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An overview of plant tissue culture research trends at Areka Agricultural Research Center, Southern Agricultural Research Institute, Ethiopia 2016-2019

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Abstract

Plant tissue culture techniques have encountered a lot of obstacles and breakthroughs as a life-giving technology in numerous field of biotechnology research. Tissue culture technology has evolved throughout times in the world, from clonal propagation to variety creation. It has found widespread application in mass propagation, in vitro conservation, hybridization, embryo rescue, double haploid development, and bio-factory. Plant tissue culture techniques are widely used in Ethiopia, both in research institutes and in academia, as well as in commercial enterprises. The Areka tissue culture research laboratory, on the other hand, was established with the goal of conducting comprehensive plant biotechnology research. Regardless of the difficulties encountered while attempting to conduct tissue culture experiments, the lab has highlighted several accomplishments in a few key areas. Among the research's major accomplishments are mass propagation, in vitro conservation, and the production of disease-free planting materials from infested mother plants. As a result, this walk-through review provides an overall picture of the lab as well as trends that may play a significant role in future study advancement.

Keywords: antibiotics, clean culture, in vitro conservation, large-scale propagation, and shoots tip culture,

Introduction

Since 1962, when Murashege and Skoog devised a full protocol for plant regenerations, plant tissue culture techniques have encountered a lot of obstacles and breakthroughs as a life-giving technology in numerous areas of biotechnology research. The technique has been used in large-scale ornamental plant production, conservation of endangered plant species, large-scale production of plant-derived secondary metabolites and recombinant proteins in liquid culture of plant cells in bioreactors, production of novel hybrids by fusing protoplasts of distantly related species or embryo-rescue technique, production of virus-free plants from virus-infected stock by culturing meristem/ shoot tip (Kumar, 2016). It has also acted as an intensive care room for cells that have undergone severe genetic transformation technique like protoplast fusion, somatic hybridization (particle bombardments), embryo rescue, and double haploid development. Plant tissue culture may be thought of as a collection of techniques/methodologies used to cultivate or sustain plant cells, tissues, or organs in a nutritional culture medium under aseptic conditions of growth (Kumar, 2016). Whatever the goal, any tissue culture laboratory must include distinct rooms and a number of essential facilities or materials (Wakil & Mbah, 2012). These include (I) washing and storage of glassware (ii) preparation, sterilization and storage of nutrient media, (iii) aseptic manipulation of plant material, (iv) maintenance of cultures under controlled conditions of temperature, light and humidity, (v) observation of cultures and (vi) hardening of in vitro developed plants (Requirements, 2013). Plant tissue culture techniques have been used in Ethiopia for decades, ever since the government established national biotechnology research road maps (Abrar et al., 2019). However, in recent years, plant tissue culture techniques have become widely used throughout the country, including in federal and regional agricultural research institutes, some national universities, and private enterprises (Abrar et al., 2019). Areka Agricultural Research Center is one of the branches of Ethiopia's Southern Agricultural Research Institute, and it is tasked with conducting plant biotechnology research, including plant tissue culture. In this regard, the tissue culture research laboratory was established with the goal of conducting comprehensive research on the in vitro propagation of selected horticultural crops, in vitro conservation and generating disease free planting materials from infested mother plants (mristem culture). As a result, this walk-through review provides an overall picture of the lab in relation to the research objectives and implementation trends. As a result, it contributes to the provision of baseline data for future study advancement.

Micro propagation of some selected horticultural crops: Clonally propagation is the most commonly used type of plant tissue culture technology (Sharma et al., 2015). When conventionally propagated, certain vegetatively propagating crops generate just a few suckers per mother plant (Dagnew et al., 2012). Another issue with traditional propagation is that economic components (storage) can be used as planting material for vegetatively propagating crops, particularly root and tuber crops. Approximately half of the output is used for planting (Sanginga, 2015). Consequently, significant portion of the produce from potato, yam and taro could be used as seed for the following production season, which is wasteful from an economic standpoint. Nowadays, tissue culture techniques can produce hundreds of thousands of plantlets from a single ex-plant in a relatively short period of time and space under controlled conditions year-round, regardless of season or weather (Singh & Kumar, 2020). In vitro multiplication, when done correctly, is a guaranteed approach that has the added benefit of lowering production costs by providing alternate seed sources. However, in vitro multiplication of pineapple, ginger, and banana on a relatively large scale has been performed to increase planting material supplies (figure 3).

Producing disease free in vitro plantlets: Ginger is a commercial crop grown in many sections of Ethiopia's southern region, mainly in Wolayta, Hadiya, and other western regions (Geta & Kifle, 2011). Not long ago, a wilt disease epidemic decimated the crop in these prospective producing zones. However, in vitro regeneration and multiplication of disease-free plantlets, as well as seed rhizome development, have been carried out on a constant basis to aid resilience. The same technique has been used for sweet potato to produced virus-free planting materials through shoot tip culture:

In vitro conservation of Ensete (*Ensete ventricosum*) plant: Ensete (*E.ventricosum*) is a perennial crop produced primarily for its corm and multi-layered pseudo stem (flesh) for human and animal use. The domesticated form of the plant is only grown in Ethiopia, where it is a staple food for approximately 20 million people (Wilkin et al., 2019). The crop is most likely grown in east Africa, specifically in Ethiopia's south and southwest (Yemata, 2020). Ensete is also widely distributed geographically in tropical Africa, Madagascar, and parts of Asia (Demissew et al., 2018) in Ethiopia, over 600 ensete landraces collected from major ensete-growing areas have been conserved *ex situ* in the gene bank at Areka Agricultural Research Center(Yemataw et al., 2017). As a national coordinating center and a center of excellence for root and tubers crop research in Ethiopia (Wandui et al., 2013), devising an in vitro regeneration, multiplication, and conservation technique for released types of ensete is one of the most important duties entrusted to the Areka tissue culture lab. As a result, the variety Miaziya has been successfully entirely in vitro regenerated (figure1.), while additional varieties like as Yambule and Zereta have been maintained in a green house for subsequent regeneration.

Screening and evaluation of antibiotics against microbial contaminants: Microbial contamination is a major risk in tissue culture lab (Wakil & Mbah, 2012). Contamination in tissue culture can originate from two sources, either through carryover of microorganisms on the surface or in the tissues of explants, or through faulty procedures in the laboratory (Cassells, 1988). Plants that sprout from subterranean shoot buds, in particular, carry both epiphytic and endophytic microbial contaminants. Endophytic bacterial contamination is one of the most significant issues that ginger production faces when employing tissue culture techniques. Antibiotics with a broad spectrum of activity against bacteria are expected to be low in toxicity to eukaryotes and have been used as selection markers in plant transformation techniques (Eziashi et al., 2014). As a result, antibiotics are likely to be tested for their capacity to limit or eradicate microbial growth in in vitro cultures of banana, ensete, and ginger.

Achievements: The lab's overall production capacity has a tendency to rise with time. However, depending on the region's demands, the quantity of in vitro plantlets produced by different crops varied over time. As a result, the number of plantlets in vitro propagated by pineapple grows dramatically in 2019 (see figure3). During the periods, over 6000 in vitro generated planting materials of improved pineapple types (smooth cayenne) were delivered to producers, and over 30 thousand in vitro pineapple plantlets were in the growing chamber, ready for acclimatization and distribution to farmers. About 2500 banana plantlets (variety poyo) were in vitro reproduced and distributed to farmers. In addition, two banana varieties (Grand naine and Dwarf Cavendish) have been initiated and are being multiplied (unpublished work).

A series of activities were accomplished phase by phase for the production of disease-free ginger planting material, including shoot tip in vitro regeneration, in vitro multiplication, acclimatization, seed rhizome production in a greenhouse, and extended field production in two marginal areas (out of disease spots), Humbo and kucha (figure2). Ginger seed rhizome multiplication is being done in collaboration with a youth cooperative to provide a continuous and sustainable seed supply.

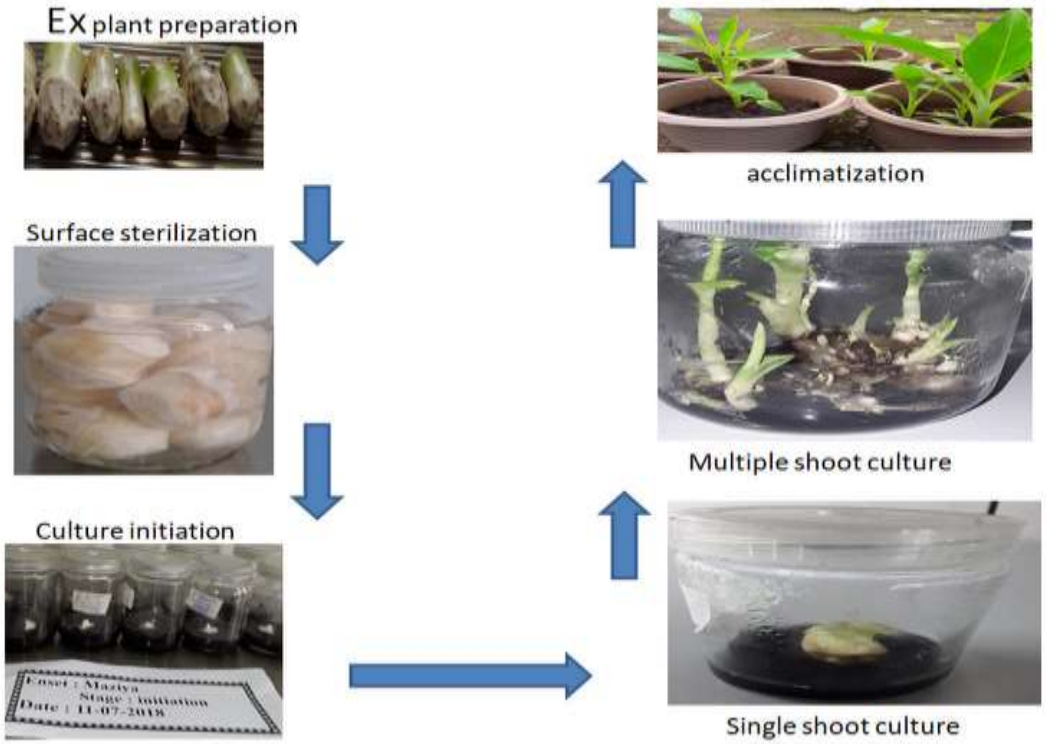

Figure1. In vitro regeneration of Enset (*E.ventricosun*) at Areka tissue culture lab.

Figure2. Bacterial wilt free ginger planting material production processes

Sweet potato clean planting materials were multiplied in vitro. Clean materials of several local varieties of sweet potato /kulfo, erihae, Erica, beletech, and hawassa-83/ were grown and acclimatized in a green house for vine production. A virus indexing experiment was carried out on samples obtained from various zones of production and TC plants, and suitable information was delivered to the area's farmers.

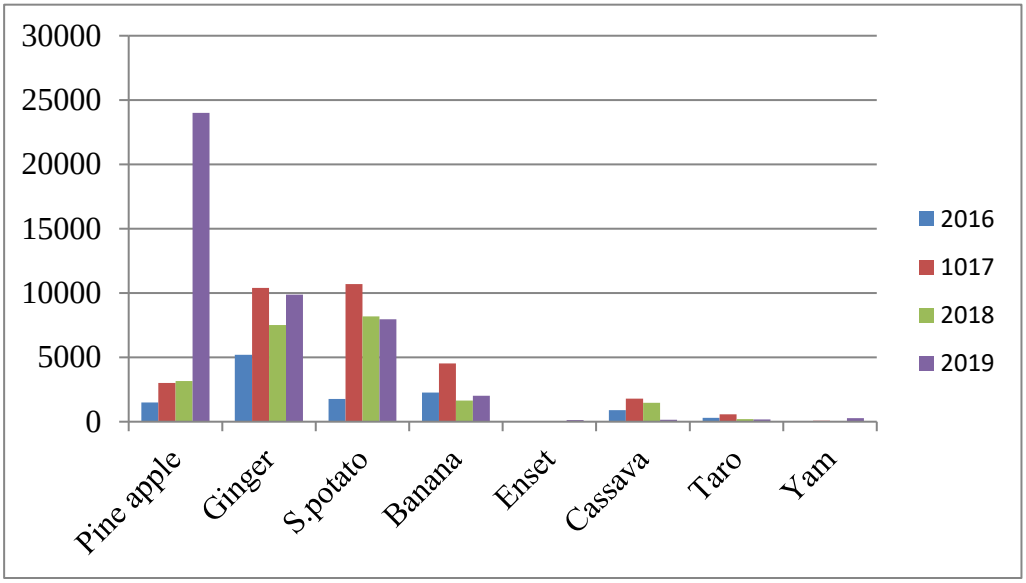

Figure3.

In vitro production trends from the year 2016 to 2019

Challenges and opportunities: Uniform light dispersion in the growth room for at least the minimum needed duration is a pre-requisite for having healthy plants. Because of a lack of photosynthesis due to low light quality and insufficient light availability, blacking and plant death have been recorded. Power fluctuations have resulted in not just plant death but also damage to sensitive equipment. Microbial contamination arises in growth media as a result of autoclave malfunction or improper handling of temporary media storage (Requirements, 2013). Medium contamination is a significant loss in tissue culture because seemingly clean media acquires latent contamination, which can result in total culture loss (Ogunsanwo, 2014). Prolonged culture media in autoclave, on the other hand, have resulted in color change and bad media quality in attempt to compensate for inadequate sterilizing conditions. standard green house features a balanced atmosphere for adapting plants grown in vitro (Kshitij, 2012). Controlled humidity and temperature are often the most important variables to meet during acclimatization for in vitro plants, which lack structured cell components, particularly hard cuticles to prevent fast evapotranspiration (Donnelly, 2015). Green house installation with poor materials (poly house) has a greater effect on raising temperature than the outside environment, leading the plants to experience rapid evapotranspiration. Lab setup and extra rooms for large-scale in vitro multiplication have been identified as promising potential. Expansion of agro industrial parks in the region necessitates healthy and high-quality output, which has raised demand for in vitro plants of various fruit crops such as banana, pineapple, and mango. Thus, future research objectives cover everything from lab development to semi-commercialization.

Conclusion

Tissue culture techniques vary depending on the scope of the research, the objectives, and the internal facilitations. To conduct any type of tissue culture research, the plant tissue culture lab requires structurally standard set ups and well-organized facilitations. Microbial contamination, which can occur from plant sources, media, and equipment, is a major risk in tissue culture labs. In order to conduct any type of tissue culture research, any tissue culture lab must maintain an aseptic condition. As a result, in vitro regeneration of ginger, ensete, and taro was extremely difficult because the crops' ex plant sources were from underground buds, which harbored microbial contaminants. However, there are always techniques and trends that can be used depending on the researcher and the lab conditions. Nonetheless, the challenges have been overcome through techniques such as surface sterilization and the use of antimicrobial agents. Despite the challenges and limitations, efforts to fully exploit the lab potentials to generate distinct in vitro plants, particularly disease free planting materials from infected plants, are encouraging trends that can be used as a lesson for future progress. Therefore, the overall trends illustrate that tissue culture technique has a vital role in large-scale multiplication, germplasm conservation and in vitro production of disease free planting materials.

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