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Plant tissue culture biotechnology in Ethiopia: Challenges and opportunities

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ABSTRACT

Ethiopia is the second most populous nation where 80% of its inhabitants acquire their livelihood from agriculture. However, the long-lasted subsistence agricultural mode of production of the country could not enabled the population to increase agricultural productivity and boost the national economy. In this regard, plant tissue culture technology is the likely opportunity for Ethiopian agricultural system towards improving agricultural yields. Indeed, so far, conventional breeding and selection of plants and animals played a vital role in agricultural productivity. Yet, unlike many developed nations, Ethiopian is at initial stages of getting benefits from modern biotechnological products. But, nowadays, Ethiopian government is giving due attention towards designing and implementation of policies and strategies related to biotechnology, particularly in agricultural sectors. In fact, it remained difficult to use such tools to obtain solutions for major agricultural problems due to lack of resources and well skilled personnel. But, state universities and research institutes, and some private enterprises, are playing a promising role in conducting research and producing skilled manpower in the area of biotechnology. In this regard, Ethiopian Institute of Agricultural Research (EIAR) and Addis Ababa University take the leading role in doing researches related to plant tissue culture biotechnology in agricultural sectors so as to produce drought and diseases resistant crop varieties. Though biotechnology and genetic engineering has a significant contribution by addressing environmental and food safety concerns with rigorous bio-safety regulations, it could also cause hazardous problems upon human health and the ecosystem when it is applied irresponsibly and unsafely.

KEYWORDS

Ethiopia;
Tissue culture;
Biotechnology;
Micropropagation;
Agriculture;
Explants;
GMOs.

INTRODUCTION

Ethiopia is the second most populated nation in Africa where agricultural sector is the leading national income supporting more than 80% of the population[1]. Ethiopian agricultural system is predominantly characterized by high level of subsistence production and low improvement of traditional farming practices resulting in declining of agricultural productivity. In fact, persistent dependency on rainfall and frequent occurrence of

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drought and other natural calamities add up for low agricultural production.

Hence, application of tissue culture biotechnology in the field of agriculture seems very crucial so as to increase agricultural productions for the purposes of feeding the population with no need of international aids.

Tissue culture is an *in vitro* culture of cells, tissues, organs or the whole plant parts under aseptic and controlled nutritional and environmental conditions often to produce the clone of plants. Nowadays, plant tissue culture is globally being widely used for large scale plant propagation and improvement as well as production of secondary metabolites[11].

In Ethiopia, application of tissue culture is premature. At this time, conventional plant and animal breeding and selection techniques are widely used as major tools for increasing agricultural productivity[17]. Until recently, research guidelines related to genetic engineering do not exist in the country discouraging Ethiopian scientists from conducting projects of genetic engineering and consequently significantly hampers the research and capacity building process in modern biotechnology research and development in the country. But, nowadays, the government has approved biosafety law that encourages genetic engineering and marketing of its products in the country. In this regard, a wide range of crop production problems that are either difficult to address using conventional research techniques are likely to be solved using crops genetically engineered for specific traits and adapted to local conditions.

Historical development of tissue culture technology

The science of plant tissue culture takes its roots from the discovery of cell followed by setting out the cell theory. In 1838, Schleiden and Schwann proposed that cell is the basic unit of all living organisms. They hypothesized that cell is capable of autonomy and therefore is capable of regenerating or differentiating into whole organisms (plants, animals or microorganisms) if given an appropriate environment to grow. Based on this premise, in 1902, Gottlieb Haberlandt, a German physiologist, attempted to culture isolated single palisade cells from leaves in knop's salt solution enriched with sucrose for the first time. The cells remained alive for up to one month, increased in size, accumulated starch but failed to divide. Though he was unsuccessful but laid down the foundation of tissue culture technology for which he is regarded as the father of plant tissue culture. After that some of the landmark discoveries took place in tissue culture are summarized under TABLE 1 below.

Techniques of plant tissue culture

Micropropagation

Micropropagation starts with the selection of plant tissues (explant) from a healthy, vigorous mother plant[14]. Any part of the plant (leaf, apical meristem, bud and root) can be used as explant.

Preparation of donor plant

Any plant tissue can be introduced *in vitro*. To enhance the probability of success, the mother plant should be *ex vitro* cultivated under optimal conditions to minimize contamination in the *in vitro* culture[4].

Initiation stage

In this stage an explant is surface sterilized and transferred into nutrient medium. Generally, the combined application of bactericide and fungicide products is suggested. The selection of products depends on the type of explant to be introduced. The surface sterilization of explant in chemical solutions is an important step to remove contaminants with minimal damage to plant cells[10]. The most commonly used disinfectants are sodium hypochlorite[13,19], calcium hypochlorite[9], ethanol[18] and mercuric chloride (HgCl2)[10]. The cultures are incubated in growth chamber either under light or dark conditions according to the method of propagation.

Multiplication stage

The aim of this phase is to increase the number of propagules[16]. The number of propagules is multiplied by repeated subcultures until the desired number of plants is attained.

Rooting stage

The rooting stage may occur simultaneously in the same culture media used for multiplication of the explants. However, in some cases it is necessary to change media, including nutritional modification and growth regulator composition to induce rooting and the development of strong root growth.

Acclimatization stage

At this stage, the *in vitro* plants are weaned and

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TABLE 1 : Summary of historical development of tissue culture

Years	Events in the history of tissue culture
1902	Haberlandt proposed concept of *in vitro* cell culture
1904	Hannig cultured embryos from several cruciferous species
1922	Kolte and Robbins successfully cultured root and stem tips respectively
1926	Went discovered first plant growth hormone –Indole acetic acid
1934	White introduced vitamin B as growth supplement in tissue culture media for tomato root tip
1939	Gautheret, White and Nobecourt established endless proliferation of callus Cultures
1941	Overbeek was first to add coconut milk for cell division in *Datura*
1946	Ball raised whole plants of *Lupinus* by shoot tip culture
1954	Muir was first to break callus tissues into single cells
1955	Skoog and Miller discovered kinetin as cell division hormone
1957	Skoog and Miller gave concept of hormonal control (auxin: cytokinin) of organ Formation
1959	Reinert and Steward regenerated embryos from callus clumps and cell suspension of carrot (*Daucus carota*)
1960	Cocking was first to isolate protoplast by enzymatic degradation of cell wall
1960	Bergmann filtered cell suspension and isolated single cells by plating
1960	Kanta and Maheshwari developed test tube fertilization technique
1962	Murashige and Skoog developed MS medium with higher salt concentration
1964	Guha and Maheshwari produced first haploid plants from pollen grains of *Datura* (Anther culture)
1966	Steward demonstrated totipotency by regenerating carrot plants from single cells of tomato
1970	Power *et al.* successfully achieved protoplast fusion
1971	Takebe *et al.* regenerated first plants from protoplasts
1972	Carlson produced first interspecific hybrid of *Nicotiana tabacum* by protoplast Fusion
1974	Reinhard introduced biotransformation in plant tissue cultures
1977	Chilton *et al*. successfully integrated Ti plasmid DNA from *Agrobacterium tumefaciens* in plants
1978	Melchers *et al*. carried out somatic hybridization of tomato and potato resulting in Pomato
1981	Larkin and Scowcroft introduced the term somaclonal variation
1983	Pelletier *et al.* conducted intergeneric cytoplasmic hybridization in Radish and Grape
1984	Horsh *et al*. developed transgenic tobacco by transformation with *Agrobacterium*
1987	Klien *et al.* developed biolistic gene transfer method for plant transformation
2005	Rice genome sequenced under International Rice Genome Sequencing Project

(adopted from Hussain et al., 2012)

hardened. Hardening is done gradually from high to low humidity and from low light intensity to high light intensity. The plants are then transferred to an appropriate substrate (sand, peat, compost etc.) and gradually hardened under greenhouse.

Current status of tissue culture biotechnology in Ethiopia

Several developed nations in the Western hemisphere and the Asia-Pacific region are already benefiting significantly from modern biotechnology[6]. However, the majority of the developing countries, including Ethiopia, have very little access to this emerging economic sector.

Ethiopian government development strategy recognizes the leading role of agriculture in the economy and stipulates that for the country to record rapid economic prosperity. The strategy identifies information and communication technology and biotechnology as essential tools for rapid transformation of largely subsistence mode of production to market-oriented production enterprises that ultimately lead to industrialization[1,7].

Modern agricultural biotechnology research and development are recently being developed along with long established conventional agricultural research in Ethiopia. In this regard, public institutions with significant activities in the area of biotechnology include the Ethiopian Institute of Agricultural Research (EIAR), Addis Ababa University, National Veterinary Institute, The National Animal Health Research Laboratory, Institute

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TABLE 2 : Development status of tissue culture protocols at Ethiopian institute of agricultural research (adopted from Abraham, 2009)

Plant Name	Explant Used	Main Purpose	Status
Banana	Shoot tips	Micropropagation, Virus cleaning	Completed and being scaled up
Black pepper	Shoot tip	Micropropagation	Ongoing and in good progress
Brassica spp.	Anther	Double haploid line development	Initial stage
Cardomom	Rhizome lateral bud	Micropropagation	Completed and being scaled up
Cassava	Meristem	Micropropagation, Virus cleaning	Initial stage
Citrus	Seed	Micropropagation, virus cleaning	Ongoing and in good progress
Coffee	Leaf	Micropropagation, *in vitro* disease screening	Completed and being scaled up
Enset	Shoot tip	Micropropagation	Ongoing and in good progress
Garlic	Meristem	Micropropagation, virus cleaning	Initial stage
Geranium	Shoot tip	Micropropagation	Completed
Ginger	Rhizome lateral bud	Micropropagation	Initial stage
Grapevine	Shoot tip	Micropropagation	Completed
Cardamon aframomum	Rhizome lateral bud	Micropropagation	Completed
Niger	Anther	Double haploid line development	Ongoing and in good progress
Pineapple	Shoot tip	Micropropagation	Completed and being scaled up
Potato	Node	Micropropagation, virus cleaning	Completed and being scaled up
Sweet potato	Shoot meristem	Micropropagation	Initial stage
Tef	Floral part	Double haploid line development	Ongoing and in good progress

of Biodiversity Conservation, the International Livestock Research Institute and the Regional Agricultural Research Institutes.

In Ethiopia, plant tissue culture has been given the highest priority in biotechnological research and development. This is because tissue culture provides large quantities of disease-free plant materials in short time. Tissue culture activities first started in Ethiopia in 1980's at Addis Ababa University with focus on micropropagation of indigenous forest species like *Podocarpus* sp., *Cordia africana, Hagenia abyssinica* and *Annengeria sp*. that are either difficult to regenerate vegetatively or require long time propagation[8]. This was followed by micropropagation works carried out on some Ethiopian plant species like *Phytolacca dodecandra*[5], *Eragrostis tef*[2] and *Enset ventricosum*[3,15].

In 2000, a more comprehensive and concerted emphasis was given for a protocol of optimization for mass propagation, disease cleansing and *in vitro* conservation of economically important crop species. The major achievements from these works include the distribution of large number of tissue culture plants of banana, hybrid coffee, pineapple and potato to farmers in various parts of the country (see TABLE 2). In addition to micropropagation and virus cleansing, *in vitro* techniques are also being used for production of dihaploid plants to reduce breeding cycle by obtaining pure line for further improvement in crops like tef and Niger[1] and for *in vitro* screening of crops like coffee for resistance to coffee berry diseases.

Challenges and opportunities of application of tissue culture in Ethiopia

In Ethiopia, application of technologies generated by conventional research has significantly improved the country's agricultural productivity in the past[1]. If biotechnology is properly integrated into these technologies, it would complement these efforts by providing opportunity to speed up such processes giving new solutions to the old and emerging problems in a more precise and cost-effective manner. The potential of tissue culture biotechnology in improving crop and livestock productivity is very huge and rapid progress is being made worldwide on its application. In this regard, Ethiopia has developed favorable policies to facilitate the safe application of biotechnology in agricultural sectors in particular. Yet, nowadays, it remained difficult to use such tools to acquire solutions to major agricultural problems owing to lack of resources (infrastructures) and well skilled personnel.

The advantage of tissue culture for rapid and large

scale multiplication of plants has been widely recognized in the country in recent years and research efforts are now being extended to other institutions as well. For instance, regional agricultural research institutes namely the Amhara Agricultural Research Institute and Southern Agricultural Research Institute have recently developed their capacity and initiated tissue culture work for micropropagation of selected crops of importance in the respective geographical area. Furthermore, private enterprises like Mekele Plant Tissue Culture Laboratory have recently engaged in large scale multiplication and propagation of tissue culture of crops including banana, sugarcane, grapevine and flowers to producers in northern Ethiopia.

Genetic engineering offers several benefits when used responsibly by addressing the environmental and food safety concerns with rigorous biosafety regulations. Until recently, guidelines with genetic engineering research and deployment of genetically modified organisms (GMOs) do not exist in the country. This situation discouraged Ethiopian scientists from initiating genetic engineering projects and participating in similar network activities at regional and international level and consequently significantly hampering the research and capacity building process in modern biotechnology research and development in the country. Three years back, the Ethiopian government has approved biosafety law which is expected to encourage genetic engineering research as well as marketing of its products in the country. A wide range of crop production problems that are either difficult or impossible to address using conventional research techniques are likely to be solved using crops genetically engineered for specific traits and adapted to local conditions. For some of these constraints, transformation technology is already developed elsewhere and commercially available and only needs to be introduced and adopted to local conditions with minimum value inputs. Recently, the private sector has expressed keen interest in introducing *Bt* cotton to boost its production and thus satisfying the booming textile industry in the country[1].

It is also possible to use transgenic plants as parents to transgress the desired genes to locally preferred cotton varieties by conventional breeding. On the other hand, there is a need to develop local capacity in genetic engineering technologies in terms of infrastructure and manpower to address constraints on indigenous crops like *Eragrostis tef* and *Enset ventricosum*.

Potential impacts of GMOs

Genetic engineering, if not managed safely and responsibly, could impose some dangerous problems on the environment and wellbeing of human. For instance, transgenic plants harm non-target species directly and indirectly down the food chain. For instance, Bt-cotton harms bees which are major pollinators, Bt-maize harms lacewings fed on pests that have eaten Bt-maize, and Bt-maize pollen also harms larvae of Monarch butterflies. Transgenic potatoes with snowdrop lectin harms ladybirds fed on aphids that have eaten transgenic potato[12].

Transgenic varieties are unstable, do not breed true, and do not perform consistently. Herbicide tolerant transgenic crops are incompatible with sustainable agriculture dependent on mixed cropping and crop rotation. Broad-spectrum herbicides harm earthworms and microoragnisms that maintain natural soil fertility in organic farming. Transgenic plants with bt-toxin undermine pest control for organic farming and are toxic to major pollinators and other beneficial insects.

Transgenic lines are even more genetically uniform than conventional monoculture crops and may hence be more susceptible to diseases and environmental exigencies. Viral resistant transgenic plants can generate new, often super infectious viruses. Terminator technologies destroy seed fertility[12].

The hazards are inherent to the hit or miss technology. Random gene insertions give random genetic abnormalities and unexpected effects. New genes, gene constructs and products from viruses, bacteria and non-food species are introduced into our food for which no safety tests exist. Interaction between introduced gene and host genes increases unexpected effects including toxins and allergens. The technology enhances horizontal gene transfer and has the potential to generate new viruses and bacteria that cause diseases and spread drug and antibiotic resistance.

Horizontal gene transfer and recombination spread antibiotic resistance genes and have created new pathogens in recent years. Strains of four dangerous bacteria, including the one causing tuberculosis, are resistant to all antibiotics and hence are untreatable. So far, reports reveled that at least 40 new viruses that cause disease in human beings have emerged between 1988

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and 1996. Transgenic plants were found to transfer transgenes and antibiotic resistant marker genes to soil microorganisms and fungi[12]. Usually, DNA released from dead or viable cells persists in all environments and remain infectious.

CONCLUSION

Biotechnology is a scientific technique based on biology, especially used in agriculture, food and medicine, which uses living organism or substance of organism to make or modify product, to improve plants or animal or to develop microorganism for specific uses. The genetic modification leading to improvement of organism is necessary for production of certain products. The most common techniques of biotechnology include tissue culture, *in vitro* regeneration, organogenesis, genetic engineering (transformation) and embryogenesis.

Tissue culture biotechnology is the most widely used tool in agricultural sectors for improving and producing disease-free plants and animals, and their products. In this regard, developed nations have significantly benefited from the product of biotechnology in general and tissue culture technology in particular.

Unfortunately, underdeveloped countries including Ethiopia missed such opportunities so far as the result of lack of resources and required knowledge, and lack of awareness. However, nowadays, the Ethiopian government started planning and implementing policies and strategies related to biotechnology in agricultural sectors.

In Ethiopia, application of plant tissue culture biotechnology is at initial stage. In fact, presently, many government universities and research institutes are taking initiatives towards developing curricula, educating skilled manpower and conducting problem solving researches in the area of biotechnology. Despite the fact that biotechnology would solve societal problems, irresponsible and unsafe application and management of biotechnological products could severely harm the wellbeing of human, his possession and the environment at large. So, application of biotechnology and its product should be handled in a responsible and safe ways.

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