

# Plant Tissue Culture: Beyond Being a Tool<br>for Genetic Engineering

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#### Abstract

Plant tissue culture has been in practice for more than 100 years since its conception by Haberlandt in 1902. The development of widely used media composition by Murashige and Skoog in 1962 forms the backbone of most of the tissue culture protocols. Besides micropropagation of economically important species by large- and small-scale companies worldwide, plant tissue culture is widely used to develop crops with economically important traits by transforming different explants and thereafter regenerating them under optimized culture conditions; it has been widely perceived and used as the workhorse for plant genetic engineering. This fitted the growing Plant Biotechnology arena in the late 1990s where commercial companies tried to develop protocols to deliver commercial traits into economically viable crops. However, plant tissue culture's untapped potential is getting revealed now during the changing climatic conditions and rising needs of the human population. Not only fulfilling the need to feed, plant tissue culture could also be used to develop a sustainable future under harsh conditions by multiplication of endangered plant species, developing heavy metal scavenging plant populations, replanting eroded lands and forests by tissue culture generated trees, developing viral free plant populations and establishment of ocean farms where one unit could be dedicated to plant tissue culture/hydroponic system. Thus, plant tissue culture has the potential to impact the future of mankind in many ways.

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#### 9.1 Introduction

Plants have pre-existed animals on earth; cyanobacteria and other photosynthetic eukaryotes existed 1 billion years ago. Flowering plants evolved around 200 million years ago; plants have endured and evolved through several epochs of harsh conditions and developed traits highly diverse and valuable. Plants have been a source of sustainability to aerobic life on earth; they have been used since ancient times as a source of valuable products: food, feed, fiber, oil, sugar, etc. for humans and animals. However, in last two centuries, due to ever-increasing population, urbanization, and industrialization, there has been an extreme pressure on this "Treasure of Earth." Earth has lost a considerable area of arable land, soil and water quality have deteriorated, forest areas have shrunk, and pollution levels are high, endangering many plant species. Increased demand for value crops like rice, wheat, cotton, sugarcane, etc. to sustain the economies have led to almost wiping out of some of the indigenous plant species. In this regard, plant tissue culture can offer a way to tackle the challenges mentioned above. There have been some successful examples of applied tissue culture to endangered species, economically important crops, fruits, flowers, and trees, but in today's time of climate change such efforts do not give a chance to sustain human race. Thus, plant tissue culture currently needs to be looked at from a different perspective and certainly above the economic gains. We need to understand the real basis of cellular totipotency of the plant kingdom and apply it to save and sustain the life on earth.

The term totipotency or the ability to regenerate plants from single cells was developed by Haberlandt in 1902. Now after more than 100 years of the first report and more than 58 years after the development of universally used Murashige and Skoog ([1962\)](#page-25-0) medium for culturing plants, plant tissue culture is still being used for generating transgenics and commercial micropropagation of some plant species.

The idea of totipotency itself is not utilized to its full scale mostly due to the plasticity of the plant system and unavailability of protocols to culture and regenerate plants from almost all the species. Plant developmental biology research is considerably slow due to long time taken for tissue culture protocol development. Protocols are highly dependent on explant type, species, medium for different phases for cell proliferation, embryo/organ formation, and regeneration. No two species are alike in terms of hormone regime, explant response, media requirements, etc. Large number of variables need long and steady optimization steps. Besides, lack of research funds for plant developmental research compared to the counterparts in animal biology and lack of highly reproducible and flexible protocols that can work across different plant species limit the horizons of plant tissue culture. There are several examples of tissue cultured plant species and genetic engineering products but mostly in cultivated crops/trees. Considering the size and diversity of the Plant Kingdom and inherent value traits available in plants growing in different geographies, in harsh or adverse conditions, might give us a glimpse into the untapped potential of plants.

In this chapter, we will cover these points and try to look beyond the normal process of plant propagation and see how academic and industrial collaboration and investments in this industry can contribute towards saving environment, minimizing risks associated with genetic engineering and improving the livelihood of the farmers.

## 9.1.1 Plant Tissue Culture in Modern Agriculture

Micropropagation—tools and techniques, rapid protocols for multiplication of plantlets. Micropropagation is the method used to produce plantlets from a plant part or explant. It is the process to rapidly multiply explants material to regenerate many progeny plants. Micropropagation of plant material was started by Fredrick Campion Steward in 1950s and off late the technique has been extremely popular to bulk produce and regenerate plant material in species which happen to be difficult to produce seeds. It is also an effective way to regenerate tissues of genetically transformed material. The process involves isolation of a part of plant tissue e.g. leaf, bud, meristem under sterile condition and propagation under different source of hormone and media regime. This method happens to be fast and costeffective as compared to conventional method of cell/tissue culture and has the potential to generate true to type plant material if meristem culture is involved. Below are the advantages of micropropagation:

- a. Rapid multiplication of plants within a short period and on small space.
- b. Plants are obtained under controlled conditions, independent of seasons.
- c. Sterile plants or plants which cannot maintain their characters by sexual reproduction are multiplied by this method.
- d. The rare plant and endangered species are multiplied by this method and such plants are saved.
- e. Production of virus free plants like potato, banana, apple, papaya, etc.
- f. Production of rare and endangered species like Taxus and other medicinal plants.

# 9.1.2 Source of True to Type Plant Material

Generally, tissue culture or micropropagation of species give rise to somaclonal variations especially when the plant tissue undergoes a callus phase. This is mainly due to the genetic or epigenetic changes that occur when the cells undergo a phase of dedifferentiation to form the callus (simple parenchymatous cells) and redifferentiation again when the callus cells give rise to complex tissue for regeneration. However, there are tissues or cell types that can give rise to true to type plants e.g. meristem cells. These specific cells are responsible for organ generation from simple cell types. In normal plants, meristematic zones are formed in areas of growth e.g. cambium tissue in stem and root, bud axis that can give rise to either a floral bud or branch. These cells are similar to the stem cell tissue of animals and have the potential to give rise to true to type plants under optimal conditions. However, optimizing those conditions whereby these cells directly regenerate into complete plants in a short period is difficult and need to be optimized for each species and subspecies or varieties.

# 9.1.3 Generation of Disease-Free Plant Material

Micropropagation is a means to generate disease-free plant material. A two-step process is necessary in order to generate disease-free plants:

# 9.1.3.1 Selecting the Best or Disease-Free Source Material

This can be done by selecting vigorous or healthy source material and growing it in a nursery or controlled field or Greenhouse conditions in order to evaluate the growth and disease in the primary stock. Methods are available which utilize molecular kits to check the presence of disease-causing microorganisms e.g. bacteria, certain endophytes, etc. Assays for many viruses are not available so it is better to grow one generation of non-infected plant tissue in greenhouse and use it as a source to generate virus free or disease-free sapling.

# 9.1.3.2 Disease-Free Plantlet Regeneration Under Tissue Culture Conditions

In tissue culture aseptic conditions are utilized to multiply cells or tissues; these cells go through multiple cycles or multiplication and under certain conditions using antibiotics, etc. can be made disease-free. In another method, by using meristem culture, spreading of any viral disease could be avoided, as viruses cannot move through meristematic tissues. These tissues lack plasmodesmata or cell connectors and hence, movement of any infectious organisms from one cell to the other is impossible. Thus, selecting meristematic tissue and culturing it to develop into a complete plantlet is one of the popular means to make disease-free plants. Many micropropagation companies utilize this approach to bulk produce disease-free plants.

# 9.1.3.3 Cryopreservation for Generating Disease-Free Material

Recently storing plant parts or tissues under liquid nitrogen  $(-196 \degree C)$  for a short time has been found to be useful to render plant tissues virus free. Classical cryopreservation techniques involve slow cooling down to a defined pre-freezing temperature, followed by rapid immersion in liquid nitrogen. Some of the examples to use Cryopreservation for virus elimination are mentioned in Table [9.1.](#page-4-0)

# 9.1.4 Developing Improved Varieties via Wide Hybridization

A critical requirement for crop improvement is introduction of new genetic material into cultivated lines of interest, either by single or multiple genes through genetic engineering, through conventional hybridization or other tissue culture techniques. Normal fertilization process of embryo fusion or fusion of egg cell with pollen

<b>Species</b>	Virus			
Woody plant				
Grapevine	Grapevine fanleaf virus (GFLV) and grapevine leaf roll-associated virus-1 $(GLRaV-1)$			
Banana	Banana bract mosaic virus			
Citrus	Citrus psorosis virus			
Cocoa	Cocoa swollen shoot virus			
Rose	Rose mosaic virus			
Herbaceous plants				
Sugarcane	Sugarcane mosaic virus (SCMV) and sugarcane yellow leaf virus (ScYLV)			
Garlic	Leek yellow stripe (LYS) and onion yellow dwarf virus (OYDV)			
Potato	Potato leafroll virus (PLRV) and potato virus (PVY)			
Carnation	Carnation latent virus (CLV)			
Chrysanthemum	Cucumber mosaic and tomato aspermy virus			
Dahlia	Dahlia mosaic virus			
Peanut	Peanut mottle potyvirus (PMV) and peanut stripe potyvirus (PStV)			
Pumpkin	Zucchini yellow mosaic virus, cucumber mosaic virus, alfalfa mosaic virus, bean yellow mosaic virus			

<span id="page-4-0"></span>Table 9.1 Representative examples of tissue culture technique used for virus elimination in selected woody and herbaceous plants (Source: Cruz-Cruz et al. ([2013\)](#page-24-0))

nucleus could be hindered in some species due to multiple factors. These could be categorized either as pre-fertilization barriers e.g. failure of pollen to germinate or poor pollen tube growth or post fertilization barriers e.g. lack of endosperm development. Tissue culture presents an opportunity for another, pollen or embryo culture to overcome such barriers. When fertilization cannot be induced by in vitro treatments then protoplast fusion can be implemented to produce desired hybrids. Some of the techniques used are described below:

#### 9.1.4.1 In Vitro Fertilization

IVF has been applied to produce many crosses by transferring pollen from one species to other leading to wide crosses. Interspecific and intergeneric hybrids of a number of agriculturally important crops have been successfully produced, including cotton, barley, tomato, rice, jute, Hordeum X Secale, Triticum x Secale, Tripsocumto and some Brassicas. However, with the advent of new editing technologies, these hybridizations would be possible in future whereby, recombination leading to the formation of an embryo by fusing egg and pollen nucleus would be done in vitro. One similar technique involves the fusion of protoplast. However, recovery of plants from this technique is pretty low suggesting that more optimizations are required to make it a reality similar to animal IVF techniques.

## 9.1.4.2 Embryo Culture

Recently embryo rescue has been utilized to overcome embryo fatality or abortion in some of the crop species. Some crops undergo normal fertilization but due to some genetic factors or due to poor embryo development the embryo is aborted thus

leading to no progeny. In this case, fertilized egg cell or embryo is removed from the plant and cultured under aseptic conditions leading to recovery of the plantlets. This technique has been successfully applied to crops like maize, rice, and wheat.

#### 9.1.4.3 Protoplast Fusion

Any two plant protoplasts can be fused by chemical or physical means; production of unique somatic hybrid plants is limited by the ability to regenerate the fused product and sterility in the interspecific hybrids rather than the production of protoplasts. Although success of protoplast fusion has been limited mostly to Nicotiana and few other species, trials in other important species need to be optimized especially in relation to protoplast regeneration or formation of somatic hybrids after protoplast fusion. Protoplast fusion products are presently grown on approximately 42% of the flue-cured tobacco acreage in Ontario, Canada. This represents a value of approx. US\$199,000,000.

#### 9.1.4.4 Producing Homozygous Pure Breeding Lines for Hybrid Production

Demand of homozygous lines in breeding program has enhanced a lot. These homozygous lines have been achieved by producing doubled monoploid plants via haploid cultures. This is done by treating the plant cell with colchicine which causes doubling of chromosome number.

# 9.2 Plant Genetic Engineering

Plant genetic engineering refers to the process of genetic manipulation of any plant species. Over last 50 years, the field of genetic engineering has developed rapidly due to greater understanding of deoxyribonucleic acid (DNA) as the chemical double helix code from which genes are made. The term genetic engineering is used to describe the process by which the genetic makeup of an organism can be altered using "recombinant DNA technology." This involves the use of laboratory tools to insert, alter, or cut out pieces of DNA that contain one or more genes of interest. Genetic Engineering has been used to transfer or introduce new and valuable traits into plants. Unlike conventional breeding technology, where a trait is selected and bred into other varieties, genetic engineering allows direct transfer of one or just a few genes of interest between either closely or distantly related organisms to obtain the desired agronomic trait. Not all genetic engineering techniques involve inserting DNA from other organisms. Plants may also be modified by removing or switching off their own genes (ISAAA). A variety of genetic engineering techniques are described in the following text:

#### 9.2.1 Agrobacterium Mediated Genetic Transformation

Agrobacterium tumefaciens is a naturally occurring soil microbe best known for causing crown gall disease on susceptible plant species. Agrobacterium has the potential to transfer its own DNA into the plant species. The transferred DNA is stably integrated into plant DNA, and the plant then reads and expresses the transferred genes as if they were its own. The transferred genes then direct the production of several substances that mediate the development of a crown gall. These tumor causing genes (opines and nopalines) are harbored onto the Ti plasmid of the Agrobacterium. In the early 1980s, strains of Agrobacterium were developed that lacked the disease-causing genes or engineered Ti plasmid DNA but maintained the ability to attach to susceptible plant cells and transfer DNA.

By substituting the DNA of interest for crown gall disease-causing DNA, scientists derived new strains of Agrobacterium that deliver and stably integrate specific new genetic material into the cells of target plant species. If the transformed cell then is regenerated into a whole fertile plant, all cells in the progeny will also carry and may express the inserted genes. Agrobacterium is the workhorse for delivering any desired genes into the plant and its flexibility to transform many plant species makes it a favorite natural plant genetic engineer.

## 9.2.2 Particle Bombardment

Since Agrobacterium has a limited host range in terms of plant species to be infected, an alternative was developed by Klein et al. ([1987\)](#page-24-1). They bombarded plant tissues with micro particles to which the DNA was precipitated and adhered using a technical device called as the Gene Gun. This device operates by compressing helium gas under vacuum and using this pressure to deliver the particles called as microprojectiles into the plant tissue. Since the projectiles carry the coated DNA with the gene of interest and are bombarded onto the plant tissue with great force, this method is widely utilized to transform tissues of recalcitrant species such as corn, rice, and other cereal grains which show a low transformation efficiency with Agrobacterium. The method has certain limitations as it is not very cost-effective, it also might cause DNA rearrangements or undesired insertions in the host. Several manipulations are required to optimize the efficient delivery of DNA and recovery of plants.

#### 9.2.3 Electroporation

Electroporation involves the use of plant protoplasts (plant cells lacking the cell walls) to directly take up macromolecules/DNA from their surrounding fluid using an electrical pulse or other reagents. Plant protoplasts are generated by dissolving the cell walls using enzymes like pectinase, cellulase, and macerozyme; they are kept in a medium and then under an influence of electrical pulse or other reagents e.g. Polyethylene Glycol (PEG) that can alter the mechanics i.e. ion uptake of cell membrane can take up macromolecules like DNA/RNA. Further these protoplasts are regenerated to form complete plants. This method is not widely used due to poor efficiency of macromolecule delivery and regeneration of the protoplast.

# 9.2.4 Microinjection

DNA can be injected directly into anchored cells. Some proportion of these cells will survive and integrate the injected DNA. However, the process is labor intensive and inefficient compared to other methods.

# 9.3 Non-transgenic Method for Genetic Manipulation

Use of virus to transiently deliver DNA that can result in genetic manipulation has been observed; however, this method needs more research to be used frequently. The other method includes epigenetic regulation of genes by transient delivery of proteins, RNA to manipulate the expression of some genes. This method has also been applied in animal studies and recently been tried in some of the plant species.

# 9.4 New Tools to Mitigate Environmental Risks Using Technologies Like CRISPR-Cas and Other Genome Editing Tools

CRISPR/Cas9 is a rapidly growing genome editing technology which has been successfully applied in many model and crop plants. CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats and Cas9 is a nuclease associated with CRISPRs. Lately the system has been modified as an application tool for genome editing in different organisms. The main components of this system are Cas9 protein and sgRNA (single guide RNA) which recognizes the PAM sequence in the genome and guides Cas9 to it, whereby it forms a complex and the Cas9 protein cuts the DNA in a highly specific manner. This technology is used to create simple additions/deletions, editing or replacement of nucleotide sequences, or insertions of large DNA sequences or genes.

# 9.4.1 Use of CRISPR Technology to Save Endangered Species

To meet the need of the growing population, mankind has focused on cultivating crops for food and feed; numerous species were rendered endangered or wiped out due to the expanding agricultural land use which included clearing of forest land. Also, an emphasis on cultivating economically important trees and other vegetable crop has put a pressure on the natural or indigenous varieties that were cultivated in the past and has led to loss of many such crop varieties over a period of time. Some

	Area (ha/village)					
Crop/cropping			Area decline			
season	1970-1974	1990-1994	$(\%)$	Probable reasons for decline		
Kharif season crops (April–October)						
Panicum miliaceum	14.2	4.9	65.5	Cultivation/introduction of high yielding varieties (HYVs)		
Oryza sativa (Irrigated)	14.2	14.2		Cultivation/introduction of HYVs		
Avena sativa	15.8	3.4	78.5	Cultivation/introduction of potato		
Fagopyrum tataricum	8.6	1.5	82.5	Cultivation/introduction of potato + kidney bean		
Perilla frutescens	1.3	$\overline{\phantom{0}}$	100.0	Cultivation/introduction of soybean		
Setaria italica	2.3	0.8	65.2	Cultivation/introduction of soybean		
Oryza sativa (rainfed)	11.2	11.2	$\overline{\phantom{0}}$	Cultivation/introduction of HYVs		
Eleusine coracana	9.6	6.1	36.5	Cultivation/introduction of soybean + amaranth		
Echinochloa frumentacea	2.5	0.7	72.0	Cultivation/introduction of pigeon bean		
Vifna spp.	3.3	-	100.0	Cultivation/introduction of potato, amaranth +kidney bean		
Rabi season crops (October-April)						
<b>Triticum</b> aestivum	14.2	14.2		Cultivation/introduction of HYVs + <i>Brassica</i> spp.		
Hordeum himalayense	17.1	4.7	72.5	Cultivation/introduction potato, amaranth + kidney bean		
Hordeum vulgare	7.0	1.1	84.3	Cultivation/introduction of HYVs		
<b>Brassica</b> campestris	2.0	2.0	$\overline{\phantom{0}}$	-		

<span id="page-8-0"></span>Table 9.2 Area in ha/village under different traditional crops in Kharif and Rabi seasons during 1970–1974 and 1990–1994 in central Himalaya (Source: Maikhuri, Rao & Semwal [2001\)](#page-24-2)

of these nutritious/other value trait varieties lost to cultivated varieties that farmers chose to boost their economic status. In the paper entitled 'Changing scenario of Himalayan agroecosystems: loss of agrobiodiversity, an indicator of environmental change in Central Himalaya, India', Maikhuri et al. [\(2001](#page-24-2)) described the loss of certain valuable crop species to other economically important species due to the changing ecosystem and socioeconomic status (Table [9.2\)](#page-8-0).

India was once home to more than 100,000 indigenous rice varieties with valuable traits like high fiber, nutrient like Zn, Fe and other micronutrients, pests, disease resistance, drought and even lodging. However, most of these varieties were lost in last 30 years due to the cultivation of other economically viable varieties (The guardian, 2017). There have been some conventional methods tried in last few years by the farmers to bring back these heirloom varieties. With the advent of new technology like CRISPR, it is possible to engineer native species or edit a particular trait to create value in an economically important species.

Since CRISPR based technology is highly precise as the guide RNAs are designed specific to the genomic sequences of the target organism, it presents high potential for improving genetic traits in a much precise manner as compared to other genetic engineering technologies. Also, there have been reports of engineering new traits in the target organism without leaving any traces of foreign DNA; this involves delivering Cas9 protein and sgRNA complex directly into the cell to edit, insert, or delete or epigenetically regulate certain genes by transiently upregulating or downregulating the gene expression. Recently many crops like rice, wheat, sorghum, millets, citrus, apple, etc. have been edited to improve economically important traits like herbicide tolerance, insect resistance, drought tolerance, and yield, using CRISPR technology. In the same way, this technique can be used to engineer crops for increasing metal uptake in soil polluted with heavy metals, mining areas, editing woody plants for virus and disease resistance and other crops for traits like water use efficiency, drought tolerance for arid region, improved root system for areas prone to soil erosion, high carbon assimilation, etc.

## 9.5 Plant Tissue Culture as an Industry

## 9.5.1 Value Crops

Large-scale production of medicinal, ornamental, and food crops—over the century, plant tissue culture has been established not only as a tool for genetic engineering but also for large-scale production of medicinal, ornamental, and food crops. These tissue culture grown ornamental plants form the bulk of large-scale production and have commercial value. Ornamental industry has applied immensely in vitro propagation approach for large-scale plant multiplication of elite/or superior varieties. As a result, hundreds of plant tissue culture laboratories have come up worldwide, especially in the developing countries due to cheap labor costs. There have been different methods applied for propagation of these value crops e.g. meristem culture, shoot tip culture, embryogenesis, etc. Of these, micropropagation has been most cost-effective in large-scale commercial cultivation of these crops. Majority of the pot plants such as Begonia, Ficus, Anthurium, Chrysanthemum, Rosa, Saintpaulia, and Spathiphyllum are produced in the developed countries and the Netherlands leads the export of ornamental and pot plants (Anonymous [2003](#page-24-3)). About 156 ornamental genera are propagated through tissue culture in different commercial laboratories worldwide. Four leading exporters (The Netherlands, Colombia, Italy, and Israel) constitute about 80% of the world market and the developing countries of Africa, Asia, and Latin America contribute only less than 20% (Rajagopalan [2000;](#page-25-1) Schiva [2000;](#page-25-2) Rout and Mohapatra [2006\)](#page-25-3).

#### 9.5.2 Medicinal Plants

The products derived from medicinal plants act as a source of drugs in many traditional and other medication systems. The percentage of people using traditional medicines has observed a decline in developed countries: 40–50% in Germany, 42% in the USA, 48% in Australia, and 49% in France (FAO). The global market for Herbal drugs is estimated to be about 72 billion USD, of which the revenue generated from extracts was reported to be USD 27.1 billion in 2016 and is expected to grow approximately by 65% and reach USD 44.6 billion by 2024. Species like Dioscorea deltoidea, Papaver somniferum, Atropa belladonna, Rauvolfia serpentina, Hyoscyamus niger, Digitalis lanata, Datura metel, Digitalis purpurea, Pilocarpusa bonandi, Cinchona ledgeriana are direct contributors to several prescribed medicines. This ever-increasing trend in use of medicinal herbs and herbal products in therapeutic purpose, research, and trade has created tremendous pressure on supply from their wild source. The increasing demand of chemical compounds from these species, indiscriminate extraction, and destruction of their wild habitat have put them at a risk of extinction. A viable alternative to overcome this unsustainability problem of medicinal plants would be the systematic cultivation of medicinal herbs from the wild source and an opportunity to optimize yield to achieve a uniform, high-quality product. Several drugs from species like cardamom, cannabis, cinnamon, ginger, cinchona, opium, linseed, and fennel are now obtained almost exclusively from cultivation source (Alamgir [2017\)](#page-24-4). Since the species growing under different geographies and environmental conditions have different levels of chemical compounds, a profitable alternative could be cultivation of these species under uniform controlled conditions. However, micropropagation or tissue culture of medicinal plants is quite difficult due to high production of alkaloids and phenols which drastically affects the cell proliferation and regeneration of these plants. Unavailability of protocols for culture, long time to establish cell culture makes it a less lucrative system for commercial production. However, with the recent advances in cannabis extracts for medicinal use and the established market size (20 billion USD), medicinal marijuana has made new waves in last one year to look back at the medicinal plant industry and has opened new prospects in this area.

Plant tissue culture also provides interesting prospects for commercial production of food crops. Many fruit and vegetable crops like banana, tomato, potato, sweet potato, capsicum, strawberry, lettuce, etc. are being commercially propagated. Use of meristem culture, shoot-tips culture, cell or embryo culture is being deployed to disease-free cultivation of food crops. Maintenance of controlled conditions during the culture period for temperature, photoperiod, light spectra also has added advantage in maintaining nutrition, flavor, color, and texture for food crops. Such crops have high value in the consumer market as compared to conventionally grown crops. Recently many startup companies have invested in micropropagation of food crops to supply fresh fruits and vegetables to the consumer in big cities. Also, companies and government agencies are culturing and propagating these species in indoor farms to maintain the quality.

#### 9.6 Land Reclamation Projects

Reclamation of Saline, drought stricken, and effluent contaminated lands and fields through propagation and planting of tolerant plant species—Over the period of time due to the increasing population and human activity, most of the forest and agricultural land has been rendered eroded or infertile. In order to reclaim the land, many projects have been started by NGOs and government agencies that involve planting of species that could be grown in adverse conditions; some of these species include N-fixing species of legumes, grasses, and trees.

#### 9.6.1 Controlling Soil Erosion and Forest Lands

Propagation of woody and grass species for reforestation and controlling soil erosion. Soil erosion has been a problem due to the deforestation of land which then tends to be exposed to wind and water erosion. Species that have strong root system tend to hold the soil together. Half of the topsoil on the planet has been lost in the last 150 years. In addition to erosion, soil quality is affected by other aspects of agriculture like compaction, loss of soil structure, nutrient degradation, and soil salinity. Sustainable land use can help to reduce the impacts of agriculture and livestock, preventing soil degradation and erosion and the loss of valuable land to desertification (WWF). Bodies like WWF and other national agencies along with the government are running projects like zero net deforestation to save the current forest species and control erosion; however, for the already eroded land, selection of plant species that can hold the soil and bulk production of the same depending on the geographic location is also an alternative that needs to be considered. Cover crops, such as vetch, rye, and clover are the excellent plants for erosion control. These hardy easy to grow plants send out nets of roots that help hold topsoil in place while also reducing competitive weeds. Some small-scale efforts in this area are known but the true potential of tissue culture to either propagate or engineer such species is yet to be realized.

# 9.7 How Can We Use Plant Tissue Culture to Address Climate Change?

Propagation of endangered or rare plant species—The study, conducted by the World Wildlife Fund, University of East Anglia and the James Cook University, warned that rising temperatures could have a disastrous impact on areas such as the Amazon, Galapagos islands, and Madagascar, as well as European coasts and the Caribbean. Various plant species in the different geographies have been classified as endangered. Botanical garden conservation international (BGCI) describes temperature and rainfall to be the main determinants of the loss of change in behavior of many plant species. Some isolated or disjunct species are particularly vulnerable, as they may have "nowhere to go" e.g. Arctic and alpine species, and Island endemics, Coastal species which will be "squeezed" between human settlements and rising sea levels. Plant genetic composition may change in response to the selection pressure of climate change. Some plant communities or species associations may be lost as species move and adapt at different rates.

As conditions become more suitable for exotic species and less well suited for the native species, an increase invasion trend by alien species may be observed (for example, Bromus is more invasive in wet years (Smith et al. [2000](#page-25-4))). This is especially true given human interventions which have deliberately and accidentally facilitated the spread of species across the globe. Many plant communities act as "sinks" (store carbon), which helps to offset carbon emissions. However, over the next 70 years, the effects of climate change on plants may result in many terrestrial sinks turning into sources.

#### 9.7.1 Conservation of Plant Species Using Tissue Culture Practices

Tissue culture is the technique to culture plant parts or tissues under in vitro conditions. As mentioned earlier in the chapter, research in this area is limited and most of the efforts have been directed towards micropropagation or culture of economically important plant species. The other issues include lack of expert manpower to deal with complicated plant species, lack of complete protocols, and genetic complexity of the plant species itself, some of the species happen to be recalcitrant or do not respond to tissue culture.

Advances in plant biotechnology provide new options for collection, multiplication, and short- to long-term conservation of plant biodiversity, using in vitro culture techniques. Significant progress has been made for conserving endangered, rare, crop ornamental, medicinal, and forest species, especially for non-orthodox seed and vegetatively propagated plants of temperate and tropical origin. Cell and tissue culture techniques ensure the rapid multiplication and production of plant material under aseptic conditions. Medium-term conservation by means of in vitro slow growth storage allows extending subcultures from several months to several years, depending on the species.

Cryopreservation (freezing in liquid nitrogen,  $-196$  °C) is the only technique ensuring the safe and cost-effective long-term conservation of a wide range of plant species (Table [9.3](#page-13-0)). Cryopreservation of plant germplasm has obvious advantages over in vitro storage in terms of space saving and improved phytosanitation (Towill [1991;](#page-25-5) Engelmann [1997\)](#page-24-5). Cryopreservation banking for long-term germplasm storage can be applied to a variety of propagules, including seeds, embryos, spores, pollen, gametophytes, shoot tips, and embryogenic callus cultures. However, longterm storage and culture of plant tissues often present a problem of somaclonal variation (Scowcroft [1984](#page-25-6)); variation have been reported in Musa spp. (Vuylsteke et al. [1990\)](#page-25-7), Solanum tuberosum L. (Harding [1991\)](#page-24-6), Vitis vinifera L. (Harding et al. [1996\)](#page-24-7), and others. The risk of genetic instability may be minimized through the selection and optimal use of organized tissues such as meristems or shoot tips (Kartha [1985](#page-24-8)). Over 110 accessions of rare or threatened species are stored under

Gene bank/country	
The National Bureau for Plant Genetic	
Resources (NBPGR). New Delhi, India	
Kings Park and Botanical Garden, Perth,	
Australia	
IRD Montpellier, France	
National Center for Genetic Resources	
(CNGR), Fort Collins, USA	
National Institute of Agrobiological	
Resources (NIAR), Yamagata, Japan	
<b>INIBAP</b> International Transit Center,	
Catholic University of Leuven, Belgium	
International Center for Tropical	
Agriculture (CIAT), Call, Colombia	
National Center for Genetic Resources	
(CNGR), Fort Collins, USA	
College of Landscape Architecture, Beijing	
Forestry University, Beijing, China	
Phytera, Sheffields, UK	
Sylvagen, Vancouver, Canada	
Biotechnology Laboratory of the Nestle	
Company, Notre Damedoe, France	

<span id="page-13-0"></span>Table 9.3 Efforts in cryopreservation of plant samples (Source: Cruz-Cruz et al. [2013\)](#page-24-0)

cryopreservation at the Perth Royal Botanic Garden in Australia (Touchell and Dixon [1994\)](#page-25-8) and the Cincinnati Botanic Garden in the USA conserves seeds of rare and endangered native species in liquid nitrogen (Pence [1999](#page-25-9)).

## 9.7.2 Bio Cleaners or Development of Plants Scavenging Heavy Metals, Metabolizing Herbicide and Chemicals

Metal tolerant plants can be effective for acidic and heavy metals bearing soils—a drawing of combined effect of human activity and climate change affecting the plants. Excessive human activity, changes in agricultural practices, and industrialization has led to decrease in soil quality, erosion of the top layer often leads to infertile or barren land. With modern day urbanization and industrialization, heavy metal (HM) contamination has become a prime concern. The heavy metals (HMs) and metalloids, including Cr, Mn, Co, Ni, Cu, Zn, Cd, Sn, Hg, Pb, among others, can result in significant toxic impacts. Metals/metalloids concentrations in the soil have been raising at an alarming rate and affect plant growth, food safety, and soil microflora. Heavy metals are extracted from their ores during mineral processing. During this process, some parts are left in the open and transported to other places via wind and flood, causing severe threats to the environment (Lenntech [2004;](#page-24-9)

Nagajyoti et al. [2010](#page-25-10)). The biological and geological reorganization of heavy metal depends mainly on green plants and their metabolism. Metal toxicity has direct effects to flora that forms an integral component of ecosystem. Altered biochemical, physiological, and metabolic processes are found in plants growing in regions of high metal pollution and some of the plants can either detoxify or accumulate metals in their organs like roots, stem, or leaves thus removing excess metals from the soil. For example, under Al-stress, the roots of buckwheat secrete oxalic acid to form non-toxic Al-oxalate complexes which get transferred into the leaves (Ma et al. [1998\)](#page-24-10). A Chinese brake fern (Pteris vittata) can hyper-accumulate As (more than 1000 mg As/kg shoot dry weight, DW). This plant has the capacity to transform As (V) to As (III) and transports it via xylem as an As (III)-S compound along with water and minerals, and gets accumulated in the fronds as As (III) (Ma et al. [2001\)](#page-24-11). The other plants that can volatilize heavy metals include Thlaspi caerulescens for Zn, Cd, Pb, Achillea millefolium for Zn, Alyssum sp. for Ni, cabbage for Pb and Cd (Argint [2003\)](#page-24-12).

In case of eroded soil, drought-resistant, fast growing crops or fodder are chosen which can grow in nutrient deficient soils. Selected plants should be easy to establish, grow quickly, and have dense canopies and root systems. Grasses, particularly C4, can offer superior tolerance to drought, low soil nutrients, and other climatic stresses. Roots of grasses are fibrous that can slow down erosion and their soil forming tendencies eventually produce a layer of organic soil, stabilize soil, conserve soil moisture, and may compete with weedy species. Nitrogen fixing species have a dramatic effect on soil fertility through production of readily decomposable nutrient rich litter and turnover of fine roots and nodules. Reclamation of soil involves introduction of both microbial and plant species into the damaged soil. Plant cell and tissue culture is considered as an important technique for fundamental studies that provide information about the plant–contaminant relationships, which helps to predict plant responses to environmental contaminants, and improve the design of plants with enhanced characteristics for phytoremediation. Callus, cell suspensions, hairy roots, and shoot multiplication cultures are used to study the interactions between plants and pollutants under aseptic conditions (Couselo et al. [2012\)](#page-24-13). Besides being a source of basic research to study phytoremediation, genetically engineered plants with traits for metal chelating, transport, deep root system, etc. are the step forward. Recently, a new transgenic for cotton has been developed at Texas A&M by a research group led by Dr. Keerti Rathore that can detoxify phosphite residues from the soil (The News & Observer 2018). More such research would involve understanding biochemical and molecular basis of phytoremediation, drought, or stress tolerance in native or wild species and genetically engineer these traits in plants that could be used for planting in contaminated or eroded soil. Micropropagation of such plants that possess native traits to scavenge metals, herbicides, and other chemicals is an option that could be considered for future research, besides engineering plants with these traits and improving the genetics of such species involves the use of tissue culture thus presenting the true potential this technique possesses.

#### 9.7.3 Ocean Farms

It is estimated that by 2050, the world population will be close to 9.1 billion and the demand for food will rise by 70%. To tackle these problems, some of the companies are coming up with the idea of Floating Farms. The design suggests building light weight, multilevel, buoyant rigs floating in the oceans. The farms will harvest fish, crops, and sunlight and will be located near areas where food is most needed or there is scarcity of cultivable land. This kind of concept is interesting from environmental perspective as it runs on clean energy i.e. solar energy. The farms will require much less fresh water and fertilizer for producing crops, as the nitrogenous excreta from fish could be used to fertilize crops. The "Smart Floating Farm (SFF)" concept was the brainchild of Barcelona based designers Javier Ponce and Jacob Dycha, who look forward to work with traditional farmers on this [\(www.dailymail.co.uk\)](http://www.dailymail.co.uk). Nearly 40% of the world's oceans are severely affected by human activities, resulting in pollution, exhausted fisheries, and loss of coastal habitats. Here are some of the pictures of Floating Farms (Picture Credit: Mr. Javier Ponce, Founder, Floating Farms) (Figs. [9.1,](#page-15-0) [9.2](#page-16-0), [9.3](#page-16-1), and [9.4\)](#page-17-0).

Another company "Green Wave" came up with similar idea for ocean farms as systems of underwater vertical gardens, it builds 3D vertical ocean farms, where kelp, scallops, and mussels grow on floating ropes, stacked above oyster and clam cages. Green Wave's ocean systems produce healthy and local foods, while capturing carbon and providing a source for biofuel. That is because its farms can grow between 10 to 30 tons of sea vegetables and 250,000 shellfish per acre per year crops that can be used to produce food, fertilizer, animal feed, and sustainable biofuels. According to the company, the kelp produced absorbs five times more carbon than land-based plants, and the crops do not require any fertilizers, fresh water,

<span id="page-15-0"></span>

Fig. 9.1 Top level: solar panels for renewable energy (Photo courtesy: Mr. Javier Ponce, Founder, Floating Farms)

<span id="page-16-0"></span>

Fig. 9.2 Level 2 for hydroponics of vegetables and other crops (Photo courtesy: Mr. Javier Ponce, Founder, Floating Farms)

<span id="page-16-1"></span>

Fig. 9.3 Level 1 is used for fish farming (Photo courtesy: Mr. Javier Ponce, Founder, Floating Farms)

antibiotics, or pesticides ([https://www.virgin.com/virgin-unite/how-vertical-ocean](https://www.virgin.com/virgin-unite/how-vertical-ocean-farming-could-restore-marine-ecosystems)[farming-could-restore-marine-ecosystems](https://www.virgin.com/virgin-unite/how-vertical-ocean-farming-could-restore-marine-ecosystems)). This design has other benefits as well e.g. instead of growing vulnerable monocultures, they create biodiverse ecosystems using the entire water column in production. They also help in rebuilding natural reef systems, using native and restorative species and sequester the nitrogen runoff from farms, factories, and homes, and help prevent oxygen-depleted marine dead zones. Such sustainable units combined with tissue culture units for marine bio-algae or seaweeds could be used in future ocean farms. Some of the chemicals produced by marine bio-algae are biologically active and some possess potent pharmacological

<span id="page-17-0"></span>

Fig. 9.4 Interior of level 2 of floating farm (hydroponic cultivation of vegetable crops) (Photo courtesy: Mr. Javier Ponce, Founder, Floating Farms)

activity. For example, the tropical red alga Portieria hornemannii contains halomon, a halogenated monoterpene with potent anti-tumor activity (Fuller et al. [1992\)](#page-24-14). Another example is temperate brown alga Laminaria saccharina, which contains eicosanoid and oxylipin compounds involved in the mediation of inflammation (Gerwick and Bernart [1993](#page-24-15); Rorrer et al. [1997\)](#page-25-11). Some of advantages and limitations of Seaweed tissue culture and description of marine algal culture in bioreactor units have been described (Rorrer and Cheney [2004](#page-25-12); Baweja et al. [2009\)](#page-24-16).

# 9.7.4 Sustainable PTC Units Running on Solar Panels or Wind (Green Energy)

Commercial Plant Tissue Culture units are operated around the globe by public and private companies. Normally for the developed countries the energy cost for running such labs is low due to the low cost of electricity and uninterrupted power supply; however, for developing nations where the energy cost is high and power supply is interrupted frequently, the cost for running a commercial tissue culture unit could be high. The other alternatives could be use of solar panels or solar roofs; most of the developing nations like India, China, Brazil, Colombia, and some African countries have abundant sunlight for most part of the year. Solar cells are an alternative in these nations as a renewable source of energy. With the new developments in improved solar panels, tiles, or roofs whereby the total cost has been reduced significantly over last 3 years running such laboratories is cost-effective.

Recently University of British Columbia Researchers developed a Bacteria Powered Solar Cell; they engineered E. coli cells to produce high levels of lycopene to act as a light harvesting molecule and coated the bacterial cells with a mineral that

acts as a semiconductor and applied to the glass surface that acted as an anode. They harvested the current density of 0.6 mA/cm<sup>2</sup> which is higher than others in the field. This innovation with further optimizations in engineering and cost production for these bacterial cells could be used in countries that receive less amount of solar energy per year e.g. Europe, Canada, North America. Overall, combining the innovation and improvement in energy sector, material research and engineering can make these PTC units run at very low cost in near future and could be a costeffective alternative to traditional farming that involves large fields or land, chemicals, and water to produce crops. With these applications, in next 20 years commercial crop production can see a dramatic switch.

#### 9.7.5 The Rainmakers

Trees for cloud seeding in deserts—Greenery can have a number of effects on a local climate. Plants are thought to transfer moisture from the soil into the air by evaporation from their leaves, and hold water in the soil close to the surface, where it can also evaporate. What is more, the darker surfaces of plants compared to sandy deserts also absorb more solar radiation, which, along with their rough texture, can create convection and turbulence in the atmosphere. This might create more—or less rainfall (Noorden [2006\)](#page-25-13). It has been shown that vegetation effects account for 30% of annual rainfall variation in Africa's Sahel region (Los et al. [2006\)](#page-24-17). Trees are capable of seeding or cloud formation, and clouds are made of microscopic droplets of liquid water or, in some cases, of small ice crystals. But in the atmosphere, water vapor cannot simply turn into a cloud: it needs solid or liquid particles, known as aerosols, on which to condense. Some of the research studies done in Amazon forests describe that trees help seed the cloud or rain by releasing salt or other chemicals from their leaves; this helps in aerosol formation that acts later as the cloud seed and thus resulting in rain. In one of the studies published in Nature, it was reported that aerosols can form and grow to the size needed to seed a cloud from compounds emitted by trees—without any sulfuric acid and accelerated by simulated cosmic rays.

In another study, it was revealed that Amazonian rainforest not only help in cloud formation, but they also trigger the shift in wind pattern drawing moisture from the ocean and actually causing the rainy season. It was observed that vapor rising from the sea is light as the isotope of H2 deuterium is left behind in the ocean, while in rainforests, high temperatures result in high rate of transpiration during high heat or dry season, the vapors released during transpiration during this period has deuterium which is heavy as compared to the water vapors from the sea. This deuterium acts as the seeder resulting in cloud formation, as these clouds get heavier, it rains causing the atmosphere to heat up resulting in circulation and drawing the movement of water vapors from the sea towards the forest and resulting in rainy season (Wright et al. [2017](#page-25-14)). This action could be replicated in deserts by growing small patches of vegetation involving seeds with higher rate of transpiration.

Along with the artificial cloud seeding these areas can further be transformed into green oasis. Culturing such plants could be possible by micropropagation; on the other hand, new technologies in genetic engineering could lead to development of tree species that can survive arid or semi-arid conditions. Overall, natural cloud seeding by trees is an exciting phenomenon and with the studies mentioned above, it is proven that trees or vegetation can bring about not only the change in rainfall but can drive changes in the season. The matter is thought provoking and calls for projects and plans involving multidisciplinary applied research to save land from desertification.

# 9.8 Policies, Guidelines, and Connectivity Between Environmentalists and Plant Biotechnologists

Environmental Biotechnology is a multidisciplinary branch of environmental science that includes applied research in microbiology and biotechnology with a view to address the issues related to environmental pollution. However, it needs to be extended to areas like plant sciences and agriculture as well. As we have discussed in the above topics, agriculture and forest lands are one of the main affected areas due to increasing population and industrialization. Thus leading to increasing pollution in land, natural water, oceans and air. We have also discussed about some of the technologies like plant tissue culture and genetic engineering that could be applied to address some of the issues e.g. phytoremediation by plants for mined areas, reforestation for eroded soil, land revival by planting suitable species. This calls for establishing more connectivity between environmental scientists, plant biologists, and microbiologists. Instead of conducting research in silos or individual areas, a networked research spanning the discussed areas would lead to faster solutions for such problems.

In an excerpt from The Economic and Social Council of the United Nations (ECOSOC), diffusion of both endogenous and exogenous innovations is a key factor for agricultural growth, hunger eradication, and poverty reduction. The Economic and Social Council of the United Nations (ECOSOC) underscored in 2004, that most developing countries are unlikely to meet the internationally agreed Millennium Development Goals (MDGs) of reducing poverty and hunger without a clear political commitment to making science and technology top priorities in their development agenda and increasing the related budget up to at least 1% of the gross domestic product (GDP).

Government agencies along with Universities and Industries need to develop and launch programs related to multidisciplinary research in these areas of environmental biotechnology and agriculture science or plant biotechnology. In Europe, the kBBE concept has been translated in kBBE specific European Technology Platforms (ETP) and the implementation of several European Research Area (ERA) networks to reduce fragmentation and improve the coherence and coordination of national research programs. Along with this, several European Commission expert groups have been established. The rising need for a sustainable supply of food, raw

materials and energy, together with tremendous progress in the life sciences has led to the concept of the knowledge-Based Bio-economy (kBBE) (2007) or "bioeconomy" with emerging key technologies as major drivers of innovation Research in the different areas of the kBBE has been promoted and financed through the Commission's Framework Programme 7 (FP7) and several Member State initiatives (The knowledge Based Bio-Economy kBBE in Europe: Achievements and Challenges 2010). Some interesting examples of government agencies driving the implementation of such programs are seen in Africa e.g. in Gabon, Centre national dela recherche scientifique et technologie (CENAREST) was established in 2002 and is the biggest in vitro propagation facility in Gabon. By 2004, this laboratory was already producing micropropagated pineapples, bananas, and plantains that were expected to be distributed to farmers by 2005. In Nigeria, vegetatively propagated crops (cassava, yams, sweet potato, pineapple, plantain, banana, etc.) have a significant relevance for food security and poverty reduction. The Federal government is making efforts to rapidly apply biotechnologies for the propagation of some of these important crops, especially cassava, the staple food for most Nigerians. A national program code named "Presidential Initiatives for Cassava Production in Nigeria," aims at replacing local cultivars of cassava with improved ones. Such initiatives are going to drive the socioeconomic development for the countries and reduce the expenditure on research by investing in overlapping research areas towards a common goal.

## 9.9 Societal Impact and Future Path

Science has provided solutions to some of the most challenging problems of the century. The last few decades of investment in biotechnology, agriculture, and environmental sciences research has created awareness in the masses towards scientific solutions to some of the most pressing problems of today e.g. increasing population and decreasing food security, degradation and reduction of agricultural land, pollution of water bodies, land, and oceans due to increased industrialization and urbanization, climate change, etc. All these problems have a direct impact on human livelihood, health, and pose a risk to sustainability of human civilization in near future. However, some of the programs and scientific research have made a positive impact on the society by either providing short term and long-term solutions to some of these issues. We will cover the impact of tissue culture or micropropagation of some of the economically important plants had on raising the economic status of the farmers in developing poor countries and how this can impact other future programs to drive humanity towards a better and sustainable future. In Shandong Province of China, the economic impact of micropropagated virus free sweet potato has been assessed and results indicated that 80% of the farmers have taken up the technology because of its proven ability to increase yields by up to 30%; the IRR was estimated to be 202%, with a NPV of USD 550 million (assuming a 10% real discount rate). By 1998, the annual productivity increases were valued at USD 145 million, with an increase in agricultural income of the province's seven

million sweet potato growers by 3.6 and 1.6%, in relatively poor and better-off districts, respectively (Fuglie et al. [1999](#page-24-18)). In Kenya, the commercial micropropagation of disease-free bananas had been adopted by over 500,000 farmers (Wambugu [2004](#page-25-15)) and was predicted (Qaim [1999](#page-25-16)) and shown (Mbogoh et al. [2003](#page-24-19)) to offer relatively higher financial returns than traditional production. In Vietnam, the introduction of improved high yielding and late blight resistant potato varieties and the subsequent adoption of micropropagation by farmers have seen potato yields increasing significantly from 10 to 20 tons/hectare. The self-supporting plantlet production by the farmers has made the seed more affordable and the rate of return on investment in this new seed is system highly favorable.

Micropropagation not only increased farmer's yields and incomes, but also led to the creation of rural micro-enterprises that have specialized in the commercial provision of disease-free seed (Uyen et al. [1996](#page-25-17)). In India the "Revolving Fund Scheme for Potato Breeders Seed Production" integrated micropropagation and virus detection in the initial stages of potato breeders seed production, leading to two- to threefold improvement of health standards of the seed produced. The scheme generated total revenue of over USD 4 million, over a period of 10 years, with a cumulative balance of USD 0.735, deducting the total expenditures for the development of infrastructure and for the recurring costs (Naik and Karihaloo [2007\)](#page-25-18). Adoption of micropropagated sweet potato varieties in Zimbabwe has brought about a change in the socioeconomic status of the farmers livelihood. Improvements associated with adoption of new sweet potato varieties include diet diversification, food security, increased capacity of investing in other agricultural activities (inputs, etc.), purchasing equipment and animals, paying school fees, and coping with vulnerability factors (drought, disease, inflation). The success in these projects depended on the increase in yield with low inputs thus decreasing the cost of production. Policy decisions e.g. subsidies provided by the government and the service package in terms of not only the dissemination of the technology but also enablement of the implementation of a new technology by providing guidance and solution to short term problems arising during the implementation phase of such projects also made these projects successful. Adaptation of these technologies depends on the ease associated with the above-mentioned factors and the government policies and support. A synergistic approach from the scientific experts, government agencies, policy makers, and farmers can truly bring about a huge benefit to not only the individual farmer or the farming communities but to poor and developing countries, leading to stability and progress of the society.

#### 9.10 Conclusion

Though plant tissue culture has been practiced for more than a century now and significant advances have been made over the last 50 years in research and commercial development of this area, its full potential has still not been realized. There are some roadblocks in the field of plant tissue culture due to reasons discussed earlier in the chapter that happen to have a significant impact on the development and

application of this area. Most of the academic research in Plant Tissue Culture is conducted with a view to publish the findings of the research rather than with a vision to apply the findings of the research to benefit the unmet needs of the farmers. Hence most of the published protocols from plant tissue culture research are hardly reproducible or lack enough data in terms of fine observations of the tissue or cell growth that could lead to have a stabilized protocol or could become a process. More than this, most of the research involving genetic engineering of plants whereby plant tissue culture is used just as a tool to test the effect of the newly engineered trait in plant cells deploys the use of easy to transform tissue or variety. The wild species cultivated indigenously or elite varieties that are cultivated by conventional means are normally left out even from tests and only few research institutes or companies focus on such varieties. These significant gaps hinder in the application of this technology to a broad range of varieties or species. However, with the right kind of scalable protocols that work for more than one variety, the technology demonstrates high potential to be used for areas in commercial production of fruits, vegetables, cereals, and woody plant species. The topics covered in the chapter about large-scale production of woody plants or trees for reforestation, eroded lands, phytoremediation, etc. can be realized by investing in research in this area leading to cost-effective large-scale production of such species.

Another important need is to break the silos i.e. instead of providing funds and having multiple research programs for each scientific discipline due to the expertise, it would be valuable to have network research programs in multidisciplinary areas towards a common goal; these programs need to be launched by Government Organizations, Industries, and other private funding agencies e.g. engineering "Poplar" for phytoremediation could be a network project with experts from genetic engineering, environmental science, physiology, and forestry. Such sporadic initiatives are launched but the numbers of such network programs are too few and often involve experts located in far off places. This can be curbed by short term transfer of experts to the relevant area or country to facilitate the project. This kind of initiatives will cut down the costs and timeline of a project.

Technology transfer or implementation of large-scale propagation of plants that could be viable for farmers or other industries involves not only a robust scalable technology but also support in form of subsidies from the government. Farmer awareness programs by Industry experts or government officials are also important to make this a success. Examples of commercial micropropagation of crops in Africa involved initiatives and support from the government, funds from different agencies, and awareness of the farmers made these initiatives successful and led to an upliftment of the socioeconomic status of the farming communities. Initiatives like Floating Farms are building the commercial agriculture platforms for the future. Investment in such technologies and spreading awareness about building a sustainable ecosystem and guaranteeing food security at low cost by intelligent use of clean energy and technology is the need of the hour. Overall, despite many challenges, tissue culture is not only a workhorse for genetic engineering, but it also presents tremendous opportunities for other disciplines e.g. environmental sciences and forestry, and farming communities. In this time of climate change, decreasing food

security and sustainability of the human kind investments in tissue culture can provide a means of livelihood as an alternative to conventional agriculture that involves land, high input of chemicals, and overall cost.

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