postulated that true *in vitro* cultures could be raised from meristematic cells from root tips and shoot tips. Kotte (1922) could cultivate root tips of pea and maize in nutrient media for long, but no sub-culturing were done. Robbins (1922) could subculture his maize root cultures. White (1934) obtained unlimited growth of tomato roots, using the same medium as Robbins (1922), with yeast extract. Root cultures are useful in: (i) secondary metabolite production, and (ii) in basic studies on nematode infections, mycorrhizal associations, and root nodulation by *Rhizobium* bacteria.

4.11. Organogenesis

Organogenesis is the process of initiation and development of a structure that shows natural organ form and/or function. It is the ability of non-meristematic plant tissues to form various organs *de novo;* the production of roots, shoots or leaves. These organs may arise out of pre-existing meristems or out of differentiated cells. Indirect pathway includes a callus stage.

Direct pathway bypasses a callus stage. The cells in the explant act as direct precursors of a new primordium, an organ or a part in its most rudimentary form or stage of development.

4.12. Somatic embryogenesis

In plants, embryo-like structures can be generated from non-germ cells (somatic cells), by circumventing the process of normal fertilization. As somatic embryos are formed without fertilization event, they are genetically identical to the parent tissue, and are therefore clones.

Somatic embryogenesis may be direct or indirect. Indirect somatic embryogenesis involves a callus phase prior to embryo production. Direct somatic embryogenesis involves production of embryos from organized tissue without an intervening callus phase. Irrespective of the mode of production, anatomical and physiological features of somatic embryos are highly comparable to zygotic embryos. The morphological and temporal developments of somatic embryos are very similar to that of zygotic embryos. They both proceed through a series of distinct stages, namely, globular, heart, torpedo and cotyledon or plantlet stages for dicotyledons, and globular, elongated, scutellar and coleoptilar stages for monocotyledons. These stages typically span a period of several days. In dicots initially small globular embryos form which undergo isodiametric growth and establish bilateral symmetry. In monocots, especially in grasses, the transition from globular stage follows a series of events occurring simultaneously; such as the development of scutellum, initiation of the coleoptilar notch, tissue differentiation with the development of embryogenic vascular system and accumulation of intracellular storage substances. Somatic embryogenesis is used for: large-scale clonal propaga-

tion of elite cultivars, as an alternative to conventional Micropropagation, producing synthetic (artificial) seeds. Indirect somatic embryogenesis (via callus) or secondary embryogenesis is used in gene transfer. Somatic embryogenesis also offers potential model for the study of molecular, regulatory and morphogenetic events in plant embryogenesis.

4.13. Micropropagation

Micropropagation is the tissue culture method of clonal propagation of plants. Plant tissue culture is rapidly becoming a commercial method for propagating difficult-to-propagate plants, new cultivars (selections, hybrids, transgenic), rare/endangered species. Micropropagation is usually achieved by the release (from dormancy), and growth of pre-existing (axillary/ lateral) meristems in the initial culture. This is followed by repeated enhanced formation of axillary shoots by sub-culture on medium supplemented with plant growth regulators. The shoots produced are rooted either *in vitro* or *ex vitro* (out of culture).

There are many advantages of Micropropagation. Shoot production is reliable and consistent. Multiplication rates can be three-fold to eight-fold a month. Plants produced via shoot culture are usually true-to-type and uniform. Allows propagation of rare/ endangered/ hybrid/ induced mutant/ genetically transformed plants. There also are few disadvantages. PGRs do not release apical dominance in all species. There may be a difference in results between juvenile and mature tissue of perennial species; shoot cultures may require a reversion to juvenility. Rooting of the micro-shoots may be difficult. Getting uniform shoot production *in vitro*, which is very important in commercial operations, may not be possible in some instances. The procedure is relatively labor intensive, with high upfront costs to get started.

4.13. 1. Applications of micropropagation

Rapid and large-scale clonal (genetically uniform) propagation of plants (micropropagation) may allow faster production of plants that are slow to propagate *in vivo*.

The time required for bulking-up of new cultivars before they are commercially introduced can be drastically decreased. Storage of germplasm, e.g. *Cryopreservation.*

4.13.2. The process of micropropagation

a. A small piece of the plant to be cloned (the explant) is removed from a healthy, well-maintained stock plant and surface sterilized (explant varies with species, but shoot tips, leaves, stem pieces, lateral buds, and young flowers or floral parts are used).
b. Surface sterilized explants are rinsed with sterile water, and placed aseptically in/ on specially formulated and sterilized medium in culture vessels.
c. The explant may proliferate directly by enhanced lateral branching, or the tissue may undergo a certain period of unorganized growth (callus) prior to shoot differentiation.
d. The growth of the cultures is principally determined by the plant growth regulator (PGR) content of the culture medium (the auxin and cytokinin alone or in combination and concentration/s). Most cultures are established within 4 to 12 weeks depending on the species/ cultivar.
e. A proliferating shoot culture can be sub-cultured to produce divisions which will multiply rapidly.
f. Rate of multiplication vary and are affected by many factors. Production of thousands, and in some cases millions of plants a year from a single explant has been demonstrated

5. Role of plant tissue culture in sustainable agriculture

Sustainable agriculture requires efficient, biotic and abiotic stress resistant crop varieties. Germplasm collection and