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The Role of Plant Tissue Culture to Supply Disease Free Planting Materials of Major Horticultural Crops in Ethiopia

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Abstract
Agricultural diversification to meet our future needs call for the adoption of new technologies in agriculture**.** Utilization of the best cultural practices, fertilization, pest control measures will not give the necessary results without the use of best planting material. Tissue culture has been significant role in producing disease free planting materials of vegetative propagated crops in horticulture industry of many countries. Among these crops potato, sweet potato, banana and citrus are the major crops being propagated intensively to produce disease free planting materials. Many research works have being conducted on the potential of *in vitro* plantlets over conventional one on productively per unit area particularly in horticultural crops. Almost all research results revealed that *in vitro* plantlets significantly perform better than conventional in terms of uniformity, earliness, yield, and quality due to free from disease load. The limiting factor of using *in vitro* plantlets from the point of view of farmers is the higher price of the material if compared to conventional and TC plants require additional care and improved management. Higher cost is justified by achieved high economic returns. In addition, the technology has created several employment opportunities and opened up many entrepreneurial fields. This industry has made available different unique commercial plant species such as ornamentals and foliages in large scale, which were not produced earlier by the conventional methods.
Keywords: Tissue culture, *in vitro* and plantlets.

1. Introduction

Agricultural diversification to meet our future needs call for the adoption of new technologies in agriculture. Utilization of the best cultural practices, fertilization, pest control measures will not give the necessary results without the use of best planting material. Tissue culture is now a significant horticultural propagation method which has revolutionized the horticultural industry. Use of this technique should be considered for mass propagation and the establishment of disease free stock material[1].

Plant tissue culture is now a well established technology. Like many other technologies, it has gone through different stages of evolution; scientific curiosity, research tool, novel applications and mass exploitation. Initially, plant tissue culture was exploited as a research tool and focused on attempts to culture and study the development of small, isolated segments of plant tissues or isolated cells[2].

Tissue culture is a method of vegetative propagation based on biotechnology. The plants are derived from stem, root or leaf tissues and the technology generally aids in mass production of desired crop varieties. Tissue culture is also useful in regeneration of genetically modified cells into whole plants as well as in embryo rescue techniques[3]. The advantages and disadvantage of this method are stated by Ilan, and Workafes [4], are controlled environment and controlled development of the plants that enable very rapid multiplication rate and clean conditions for plant development that produce micro-plants free of many pests and diseases. The small size of the propagated plants saves nursery space and plant transport costs. The main disadvantage of TC plants is their high production costs. This difficulty limits the number of plant species in commercial TC propagation.

Reproducing the planting materials of vegetatively propagated crops presents complex problems and many logistical issues for their extensive use. This is particularly an issue for smallholder farmers because of absence of formal seed systems, lack of knowledge of phytosanitary measures and quarantine issues related to safe movement of germplasm, plants and planting material across national borders, lack of consistent supplies of good quality planting material, variable demand for clean planting material, bulkiness and perishability of planting materials and use of traditional varietal mixtures, including local varieties [5].

In Ethiopia, tissue culture technology is being used in the mass propagation of beverage crops, fruits, spices, root crops, industrial crops, medicinal and indigenous trees.These includes Arabica coffee, Pineapple, Banana, Cardamom, Vanilla, Garlic, Black Pepper, Ginger, Cassava, Sweet potato, Enset, Grape vine, Sugarcane, Endod, *Artimessia, Hygenia* and *Podocarpus* tree spp. and recently potato. Indeed the demand for tissue culture plantlets has in recent years outstripped supply of tissue culture plantlets in the market and government is now starting new programmes to establish basic facilities to enhance the use of the technology [6]. Therefore, the objective of the review is to briefly assess the role of plant tissue culture to supply disease free planting materials of vegetative propagated horticultural crop.

2. Plant tissue culture (PTC) technique

Meristem culture: The propagation from meristematic tissue generally provides a method of cleaning up material from viruses and other systemic pathogen infections. Micro-propagation is a tissue culture *(in vitro*) method used for rapid and true to type multiplication of plants on artificial nutrient media under controlled environment. Micro propagation is the most commercially exploited area of plant tissue culture, having been widely used for production of quality planting material in vegetative propagated species. The most significant advantages offered by micro propagation are large numbers of disease free propagules can be obtained from a single plant in a short period, propagation can be carried out throughout the year and the propagating material can be accommodated in a small space, reduction of labour costs for germplasm maintenance, avoidance of field inspections & environmental hazards, easy availability of material for micro propagation and rapid multiplication [36].

According to Chadha and Choudhary [7], various steps involved in production of pathogen free plantlets by meristem tip culture are: testing of parent material for the presence of viruses and similar pathogens (viroids and phytoplasmas), Thermotherapy/chemotherapy of parent material if disease-free material is not available, excision of meristem tip under aseptic conditions, culture of apical dome plus one or two leaf primordia on suitable medium to produce plantlets, Indexing of plantlets for presence or absence of viruses, plantlets transferred to soil, maintenance of pathogen free nuclear plant stocks, meristem culture is then followed by *in vitro* mass propagation of the virus-free plants thus obtained.

3. Applications of micro propagation

Plant tissue culture is used widely in plant science; it also has a number of commercial applications. These include: i) Screening cells rather than plants for advantageous characters, e.g. herbicide resistance/tolerance ii) Large scale growth of plant cells in liquid culture inside bioreactors as a source of secondary products, like recombinant proteins used as biopharmaceuticals iii) To cross distantly related species by protoplast fusion and regeneration of the novel hybrid iv) Embryo rescue (the resulting embryo as a result of cross pollination which would otherwise normally die is cultured in a medium to rescue it) v) For production of doubled monoploid plants from haploid cultures to achieve homozygous lines more rapidly in breeding programs, usually by treatment with colchicine which causes doubling of the chromosome number. vi) As a tissue for transformation, followed by either shortterm testing of genetic constructs or regeneration of transgenic plants. vi) *In vitro* conservation of germplasm. This technique is mainly used to conserve plant which do not produce seeds or which have recalcitrant seeds which cannot be stored under normal storage conditions in seed gene banks. Hence, vegetative propagated crops such as root and tubers, ornamentals, medicinal plants and many other tropical fruits have to be conserved using *in vitro* methods [37].

4. Advantages of micro propagation

Micro propagation offers several distinct advantages not possible with conventional propagation techniques. i) Rapid multiplication of genetically uniform plants (clones) that possess desirable traits. Single explants can be multiplied into several thousand plants in a very short time. Once established, actively dividing cultures are a continuous source of micro cuttings which can result in plant production under greenhouse conditions without seasonal interruption. ii) The production of multiples of plants in the absence of seeds or necessary pollinators to produce seeds. iii) The regeneration of whole plants from plant cells that have been genetically modified. Using methods of micro propagation, the nurseryman can rapidly introduce selected superior clones of ornamental plants in sufficient quantities to have an impact on the landscape plant market. iv) The production of plants in sterile containers that allows them to be moved with greatly reduced chances of transmitting diseases, pests and pathogens. v) The production of plants from seeds that otherwise have very low chances of germinating and growing. vi) To clean particular plant of viral and other infections and to quickly multiply these plants as 'cleaned stock' for horticulture and agriculture.

5. Disadvantages of tissue culture plantlets

The first limiting factor of using *in vitro* plantlets from the point of view of farmers is the higher price of the material if compared to conventional**.** TC plants require additional care and improved management. Since they have no nutrient reserves when transplanted, external stress is particularly harmful in the first months after transplanting and most of the pathogens are removed in the tissue culture procedure, viruses can still be transmitted through in vitro plants in some crops like banana [37].

6. The capacity of plant tissue culture laboratories in Ethiopia

Many of EARO's biotechnology researches are confined to conventional ones. The works include tissue culture techniques of virus-free potato varieties, somatic embryo regeneration techniques for coffee, false banana and banana, regeneration through callusing of Tef (Eragrostis tef), *in vitro* propagation of spices, medicinal and oil plants, double haploid breeding of mustard, rape seed and barley, embryo transfer technology, multiple ovulation

and embryo transfer and microbial inoculums selection and molecular characterization of soybean, grass pea and field peas [8].

The lab at Jimma Agricultural research Center is developing protocols for rapid multiplication of high yielding and coffee berry disease resisted hybrids of coffee whilst the lab at Melkassa Agricultural research center is micro propagating banana. The tissue culture lab at Debre Zeit Agricultural Research Center (established through the technical assistance project of IAEA) is developing double-haploid protocols for tef and is also developing micro propagation techniques for endod (*Phytolacca dodecandra*), and grape vine. At Holetta Agricultural Research Center, the lab is working on potato micro propagation and disease cleaning [9].

The demand in Ethiopia for plants that can and should be propagated by TC is large. For example, the area of banana fields in Ethiopia is about 40,000 hectare. Accordingly, the number of banana plants in Ethiopia can be estimated as 80 millions. Under favorable conditions we can assume the renewal of plantation every five years. Consequently, the annual banana planting in Ethiopia can be estimated as 16 million plants. The current micro propagation capacity in Ethiopia is about 3 millions plants per annum. This is based on one medium commercial TC lab (MIT Mekele) and several small research centers TC laboratories that recently began mass production [10].

Jimma Agricultural Research Center were delivered the first 2000 pineapple plantlets to Teso pineapple cooperatives in SNNPR through SNNPR BoARD and another 5,500 plantlets are ready for dispatch to each of the Dara and Chuko Woreda farmers by SNV project financial and technical support. The research centre has expanded its facilities with additional four growth centers. They plan to prepare 500,000 plantlets within six months up to August 2009 [11].

7. Tissue culture laboratory for production of potato tuber seeds in Ethiopia

Potato requires incessant efforts to meet the ever increasing demand of the growing population of the world. In all the potato growing regions the availability of high quality clean seed tuber has been the most limiting factor owing to the conventional clonal propagation that favors disease build up that drastically reduces yield. Seed alone accounts for 40-50% of the total cost of the cultivation, hence quality seed is a vital input for obtaining high yields [12]. The potato, mainly due to its amenability for micro propagation has moved it from test tubes to field. A few private companies are also engaged in potato seed production through biotechnological methods but this quantity is not sufficient to meet the growing demand. In order to reduce the demand and supply gap, micro propagation by shoot culture technique is used for the mass propagation of potato. This method is used as a control approach to viral and bacterial diseases which are commonly spread through propagative materials [13]. In 2007 over 720,000 mini tubers (Nuclear) were produced in Burundi, Ethiopia, Kenya, Madagascar, Rwanda and Uganda for further multiplication of pre basic and basic seed with PRAPACE support. During the same time, about 1,700 tons clean potato seed of the region's most important seven improved varieties were produced and availed to decentralized informal seed multipliers for further multiplication and dissemination [14].

The Holetta biotech labs commenced TC researches of potato with objective of optimize in vitro propagation protocol for released potato varieties to assist efforts to produce quality seed tubers. The application of the protocol is mass production of clean released varieties for mini tuber production in Aeroponics and screen house (pots). Over 20,000 in vitro plants produced since of Gudene, Jalene, Belete and Awash. The system is supplemented with virus testing**.** In 2011/12 large number of mini tubers produced for distribution and further research work [15].

Table 1: Number of Mini tubers produced from *in vitro* plantlets (G1) in 2012

Variety	No of plants/pots planted	No of plants harvested	Number of tubers harvested	Average tuber /plant
Belete (Black Screen)	1213	1158	7806	6.74
Belete (white screen)	638	630	6640	10.54
Awash	301	286	1625	5.68
Gudanie	762	730	2725	3.73
Total	2914	2804	18,796	6.70

Amhara Region Agricultural Research Institute (ARARI) TC lab produced 480000 mini tubers in 2011/12 and 47000 mini tuber of different released potato varieties in 2010/11 [16].

8. Tissue culture: successful technology in banana production in Kenya

Currently in Kenya, over 500,000 farmers have planted tissue culture bananas, an average of about 50,000 new farmers per year since the introduction of the technology. While more than 2 million Kenyans faced famine in 2008-09, none of the tissue culture banana farmers needed emergency food assistance. The demand for tissue culture banana seedlings continues to surpass existing supply as more and more farmers adopt the technology. By applying a similar model, the tissue culture banana has been successfully introduced in Tanzania, Uganda and Senegal although the extent of the impact in these countries is not well documented. Interest has also been expressed in Malawi, Mozambique and Zambia, but again the level of success is not well documented. It increased the availability of large quantities of clean superior planting materials, reduced the harvest cycle of the bananas to 12-16 months compared to 18-24 months for traditional bananas due to early maturity, It yielded bigger bunches of at least 30kg compared to 10-15 kg for traditional types, offered a uniform plantation development that eased marketing coordination, the uniform bunches also enhanced market acceptance, and increased farm net profits from about US$ 660 for traditional bananas to US$ 1,800 for tissue culture per average 0.2 ha land per year due to increased outputs, quality and reduced marketing costs [17].

9. Production and Productivity of TC disease free planting material in different horticultural crops

9.1. Sweet potato

Mutandwa, [18], reported that the use of tissue cultured sweet potatoes by smallholder farmers in Zimbabwe has been shown to improve household food security. Farmers who used tissue cultured sweet potato varieties, obtained yields of up to 25 tons per hectare against a national average of 6 tons per hectare. Sweet potato farmers in Zimbabwe were able to achieved high economic returns per ha base using tissue culture derived planting material which is replaced every 3 years whereas growers using unimproved planting material made a loss.

Fuglie *et al*, [19], concluded that the rapid diffusion of virus free sweet potato planting material in Shandong province of china, reaching 80% of the province`s small growers within the only four years, can be explained by several factors. Most importantly, users of the new roots showed yield increment by 10 t/ha, or 30%. Further the technical package was simple and required only one small change in the farmers' production system.

The majority of smallholder farmers interviewed, 93% respond that they used disease free vines in sweet potato production, while only 7% still used the traditional sweet potato vines in Zimbabwe. Average yields for the 2003/04 cropping seasons were 18 tons per hectare for tissue cultured sweet potatoes, while unimproved sweet potato varieties yielded 5 tons per hectare [20].

Conventional propagation method gave highest growth rates however the difference in yield between the conventional propagation and tissue culture regenerated plants did not vary significantly ($P<0.05$). The ELISA results revealed that leaf samples obtained from symptomatic conventional plants had higher photometer readings than plants obtained from the TC regenerated propagules and the negative control. The results further revealed that under field conditions there was a high virus titre in the conventional propagated plants relative to leaf samples obtained from TC regenerated plants [21].

9.2. Potato

The production of potato seed under conventional system has not been effective in avoiding or reducing the buildup of pathogens and has consequently led to reduced quality potato seed and low crop yields. Plants once cleaned through meristem culture and induction of tuberization under aeroponics system, produce high quality potato seed tubers rapidly that are free from contamination of pathogens. Further multiplication of potato seed tubers under aeroponics also compliments tissue culture (micro propagation), as it clones mini tubers in a short time and reduces numerous labor steps associated with direct use of plantlets from tissue culture into the field in the post flask stage [22].

Due to progressive accommodation of viral diseases in seed stock, availability of good quality seed is a major constraint in potato production, which is approximately 50% of the total production cost. Besides high cost of seed potato, propagation is also characterized by low multiplication rate of only 4-6 times. To large production of clonal material to produce the uniform, identical seed material of potato, micro propagation is the better alternative over to conventional propagation methods of potato. Saving of food material, development of diseases free and clonal planting material are some of major advantages from *In vitro* propagated micro tuber. This will certainly help in the development of social and economical condition of farmers [23].

Researchers in Taiwan have reported production of 36,000 micro tubers from 1,200 culture flasks in a period of 4 months. After 3 field multiplications, these micro tubers produced 1,800 t potato seed, which was enough for 2000 ha on a schedule of one-third rotation per year. In India, the possibility of producing 264,500 basic seed tubers after one nursery bed and two field multiplications of micro tubers produced from one *in vitro* plant [24].

In the Republic of Korea, use of virus free planting material produced through tissue culture has

increased the national potato yield from 11.9 t/ha in 1980 to 20.3 t/ha in 1986. Subsequently, an in vitro tuberization system was also established and became an integral component of the potato seed industry in the country [25].

9.3. Musa species

Banana production is suffering heavy losses from virus diseases in many countries. To date, five viruses infecting *Musa* spp. have been reported ABMV, BBMV, BBTV, CMV and BSV. These viruses can be transmitted in vegetative planting material. Successful control of virus diseases should begin with virus free planting materials. The solution is to develop cheap, efficient production of "clean" planting material through tissue culture. Since the tissue culture program began in 1983, a total of 26 million banana plantlets have been produced for commercial planting in Taiwan [26].

The technology package has brought many benefits to farmers. The most important is the availability of improved, disease free planting materials. This way, the farmers can now be able to replace their degraded orchards with superior material which is early maturing 12-16 months compared to the conventional banana of 2-3 years, bigger bunch weights of more than 30 kg and a higher annual yield per same unit of land, 40-60 t/ha have been observed. This is a very significant achievement given the very small farm sizes (1-2 acres) with a majority of the farmers. The uniformity and more simultaneous plantation development of the TC plantlets further promises easier marketing and coordination of the whole production process [27].

Use of untreated suckers leads to poor crop establishment due to pests and diseases, and subsequent reduced yields. The introduction of tissue culture (TC) techniques for banana propagation was thus perceived as a measure to help reverse the situation since it can facilitate the production of clean planting material. The sterile operational nature of TC procedures excludes fungi, bacteria and pests from the production system. Thus black leaf streak and Sigatoka leaf spot, Fusarium wilt, banana weevils, and plant parasitic nematodes are not transmitted through the micro propagation process. Most of the banana pests and diseases are transmitted through suckers from infected parent plants and from one farm to another through the exchange of suckers, a common practice among small scale farmers. The use of this practice can reduce banana yields by up to 90% when compared with the use of clean, pest and disease free planting materials such as those obtained from TC. However, viruses, such as the banana bunchy top virus and banana streak virus, are not eliminated by TC unless virus indexing and other measures are undertaken to prevent their propagation [28].

Banana is a popular food crop in Kenya. Around 2 million tons are produced annually from about 80,000 hectare. However, production has been declining in recent years, mainly due to diseases and pests. Farmers traditionally use suckers from their old orchards or from their neighbors' fields for replanting. These materials are often infected by pathogens, particularly *Fusarium oxysporum* spp. *cubense*, weevils and nematodes. This practice has led to the spread of diseases and pests to many production areas, eventually leading to declining productivity. In 1997, the Kenya Agricultural Research Institute started distributing tissue cultured banana plantlets to farmers' groups. A participatory evaluation approach was used for farmers to compare the performance of their traditional materials and tissue cultured materials. Key consideration was the economic benefits of using tissue culture materials as a way of disease management and increasing productivity. By adopting the technology, the yield increased from less than 10 t/ha to about 30 t/ha. Tissue cultured materials were free of diseases and pests for a longer time than the traditional suckers. It was also found that, although tissue cultured materials were more expensive than the traditional suckers, the extra cost was more than offset by the increased yield, resulting in a 145% increase in income for farmers. It can therefore be concluded that tissue culture technology has great potential for the management of diseases and pests in bananas in an integrated approach system [29].

Agrobiotec is the first private tissue culture (TC) laboratory established in Burundi in 1998. Banana (*Musa* spp.) is the main crop micro propagated by the company. The capacity of the lab is more than 1,500,000 plantlets per year but is limited by a number of constraints, such as inadequate infrastructure, rural poverty, and poor access to improved seeds and other agro inputs. These constraints have also a negative impact on prices, which could be reduced by 30% [30].

Table 2: Annual production of banana tissue culture plants by Agrobiotec in Burundi

Year	Production
1999-2000	80,000
2000-2001	150,000
2001-2002	250,000
2002-2003	250,000
2003-2004	250,000
2004-2005	200,000
2005-2006	350,000
2006-2007	150,000
2007-2008	350,000
2008-2009	420,000

Source: Rishirumuhirwa, 2010

Per hectare average yield of tissue culture banana was 50.04 tons which was higher than the yield of sucker propagated banana 40.05 tons this was because of superiority of technology over sucker propagated banana production besides uniformity in yield [31].

Micro propagation is being commercially exploited and some 1.5 million plantlets are being produced every year in banana, sugarcane, ornamentals, spices and medicinal plants in India. Commercial exploitation through micro propagation has become vital for improved crop production. The demand for tissue culture banana is expected to increase by about 25 to 30 percent estimated to the extent of about 5.0 million every year by 2009. In 2011-2012, the anticipated demand for tissue cultured plants would be 219.67 million plants against the installed capacity of 144 million plants. Tissue culture raised banana plantlets are now commercially adopted. At present, virus free clones of cardamom and micro tubers of potato, obtained through meristem culture, are a reality [32].

9.4. Citrus

Batool *et al*. [33], concluded that, greening is one of the major causes of citrus decline throughout the citrus growing areas of the world as well as in Pakistan. The Asiatic form is prevalent in Pakistan, which may be a threat for the citrus industry of Pakistan as well as for the neighboring countries. Citrus production can be increased through nurseries run on a scientific and professional basis of tissue culture. Certified citrus nurseries are needed to solve the problem of citrus greening. Certified bud wood programs might be the best way to establish disease free citrus orchards. In addition, an IPM program should be launched for the control of this disease.

Most of the citrus growing regions are experiencing decline in the population due to different reasons. This is mainly attributed to the different viruses which are spreading through planting of infected planting material. Viruses like Tristeza, Posorosis and Xyloporosis, and Greening bacterium are causing decline in citrus and the need is felt to revive citrus plantations on sound footing with appropriate biotechnological interventions. Meristem culture and shoot tip grafting have been trend in different citrus varieties and have become an important regulation in different citrus growing countries. It is suggested that the desired scion varieties may be first made virus free using meristem culture, tested for virus detection using different serological techniques followed by shoot tip grafting (STG) to raise healthy specific-virus-tested (SVT) clones. The mother plants regenerated so be then maintained in net-house containment and then multiplied on the desired rootstocks using micro-budding method [34].

9. 5. Date palm in Morocco

At Regional Research Center, INRA, Marrakech, micro propagation of date palm carried out successfully using auxiliary buds and inflorescence as explants. National capacity of *in vitro* plants production is between 70,000 to 100,000 plantlets per year. This capacity is insufficient to fulfill the high demand for the plantlets. For instance, in year 2007, total demand for plantlets was more than three times the production capacity total demand for plant lets 3,212,000 which includes variety Mejhool 105,000 plantlets, other varieties 140,000 and other selected clones 1,075,000 [35].

10. Constraints, gaps and needs

The industry is technology driven. This technology is the amalgamation of triple alliance capital, labor and energy. Although, labor is cheap in many developing countries, the resources of trained personnel and equipment are often not readily available. Acclimatization in mass is also another expensive part of the industry where in sophisticated greenhouses are essential to generate suitable end products. Although, good progress has been made, in several countries reports still recognize a number of gaps and needs. These includes the urgent need to increase plant TC capacity worldwide in order to adapt agricultural technology to meet the rapidly expanding

demand for more and different food as well as non-food products under different climatic conditions. The training of more breeders, technicians and field workers and the provision of better facilities and adequate funds are all essential. It needs greater awareness creation on the value and the importance of crop productivity in meeting future global challenges among policymakers, donors and the general public. It need for countries to adopt appropriate and effective strategies, policies, legal frameworks and regulations that promote the use of PTC. Stronger links are needed, especially between plant breeders and those involved in the seed system, as well as between the public and private sectors.

11. Conclusion

The plant tissue culture technology has been greatly contributed to producing disease free planting materials of vegetative propagated crops in horticulture industry of many countries. Usage of tissue culture generated plants has increased productivity per unit area, particularly in horticultural crops but capacity is insufficient to fulfill the high demand for the plantlets. The technology has created several employment opportunities and opened up many entrepreneurial fields. Tissue culture has been one of the main technological tools and reasons that have contributed a lot to feed 7 billion people in the globe.

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