

Current State and Development of Micro Propagation of Selected High Value Crops in Ethiopia: A Review

Habtamu Seyoum¹, Ashenafi Ayano², Berihu Menges¹

¹Plant Biotechnology Laboratory/Department, Ethiopian Institute of Agricultural Science, Jimma Agricultural Research Center, Jimma, Ethiopia

²Coffee Breeding and Genetics Department, Ethiopian Institute of Agricultural Science, Jimma Agricultural Research Center, Jimma, Ethiopia

Email address:

htito2050@gmail.com (H. Seyoum)

To cite this article:

Habtamu Seyoum, Ashenafi Ayano, Berihu Menges. Current State and Development of Micro Propagation of Selected High Value Crops in Ethiopia: A Review. *American Journal of Life Sciences*. Vol. 9, No. 4, 2021, pp. 81-91. doi: 10.11648/j.ajls.20210904.14

Received: July 22, 2021; **Accepted:** August 6, 2021; **Published:** August 24, 2021

Abstract: Rapid and mass propagation of clean and quality seedlings in horticulture industry becomes a priority for many countries to supply increasing demand of planting materials. Attempt of exploiting the benefit of heterosis from F1 hybrid and overcoming the shortage of planting materials associated with it needs proper intervention. Tissue culture techniques offer disease free, uniform, and large amount of planting materials. The technique is playing a significant role in advancing agriculture in many developing countries in which the current production has to be doubled by 2050. Specially, countries like Ethiopia in which population growth is very high and food insecurity persist, the need for improved production and productivity through the use of modern technologies become apparent. To feed the tremendously growing population, utilization of biotechnology tools such as micro-propagation in agriculture is helpful. Currently, in Ethiopia, somehow there is a fertile ground to exploit the possible advantage which may be acquired from biotechnology as in the case of sugarcane, banana, pineapple etc. micropropagation that boosted the production. This review is therefore to bold out the development of mass-propagation of selected crops and present the current status of protocol optimization of high value horticultural and spice crops. In the meantime, the development of private tissue culture laboratories makes an encouraging advancement where it has to be largely consolidated and highly linked with innovating laboratories to take over the responsibility of multiplying improved varieties. Thus, it can be concluded that despite the development of biotechnology remains at its early stage, promising results were obtained from micro propagation work and if adequate attention given to rapid development of protocol for mass propagation of high value cash crops, there exist untapped potential of biotechnology in Ethiopia.

Keywords: Horticulture, Micro Propagation, High Value Crops, Biotechnology

1. Introduction

Micro propagation is an *in vitro* vegetative propagation of plants by tissue culture with the objective to produce large numbers of progeny plants, genetically identical to the parent. Usually it starts with the selection of explants (plant tissues) from a healthy, vigorous mother plant [1]. Apical meristems, buds (nodes), leaves, stems and roots are parts of mother plant which can be used as sources of explants. It is a procedure through which plant organs, embryos, cells or protoplast are aseptically isolated, incubated on artificial media and allowed to grow into complete plants in under controlled environmental condition. The technique made

great progress since the development of plant totipotency to many applications either for academic or applied sciences including germplasm conservation, embryo rescue and production of (haploid, transgenic and pathogen) plants and secondary metabolites. Without a doubt, plant tissue culture is currently an essential component of most crop improvement programmes.

Ethiopia has got familiar with the science of biotechnology since early 1980s when the AAU started using tissue culture techniques to propagate native tree species which are either difficult for vegetative regeneration or require long time for establishment via seed propagation [2]. However, more comprehensive and concerted tissue culture research program

was rolled out by the Ethiopian Institute of Agricultural Research (EIAR) in 2000, with the emphasis to optimize protocol for invitro propagation and virus elimination of economically important crops and/or plant species including: banana, cardamom, grapevine, citrus, garlic, potato, geranium, enset, coffee, pineapple, black pepper, sweet potato, cassava, *afmomum corrorima* and other high value crops [3]. Following progress made in tissue culture, regional agricultural research institutes like Amhara Agricultural Research Institute, Southern Agricultural Research Institute, Oromiya Agricultural Research Institute and some higher learning institutions have adopted and expanded tissue culture application in Ethiopia. In addition, private enterprises such as Mekelle Plant Tissue Culture Laboratory also joined the public institutions in undertaking tissue culture activities. Adane et al. [4] summarized concerted plant tissue culture research activities started at the EIAR (Table 1).

In the last decades, however, the development of this sector encircled by many challenges, and hence unable to

satisfy the demand for agriculture modernization which the country envisage for. The development strategy of the Ethiopian government recognizes the dominant role of agriculture in the economy and stipulates that for the country to register rapid economic prosperity, it should follow the path of Agricultural Development-Led Industrialization (ADLI). Despite many challenges, it is agriculture that still accounts for half of gross domestic product (GDP), 71% exports earning and 80% of total labour force employment [5]. The main pillar of ADLI was achieving food security thereby increasing agricultural productivity. Application of technologies generated by conventional research has significantly increased the country's agricultural productivity in the past. Biotechnology, if properly integrated into these strategies, would complement and catalyze the efforts by speeding up processes and giving new solutions to the old or emerging problems like shortage of planting materials in a more precise and cost effective manner [4].

Table 1. Status of protocol development for micropropagation of major crops species in Ethiopia.

Crop species	Sample source (explants)	Main purpose	Status
Banana	Shoot tip	Micropropagation, virus-cleaning	Completed and being scaled up
Cardomom	Rhizome	lateral bud micropropagation	Completed, being scaled up
Grapevine	Shoot tip	Micropropagation	Completed
Citrus	Seed	Micropropagation,	virus-cleaning ongoing, good progress
Garlic	Meristem	micropropagation,	virus cleaning initial stage
Potato	Node	micropropagation,	virus-cleaning completed and being scaled up
Geranium	Shoot	Tip micropropagation	Completed
Enset	Shoot	Tip micropropagation	ongoing, good progress
Tef	Floral part	Double haploid	Line development ongoing, good progress
Niger	Anther	Double haploid	Line development ongoing good progress
<i>Brassica</i> spp.	Anther	Double haploid	Line development initial stage
Coffee	Leaf	Micropropagation,	Optimization of micro propagation protocol under ways
Korerima	Rhizome	Lateral bud micropropagation	Completed
Pineapple	Shoot tip	Micropropagation	completed, being scaled up
Black pepper	Shoot tip	Micropropagation	ongoing, good progress
Sweet potato	Shoot meristem	Micropropagation	initial stage
Ginger	Rhizome	Lateral bud	virus-cleaning completed
Cassava	meristem	Micropropagation,	virus cleaning initial stage

Source: Adapted from Adane, et al. [4]

Furthermore, realizing the relative advantage and timeliness; the government of Ethiopia established Ethiopian Biotechnology Institute in 2016. It is established by the council of ministers regulation number 388/2016 as an autonomous federal institution accountable to guide biotechnology issues in the country and answerable to the Ministry of innovation and Technology. Its mandates and responsibilities span a wide array of sectors in the nation. It is responsible to formulate a policy and framework as well as establish a system to conduct problem solving research and development in Bio and Emerging Technologies in the country.

Now, in Ethiopia somehow there is a fertile ground to exploit the possible advantage which may be acquired from biotechnology especially from tissue culture. Currently some of the micro-propagation efforts came to results and commenced to solve the prevailing shortage of planting

material. The objective of the current review is therefore to highlight the major achievements of protocol development for mass-propagation of some important high value cash crops and to learn from other similar efforts made elsewhere on plant tissue culture research as well as to compile and briefly present the current state and development of protocol optimization/development in Ethiopia.

2. Objectives

- 1) To indicate the effort made by research centers and show summary of released micro-propagation protocol for some high value crops in Ethiopia.
- 2) To scan and bold out the propagation protocols already commercialized and came to planting material production.
- 3) To review and learn from other laboratories.

3. Propagation Histories in Some High Value Crops

3.1. Sugarcane

Sugarcane (*Saccharum officinarum* L.) is monocotyledonous crop plant that mostly propagates through conventional methods. It is an important agro-industrial crop that normally reproduces through sexual (flower or fuzz) and asexual by stem cuttings (sets) and in vitro propagation. However, low propagation rates, slow propagation process and the potential carrier of pathogen in seed cane for long time make conventional or sexual methods inefficient. Varieties of sugarcane are highly heterogeneous and generally multiplied vegetatively by stem cutting. Hence, rapid multiplication methods for large scale commercial productions within a short period of time are needed. In most tropics and subtropical sugar producing regions nodal

sections of sugarcane with 2 or 3 nodes are commonly used as a planting material [6].

In Ethiopia, sugarcane is widely cultivated for several purposes starting from smallholder farmers up to large scale commercial farms; the later mainly produces sugar and other byproducts [7]. The country's sugar industry is one of the major economic pillars responsible to fulfill the nation's sugar requirements despite multifaceted production challenges including lack of proper planning and implementation of sugar development project such as kuraz [8]. Recently, the state has taken some corrective measures on sugar sector such as enhancing the production capacity of old and new sugar estates and calling for partial privatization in order to increase the efficiency. With the aim to fill the chronic shortage of sugar supply across the nation, new sugar processing factories were built in different part of the country and upgrading the old one are presented (Table 2).

Table 2. Major irrigation sites of sugarcane productions in Ethiopia.

River basin	Site no.	Location	Water source	Total acreage (ha)
Awash	1	Angelele balhamo	Awash	11,000
	2	Maro gala	Awash	14,700
	3	Kasem kebena	Kasem	17,600
Blue Nile	4	Arjo dedesa	Dedesa	139,000
	5	Anger valley	Anger	65,500
	6	Upper beles	Beles	65,000
	7	Upper dinder	Dinder	80,000
	8	Rahad	Rahad	100,000
Tekeze	9	Angereb	Angereb	45,600
	10	Tekeze	Setit	68,550
Omo gibe	11	Lower omo	Omo	58,000
Baro	12	Abobo/ ubala	Gilo	46,900
	13	Itang	Baro	21,000
Nile	14	Dabus	Dabus	5,100
Omo	15	Gojeb	Gojeb	12,000

Source: Adapted from Temam and Alemayehu [9].

Sugarcane propagation through seed methods does not provide adequate planting material of a variety, particularly newly released varieties to achieve large-scale dissemination. To this effect mass propagation of sugarcane has got a priority to fill the shortage of planting materials. In order to satisfy the shortage of planting material requirement, the Ethiopian Sugar Corporation (ESC) made an agreement with Tigray Biotechnology Center (TBC) and Narus Biotechnology and Agro-Industry Private Limited Company (PLC) to produce a total of 170 million primary acclimatized sugarcane planting materials of 14 different sugarcane genotypes [7].

Below are the sugarcane tissue culture achievements.

The development of micropropagation technology made possible to produce quality sugarcane planting materials. Adoptions of mass propagation methods for sugarcane production programs appeared to be best fit solutions for rapid expansion of sugar factories in Ethiopia. Here, sugarcane tissue culture activities are dealt with somaclonal variation, optimizing somatic embryogenesis, germplasm preservation [6]. Standardization of protocol for

micropropagation of sugarcane focuses on obtaining an optimum condition through studying the effect and type of medium, concentration of plant growth regulators viz. 6-Benzyl Aminopurine, Naphthalene Acetic Acid, Benzyl Adenine, Kinetin, Indol Acetic Acid, Giberrellic Acid on callus formation, embryo development, suspension culture, and shoot regeneration [7, 9-12]. In this regard, great effort has been made by individuals and institutions like research in Sugar Corporation, Addis Ababa and Jimma Universities that developed improved sugarcane varieties and their propagation protocols [12].

3.2. Advances in Coffee Biotechnology

Conventional coffee improvement research involves long process with many different approaches beginning with selection of wild populations and undergoing series of breeding techniques: hybridization and progeny evaluation, backcrossing and interspecific crosses. Such traditional method, unfortunately, requires long time (up to 30 years) to develop new cultivar, which is extremely slow process and expensive, and the resulting seed production and distribution

is not sufficient to fulfill the needs. The advancement of in vitro propagation approaches via SE methods, proven to revolutionize the coffee production by developing superior genotypes at a relatively faster time and subsequently multiply with reasonable cost. In vitro regeneration and propagation protocols have already been optimized successfully for Arabica coffee, including somatic embryogenesis and scale-up of somatic embryogenesis using bioreactors and Erlenmeyer flasks [1, 14], apical meristem and axillary bud culture, induction and development of adventitious buds, culture of zygotic embryos, anther/pollen culture, cell suspension culture and protoplast culture [15]. The advances made to date in coffee SE techniques have opened the door for in vitro manipulation of the crop at cellular and molecular levels, demonstrate coffee a suitable crop for the application of molecular breeding and genetic engineering [16].

To exploit the maximum benefit of heterosis conserved in hybrid coffee, coffee breeders in Ethiopia have created seven hybrids by crossing different parents of Arabica coffee. The hybrid varieties can be propagated by sexual *i.e.* collecting F1 seeds after hand pollination and crossing of two parents or by asexual methods using orthotropic stem cuttings. Propagation via seed requires skilled personnel for coffee tree management operation including handling pollination at the right time. It normally yields small amount of seed per annum, as it is carried by hand pollination to get an F1 seed. Using F2 seeds is not advisable, which could counter produce uniform crop due to segregation. However, vegetative propagation through cutting yields uniform crops but generate relatively small amount of seedlings as it only derived from orthotropic braches. Alternatively, multiplication by tissue culture methods can produce numerous planting materials, and hence is valuable method for propagating hybrid coffee and other woody species. SE methods permit the production of relatively uniform plants on a massive scale in a short periods of time and particularly important for plants having long life cycle and inherently difficult to propagate through conventional methods [17].

3.2.1. Somatic Embryogenesis (SE)

Somatic embryogenesis appeared to be the most promising method among other micropropagation techniques for rapid, large-scale dissemination of elite varieties [18]. Consequent to the absence of efficient clonal propagation technology little of the genetic progress (e.g. F1 hybrid cultivars) is currently disseminated to growers. The recent development of highly heterozygous F1 hybrids in *C. arabica* made necessary to implement an efficient vegetative system for their mass propagation [19]. Using micropropagation techniques, particularly SE one can significantly reduce the time laps needed between the development of improved variety and its subsequent dissemination. Besides, SE guarantees the production of relatively uniform plants on a massive scale within short time. In this regard, highly segregating and transgenic crops could be efficiently produced for large-scale commercial purposes.

(i) Direct and Indirect Somatic Embryogenesis

There are two ways by which somatic embryo regenerated: Direct and indirect method. In direct method (DSE), the somatic embryo develops directly from the explant without undergoing callus production. More often somatic embryos generated via indirect method in which the somatic embryo develops after the production of embryogenic friable callus [20]. Obtaining somatic embryos are normally quicker (approx. 10 weeks) and less numerous in DSE compared to indirect method. These could represent an advantage for the rapid release of cloned material. In indirect SE an embryogenic friable callus is formed either in semi solid or in liquid media (cell suspensions) from which the somatic embryos arise. In this method, secondary regeneration medium are required to produce embryogenic friable callus regenerating several hundred thousand somatic embryos per gram of callus [21]. This process uses liquid medium for embryogenic tissue proliferation and plant regeneration, and consequently preferred for large scale micro propagation procedures. In general, the major advantages of somatic embryogenesis over other vegetative propagation systems relies its higher regeneration rate than organogenesis providing an alternative approach to conventional clonal propagation for mass multiplication of elite cultivars (interspecific or intraspecific hybrids).

(ii) Somatic Embryogenesis in Bioreactors

Coffee somatic embryogenesis can be achieved in liquid medium (cell suspensions), and it is the best alternative to other propagation techniques for Arabica F1 hybrid varieties mass propagation. Coffee SE in liquid media is mainly applied along with bioreactors technology. The term “bioreactor” is generally used to describe a vessel carrying out a biological reaction for the production of microbial, plant or animal secondary metabolites (e.g. alkoids, terpenoids, phenylpropanoids) [22]. CIRAD is a pioneer in the development and use of temporary immersion bioreactor, whose benefits for micro propagation were clearly demonstrated in the coffee SE model through high yielding and reliable processes [23, 24].

The uses of bioreactor present several advantages over solid media SE systems such as; (1) higher multiplication rates of cell or embryos (*i.e.* secondary embryogenesis) (2) during regeneration in liquid media, root and shoot formation is simultaneous, thus, eliminating the need of root induction phase as with conventional micro-propagation methods (e.g. solid media SE or organogenesis) [25]. (3) Cultures can be manipulated such that embryo formation and germination can be synchronized maximizing plant output while reducing labour cost [26]. (4) Liquid cultures are easier to scale-up and sub culturing than solid media based systems. (5) It enables a high degree of control over culture conditions (pH, aeration rate, oxygen, ethylene and carbon dioxide concentrations) and are compatible with the automation micropropagation procedures, particularly using robots, improving the efficiency of embryo production in a short period of time and reducing labor costs [27].

However, lengthy and continuous contact of plant tissues

with the liquid medium could result tissue hyperhydricity [28]. Thus, the phenomena characterized by different degrees of morphological and physiological disorders including a glassy, water logged tissue appearance, disordered growth in the shoot system, and more specifically in the leaves [29]. Hyperhydricity, caused by excessive hydration, is responsible for physical malformation or poor growth and substantial losses during and after in vitro culture.

3.2.2. Hybrid Coffee Nodal Explants Propagation

Nodal culture is one of the possible tissue culture methods allowing the production of plantlets in a short period, and with a narrower genetic base than those under the conventional methods [17]. Several studies have been carried

out with the aim of micro-propagation of superior coffee genotypes by using apical or axillary meristem and nodal cultures [30]. The somatic embryogenesis initiated directly from the stem segment for shoot regeneration of *C. arabica* hybrid has been demonstrated at Jimma Agricultural Research Center (JARC). It was observed that the plantlets with roots shoots are acclimatized in the green house with 87% survival and are successfully transferred to field conditions [31]. Furthermore, JARC biotechnology lab have been propagating and disseminating for quite few years just to familiarize growers with seedlings produced through tissue culture while in the meantime undertaking protocol optimization (Figure 2 and Figure 3).

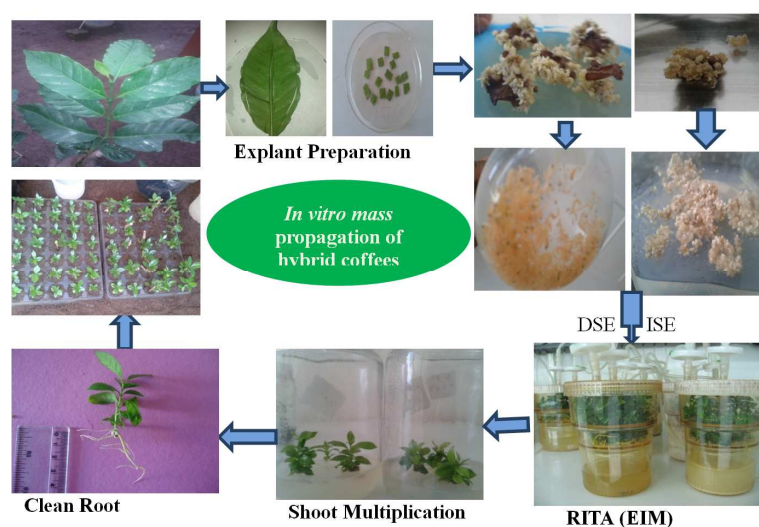


Figure 1. Life cycle of hybrid coffee tissue culture. Source: photo by Elias G. & Berihu M.



Figure 2. In vivo coffee acclimatization. Germinated embryos in RITA (a), Primary acclimatization (b), somatic embryo derived seedlings distributed to farmers (c).



Figure 3. Coffee tissue culture of nodal culture. Source: Elias, 2017.

3.3. Ginger Tissue Culture

Ginger (*Zingiber officinale* Rosc.) is widely cultivated crop in Ethiopia, serving as source of spice ingredient for various recipes. It is also a source of income for many small-holder farmers. In Ethiopia, particularly south and southwest of rift valley areas are suitable for producing the crop. Over the past decade there has been huge demand for clean planting material of improved ginger cultivars [32]. The prevailing demand of planting materials using the conventional propagation techniques is inefficient to have large number of disease free planting material. Application of tissue culture techniques played significant role in producing disease free planting materials and subsequent introduction to uninfected pocket areas,

especially when ginger was severely attacked by bacterial blight (*Ralstonia solanacearum*) in Ethiopia (unpublished data).

To solve such critical problems and even to maintain the germplasm from total loss; *in vitro* conservation and propagation were undertaken in Jimma Biotechnology laboratory. After successful protocol optimization, ginger mass multiplication of commercially released elite ginger cultivars; Yali and Boziab were undertaken to disseminate to farmers. In this review, as part of our previous work, we give highlight on the major steps starting from identification of explant type and determination of suitable hormone combination for *in vitro* propagation [33, 34]. Shoot tip explants on MS basal medium with 2 mg l⁻¹ BA and 1 mg l⁻¹ kinetin gave higher number of shoots within six weeks of culture [34, 35], as well as better performing plantlets with respect to other growth and development parameters. This includes number of leaves and dry weight of plantlets regardless of varietal difference in comparison to axillary bud. In this study shoots cultured on MS medium with 1 mg l⁻¹ NAA alone developed numerous longer roots. Plantlets produced using this propagation protocol was successfully acclimatized within four weeks of hardening period. The acclimatization procedure has been supported with the application of shade nets (at 30 and 70% shade level) and polythene under the greenhouse condition. Subsequently, the seedlings have survived under field condition well.

In vitro developed clean ginger plantlets have also been tested by biochemical test using selective media and serological test (ELISA) to confirm the complete removal of *R. solanacearum* at National Agricultural Biotechnology Research Center and Ambo plant Protection Research Center. It was confirmed that successful optimization of protocol for clean ginger plantlets were developed and subsequent commercialization being commenced [36]. On the other hand to speed up the production of disease free and large number of planting material, agreement was done between EIAR and Tigray Biotechnology Center (TBC) via memorandum of understanding to multiply large number of nationally released ginger varieties. In-vitro propagation procedures and acclimatization of in-vitro raised ginger plantlets demonstrated in figure 4 and Figure 5 respectively.



Figure 4. Ginger in-vitro mass propagation procedure. Adapted from Mengs et al., [34].



Figure 5. Acclimatization of ginger in the greenhouse with forest soil, sand, and coffee husk at 2:1:1 ratio respectively A) in single polyethylene bag for two months B) planted on pot for three months C) seedlings survival under field condition for five months D) rhizomes setting in pot and field after eight months. From our experimental plot.

Using the protocol, multiplication was started in JARC plant biotechnology laboratory to produce ginger plantlets. It is tried to make available initial material as problem solving research results and many of them distributed to research centers working on ginger and submitted to different growers to compensate the ginger planting material that was lost due to bacteria wilt (*Ralstonia solanacearum*), highly devastating disease which affected ginger production in Ethiopia.

3.4. Pineapple in Vitro Propagation

Pineapple (*Ananas comosuss* L.) is one of the most liked tropical fruit with delicious taste. It is propagated through vegetative using suckers, slips or crowns [37]. However, seedlings produced through vegetative method have certain limitations including transmission of diseases, less uniformity and inadequacy for commercial productions. For instance suckers developed from leaves of fully-grown pineapple plants can be used for next plantation after mother plants are harvested. Nevertheless, the suckers (ratoon crops) can be used for only one cycle of culture to limit contaminations and always have to be renewed [38].

The demand for planting materials had long been the bottleneck to produce pineapple in large-scale in Ethiopia. Different possible solutions were hence forwarded to avert the problem, including undertaking the expansion program step by step using the propagules from the existing plantations, and also to undertake *in vitro* propagation. To this effect, Jimma plant biotechnology lab has so far produced over a million seedlings using in vitro techniques and delivered to various users (pers. communication). Pineapple *In vitro* propagation protocol has been successfully optimized and documented by Jimma research center [37]. Besides, mass multiplication of pineapple is taking place as per the development request. In vitro method has comparative advantage over the traditional methods as it leads to the production of large numbers of disease-free uniform planting materials in a relatively shorter period independent of the season.

The demand and availability of planting materials through conventional pineapple production (suckers, slips and crowns) during planting period are not well synchronized since the planting time in the country relies on the rain fed season alone. Hence, micropropagation techniques offer year

round plantlet production, and it is efficient to overcome the problem of planting material shortage to attain the targeted extensive pineapple [39]. The following figure illustrated the process of pineapple propagation at Jimma plant biotechnology lab.

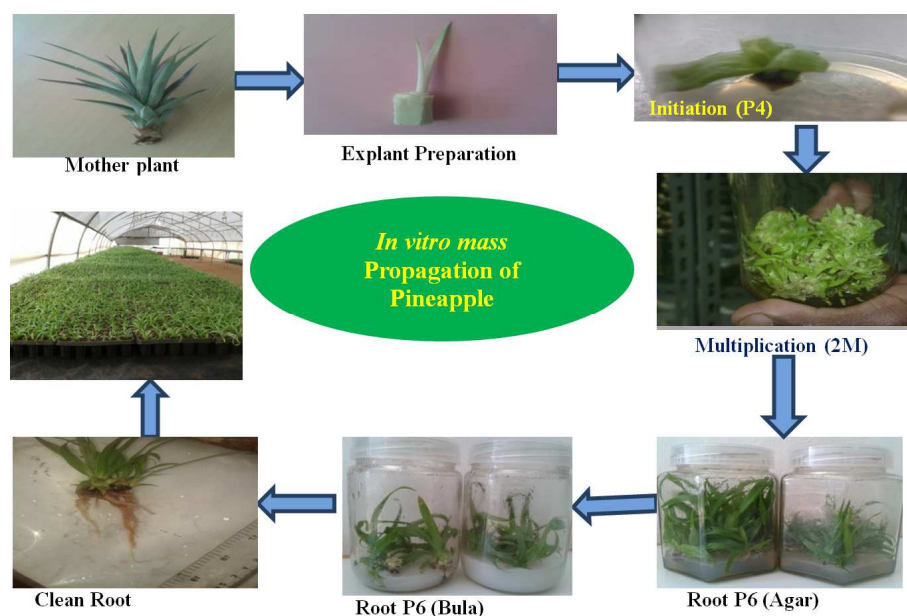


Figure 6. Life cycle of pineapple (var. smooth cayenne) micro propagation adapted from Abebe *et al.* [40].

3.5. Micro Propagation of Korarima

Ethiopian cardamom, also called Kororima [*Aframomum corrorima* (Braun) P. C. M. Jansen], is a prominent spice and medicinal crop growing native and widely distributed in southwestern Ethiopia. Korarima can be propagated either by seed or by cutting of its clumps. Propagation by clump practiced more often than the seed method, as it yields earlier and ensures a true-to-type crop. Nevertheless, the use of seed for *in vivo* propagation of korarima found to be suitable alternative over traditional propagation techniques despite of long dormancy associated with it [41]. The conventional method used to multiply kororima via vegetative techniques is done by splitting the rhizome into one old and young sucker and there by shortens juvenile phase of the stand and also enables propagation of true-to-type plants of a desired clone. However, this method has a drawback of achieving sufficient number of planting materials to cover up large areas of land and involves sacrifice of potentially productive stands [42]. Similarly, vegetative propagation through cuttings also results in the destruction of the productive garden, which is commonly associated with shortage of planting materials to cover wider areas of land.

To overcome such limitation, efforts were made to improve the crop by applying modern biotechnological techniques. The use of micro propagation can be a powerful alternative to mass propagate improved kororima varieties in a short period of time [43].

An efficient micro propagation method was developed for korarima using axillary bud explants obtained from the

rhizome. Basal MS medium proved to be the best for invitro kororima growth and development. According to Wondyifraw and Wannakraioj [43], addition of 5% coconut water to the culture medium was effective in enhancing shoot proliferation. Basal medium supplemented with 2 mg/l imazalil in combination with 0.5 mg/l thidiazuron gave 7.5-fold higher shoot multiplication compared to Plant Growth Regulator (PGR)-free medium within eight weeks of culture period. The shoots developed roots readily when transferred to PGR-free MS medium. Rooted plantlets were easily acclimatized by transplanting them to potting mix substrate of river sand and peat moss (1:1), and then covered with polythene bags for a week. Acclimatized plants successfully grew (93%) when transferred to screen-shaded nursery [42].

In general, *in vitro* techniques guarantee the production of sufficient amount of disease free planting materials to growers who are planning to start commercial production as the price of kororima becoming damn expensive in Ethiopia.

3.6. Banana Tissue Culture

Banana is staple food for millions of people throughout the tropics and subtropics. Being grown in over 150 countries, banana accounts for one of the most widely exported fruits in the world [44].

In Ethiopia, where vast majority areas are suitable for banana cultivation, and production ranges from small scale to commercial plantations. Commercial production systems employ both rainfed systems and/or supplementation irrigation that give the opportunity for exporting fresh banana fruits [45]. At present, bananas are the leading fruit crop

produced in the country both in terms of area coverage and production where the bulk is produced in traditional agricultural system. Most commercial bananas are propagated vegetatively due to the high degree of sterility and polyploidy of the edible varieties [46]. Since, on average only 5 to 10 suckers can be obtained per plant per year, the traditional clonal propagation method appears to be unable to supply the increasing demand for healthy planting materials of banana [47]. The materials used for conventional propagation are corms, and small and large suckers [46]. However, these conventional materials are not the ideal propagule, because they often carry weevils or borers, fungal pathogens, nematodes, and viruses and also suffer from slow multiplication, bulkiness, and poor phytosanitary quality.

The protocol reported by Karule et al., [48] on three different varieties clearly described as follows; (1) Initiation for Dwarf (MS medium + 3 mg/l BAP), Giant Cavendish

(MS medium + 3 mg/l BAP) and for Poyo (MS medium + 2 mg/l BAP). (2) Shoot multiplication for Dwarf (MS medium with 3 mg/l BAP + 0.4 mg/l IAA), Giant (MS medium with 4 mg/l BAP + 0.4 mg/l IAA) and Poyo (MS medium with 3 mg/l BAP + 0.2 mg/l IAA). Furthermore, well rooted plantlets can be obtained when the shoots are cultured on MS medium with 2.12 mg/l NAA for Dwarf and Giant while 1.74 mg/l IBA for Poyo. The necessary vegetative growth can be attained for plantlets that are hardened and acclimatized on potting media with sugarcane filter cake and sand at 3:1 ratio (v/v). On the other hand banana *in vitro* propagation technique as described by Manguatosha Ngomuo [49], technical challenges related to lethal brown and other constraints should be managed to ensure the development of safe and reliable planting material of banana. Figures 7 shows the general procedure from explant initiation to *in vitro* derived banana plant.

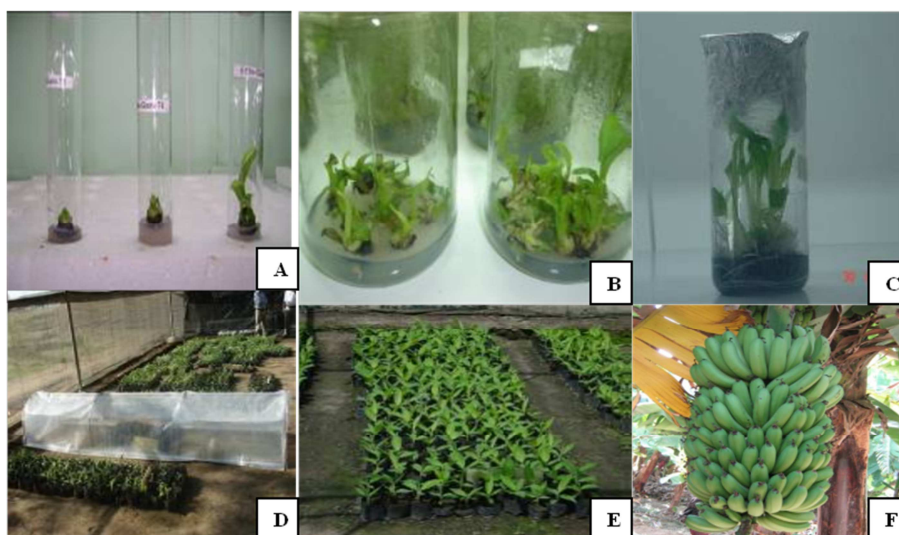


Figure 7. *In vitro* and *ex vitro* banana: (A) Shoot tip initiation; (B) Shoot multiplication; (C) Shoot elongation and rooting; (D) Acclimatization inside chamber; (E) Acclimatized and hardened plants; and (F) *In vitro* derived plant at fruiting in the field. Source: adapted from Asmare Dagne et al., [47].

4. Tissue Culture in Agriculture

As one technique of biotechnology, the plant tissue culture has a great impact on both agriculture and industry, through providing initial seedlings/plantlets needed to meet the ever increasing world demand. It has made significant contributions to the advancement of agricultural sciences in recent times and today they constitute an indispensable tool in modern agriculture [50]. Biotechnology has been introduced into agricultural practice at a rate without pattern. Tissue culture allows the production and propagation of genetically homogeneous, disease-free plant material [51]. Application of biotechnological approaches for *in vitro* regeneration, mass micro propagation techniques and gene transfer studies in many high value crop species revolutionized the agriculture.

Especially countries like Ethiopia where the population growth is very high and food insecurity prevails constantly, the demand to use such biotechnological tools increases than

ever. Recently, the country is gearing toward using modern tools, including artificial intelligence to key areas like agriculture, health and environment. An efforts made to boost agricultural productivity through availing numerous amount of seedlings derived from tissue culture.

Research centers and universities which have been working on biotechnology in Ethiopia are expected to collaborate and create synergy for the betterment of scientific implementation and targeted research to achieve food security. To overcome redundancy of effort and misuse of limited national resources best collaborative work is mandatory. In this regard, the Ethiopian Biotechnology Institute has a national mandate and responsibility to facilitate and/or coordinate focused works. On the other hand the emerging of private tissue culture laboratories is an encouraging advancement where it has to be largely consolidated and highly linked with innovating laboratories to take over the responsibility of mass propagation of high value crops.

5. Conclusion and Recommendation

Shortage of planting materials of high value horticultural crops is one of the major critical problems. So, thinking of rapid multiplication of improved crop varieties through micro propagation is sensible. Thus, we highlight overview about the current state and development of biotechnology research in general and micropropagation of high value crops in particular and also indicate the constraints or key challenges of research and development in Ethiopia.

Despite prevailing factors, the research results available in the country are good indicators to solve production challenges. The progresses made in multiplication of high value crops like sugarcane, pine-apple, banana, ginger and other crops is an encouraging development for horticultural sector. Thus, the authors recommend that, consolidated efforts and synergy between public and private institutions is required to get better results. It is also recommended that each tissue culture laboratories in the country support each other and exchange ideas for a common goal. Furthermore, public laboratory should have a focus on few very important crops in their vicinity rather than having dismantled efforts to work on stretched over many crops.

Research centers, Universities and Private laboratories should create a very good platform and collaboration that enables to solve the key challenges: Limited public investment in biotechnology research. Most laboratories and/or institutions heavily rely on external funding from development partners that may not satisfy our need; shortage of well-trained and experienced tissue culture personnel and high staff turnover and regrettably, skills for maintenance and repair of tissue culture equipment is also limited; Lack of appropriate tissue culture infrastructure which aggravate by unreliable support services (power and water supplies); bureaucratic procurement procedures particularly for the purchase of consumables; Inadequate national and/or regional policy frameworks to support private sector involvement in biotechnology research; The comparatively high cost of tissue culture products compared to their conventional counterparts; Limited public awareness about proven tissue culture products and technologies; Weak collaborative linkages and/or partnership among the different stakeholders along with the tissue culture development and delivery pipeline.

Thus, from this, though not covered much, we can conclude that out of existing piece of works still possible to exploit more.

References

- [1] Murashige T., Skoog F., (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Plant Physiology*, 15: 473-497.
- [2] Feyissa T, Welander M, Negash L (2005) Micropropagation of *Hagenia abyssinica*: a multipurpose tree. *Plant Cell, Tissue & Organ Culture*. 80: 119-128.
- [3] Clet W. M., Emmarold M., Francis W., and Charles M., (eds.) 2013.
- [4] Adane Abraham (2009) Agricultural biotechnology research and development in Ethiopia (Review) *African Journal of Biotechnology* Vol. 8 (25), pp. 7196-7204, 29 December, 2009 Available online at <http://www.academicjournals.org/AJB>. ISSN 1684-5315 © 2009 Academic Journals.
- [5] Ministry of Finance and Economic Development, 2013. Annual progress Report of the GTP (Growth and Transformation Plan).
- [6] Tolera B. and Shimelis D. (2016). Comparison of Micropropagated and Conventional Raised Sugarcane Planting Materials as Initial Source of Seed Cane at Metahara Sugar Estate, Ethiopia. *Advances in Tissue Engineering and Regenerative Medicine*, 1 (2): 1-3.
- [7] Tena, E., Mekbib, F., Shimelis, H., & Mwadzingeni, L. (2016). Sugarcane production under smallholder farming systems: Farmers preferred traits, constraints and genetic resources. *Cogent Food & Agriculture*, 2 (1), 1191323.
- [8] Benedikt Kamski (2016) The Kuraz Sugar Development Project (KSDP) in Ethiopia: between 'sweet visions' and mounting challenges, *Journal of Eastern African Studies*, 10: 3, 568-580, DOI: 10.1080/17531055.2016.1267602.
- [9] Temam A., Alemayehu LA., 2017. Sugarcane (*Saccharum officinarum* L.) Tissue Culture In Ethiopia: Opportunities For Ethiopia's Sugar Industries.
- [10] Tolera B, Diro M, and Belew D (2014a). In vitro aseptic culture establishment of Sugarcane (*Saccharum officinarum* L.) varieties using shoot tip explants. *Advances in Crop Science and Technology*, 2 (3): 1-6.
- [11] Tolera B, Diro M, and Belew D (2014b). Effects of 6-Benzyl aminopurine and Kinetin on In vitro Shoot Multiplication of Sugarcane (*Saccharum officinarum* L.) Varieties. *Advances in Crop Science and Technology*, 2 (3): 1-5.
- [12] Mekonnen, T., Diro, M., Sharma, M., & Negi, T. (2014). Protocol optimization for in vitro mass propagation of two sugarcane (*Saccharum officinarum* L.) clones grown in Ethiopia. *African Journal of Biotechnology*, 13 (12).
- [13] Berthouly, M., & Etienne, H. (1999). Somatic embryogenesis of coffee. In *Somatic embryogenesis in woody plants* (pp. 259-287). Springer, Dordrecht.
- [14] Etienne, H. (2005). Somatic embryogenesis protocol: coffee (*Coffea arabica* L. and *C. canephora* P.). In *Protocol for somatic embryogenesis in woody plants* (pp. 167-179). Springer, Dordrecht.
- [15] Etienne, H., & Bertrand, B. (2001). Trueness-to-type and agronomic characteristics of *Coffea arabica* trees micropropagated by the embryogenic cell suspension technique. *Tree Physiology*, 21 (14), 1031-1038.
- [16] Landey, R. B., Cenci, A., Georget, F., Bertrand, B., Camayo, G., Dechamp, E., & Etienne, H. (2013). High genetic and epigenetic stability in *Coffea arabica* plants derived from embryogenic suspensions and secondary embryogenesis as revealed by AFLP, MSAP and the phenotypic variation rate. *PLOS one*, 8 (2), e56372.
- [17] Kumar V, Naidu M. M, Ravishankar G. A (2006) Developments in coffee biotechnology—in vitro plant propagation and crop improvement. *Plant Cell Tiss Organ Culture* 87: 49-65.

- [18] Landey Roberto Bobadilla (2013) Influence of micropropagation through somatic embryogenesis on somaclonal variation in coffee (*Coffea arabica*): assessment of variations at the phenotypical, cytological, genetic and epigenetic level. *Vegetal Biology*. Université Montpellier II - Sciences et Techniques du Languedoc.
- [19] Etienne, H., Breton, D., Breitler, J. C., Bertrand, B., Déchamp, E., Awada, R., & Courtel, P. (2018). Coffee somatic embryogenesis: how did research, experience gained and innovations promote the commercial propagation of elite clones from the two cultivated species? *Frontiers in plant science*, 9, 1630.
- [20] Namasivayam P (2007) Acquisition of embryogenic competence during somatic embryogenesis. *Plant Cell. Tissue Organ. Culture*. 90: 1-8.
- [21] Söndhal MR & Sharp WR (1977) High frequency induction of somatic embryos in cultured leaf explants of *Coffea arabica* L. *Z. Pflanzenphysiol.* 81: 395-408.
- [22] Takayama, S., & Akita, M. (2008). Bioengineering aspects of bioreactor application in plant propagation. In *Plan Tissue Culture Engineering* (pp. 83-100). Springer, Dordrecht.
- [23] Teisson, C. & D. Alvard, 1995. A new concept of plant in vitro cultivation liquid medium: Temporary immersion. In: M. Terzi et al. (eds.) *Current Issues in Plant Molecular and Cellular Biology* (pp 105-110). Kluwer Academic Publishers, Dordrecht, The Netherlands.
- [24] Etienne, H., Lartaud, M., Michaux-Ferrière, N., Carron, M. P., Berthouly, M., & Teisson, C. (1997). Improvement of somatic embryogenesis in *Hevea brasiliensis* (Müll. Arg.) using the temporary immersion technique. *In Vitro Cellular & Developmental Biology-Plant*, 33 (2), 81-87.
- [25] Deo, P. C., Tyagi, A. P., Taylor, M., Harding, R., & Becker, D. (2010). Factors affecting somatic embryogenesis and transformation in modern plant breeding. *The South Pacific Journal of Natural and Applied Sciences*, 28 (1), 27-40.
- [26] Von Arnold, S., Sabala, I., Bozhkov, P., Dyachok, J., & Filonova, L. (2002). Developmental pathways of somatic embryogenesis. *Plant cell, Tissue and Organ culture*, 69 (3), 233-249.
- [27] Etienne, H., Dechamp, E., Barry-Etienne, D., and Bernóit Bertrand (2006). Bioreactors in coffee micropropagation, *Braz. J. Plant Physiol.*, 18 (1): 45-54, 2006.
- [28] Debergh P, Harbaoui Y, Lemeur R (1981) Mass propagation of globe artichoke (*Cynara scolymus*) / evaluation of different hypotheses to overcome vitrification with special reference to water potential. *Physiol. Plant.* 53: 181-187.
- [29] Ziv M (1995). The control of bioreactor environment for plant propagation in liquid culture. *Acta Hort.* 393: 25-38.
- [30] Berthouly, M., Alvard, D., Carasco, C. and Teisson, C., 1994. In vitro micropropagation of coffee sp. by temporary immersion.
- [31] Elias Gebremariam. 2017. In Vitro Propagation of *Coffea arabica* cv. MCH2 Using Nodal Explants. *Journal of Natural Sciences Research* www.iiste.org ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online) Vol. 7, No. 1, 2017.
- [32] Ministry of Agriculture and Rural Development (MoARD) (2008). A proceeding report on status and challenges of spice production in Ethiopia. National Workshop in United Nations for Economic Commission for Africa November 6, 2008 Addis Ababa, Ethiopia. pp. 17-26.
- [33] Mengs, B., Chimdessa, M., & Abraha, E. 2017. Sterillization of media via sodium hypochlorite (nacllo) without autoclaving in-vitro micro-propagation of ginger (*Zingiber officinalerose*.) *Culture*. pp. 2347-2154.
- [34] Mengs B., G. 2018. Control of Contamination and Explants Phenolics in Ginger Accession (*Zingiber officinale* Rosc.) *In Vitro Cultures. Journal of Biology, Agriculture and Healthcare*. (8) 2224-3208.
- [35] Biruk A, Wondyifraw T. and Kassahun B., 2012. In vitro propagation of Ethiopian ginger (*Zingiber officinale* Rosc.) cultivars: Evaluation of explant types and hormone combinations. *African Journal of Biotechnology* Vol. 11 (16), pp. 3911-3918, 23 February, 2012 Available online at <http://www.academicjournals.org/AJB>
- [36] Jimma Agricultural Research Center (JARC). 2018. Research Progress Report for the period 2017/18. Girma Hailemichael, Habtamu Seyoum, Yalemtehay Debebe, Fekadu Tefera and Dejene Kebede (eds.), EIAR/JARC, Jimma, Page 22/236.
- [37] Jackson, D., Williams, S., Newby, D. M., Hall, S., & Higgins, S. (2016). Tissue Cultured Versus Traditionally Grown Pineapples: Growth and Nutrient Profile. *J Biotechnol Biomater*, 6 (237), 2. · DOI: 10.4172/2155-952X.1000237.
- [38] Megersa, H. G. (2017). Propagation methods of selected horticultural crops by specialized organs: review. *J Hortic*, 4 (198), 2376-0354.
- [39] Mengesha, A., Ayenew, B., & Tadesse, T. (2013). Acclimatization of in vitro propagated pineapple (*Ananas comosus* (L.), var. smooth cayenne) plantlets to ex vitro condition in Ethiopia. (2): 2-9. DOI: 10.4236/ajps.2013.42042.
- [40] Abebe, Z. Tefera, W., Fellipe, M., Teressa, A., and Mengesha, A. 2003 "In Vitro Multiplication of Pineapple (*Ananas comosus* (L.)) and Cardamom (*Elletaria cardamomum*) in Ethiopia," Proceeding of the Second Biennial Conference of Ethiopian Horticultural Science Society, Addis Ababa, 22-23 January, 2003, pp. 9-18.
- [41] Eyob, S. (2009). Promotion of seed germination, subsequent seedling growth and in vitro propagation of korarima (*Aframomum corrorima* [Braun] PCM Jansen). *Journal of Medicinal Plants Research*, 3 (9), 652-659.
- [42] Tefera, W., & Wannakrairoj, S. (2004). A micropropagation method for korarima (*Aframomum corrorima* [Braun] Jansen). *Sci Asia*, 30, 1-7.
- [43] Hagos, R., & Gebremdhin, H. (2015). Effects of cytokinin types and their concentration on in vitro shoot induction and multiplication of korarima. *Int J Genet Mol Biol*, 7, 8-14.
- [44] Singh, H., Uma, S., Selvarajan, R. and Karihaloo, J. (2011) Micropropagation for production of quality banana planting material in Asia-Pacific. *Asia-Pacific Consortium on Agricultural Biotechnology (APCoAB)*, New Delhi, Vol. 92.
- [45] Alemu, M. M. (2017). Banana as a cash crop and its food security and socioeconomic contribution: the case of southern Ethiopia, Arba Minch. *Journal of Environmental Protection*, 8 (3), 319-329. doi: DOI: 10.4236/jep.2017.83024.

- [46] Kaçar Y. A., Faber B. (2012) Micropropagation of Banana. In: Loyola-Vargas V., Ochoa-Alejo N. (eds) *Plant Cell Culture Protocols. Methods in Molecular Biology (Methods and Protocols)*, vol 877. Humana Press, Totowa, NJ DOI: 10.1007/978-1-61779-818-4_11.
- [47] Dagnew, A., Shibru, S., Debebe, A., Lemma, A., Dessalegn, L., Berhanu, B.,... & Sierra, Y. M. (2012). Micropropagation of banana varieties (*Musa* spp.) using shoot-tip culture. *Ethiopian Journal of Agricultural Sciences*, 22 (1), 14-25.
- [48] Karule, P., Dalvi, V., Kadu, A., Chaudhari, R., Subramaniam, V. R., & Patil, A. B. (2016). A commercial micropropagation protocol for virupakshi (AAB) banana via apical meristem. *African Journal of Biotechnology*, 15 (11), 401-407. DOI: 10.5897/AJB2015.14883.
- [49] Ngomuo, M., Mneney, E. and Ndakidemi, P. A. (2014) The *in Vitro* Propagation Techniques for Producing Banana Using Shoot Tip Cultures. *American Journal of Plant Sciences*, 5, 1614-1622. DOI: 10.4236/ajps.2014.511175.
- [50] Garcia-Gonzales R, Quiroz K, Carrasco B, Caligari P (2010) Plant tissue culture: Current status, opportunities and challenges. *Cien. Inv. Agr.* 37 (3): 5-30.
- [51] Idowu, P. E., Ibitoye, D. O., & Ademoyegun, O. T. (2009). Tissue culture as a plant production technique for horticultural crops. *African Journal of Biotechnology*, 8 (16).